Handbook of Zoo & Aquarium Research

Guidelines for conducting research in zoos and aquariums

Editors: Joanna Bishop, Geoff Hosey and Amy Plowman

Dedicated to Dr Miranda Stevenson OBE, Director of BIAZA 2003-2013, an inspirational advocate for zoo- and aquarium- based science and research

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Introduction

There has been a great increase in research in zoos over the past few years. The management and husbandry of exotic animals in zoos is made complicated by the sheer variety of different species involved, and the fact that we know so little about the basic biology of so many of them; and a lot of recent zoo research has been designed to try and fill some of those gaps in our knowledge, and to enhance the management and welfare of those species. But there has also been a growing recognition that zoo collections provide an amazing resource, where we can find out more about species which most of us would have little hope of researching in the wild. And finally, we have become more interested in the social aspects of zoo visiting and the reasons why people visit, not to mention the educational and awareness-raising potential that these visits offer.

But undertaking research in a zoo can present challenges which are rather different from those we encounter in laboratory or field research. These challenges can sometimes take even a seasoned researcher by surprise, let alone someone who is unfamiliar with the zoo environment. In this Handbook we aim to provide guidance for those who already are involved, as well as those who want to become involved, in zoo research. In using the word 'zoo', we also include aquariums and other sorts of collections of exotic animals, and have tried to point out in the Handbook where these present their own unique challenges. Our approach is based on the importance of recognising that zoo research, like any other scientific research, follows the rigorous principles of formulating questions to ask, or hypotheses to test, the collection and analysis of data, and the placing of the research within a wider body of literature.

This Handbook is the result of themed research workshops which have been held at each of the annual BIAZA Research Symposia since their start in 1999. Previously the individual workshop reports have been available as separate guidelines on the BIAZA website. Inevitably this has led to some fragmentation and duplication, and our intention with this Handbook is to replace all of these individual guidelines with one coherent guide to undertaking research in zoos. The first four chapters present the basic principles of zoo research, in terms of project planning (Chapter 1), ethics and legislation (Chapter 2), data analysis (Chapter 3) and the presentation of results (Chapter 4). These general chapters are then followed by more specialised chapters which each deal with a specific aspect of zoo research. The workshops still happen, so hopefully more chapters will be added in future as they become available.

All of us who have been involved in this have been delighted at how much the original guidelines have been welcomed and used by researchers, and we hope that this combined Handbook will be at least as successful.

Geoff Hosey

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Chapter 1

Planning and Carrying out a Zoo-based Research Project

Editor: Geoff Hosey

Contributing authors: Geoff Hosey, Amy Plowman, Vicky Melfi, Stephanie Wehnelt, Lottie Hosie, Anna Feistner, Miranda Stevenson.

In principle, undertaking a research project in a zoo is not particularly different from carrying out a scientific project anywhere else. As with other projects, zoo-based projects require the identification of an initial question to be answered or hypothesis to be tested; identification of the variables to be measured and the ways in which they are to be measured; and a procedure for collecting and analysing those data. Again, as with other projects, the most successful projects are those which set achievable goals, and this means careful planning before the data collection starts. For zoo work, there are particular things to be borne in mind at each of these stages, and the aim of this chapter is to try and guide the prospective zoo researcher through the planning stage of their project.

1.1. Choosing a Research Topic

Many people who intend to do zoo research know exactly what project they want to do; others may only have a vague idea of the sort of research they would like to do, while others again may know what animals they want to work with, but not what they want to investigate. Some may have no idea of either topic or species, but just know that they want to do their research in the zoo. So let’s start by looking at the kinds of research projects that can be done in zoos, before coming to the question of what topic and what species.

1.1.1. The scope of zoo research

Zoos and aquariums are not just places to go and see animals. In order to attract and inspire visitors with the wonders of the natural world, they have to be efficiently run businesses, and if they are to practise what they preach, they also have to be run on sound environmental principles. Zoos and aquariums in Britain and Ireland are mainly Small and Medium Sized Enterprises (SMEs), that depend on paying visitors for most of their income. They also (by law) have to carry out conservation and educational activities (Zoo Licensing Act 1981). As visitor attractions they require good general facilities, well trained and welcoming staff, excellent eating places, toilets and retail outlets – a wide remit and one which encompasses the potential for a range of research topics, most successfully carried out in partnership with Higher Educational Institutions (HEIs). Zoos and aquariums also attract historians, and there is an impressive body of research on zoo history and changes in people's attitudes towards keeping wild animals in captivity.

Business schools can carry out projects on running SMEs in competitive environments. This research can range from marketing and PR projects, catering and retail to consumer studies. As zoos and aquariums are increasingly being run on sound environmental principles, there is scope for considerable research on ethical consumerism, the factors that affect a person's decision to visit, and on the expectations of those visitors.

