CHAPTER 16

The conservation biologist's toolbox – principles for the design and analysis of conservation studies

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"Conservation biology" is an integrative branch of biological science in its own right (Chapter 1); yet, it borrows from most disciplines in ecology and Earth systems science; it also embraces genetics, dabbles in physiology and links to veterinary science and human medicine. It is also a mathematical science because nearly all measures are quantified and must be analyzed mathematically to tease out pattern from chaos; probability theory is one of the dominant mathematical disciplines conservation biologists regularly use. As rapid human-induced global climate change (Chapter 8) becomes one of the principal concerns for all biologists charged with securing and restoring biodiversity, climatology is now playing a greater role. Conservation biology is also a social science, touching on everything from anthropology, psychology, sociology, environmental policy, geography, political science, and resource management (Chapter 14). Because conservation biology deals primarily with conserving life in the face of anthropogenically induced changes to the biosphere, it also contains an element of economic decision making (Chapter 14). This is a big toolbox indeed, so we cannot possibly present all aspects here. We therefore focus primarily in this chapter on the ecological components of conservation biology (i.e. we concentrate on the biology *per se*).

Conservation biology, and the natural sciences in particular, require simplified abstractions, or models, of the real world to make inferences regarding the implications of environmental change. This is because ecosystems are inherently complex networks of species interactions, physical constraints and random variation due to stochastic (random) environmental processes. The conservation biologist's analytical toolbox therefore comprises methods that mainly serve to simplify the complexity of the real world such that it is understandable and (partially) predictable. The quantification of these relationships – from the effects of habitat loss on biodiversity (Chapter 4) to the implications of small population size for extinction risk (Chapter 10) – is the backbone of analytical conservation biology and evidence-based decision making. Without quantified relationships and robust measures of associated uncertainty, recommendations to improve biodiversity's plight via management intervention or policy change are doomed to fail.

Even though we have chosen to focus on the techniques dealing with the biological data in the conservation realm, we can by no means be comprehensive; there are simply too many ideas, metrics, tests, paradigms, philosophies and nuances to present within a single chapter of this book. However, we have striven to compile a compendium of the major approaches employed along with a list of the best textbook guides and peer-reviewed scientific papers providing the detail necessary for their implementation. We first present measures of biodiversity patterns followed by a general discussion of experimental design and associated statistical paradigms. We then introduce the analysis of abundance time series followed by assessments of species' fate risks. The final section is a brief introduction to genetic tools used to assess a species' conservation status. Although issues of reserve

design and their associated algorithms are an essential part of the conservation biologist's toolbox, they have been discussed in detail elsewhere in this book (Chapter 11) and so do not feature in this chapter.

16.1 Measuring and comparing 'biodiversity'

Chapter 2 provides an excellent overview of the somewhat nebulous concept of 'biodiversity' and a brief mention of how it can be measured, and Chapter 11 introduces the concept of 'surrogacy' (simplified measures of biodiversity patterns) in conservation planning. Here we develop these concepts further with particular emphasis on practical ways to obtain comparable and meaningful metrics over space and time. It should be noted that regardless of the logistic constraints, biological consideration and statistical minutiae driving the choice of a particular set of metrics for biodiversity, one must not forget to consider the cost-benefit ratio of any selected method (Box 16.1) or the difficulties and challenges of working across cultures (Box 16.2).

16.1.1 Biodiversity indices

It is simply impossible to measure every form of life (Chapter 2), regardless of the chosen metric or focal taxon, due to the sheer number of species and the difficulty of sampling many of the Earth's habitats (e.g. ocean depths and tropical forest canopies). We are therefore required to simplify our measurements into tractable, quantifiable units that can be compared across time and space. The simplest and perhaps easiest way to do this has traditionally been to use organism-based metrics that count, in one way or another, the number of 'distinct' species in a defined area. Species richness is therefore the base currency used for most biodiversity assessments, but it can be complicated by adjusting for relative abundance, uniqueness, representativeness, spatial scale or evolutionary history.

As mentioned above, a direct count of the number of species within a defined area is known as *species richness* ($\hat{S}$). Species richness can be corrected for total abundance (number of individuals) to produce the *diversity* index better known as *Simpson's Diversity Index* $(1 - \hat{D})$ (Simpson 1949):

Box 16.1 Cost effectiveness of biodiversity monitoring
Toby A. Gardner

There is a shortage of biological data with which to meet some of the primary challenges facing conservation, including the design of effective protected area systems and the development of responsible approaches to managing agricultural and forestry landscapes. This data shortage is caused by chronic under-funding of conservation science, especially in the species-rich tropics (Balmford and Whitten 2003), and the high financial cost and logistical difficulties of multi-taxa field studies. We must therefore be judicious in identifying the most appropriate species groups for addressing a particular objective. Such focal groups are varyingly termed 'surrogates' or 'indicators'. However, indicators are often chosen subjectively on the basis of anecdotal evidence, 'expert' opinion, and ease of sampling. This common approach has resulted in finite resources being wasted on

the collection of superficial (including the 'record everything' mantra) and unrepresentative biodiversity data that may be of only limited value. This failing threatens to erode the credibility of conservation science to funding bodies and policy makers.

To maximize the utility of biodiversity monitoring, it should adhere to the concepts of *return on investment*, and *value for money*. In essence this means that field-workers need to plan around two main criteria in selecting which species to sample: (i) what types of data are needed to tackle the objective in hand; and (ii) feasibility of sampling different candidate species groups. Practical considerations should include the financial cost of surveying, but also the time and expertise needed to conduct a satisfactory job. Species groups that satisfy
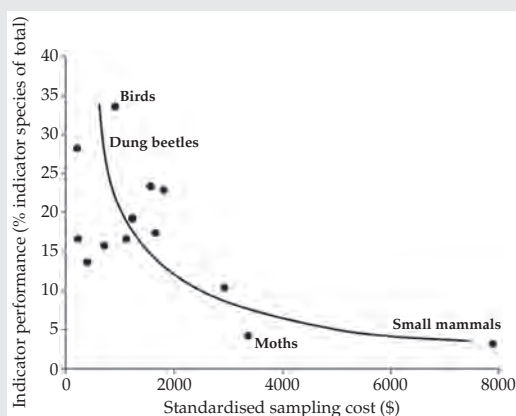

Box 16.1 (Continued)

both demands can be thought of as having a 'high performance'.

Using a large database from work in the Brazilian Amazon, Gardner *et al.* (2008) recently presented a framework and analytical approach for selecting such high performance indicator taxa. The objective of that study was to provide representative and reliable information on the ecological consequences of converting tropical rainforest to *Eucalyptus* plantations or fallow secondary regeneration. An audit was conducted of the cost (in money and time) of sampling 14 groups of animals (vertebrates and invertebrates) across a large, managed, lowland forest landscape. Notably, survey costs varied by three orders of magnitude and comparing standardised costs with the indicator value of each taxonomic group clearly demonstrated that birds and dung beetles (Coleoptera: Scarabaeinae) are high-performance groups – they provide the most amount of valuable information for the least cost. By contrast, other groups like small mammals and large moths required a large investment for little return (see Box 16.1 Figure). The fact that both birds and dung beetles are well-studied and perform important ecological functions gives further support to their value for biodiversity monitoring and evaluation. This important finding will help conservation biologists in prioritising the study of the effects of deforestation on land-use change in the Amazon, allowing them to design cost-effective field expeditions that will deliver the most useful information for the money available.

Finally when planning biodiversity surveys it is also important to consider how the data may be used to address ancillary objectives that may ensure an even greater return on investment.

One example is the opportunity to synthesise information from many small-scale monitoring programs to provide robust nation-wide assessments of the status of biodiversity without needing to implement independent studies. A better understanding of the distribution of species in threatened ecosystems will improve our ability to safeguard the future of biodiversity. We cannot afford to waste the limited resources we have available to achieve this fundamental task.

Box 16.1 Figure Cost effectiveness of different species groups for indicating habitat change in a multi-purpose forest landscape in Brazilian Amazonia.

REFERENCES

Balmford, A. and Whitten, T. (2003). Who should pay for tropical conservation, and how could the costs be met? *Oryx*, **37**, 238–250.

Gardner, T. A., Barlow, J., Araujo, I. S., *et al.* (2008). The cost-effectiveness of biodiversity surveys in tropical forests. *Ecology Letters*, **11**, 139–150.

$$1 - \hat{D} = \frac{\Sigma_{i=1}^{S} n_i(n_i - 1)}{N(N - 1)}$$

where S = the number of species, N = the total number of individual organisms, and n_i = the number of individuals of species i. The unique-

ness of species in a sample can be incorporated by using indices of *evenness* (also known as *equitability*), of which *Shannon's Index* (H; also known mistakenly as the *Shannon-Weaver* index, or correctly as the *Shannon-Weiner* index) is the most common:

Box 16.2 Working across cultures
David Bickford

Establishing conservation projects in countries with cultures and languages that are different from your own can be both daunting and challenging. Without proper thoughtfulness, openness, flexibility, and (most importantly) humour, these projects fail for reasons that are often difficult to distil. All conservation projects involve a mix of stakeholders (local people, scientists, conservation practitioners, governmental and public administrators, educators, community leaders, etc.) that may have widely different expectations and responsibilities for the project. Having worked on both successful and failed projects with a diversity of people in nine countries and six languages, much of what I have learned can be summed up in two simple yet powerful ideas for all stakeholders: *clear communication* and *equity*. The two are intricately linked.

Clear communication is an ideal often sought after, yet rarely achieved. No matter the socio-cultural context, a common denominator of transparency is necessary for a successful conservation project. Having stakeholders explicitly state their intentions, desires and goals is a good start. It also helps elicit traditional or anecdotal knowledge that can be useful in formal analysis (e.g. as Bayesian priors, see Box 16.4). Methods, benefits, and responsibilities should be outlined and agreed upon, as well as limits of what objective(s) each stakeholder perceives as 'bare minimum'. A common pitfall is an inability for leaders to communicate effectively (for many and sundry reasons), re-enforcing top-down stereotypes. Lateral communication (peer-to-peer) can be more effective and avoids many constraints imposed by translating among different languages or cultures, effectively levelling the playing field and enabling everyone to participate (at least for heuristic purposes). Activities that enhance transparent communication include small group discussions, workshops, regular and frequent meetings, project site visits and even informal gatherings such as shared meals or recreational activities.

Almost all social hierarchies involve some component of conflict based around inequity.

People want to balance their personal costs and benefits relative to others'. Conservation projects should, wherever possible, bridge gaps and narrow divides by developing equitably among stakeholders. By alleviating large disparities in cost:benefit ratios, responsibilities, and expectations between different stakeholders, the project will become more efficient because there will be less conflict based on inequity. Equity will evolve and change, with stakeholders adapting to behave fairly in a transparent system. In general, teams will reward members who treat others unselfishly and promote the overall goals of the group.

To achieve such a framework of open communication and equity, impartial leadership and long periods of interpersonal relationship building are often required. As hackneyed as they seem, capacity-building exercises, when done correctly, are excellent mechanisms of sharing information and building the competency to use it. Engaging and training local or regional counterparts is an outstanding method for ensuring clearer communication and promoting fairness, instead of forcing information from the top-down and expecting results to emerge from the bottom-up. Further links between transparency and equity can be realised through 'hands-on' applications instead of just talking about concepts. Leaders should participate at all levels, learning the most menial tasks associated with the project (e.g. an administrator should go and catch frogs for a monitoring project).

In the broadest terms, working across cultures is a high risk-high reward system. Although there are complex obstacles, the ultimate litmus for biodiversity conservation might be our ability to learn and work together across cultures to preserve nature.

SUGGESTED READING

Reed, M.S. (2008). Stakeholder participation for environmental management: a literature review. *Biological Conservation*, **141**, 2417–2431.

$$H' = \sum_{i=1}^{s} \frac{n_i}{N} \, \log \, {}_e\left(\frac{n_i}{N}\right)$$

The index provides a measure of the amount of disorder in a system, such that communities with more unique species have higher H (a system with $S = 1$, by this definition, is perfectly ordered but has no diversity). Most of these measures assume a random sampling of species within a community, but this assumption is often violated (Pielou 1966). When sampling is done without replacement, then indices such as Brillouin's H are recommended:

$$H = \frac{1}{N} \, \log \left(\frac{N!}{n_1! n_2! n_3 \dots}\right)$$

However, where representativeness is unknown, then *rarefaction* or *resampling* can be used to standardize samples from different areas or periods to a comparable metric (Krebs 1999). This includes inferring the total diversity of a community by using a statistical model to predict unobserved data (unsampled species). Of course, the measures presented here are the basic foundations of species diversity indices, but there are myriad variants thereof, many assumptions that can be tested and adjusted for, and different distributions that may be more or less important under particular circumstances. For an excellent overview of these issues, we recommend the reader refers to Krebs (1999).

16.1.2 Scale

Interpretation of the indices and their variants described above depend on the scale of measurement. Whittaker (1972) introduced the concepts of *alpha* (α), *beta* (β), and *gamma* (γ) *diversity* to measure and compare biodiversity patterns over various spatial scales. α (local) diversity refers to the quantification of species richness, etc. within a particular area or ecosystem, whereas β diversity (differentiation) is the difference in the metric between ecosystems. In other words, β diversity is a measure of species uniqueness between areas, so as β diversity increases, locations differ more from one another and sample a smaller propor-

tion of the total species richness occurring in the wider region (Koleff *et al.* 2003).

Whittaker (1972) sensibly recommended that β diversity (Whittaker's β_w) should be measured as the proportion by which the species richness of a region exceeds the average richness of a single locality within that region:

$$\beta_w = \frac{S}{\bar{\alpha}} = \frac{(a + b + c)}{\frac{(2a+b+c)}{2}}$$

where S = the total number of species recorded for all sites (regional richness) and the average number of species found within sites (local richness), a = the number of species in common in both sites (e.g. for a simple two-site comparison), b = the number of species in site 1, and c = the number of species in site 2. Since then, however, many other variants of the metric have been proposed. These include comparisons along spatial or environmental gradients, between patches of similar habitats, and the degree of similarity between sites (see references in Koleff *et al.* 2003). Indeed, Koleff *et al.* (2003) reviewed 24 different measures of β diversity and categorized them into four main groups: measures of (i) continuity (similarity in species composition among sites) and loss (fewer species relative to focal sites); (ii) species richness gradients; (iii) continuity only; and (iv) gain and loss. Not only is there lack of agreement on the most appropriate measure to use, there is also variation in the pattern of scaling applied. As such, Koleff *et al.* (2003) suggested that one should use measures that exhibit the homogeneity property (i.e. the measure is independent of the total number of species as long as the proportions comprising the different components are constant) and that when measures reveal different patterns of variation when based on absolute and proportional species numbers, both types should be examined.

γ diversity is otherwise known as "geographic-scale species diversity" (Hunter 2002), which means it is used as a measure of overall diversity for the different constituent ecosystems of a region. This metric becomes particularly valuable to explain broad-scale (regional or continental) patterns of species relative to local (site-specific) indices. Indeed, there are two theoretical types of

relationships hypothesized for local versus regional species richness (Figure 16.1). Most datasets support the existence of a proportional relationship between local and regional richness (Type I), albeit local richness always tends to be less than regional (Gaston 2000). It appears that Type II relationships (local richness reaching an asymptote) are rare because local assemblages do not seem to become saturated as one might expect from ecological mechanisms such as density dependence, parasitism and predation (Gaston 2000).

16.1.3 Surrogacy

An important goal of conservation biology, which deals with a world of limited resources and options, is to protect areas that have relatively higher biodiversity than surrounding areas. Prioritizing areas for conservation, however, does not always require a complete description of a site's biodiversity, but merely relative measures of differences among them (Margules *et al.* 2002) described using a representative taxonomic subset. The quest for a simple estimator, a *surrogate* (i.e. the number, distribution or pattern of species in a particular taxon in a particular area thought to indicate a much wider array of taxa) that is sufficiently related to the biodiversity pa-
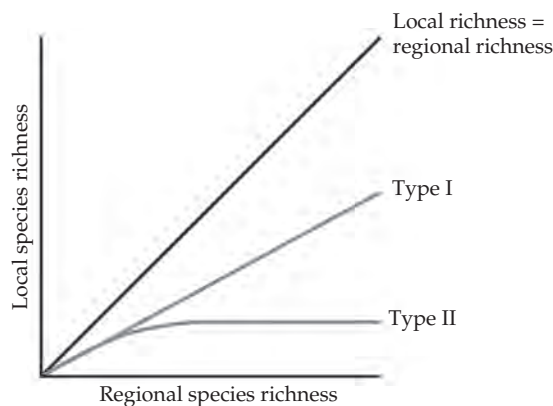
rameter of interest is an essential tool in conservation planning (see Chapter 11).

Unfortunately, there is no consensus regarding which surrogates are best for what purposes among ecosystems – many problems with current surrogate approaches remain. For instance, focusing only on a set of species-rich sites may select only a single habitat type with similar species in all areas, thus many rare species may be excluded from protection (Margules and Pressey 2000). Many methods to overcome these problems have been developed based on multivariate measures of biodiversity (e.g. multi-taxa incidence matrices) or reserve-selection algorithms (e.g. Sarkar and Margules. 2002). Advances have been made with recent work (Mellin *et al.* In review) examining surrogate effectiveness in the marine realm. It was shown that higher-taxa surrogates (taxonomic levels such as order, family or genus acting as a surrogate for some lower taxonomic level such as species) outperform cross-taxa (one taxon is used as a surrogate for another at the same taxonomic resolution) and subset-taxa (diversity in one taxonomic group is taken as representative of the entire community) surrogates. Likewise, surrogacy was least effective at broad (> 100 km) spatial scales.

16.1.4 Similarity, dissimilarity, and clustering

Although indices of biodiversity take on different aspects of species richness, abundance, evenness and scale, there are many relatively simple techniques available for comparing samples of species and individuals among sites. Most indices of similarity (> 25 types exist – Krebs 1999) are simple descriptors that do not lend themselves easily to measures of uncertainty (e.g. confidence intervals; although resampling methods can provide an index of parameter uncertainty), so their application is generally exploratory. There are two broad classes of similarity: (i) binary; and (ii) quantitative. Binary measures are applied to presence-absence data (i.e. does a species exist in a defined area?) and can be compared among sites using contingency tables using metrics such as Jaccard's similarity, Sorren's similarity, simple matching, or Baroni-Urbani and Buser

Figure 16.1 Hypothesized relationship between local and regional species richness (number of species). Type I occurs where local richness is proportional to, but less than, regional richness; Type II demonstrates situations where local richness asymptotes regardless of how much regional richness increases. Reprinted from Gaston (2000).

coefficients (see Krebs 1999). Of course, some method to assess the probability of missing species in presence-absence surveys should also be applied to account for insufficient sampling effort (e.g. MacKenzie *et al.* 2002).

Quantitative indices require some aspect of individual abundance to be assessed such as the number of individuals, biomass, cover or productivity. Distance dissimilarity indices using abundance data instead of species richness can be applied to the same binary indices listed above. Alternatively Euclidean, Manhattan, Canberra or Bray-Curtis distances between samples can be calculated using relative abundance measures between sites (see Krebs 1999). Simple correlation coefficients such as Pearson product-moment, Spearman's rank and Kendall's τ can also be used in certain situations to compare sites, but these tend to be insensitive to additive or proportional differences between community samples (Romesburg 1984) and they depend strongly on sample size (generally, $n > 30$ is sufficient for a reliable characterization of the relationship).

When many focal communities are sampled, some form of cluster analysis may be warranted. Cluster analysis refers to any technique that builds classifications, but there is no preferred method given that the choice depends on the type of data being compared. Some considerations for choice include whether the data are: (i) hierarchical (e.g. taxonomic classifications) or reticulate (overlapping classifications); (ii) divisive (sample divided into classes) or agglomerative (fine to coarse resolution); (iii) monothetic (groups distinguished by a single attribute) or polythetic (many attribute-based); or (iv) qualitative (binary) or quantitative (distance measures) (see Krebs 1999 for an overview).

16.1.5 Multivariate approaches

When the principal aim of a conservation study is to quantify the relationships between a large number of measurements, whether they be of species, individuals or abiotic predictors of ecological patterns, some form of multivariate analysis is usually required. Over thirty different multivariate techniques have been designed for various applications (Pérez *et al.* 2008), each with their own particular strengths and weaknesses. Ordination describes those methods that summarize multivariate information in a low-dimensional scatter diagram where points represent samples and distances among them are proportional to their similarity measured, for example, by Euclidean distance, Bray-Curtis or other indices. Common techniques include eigen-based principal components analysis (PCA) or correspondence analysis (CA) and distance-based multidimensional scaling (MDS), cluster analysis or polar ordination that provide coefficients quantifying the relative contribution of component variables to the reduced-dimension principal axes.

Such multivariate approaches are useful for visualizing patterns that would otherwise be difficult or impossible to discern in multidimensional space, such as ecologically related species assemblages or trophic guilds. They can also summarize the principal gradients of variation within and among communities and condense abiotic and other potential explanatory variables (e.g. climate, soil conditions, vegetation structure, chemistry, etc.) into simple gradients themselves that may be used as correlates to explain variation in species or community patterns. Their disadvantage is that they cannot be used to test the relative likelihood of alternative hypotheses, may not appropriately reflect statistical power and effect size, and if applied incautiously, can be misused to mine data for phantom 'patterns' that on closer examination turn out to be random noise or system-specific peculiarities.

16.2 Mensurative and manipulative experimental design

Conservation biology typically deals with assessments of previous environmental degradation and the quantification of its effects on biodiversity patterns. Another major aim is to design ways of preserving existing, relatively intact communities through management intervention (e.g. reserve design, control of harvest). Conservation biologists also devote a large proportion of their efforts to quantifying the most efficient and

effective methods for restoring degraded habitats to some semblance of previous ecological function. These three principal aims, and the logistical constraints on large-scale system manipulations, generally preclude the use of strict experimental design and control – there are simply too many extenuating variables modifying species patterns to control, and the systems of interest are generally too expensive to apply meaningful manipulations such as those which typify medical experimentation.

There are some notable exceptions to this rule, such as replicated microcosm experiments examining the processes of extinction in rapidly reproducing invertebrate populations. For example, the frequency of extinction times under conditions of low and high environmental variability (Drake 2006), the persistence probability of populations exposed to various spatial configurations of refugia and intensities of harvest (Fryxell *et al.* 2006) and the implications for extinction risk of chaotic and oscillatory behavior in populations (Belovsky *et al.* 1999; Hilker and Westerhoff 2007), have all been successfully examined in controlled laboratory settings. Other well-known manipulations at broader spatial scales (albeit with far less experimental control) include examining the effects of forest fragmentation on species diversity (Laurance *et al.* 2002), controlling the size and configuration of agricultural plots to test bee pollination success (Brosi *et al.* 2008), examining the effects of landscape composition on the initial dispersal success of juvenile amphibians (Rothermel and Semlitsch 2002), determining the effects of inbreeding depression on individual survival (Jimenez *et al.* 1994), measuring arthropod responses in tropical savannas exposed to repeated catchment-scale prescribed burning (Andersen and Müller 2000) and the many applications of Before-After-Control-Impact (BACI) experimental designs to detect point-source changes to systems (Underwood 1994).

The above notwithstanding, most conservation studies rely mainly on quantifying existing patterns (observational studies) or take advantage of existing gradients or measurable differences in habitat quality or type to infer mechanisms. This latter category is sometimes referred to as *mensurative* experimentation because it does not explicitly control for confounding variables (Hurlbert 1984). There has been plenty of discussion on this topic over the past twenty or so years (Hurlbert 1984; Krebs 1991; Hargrove and Pickering 1992; Oksanen 2001; Hurlbert 2004; Oksanen 2004), but it is now accepted among most conservation biologists that to make strong inferences on biological patterns and mechanisms, multiple lines of evidence, from observational, mensurative and manipulative experiments, are all required at various spatial and temporal scales (Brook *et al.* 2008).

16.2.1 Hypothesis testing

The classic scientific approach adopts the concept of *falsifiability* (Popper 1959) – that is, demonstrating that a mechanism or phenomenon is not true (null hypothesis) by controlling all other plausible determinants except the one of interest and replicating the experiment sufficiently to avoid spurious patterns that may arise simply by chance (see section below). This is still a core aspect of science because it reduces the chance of making subjective interpretations of the data collected. This is the philosophical basis for the majority of the statistical techniques used by natural scientists; we attempt to discern pattern from the 'noise' in natural systems using theory to estimate the probability that our observations could have been derived merely by chance.

Neyman-Pearson null hypothesis testing (NHT) begins with the assertion that no differences exist between experimental units (null hypothesis), with the implicit view that if the null is unsupported by the data, then one or more 'alternative' hypotheses must therefore be plausible (although these are not explicitly evaluated). Classic statistical theory that has been developed around the NHT approach provides methods to estimate the chance of making an error when rejecting the null hypothesis (*Type I or* α *error*); in other words, this is the probability of concluding that there is a difference (or effect) when in fact, there is none. The flip side to this is that classic NHT tests do not provide an estimate of

the probability of making an error when failing to reject the null hypothesis (known as *Type II* or *β* error) – this is essentially the chance one concludes there is no difference (or effect) when in fact, there is. Various *a priori* and *a posteriori* methods exist to estimate Type II errors (more precisely, the *power* of a statistical test taken as 1 – Type II error), with the latter depending on three principal elements: sample size (see below), magnitude of the difference one is attempting to detect (effect size) and the total variance associated with the measure used (see Gerrodette 1987; Osenberg *et al.* 1994; Steidl *et al.* 1997; Thomas 1997; Thomas & Krebs 1997; Thompson *et al.* 2000 for more detail on power analyses).

The disconnect between these two estimates of hypothesis-conclusion error, the implicit conflation of effect size and sample size, as well as the ambiguity related to just *how much chance of making an error is acceptable* (i.e. the moribund and bankrupt concept of statistical 'significance' beyond some arbitrary threshold), have formed for decades some of the main arguments against

using NHT (reviewed in Elliott and Brook 2007, see also Burnham and Anderson 2002; Lukacs *et al.* 2007). This is especially true in the ecological and psychological sciences, which are typically restricted to observational studies and subject to extensive variability. The alternative approaches can be classed into the general category of *multiple working hypotheses* (MWH), including best-model selection and multimodal inference (Box 16.3). MWH approaches are now becoming recognized as providing the most logical and objective approaches to assess conservation issues because they explicitly consider uncertainty in the underlying models used to abstract the real world, rather than relying on simple and arbitrarily assessed 'yes-or-no' conclusions typical of the NHT paradigm.

16.2.2 Sample size

Regardless of the statistical paradigm invoked or analysis method applied, perhaps the least controversial requirement of good scientific inference

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Box 16.3 Multiple working hypotheses
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Science is, at its core, all about evaluating the support for different ideas – working hypotheses – about how the world works. Because they never reflect the totality of real-world effects, any such hypothesis can be considered a model. But how to decide what ideas have support and which ones should be discarded?

A traditional approach has been to set up some null model (which states that there is no change or measureable effect in a variable of interest), and then proceed to evaluate whether the data conform to this model. This usually involves the arbitrary selection of a threshold probability of making Type I errors (i.e. failing to reject a null hypothesis when it is true) to conclude so-called 'significance' of effect. This line of reasoning still pervades most probabilitistic sciences today. Yet many have called for the abandonment of such subjective statistical practices (Burnham and Anderson

2004; Lukacs *et al.* 2007) in favour of a concept originally forwarded in 1890 by Thomas C. Chamberlin known as *multiple working hypotheses* (Elliott and Brook 2007). The idea is relatively simple – instead of considering a single (null) hypothesis and testing whether the data can falsify it in favour of some alternative (which is not directly tested), the use of multiple working hypotheses does not restrict the number of models considered to abstract the system under investigation. In fact, the approach can specifically accommodate the simultaneous comparison of hypotheses in systems where it is common to find multiple factors influencing the observations made (such as complex ecological systems). This is also particularly applicable to conservation biology because experimental manipulation is often technically difficult or ethically unreasonable.

The basic approach is to construct models (abstractions of complex systems) that

continues

Box 16.3 (Continued)

represent combinations of hypotheses constructed to explain variation in the metric of interest. Models (plausible hypotheses) then can be ranked or compared on the basis of relative evidential support, using methods that tend to reinforce the principle of parsimony (the simplest combination of factors providing the strongest explanatory power) via their bias correction terms. Model comparison based on information theory (usually assessed using Aikaike's information criterion – AIC – when conforming to maximum likelihood approaches – Box 16.4) immediately supposes that all models are false because they represent incomplete approximations of the truth (Elliott and Brook 2007). Weighting AICs then can be used as a means to assess the relative distance to 'truth' by approximating Kullback-Leibler information loss (i.e. measuring the relative distance between conceptual reality and the abstraction under consideration). The Bayesian information criterion (BIC) is a dimension-consistent form of model comparison that provides a measure of the weight of evidence relative to other models (the Bayes factor – see Box 16.4), assuming uninformative prior information. As sample sizes increase, BIC approaches the estimation of the dimension of a 'true' model (not necessarily embedded in the model set) with a probability = 1 (Burnham and Anderson 2004). Here the true model is one which captures main effects but ignores minor (tapering) influences.

It is generally accepted that AIC performs well when sample sizes are small (and AIC itself can be corrected to account for small samples), but it is *a priori* weighted to favour more complex models when tapering effects (biologically important signals that characterise full truth but defy reductionism) are present (Link and Barker 2006). When the aim is to determine the most important variables explaining variation in some measured 'response', BIC is recommended, especially when sample sizes are large (Link and Barker 2006). When prediction is the goal, AIC-based rankings are preferred.

Multimodel inference is gaining increasing popularity in conservation biology because it embraces the concept of multiple working hypotheses to describe complex systems. Rather than choose a single 'best' model (or not even test alternative models, as per null hypothesis testing), multimodel inference is made on the basis of all models in the *a priori* candidate set; here, each model's prediction is weighted by its relative support from the data (e.g. AIC weights or Bayesian posterior probabilities – see Box 16.4) (Burnham and Anderson 2002; Burnham and Anderson 2004; Elliott and Brook 2007). Thus, multimodel inference is advantageous because it accounts for uncertainty in the underlying choice of models used to describe the system of interest, it permits inference from different models simultaneously, and it allows for unconditional ranking of the relative contribution of variables tested (Elliott and Brook 2007). Of course, no inference is made on models/variables not included in the *a priori* model set.

The cases where null hypothesis testing can be justified (see Johnson and Omland 2004; Stephens *et al.* 2005; Stephens *et al.* 2007) are rare in conservation biology for the reasons described above (system complexity, lack of experimentation potential). It is our opinion that the multiple working hypotheses approach, even for relatively simple assessments of effect, should embrace the philosophy of estimating the *strength of evidence* and avoid the pitfalls associated with arbitrary Type I error probability thresholds. This can be usefully done even for a comparison of a null model to a single alternative, using evidence factors (the ratio of AIC or BIC weights of the two models – a concept akin to Bayesian odds ratios) and is preferable to a classic null hypothesis test because the likelihood of the alternative model is explicitly evaluated.

The basic formulae for the most common model-ranking criteria (AIC, AIC_c, QAIC and BIC) are provided below:

Box 16.3 (Continued)

$$\text{AIC} = -2L + 2k$$

where AIC = Akaike's information criterion, k = number of model parameters and L = the maximised log-likelihood function for the estimated model (MLE). Note that the variance term of a statistical model, when estimated (e.g. in a Gaussian model), is a parameter.

$$AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$$

where AIC_c = AIC corrected for small sample size and n = sample size.

$$QAIC = \frac{1}{\hat{c}} 2L + 2k$$

where QAIC = quasi-AIC and $\hat{c}$ = the variance inflation factor (when data are over-dispersed). This is commonly used in capture-mark-recapture model assessments (see White and Burnham 1999). The small-sample version of QAIC ($QAIC_c$) is calculated the same way as AIC_c. The Bayesian information criterion (BIC) is calculated as:

$$-2\log_e p(x|k) \approx BIC = -2L + k\log_e n$$

where x = observed data and $P(x|k)$ = the likelihood of x given k which is the same as the MLE used in AIC.

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in conservation biology is obtaining measurements from as many representative and unbiased *units* (individuals, plots, habitats, ecosystems, etc.) as possible. The main reason for obtaining large sample sizes is that when one measures only a few units, the chance of obtaining a good estimate of the central tendency (e.g. mean or median), variance (i.e. the spread of true values), or distribution (i.e. shape of the frequency distribution of units such as Normal, binomial, log-Normal, etc. and extreme values which characterize the tails of distributions) of a parameter is low. Without good estimates of such parameters, the ability to tease pattern and noise apart becomes increasingly intractable.

There are no rules of thumb for 'adequate' sample sizes because they depend on the hypothesis being tested, the inherent variability of the measures chosen and the temporal or spatial scales examined. The most useful generalization

is that there is no substitute for adequate sampling – more representative samples will inevitably provide more power to discern patterns (Caughley and Gunn 1996). While we generally recommend against using classic power tests (see Krebs 1999 for examples) because of their reliance on the NHT paradigm, there are techniques that can be applied to estimate adequate minimum sample size, and the sensitivity of information-theoretic and Bayesian methods (Boxes 16.3 and 16.4) to power can be evaluated

in various ways. First, resampling can be used to assess to what extent sampling should continue, but this generally requires a moderately large initial sample. The basic approach is to resample (with replacement) observations from a distribution at incrementing subsample sizes (Manly 1997). The sample size at which the desired magnitude of effect can be detected then becomes the minimum target for future studies applying the same metric. These are typically known as *saturation* or *rarefaction* curves (Heck *et al.* 1975). Other

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Box 16.4 Bayesian inference
Corey J. A. Bradshaw and Barry W. Brook

The most common statistical theory underpinning conservation (indeed, most ecological) research today is still *likelihood*-based; i.e. the likelihood of observing the data at hand based on the expected frequency (from a probability density function) that such data would be observed if the same procedure of data collection was repeated many times (McCarthy 2007). *Maximum likelihood* is therefore the optimisation process that chooses the model parameters that make the data the most likely relative to other parameter values. The process implicitly assumes no prior information on the relevant parameters, with the maximum likelihood estimate coinciding with the most probable values of that distribution. The approach essentially asks *what is the probability of observing the data given that the assumed model structure (hypothesis) is correct?*

An alternative approach is the Bayesian paradigm, which instead asks: *what is the probability the model/hypothesis is true given the data*? Bayes' theorem states that the probability of *A* occurring given that *B* has occurred is equal to the probability that both *A* and *B* occur divided by the probability of *B* occurring. Reframing *A* as a (or set of) parameter estimate θ and *B* as the data collected (*x*), then

$$P(\theta|x) = \frac{P(x|\theta)P(\theta)}{P(x)}$$

where $P(\theta|x)$ = the *posterior probability* of obtaining θ given *x*, and $P(\theta)$ = the *prior*

probability of θ and *P(x)* is the probability of the data – a scaling constant (usually derived numerically). Thus, $P(\theta)$ quantifies the available knowledge about θ prior to collecting *x*. This can often take the form of information collected during other studies that quantify the distribution (e.g. mean and standard deviation) of θ. Not only does the incorporation of prior information follow the spirit of scientific reasoning and logic (i.e. if *A* and *B*, then *C*) (McCarthy 2007), it generally provides higher certainty in parameter estimates because the model is not starting from scratch (no information). Other advantages of Bayesian approaches include: (i) errors are not assumed to follow any particular distribution, so departures from assumed data distributions are less problematic than in maximum likelihood-based models; (ii) Markov Chain Monte Carlo (MCMC) numerical optimisation (a computer-intensive method) is more flexible than maximum likelihood approaches because there is less of a tendency to become mired in local minima; and (iii) model parameters are assumed to be variable (i.e. a distribution), not fixed (a point value).

The most commonly used software to implement Bayesian models is the freely available WinBUGS (Windows Bayesian inference Using Gibbs Sampling – www.mrc-bsu.cam.ac.uk/bugs), which includes a friendly graphical user interface (GUI). While exceedingly popular, certain aspects of the software make it somewhat

continues

Box 16.4 (Continued)

cumbersome to implement, such as the requirement to re-initialise parameter settings whenever models are re-run. An alternative interface that is based on the same basic language is the BRugs library (R interface to R2WinBUGS) in the R programming language (R Development Core Team 2008 – also free, open source software). BRugs is a command-based, object-orientated implementation that can be re-run repeatedly without

having to reset parameter values each time.

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rules of thumb on sufficient sample sizes have emerged from the statistical literature based on assumptions regarding the underlying distribution of the observations (Krebs 1999), the width of Bayesian posterior credibility intervals compared to the prior distributions, or on experience from previous studies.

16.2.3 Replication and controls

One of the most common errors made when designing conservation studies is insufficient or biased *replication*. Replication essentially means *repetition* of the experiment (Krebs 1999) and is another type of sample size. Insufficient replication will inflate the estimates of error associated with any metric, so the statistical power to detect differences (or effects) even when present declines with reduced replication. Biased sampling will distort our ability to make inferences about population-level differences on the basis of finite samples. Replication is also essential to avoid the intrusion of chance events; for example, the comparison of only two sites experiencing different intensities of modification may be invalidated because some variable other than the one being tested (e.g. soil type instead of habitat quality) may drive the differences observed in, say, species richness. Only by replicating the sampling unit sufficiently will the chance of spurious events occurring be reduced.

It is important though to ensure that the appropriate statistical unit is replicated. In the above example, increasing the number of sub-samples in each of the two sites does not solve the problem of insufficient replication – the basic unit of comparison is still the 'site'. This is known as *pseudoreplication* because it may appear that increased effort leads to greater replication of the sampled unit, when in reality it is simply the reproduction of non-independent samples (see Hurlbert 1984; Underwood 1994; Krebs 1999). Without true independence among sampling units, estimates of variance, and hence, the power to detect differences (or effects), are downwardly biased, leading to higher probabilities of making Type II errors. Another form of pseudoreplication can occur when designs do not account for temporal autocorrelation among samples or repeat sampling of the same unit (e.g. multiple measures from the same animal that has been recaptured repeatedly). If sequential samples within plots are taken over time, there is a high probability that measures therein will be correlated. There are many experimental designs and statistical tests that can take temporal autocorrelation into account (e.g. Muller *et al.* 1992; Cnaan *et al.* 1997; Krebs 1999; Gueorguieva and Krystal 2004; Ryan 2007).

Another rule often broken by conservation biologists is the failure to incorporate some kind of *control* in their experimental (manipulative or mensurative) design. A control is an experimental unit that receives no direct treatment. In conservation terms, these could be, for example, sites that have

not been changed (degraded) in a particular way, areas without invasive species (i.e. the 'treatment' being the presence of the invasive species), or sites where no re-introductions of native species have occurred. While gradient studies looking for correlations between well-known predictors of biodiversity patterns (e.g. forest fragment area explaining variation in species richness; Laurance *et al.* 2002) do not necessarily require 'controls' (e.g. contiguous forest patches of equivalent size) because the relationships are so well-established, any study attempting some form of manipulative or mensurative experimental inference MUST have controls (note that controls must also be replicated) (Krebs 1999). This applies particularly to the Before-After-Control-Impact (BACI) design – contemporaneous 'controls' are essential to be able to detect any differences (or effects) (Underwood 1994; Krebs 1999).

16.2.4 Random sampling

The complexities of experimental design cannot be treated sufficiently in this chapter; however, one last element that applies to all forms of experimental design is the concept of *randomization*. Randomization refers to the process of placing a random spatial or temporal order on the sampling design such that each unit measures statistically independent values. While complete randomization is not always possible (nor entirely desirable in cases of stratified random sampling – e.g. Krebs 1999) for many conservation studies, one should always strive to maximize sample randomization wherever and whenever possible. The key point is to ensure that your sample is representative of the population parameters about which you are trying to make inference – this is the fundamental theoretical tenet of statistical sampling theory.

16.3 Abundance Time Series

if species are the currency of biodiversity assessments, then counts of individuals represent the principal unit for population dynamics models used to assess conservation risk (see following section). The restrictions imposed on comprehen-sive biodiversity assessment by the sheer number of species on Earth (Chapter 2) also apply to the quantification of population dynamics for single species – there are simply too many species to be able to obtain detailed demographic data (e.g. survival, fertility, dispersal, etc.) for the majority of them to build population models (see following section). Therefore, many types of *phenomenological* model have been developed to deal with sequential censuses (time series) of absolute or relative population size. *Phenomenological* simply means that the dynamical properties these models emulate represent the end-point *phenomenon* of total population size (number of individuals at any given point in time), that is, the emergent property of various *mechanisms* such as birth, death, reproduction and dispersal. Therefore, phenomenological models applied to abundance time series are restricted in their capacity to explain ecological mechanisms, but they certainly provide fertile ground for testing broad hypotheses, describing gross population behavior, and making predictions about population change (provided mechanisms remain constant).

One of the commonest and simplest questions conservation biologists ask is whether a population is trending or stationary. Indeed, one of the main criteria used by the World Conservation Union (IUCN) to define a population or species as threatened (i.e. either *Vulnerable*, *Endangered* or *Critically Endangered*) on its *Red List* (www. iucnredlist.org) is its rate of decline. As such, reliably determining both the direction of the trend (i.e. if declining, to highlight conservation concern, or if increasing, to indicate successful recovery) and quantifying the rate of change, are central goals of conservation biology. While it may seem superficially straightforward to determine at least the direction of population's abundance trend, factors such as the difficulty in censusing the population (counting all individuals), measurement (observation) error, and the presence of high seasonal variance in abundance due to normal environmental stochasticity (variation), are common real-world challenges that can make conclusions of population trajectory uncertain.

Many statistical tools have been developed to deal with these problems, including traditional

NHT power analyses to detect trends (e.g. Gerrodette 1987; see also Gerrodette 1993 for associated software), nonlinear models (e.g. Fewster *et al.* 2000) and the simultaneous application of multiple time series models (Box 16.3) applied to relative abundance counts to determine the direction of trend and strength of feedbacks (e.g. McMahon *et al.* 2009). We certainly recommend the multiple working hypotheses approach (Box 16.3) when querying abundance time series, but argue that much more mathematical development and empirical testing is required on this topic.

Trending, or *nonstationary* populations may be driven by *exogenous* influences ("changes in the environment that affect population change, but are not themselves influenced by population numbers" – Turchin 2003) and/or by *endogenous* influences ("dynamical feedbacks affecting population numbers, possibly involving time lags" – Turchin 2003). It is of course important to determine the interplay between such drivers (Bradshaw 2008) because either may dominate at certain times or on certain stages of the population, or short-term trends may simply represent periods of re-equilibration of longer-term cycles that are not readily apparent when sampling over too few time intervals relative to the scale of disturbance or the species' generation length.

The development of population dynamics models in ecology dates back to the early 19th century (Pearl 1828; Verhulst 1838) and has developed in the intervening 180 years into an expansive discipline in its own right, dealing with the many and complex ways in which organisms interact within and among populations and species. We cannot possibly provide a summary of all the relevant components of time series analysis here (for an excellent overview with worked examples, see Turchin 2003), but we do highlight some of the essential basics.

An important component of extinction models is the presence of density feedback, because the strength and form of such endogenous influences can strongly affect predictions of extinction risk (see below) (Philippi *et al.* 1987; Ginzburg *et al.* 1990). In situations where detailed measurements of the ways in which population density modifies demographic processes are unavailable, phenomenological models applied to abundance time series can still provide some direction. The idea that populations tend to fluctuate around an equilibrium abundance, encapsulated by the general logistic (S-shaped curve) model (Turchin 2003), was generalized for time series by Ricker's model (Ricker 1954) where the rate of population change (*r*):

$$r = \log e\left(\frac{N_{t+1}}{N_t}\right)$$

(*N* is the discrete population size estimate at time *t*), can be expressed as a simple linear function of N_t declining from an *intrinsic* (maximum) growth rate (r_m):

$$r = r_m\left(1 - \left(\frac{N_t}{K}\right)\right)$$

When *r* is positive, the population is growing; above *carrying capacity* (*K*), the population declines. Here, the environment's *K* is assumed to impose some upper limit to total abundance. There are many variants and complications of this basic model, and even more debates regarding its role in explaining complex population dynamics; however, we argue this basic model has been instrumental in defining some of the more important theoretical elements of population dynamics applied to questions of sustainable harvest and extinction risk. Indeed, Turchin (2003) goes as far as to call it a fundamental 'law' of population ecology.

In real-world situations, the negative influence of density on population rate of change is likely to apply mainly to the region around carrying capacity and be of less importance for small populations below their *minimum viable population size* (see below). For instance, as populations decline, individuals may lose average fitness due to phenomena such as inbreeding depression (see Genetic Tools section below), reduced cooperative anti-predator behavior (e.g. flocking or herding), reduced mate availability, and the loss or degradation of cooperative breeding effort (Courchamp *et al.* 2008). Thus, density feedback at these small population sizes can be *positive*, and this is generally known as an *Allee effect* (Allee 1931). Although the phenomenological evidence for Allee effects using abundance time

series is sparse – mainly because obtaining observations at low densities is logistically challenging and observation error tends to be inflated when detection probabilities are low – there are some models that can be applied, such as the Ricker-Allee model:

$$r = r_m \left(1 - \frac{N_t}{K}\right) \left(\frac{N_t - A}{K}\right)$$

where *A* represents the critical lower Allee threshold abundance below which positive feedback begins. For a comprehensive discussion of Allee effects, see Courchamp *et al.* (2008) and Berec *et al.* (2007).

16.4 Predicting Risk

A longstanding goal in conservation biology is predicting the risk a species, community or ecosystem faces when humans change the environment. Questions such as: *How many individuals are required for a population to have a high chance of persisting in the future? What species are most susceptible to human-induced changes to the environment? Are some species more likely to become invasive than others?* and *What types of species are required to maintain ecosystem function?* pervade the conservation literature from purely theoretical to highly applied perspectives. Not only do these questions require substantial data to provide realistic direction, the often arbitrary choice of the degree of risk (defined as a probability of, for example, becoming threatened, invasive, or falling below a predefined population size), can add subjectivity to the assessment.

16.4.1 Cross-taxa approaches

The ranking of species' life history traits (e.g. evolved characteristics such as generation time, mean body mass, reproductive potential; ecological attributes such as dispersal capacity, niche constraints) and environmental contexts, which together predict a species' response to environmental change, has received considerable attention in recent years (e.g. Bennett and Owens 1997; Owens and Bennett 2000; Purvis *et al.* 2000; Kolar

and Lodge 2001; Heger and Trepl 2003; Brook *et al.* 2006; Pimm *et al.* 2006; Bielby *et al.* 2008; Bradshaw *et al.* 2008; Sodhi *et al.* 2008a, b, 2009). Determining which traits lead to higher extinction or invasion risk, for instance, is important for prioritizing management to eradicate harmful invasive species or recover threatened taxa (Bradshaw *et al.* 2008). Developing simple predictive generalizations ('rules') for categorizing poorly studied species into categories of relative risk (proneness) thus becomes a tool to assist in the efficient allocation of finite conservation resources.

There is now good correlative evidence that particular combinations of life history and ecological characteristics (e.g. organism size, dispersal capacity, geographic range, and other reproductive, dispersal, morphological and physiological attributes) influence a species' risk of becoming extinct or invasive, with the strength of effect depending on the spatial scale of measurement, environmental context, and rate of change of the forcing factor (e.g. deforestation or climate change) (Bradshaw *et al.* 2008). Much of this evidence is derived from three main types of models: generalized linear mixed-effects models (e.g. Brook *et al.* 2006; Bradshaw *et al.* 2008; Sodhi *et al.* 2008a, c), generalized estimating equations (Bielby *et al.* 2008) and phylogenetically independent contrasts (e.g. Bennett and Owens 1997; Owens and Bennett 2000; Purvis *et al.* 2000). The principal reason why these complex models must be used instead of simple correlations is because of the confounding effects of shared evolutionary traits when making cross-species comparisons (Felsenstein 1985). In other words, because species are related hierarchically according to their *phylogeny* (evolutionary relationships and common ancestry), they are not strictly independent statistical units, and so their relationships should be taken into account.

Linear mixed-effects models (Pinheiro and Bates 2000) take phylogeny inferred from Linnaean taxonomy into account by using a nested structure in the random effect component of the model (Blackburn and Duncan 2001); once the variance component due to correlated relationships is taken (partially) into account, the residual variation can

be attributed to fixed effects (e.g. life history traits) of hypothetical interest. Generalized estimating equations are similar to mixed-effects models, but the parameters are estimated by taking correlations among observations into account (Paradis and Claude 2002). Phylogenetically independent contrasts (PIC) compute the differences in scores between sister clades and rescale the variance as a function of evolutionary branch length (Purvis 2008). The PIC approach (and its many variants – see Purvis *et al.* 2005; Purvis 2008) is useful, but has been criticized because of: (i) its sensitivity to errors in estimated phylogenetic distance (Ramon and Theodore 1998); (ii) incorrect treatment of extinction risk as an evolved trait (Putland 2005); (iii) overestimation of differences between closely related species (Ricklefs and Starck 1996); (iv) requirement of a complete phylogeny; (v) inability to deal with categorical variables; and (vi) its restriction of using the NHT framework (Blackburn and Duncan 2001; Bradshaw *et al.* 2008). Despite these criticisms, no one modeling approach is superior in all situations, so we recommend several techniques be applied where possible.

16.4.2 Population viability analyses

When the goal is to estimate risk to a single species or population instead of evolved life histories that may expose species to some undesirable state, then the more traditional approach is to do a *population viability analysis* (PVA). PVA broadly describes the use of quantitative methods to predict a population's extinction risk (Morris and Doak 2002). Its application is wide and varied, tackling everything from assessment of relative risk for alternative management options (e.g. Allendorf *et al.* 1997; Otway *et al.* 2004; Bradshaw *et al.* 2007), estimating minimum viable population sizes required for long-term persistence (e.g. Traill *et al.* 2007 and see section below), identifying the most important life stages or demographic processes to conserve or manipulate (e.g. Mollet and Cailliet 2002), setting adequate reserve sizes (e.g. Armbruster and Lande 1993), estimating the number of individuals required to establish viable re-introduced populations (e.g. South *et al.* 2000), setting harvest

limits (e.g. Bradshaw *et al.* 2006), ranking potential management interventions (e.g. Bradshaw *et al.* in press), to determining the number and geographical structure of subpopulations required for a high probability of persistence (e.g. Lindenmayer and Possingham 1996).

The approaches available to do PVAs are as varied as their applications, but we define here the main categories and their most common uses: (i) count-based; (ii) demographic; (iii) metapopulation; and (iv) genetic. A previous section outlined the general approaches for the analysis of population dynamics and the uses of abundance time series in conservation biology; count-based PVAs are yet another application of basic abundance (either total or relative) surveys. Briefly, the distribution of population growth rates on the logarithmic scale, constructed from a (ideally) long time series (or multiple populations) of abundance estimates, provides an objective means of projecting long-term population trajectories (either declining, increasing, or stable) and their variances. The basic premise is that, given a particular current population size and a minimum acceptable value below which the population is deemed to have gone *quasi-extinct* (i.e. not completely extinct, but where generally too few individuals remain for the population to be considered viable in the long term), the mean long-term population growth rate and its associated variance enables the calculation of the probability of falling below the minimum threshold. While there are many complications to this basic approach (e.g. accounting for substantial measurement error, catastrophic die-offs, environmental autocorrelation, density feedback and demographic fluctuations (e.g. uneven sex ratio – for an overview, see Morris and Doak 2002), the method is a good first approximation if the only data available are abundance time series. A recent extension to the approach, based on the multiple working hypotheses paradigm (Box 16.3), has been applied to questions of sustainable harvest (Bradshaw *et al.* 2006).

A more biologically realistic, yet data-intensive approach, is the demographic PVA. Count-based PVAs essentially treat all individuals as equals – that is, equal probabilities of dying, reproducing

and dispersing. In reality, because populations are usually structured into discernable and differentiated age, sex, reproductive and development stages (amongst others), demographic PVAs combine different measured (or assumed) *vital rates* that describe the probability of performing some demographic action (e.g. surviving, breeding, dispersing, growing, etc.). Vital rates are ideally estimated using capture-mark-recapture (CMR) models implemented in, for example, program MARK (White and Burnham 1999), but surrogate information from related species or allometry (body mass relationships) may also be used. The most common method of combining these different life stages' vital rates into a single model is the *population projection matrix*. While there are many complicated aspects to these, they allow for individuals in a population to advance through sequential life stages and perform their demographic actions at specified rates. Using matrix algebra (often via computer simulation), static, stochastic and/or density-modified matrices are multiplied by population vectors (stage-divided population abundance) to project the population into the future. The reader is referred to the comprehensive texts by Caswell (2001) and Morris and Doak (2002) for all the gory details. Freely or commercially available software packages such as VORTEX (www.vortex9.org) or RAMAS (www.ramas.com) can do such analyses.

Metapopulations are networks of spatially separated sub-populations of the same species that are connected by dispersal (see Chapter 5). A metapopulation can be thought of as a "population of populations" (Levins 1969) or a way of realistically representing patches of high habitat suitability within a continuous landscape. In ways that are analogous to the structuring of individuals within a single population, metapopulations 'structure' sub-populations according to habitat quality, patch size, isolation and various other measures. The mathematical and empirical development of metapopulation theory has burgeoned since the late 1990s (see Hanski 1999) and has been applied to assessments of regional extinction risk for many species (e.g. Carlson and Edenhamn 2000; Molofsky and Ferdy 2005; Bull *et al.*

2007). For a recent review of the application of metapopulation theory in large landscapes, see Akçakaya and Brook (2008).

Although genetic considerations are not nearly as common in PVAs as they perhaps should be (see more in the following section, and the book by Frankham *et al.* 2002 for a detailed overview), there is a growing body of evidence to suggest that the subtle determinants of extinction are strongly influenced by genetic deterioration once populations become small (Spielman *et al.* 2004; Courchamp *et al.* 2008). The most common application of genetics in risk assessment has been to estimate a *minimum viable population size* – the smallest number of individuals required for a demographically closed population to persist (at some predefined 'large' probability) for some (mainly arbitrary) time into the future (Shaffer 1981). In this context, genetic considerations are growing in perceived importance. Genetically viable populations are considered to be those large enough to avoid *inbreeding depression* (reduced fitness due to inheritance of deleterious alleles by descent), prevent the random accumulation or fixation of deleterious mutations (genetic drift and mutational meltdown), and maintain evolutionary potential (i.e. the ability to evolve when presented with changing environmental conditions; see following section). The MVP size required to retain evolutionary potential is the equilibrium population size where the loss of quantitative genetic variation due to small population size (genetic drift) is matched by increasing variation due to mutation (Franklin 1980). Expanded detail on the methods for calculating genetically effective population sizes and a review of the broad concepts involved in genetic stochasticity can be found in Frankham *et al.* (2002) and Traill *et al.* (2009). The next section gives more details.

16.5 Genetic Principles and Tools

The previous sections of this chapter have focused primarily on the organismic or higher taxonomic units of biodiversity, but ignored the sub-organism (molecular) processes on which

Box 16.5 Functional genetics and genomics
Noah K. Whiteman

Conservation genetics has influenced the field of conservation biology primarily by yielding insight into the provenance of individuals and the ecological and evolutionary relationships among populations of threatened species. As illuminated in the section on genetic diversity, conservation genetics studies rely primarily on genomic data obtained from regions of the genome that are neutral with respect to the force of natural selection (neutral markers). Conservation biologists are also interested in obtaining information on functional (adaptive) differences between individuals and populations, typically to ask whether there is evidence of local adaptation (Kohn *et al.* 2006). Adaptive differences are context-dependent fitness differences between individuals and are ultimately due to differences between individuals in gene variants (alleles) at one or multiple loci, resulting in differences in phenotype. These phenotypic differences are always the result of gene-environment interactions and can only be understood in that light. However, unraveling the association between particular nucleotide substitutions and phenotype is challenging even for scientists who study genetic model systems.

Adaptive differences between individuals and populations are difficult to identify at the molecular genetic level (see also Chapter 2). This is typically because genomic resources are not available for most species. However, with a set of unlinked molecular markers scattered throughout the genome, such as microsatellites, it is possible to identify candidate loci of adaptive significance that are physically linked to these markers. If the frequency of alleles at these loci is significantly greater or less than the expectation based on an equilibrium between migration and genetic drift, one can infer that this locus might have experienced the effects of natural selection. These analyses are often referred to as outlier analyses and aim to find genes linked to neutral markers that are more (or less) diverged between individuals and populations than the background (neutral) divergence (Beaumont

2005). Despite the immediate appeal of these studies, moving from identification of outlier loci to identification of the function of that locus and the individual nucleotide differences underlying that trait is a difficult task.

The genomics revolution is now enabling unprecedented insight into the molecular basis of fitness differences between individuals. Completed genome sequences of hundreds of plants and animals are available or in progress and next generation sequencing technology is rapidly increasing the number of species that will become genomically characterized. Massively parallel sequencing technology is enabling the rapid characterization of entire genomes and transcriptomes (all of the expressed genes in a genome) at relatively low cost. Currently, sequence reads from these technologies are, on average, <500 base pairs in length and so traditional Sanger sequencing still outperforms massively parallel technology at the level of the individual read. Digital gene expression (where all of the expressed genes are sequenced and counted; Torres *et al.* 2008) and microarray analysis allows one to study differences in global gene expression without *a priori* information on the identity of genes used in the analysis. Single nucleotide polymorphism (SNP) analysis is likely to be an effective tool in identifying loci and individual substitutions that are associated with differences in trait values between individuals, even when pedigree information and heritabilities of traits are not available, as is the case for most threatened species.

Although there is considerable debate over the relative importance of cis regulatory mutations (in non-coding sequences flanking protein-coding genes) versus structural mutations (in protein coding genes) in the molecular basis of phenotypic evolution across species, methods are best developed for detecting a signature of selection at codons within protein-coding genes. In this case, a conservation biologist may be interested in knowing what loci and what codons within that gene have experienced positive, adaptive selection. The redundancy of the DNA code

continues

Box 16.5 (Continued)

means that in protein-coding genes, nucleotide substitutions are either synonymous – the amino acid coded by the codon remains the same, or non-synonymous – the corresponding amino acid changes. Comparing the rates of non-synonymous/synonymous substitutions (the ω rate ratio) of a gene between species can provide evidence of whether that gene or locus is under selection (Yang 2003). A variety of methods are available to estimate ω ratios for a given gene tree. When $\omega < 1$, purifying selection is inferred because non-synonymous substitutions are deleterious with respect to fitness; when $\omega = 1$, neutral evolution is inferred because there is no difference in fitness between non-synonymous and synonymous substitutions; and when $\omega > 1$, positive selection is inferred because non-synonymous substitutions are favored by natural selection. In their most general form, ω ratios are averaged across all nucleotide sites, but because non-synonymous rates are often quite variable across a gene, ω values can also be estimated for individual codons. While it is possible to test for significant differences among ω values, the most conservative interpretation holds that adaptive evolution has occurred only when ω values are >1. However, even when ω values are >1, demographic forces can elevate ω ratios if there is an imbalance between genetic drift and

purifying selection. Because several non-mutually exclusive factors can affect ω ratios, comparisons using these data, which are always only correlative in nature, need to be interpreted with caution.

The genomics research horizon is rapidly changing all areas of biology and conservation biology is no exception. A new arsenal of genomic and analytical tools is now available for conservation biologists interested in identifying adaptive differences between individuals and populations that will complement traditional neutral marker studies in managing wildlife populations.

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Yang, Z. (2003) Adaptive molecular evolution. In D. J. Balding, M. Bishop and C. Cannings, eds *Handbook of Statistical Genetics*, pp. 229–254, John Wiley and Sons, New York, NY.

evolution itself operates. As such, no review of the conservation biologist's toolbox would be complete without some reference to the huge array of molecular techniques now at our disposable used in "conservation genetics" (Box 16.5). Below is a brief primer of the major concepts.

Conservation genetics is the discipline dealing with the genetic factors that affect extinction risk and the methods one can employ to minimize these risks (Frankham *et al.* 2002). Frankham *et al.* (2002) outlined 11 major genetic issues that the discipline addresses: (i) inbreeding depression's negative effects on reducing reproduction and survival; (ii) loss of *genetic diversity*; (iii) reduction

in *gene flow* among populations; (iv) *genetic drift*; (v) accumulation and purging of *deleterious mutations*; (vi) genetic adaptation to captivity and its implications for reintroductions; (vii) resolving uncertainties of taxonomic identification; (viii) defining *management units* based on genetic exchange; (ix) forensics (species identification and detection); (x) determining biological processes relevant to species management; and (xi) *outbreeding depression*. All these issues can be assessed by extracting genetic material [e.g. DNA (deoxyribonucleic acid), RNA (ribonucleic acid)] from tissue sampled from live or dead individuals (see Winchester and Wejksnora 1995 for a

good introduction to the array of methods used to do this).

Of these 11 themes, the first three are perhaps the most widely applicable elements of conservation genetics, and so deserve special mention here. Inbreeding depression can be thought of as an Allee effect because it exacerbates reductions in average individual fitness as population size becomes small. Inbreeding is the production of offspring by related individuals resulting from self-fertilization (e.g. the extreme case of 'selfing' in plants) or by within-'family' (e.g. brother-sister, parent-offspring, etc.) matings. In these cases, the combination of related genomes during fertilization can result in reductions in reproduction and survival, and this is known as *inbreeding depression*. There are several ways to measure inbreeding: (i) the inbreeding coefficient (F) measures the degree of parent relatedness derived from a pedigree analysis (strictly – the probability that an allele is common among two breeding individuals by descent); (ii) the average inbreeding coefficient is the F of all individuals in a population; and (iii) inbreeding relative to random breeding compares the average relatedness of parents to what one would expect if the population was breeding randomly.

The amount of *genetic diversity* is the extent of heritable variation available among all individuals in a population, species or group of species. *Heterozygosity* is the measure of the frequency of different of *alleles* [alternative forms of the same segment of DNA (locus) that differ in DNA base sequence] at the same gene locus among individuals and is one of the main ways genetic diversity is measured. Populations with few alleles have generally had their genetic diversity reduced by inbreeding as a result of recent population decline or historical bottlenecks. Populations or species with low genetic diversity therefore have a narrower genetic template from which to draw when environments change, and so their evolutionary capacity to adapt is generally lower than for those species with higher genetic variation.

Habitat fragmentation is the process of habitat loss (e.g. deforestation) and isolation of 'fragments', and is one of the most important direct drivers of extinction due to reductions in habitat area and quality (Chapter 5). Yet because fragmentation also leads to suitable habitats for particular species assemblages becoming isolated pockets embedded within (normally) inhospitable terrain (matrix), the exchange of individuals, and hence, the flow of their genetic material, is impeded. Thus, even though the entire population may encompass a large number of individuals, their genetic separation via fragmentation means that individuals tend to breed less randomly and more with related conspecifics, thus increasing the likelihood of inbreeding depression and loss of genetic diversity. For a more comprehensive technical demonstration and discussion of these issues, we recommend the reader refers to Frankham *et al.* (2002).

16.6 Concluding Remarks

The multidisciplinarity of conservation biology provides an expansive source of approaches, borrowed from many disciplines. As such, this integrative science can appear overwhelming or even intimidating to neophyte biologists, especially considering that each approach discussed here (and many more we simply did not have space to describe) is constantly being reworked, improved, debated and critiqued by specialists. But do not despair! The empirical principles of conservation biology (again, focusing here on the 'biology' aspect) can be broadly categorized into three major groups: (i) measuring species and abundance; (ii) correlating these to indices of environmental change; and (iii) estimating risk (e.g. of extinction). Almost all of the approaches described herein, and their myriad variants and complications, relate in some way to these aims. The specific details and choices depend on: (i) data quality; (ii) spatial and temporal scale; (iii) system variability; and (*iv*) nuance of the hypotheses being tested.

When it comes to the choice of a particular statistical paradigm in which to embed these techniques, whether it be null hypothesis testing or multiple working hypotheses (Box 16.3), likelihood-based or Bayesian inference (Box

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Box 16.6 Useful Textbook Guides
Corey J. A. Bradshaw and Barry W. Brook

It is not possible to provide in-depth mathematical, experimental or analytical detail for the approaches summarised in this chapter. So instead we provide here a list of important textbooks that do this job. The list is not exhaustive, but it will give emerging and established conservation biologists a solid quantitative background on the issues discussed in this chapter – as well as many more.

SUGGESTED READING

Bolker, B. M. (2008). *Ecological models and data in R*. Princeton University Press, Princeton, NJ.

Burnham, K. P. and Anderson, D. R. (2002). *Model selection and multimodal inference: a practical information-theoretic approach*. 2nd edn. Springer-Verlag, New York, NY.

Caswell, H. (2001). *Matrix population models: construction, analysis, and interpretation*. 2nd edn. Sinauer Associates, Inc., Sunderland, MA.

Caughley, G. and Gunn, A. (1996). *Conservation biology in theory and practice*. Blackwell Science, Cambridge, MA.

Clark, J. S. (2007). *Models for ecological data: an introduction*. Princeton University Press, Princeton, NJ.

Ferson, S. and Burgman, M., eds (2002). *Quantitative methods for conservation biology*. Springer, New York, NY.

Frankham, R., Ballou, J. D., and Briscoe, D. A. (2002). *Introduction to conservation genetics*. Cambridge University Press, Cambridge, UK.

Krebs, C. J. (1999). *Ecological methodology*. 2nd edn. Benjamin Cummings, Upper Saddle River, NJ.

Krebs, C. J. (2009). *Ecology: the experimental analysis of distribution and abundance*. 6th edn. Benjamin Cummings, San Francisco, CA.

Lindenmayer, D. and Burgman, M. (2005). *Practical conservation biology*. CSIRO (Australian Commonwealth Scientific and Industrial Research Organization) Publishing, Collingwood, Australia.

McCallum, H. (2000). *Population parameters: estimation for ecological models*. Blackwell Science, Oxford, UK.

McCarthy, M. A. (2007). *Bayesian methods for ecology*. Cambridge University Press, Cambridge, UK.

Millspaugh, J. J. and Thompson, F. R. I., eds (2008). *Models for planning wildlife conservation in large landscapes*. Elsevier, New York, NY.

Morris, W. F. and Doak, D. F. (2002). *Quantitative conservation biology: theory and practice of population viability analysis*. Sinauer Associates, Sunderland, MA.

Turchin, P. (2003). *Complex population dynamics: a theoretical/empirical synthesis*. Princeton University Press, Princeton, NJ.

16.4), is to some extent open to personal choice. We have been forthright regarding our particular preferences (we consider multiple working hypotheses to be generally superior to null hypothesis testing, and Bayesian outperforming likelihood-based inference), but there are no hard-and-fast rules. In general terms though, we recommend that conservation biologists must at least be aware of the following principles for any of their chosen analyses:

• Adequate and representative replication of the appropriate statistical unit of measure should be planned from the start.
• The high probability that results will vary depending on the spatial and temporal scale of investigation must be acknowledged.

• Choosing a single model to abstract the complexities of ecological systems is generally prone to oversimplification (and often error of interpretation).
• Formal incorporation of previous data is a good way of reducing uncertainty and building on past scientific effort in a field where data are inevitably challenging to obtain; and
• Multiple lines of evidence regarding a specific conclusion will always provide stronger inference, more certainty and better management and policy outcomes for the conservation of biodiversity.

This chapter represents the briefest of glimpses into the array of techniques at the disposal of conservation biologists. We have attempted to provide as much classic and recent literature to guide the reader toward more detailed information, and in this spirit have provided a list of what

we consider to be some of the better textbook guides which provide an expanded treatment of the different techniques considered (Box 16.6). A parting recommendation – no matter how sophisticated the analysis, the collection of rigorous data using well-planned approaches will always provide the best scientific outcomes.

Summary

- Conservation biology is a highly multidisciplinary science employing methods from ecology, Earth systems science, genetics, physiology, veterinary science, medicine, mathematics, climatology, anthropology, psychology, sociology, environmental policy, geography, political science, and resource management. Here we focus primarily on ecological methods and experimental design.
- It is impossible to census all species in an ecosystem, so many different measures exist to compare biodiversity: these include indices such as species richness, Simpson's diversity, Shannon's index and Brouillin's index. Many variants of these indices exist.
- The scale of biodiversity patterns is important to consider for biodiversity comparisons: α (local), β (between-site), and γ (regional or continental) diversity.
- Often surrogate species – the number, distribution or pattern of species in a particular taxon in a particular area thought to indicate a much wider array of taxa – are required to simplify biodiversity assessments.
- Many similarity, dissimilarity, clustering, and multivariate techniques are available to compare biodiversity indices among sites.
- Conservation biology rarely uses completely manipulative experimental designs (although there are exceptions), with mensurative (based on existing environmental gradients) and observational studies dominating.
- Two main statistical paradigms exist for comparing biodiversity: null hypothesis testing and multiple working hypotheses – the latter paradigm is more consistent with the constraints typical of conservation data and so should be invoked when possible. Bayesian inferential methods generally provide more certainty when prior data exist.
- Large sample sizes, appropriate replication and randomization are cornerstone concepts in all conservation experiments.
- Simple relative abundance time series (sequential counts of individuals) can be used to infer more complex ecological mechanisms that permit the estimation of extinction risk, population trends, and intrinsic feedbacks.
- The risk of a species going extinct or becoming invasive can be predicted using cross-taxonomic comparisons of life history traits.
- Population viability analyses are essential tools to estimate extinction risk over defined periods and under particular management interventions. Many methods exist to implement these, including count-based, demographic, metapopulation, and genetic.
- Many tools exist to examine how genetics affects extinction risk, of which perhaps the measurement of inbreeding depression, gene flow among populations, and the loss of genetic diversity with habitat degradation are the most important.

Suggested reading

See Box 16.6.

Relevant websites

- Analytical and educational software for risk assessment: www.ramas.com.
- Population viability analysis software: www.vortex9.org.
- *Ecological Methodology* software–Krebs (1999): www.exetersoftware.com/cat/ecometh/ecomethodology.html.
- Capture-mark-recapture analysis software: http://welcome.warnercnr.colostate.edu/gwhite/mark/mark.htm.
- Analysis of data from marked individuals: www.phidot.org.
- Open-source package for statistical computing: www.r-project.org.
- Open-source Bayesian analysis software: www.mrc-bsu.cam.ac.uk/bugs/.

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