Animal collections provide a huge resource for potential research, all of which can add new knowledge to our understanding of the biology of wild animals and their management in captivity. Examples of topics are:

- Captive-breeding programmes and zoo records
- Work on nutrition and reproduction in different species
- Veterinary and medical work, including analysis of *post mortem* data
- Use of dead animals for anatomical and taxonomic studies
- Species studies and studies of behavioural changes owing to enrichment, group and other environmental changes, or the effects of enclosure design on behaviour.
- Public/animal interactions

Plants in the Zoo are a neglected area of research, but nevertheless an important part of what the zoo looks like, and how it supports the animal collection. Plants may be used for decoration, for furnishing or enriching animal enclosures, for animal food, or as important biological specimens in their own right (and many zoos now hold important botanical collections). For all of these functions more research is needed.

Visitor Studies offer great potential and a range of topics, such as:

- Evaluation of interpretation and knowledge transfer and the potential for behavioural change
- Visitor behaviour
- Attitude of visitors to animals

Field conservation also involves research and the collection of data. Many zoos and aquariums are involved with native species projects, sometimes within the zoo grounds. Areas include:

- Native species research within zoo grounds
- Native species research with other partners, such as Natural England, Scottish Natural Heritage and the Countryside Council for Wales.
- Field conservation research in other countries, often with other non-governmental partners, both local and other
- Research into the behaviour and ecology of wild animals
- Research into human impact on wildlife

Evaluation of conservation activities of zoos and aquariums is a worthy research topic in its own right. Zoos are being encouraged to develop methods to measure their success in contributing to conservation although some areas, such as the evaluation of field-conservation projects, are notoriously difficult to study (Cambridge Conservation Forum (CCF) 2007). The Catalysts for Conservation meeting held at ZSL London Zoo in 2004 presented the results of some such research projects (Balmford *et al.,* 2007; Leader-Williams *et al.,* 2007; Mace *et al.,* 2007).

Evaluation of educational activities is another worthwhile area of research in zoos. Zoos and aquariums are increasingly pursuing research that seeks to evaluate the impact of their varied educational programmes. These studies often have a specific focus; for example, zoo exhibits (Ross et al., 2012), animal talks and demonstrations (Povey and Rios, 2002), formal education (Jensen, 2010) or interpretation (Fuhrman and Ladewig, 2008).

1.1.2. Choosing a project topic

So, you can see that zoos and aquariums offer exciting and challenging opportunities for a wide variety of potential research projects (see Anderson, *et al.,* 2008; Maple and Bashaw, 2010, for reviews of the range of research being done in zoos). Unfortunately, much of this potential is currently under utilised, but this does mean that whatever project you do in the zoo, there is a strong likelihood that you are investigating something which has not been studied before. How can you narrow this wide range down to a particular topic that you would like to research? Of course, you may have already narrowed it down through your own reading of the literature. But if you haven't, then additional ways are to look for guidance from supervisors, from previous projects, and from the zoo itself.

If you are a student, then consulting your **supervisor** is an important first step, and you should discuss the project with your supervisor before contacting the zoo or animal collection. A list of suggested projects may be available from your supervisor, or they may have ideas of suitable projects from within their own area of academic interest and expertise. Check carefully with your supervisor what your course requires with respect to the execution and write-up of the project, and whether these requirements can be fully met by the intended zoo project. For example, are descriptive statistics adequate, or is statistical hypothesis testing a requirement? It may also be useful to browse through **previous projects** which have been undertaken at zoos. Examples of these may be held in university departmental files (if you are a student, ask your supervisor), or in zoo libraries (ask the zoo research co-ordinator). If you do look at previous projects, you should be aware that most are kept without their original marks, and you shouldn't take it for granted that they are good examples. Indeed, some of them may be failed projects. You should also be aware that you cannot use any of the information or text in them without the permission of the author or their supervisor, together with due acknowledgement. Finally, the **zoo itself** may have a list of projects which it would like someone to carry out. The research co-ordinator at the zoo will be able to tell you if that is the case, and maybe suggest a topic for your research.

1.1.3. Choosing a species

Your choice of a research topic may be driven by a desire to work with a particular species; conversely, you may have a particular research question in mind, and that dictates the species that you are going to study. If you are not committed to a particular species, then it is worth bearing in mind that a large proportion of zoo research is carried out on primates, carnivorous mammals or elephants. Birds, reptiles, amphibians, fish, invertebrates, and some mammal species (such as rodents and ungulates) are fascinating animals, but relatively neglected in zoo research. If you undertake a project on one of these, you are likely to be adding new knowledge and discovering new things that we didn't previously know.

1.1.4. Finding out where the animals are

You may choose to work on a particular species because you know it is one that is kept at your local zoo. You can find out about the group structure and numbers of individuals of your selected species from the zoo's research co-ordinator. They may be able to supply you with identification sheets, which will help you to recognise individuals within the group; or with a taxon report for the species, which gives information on the age, sex and parents of each individual. At this stage you can determine whether identification of individuals is possible within the time constraints of your research. It might, of course, be sufficient for your particular research question to sub-divide the population by sex or age group.

For research projects involving some species, it may be necessary to visit more than one zoo, perhaps because the number of individuals kept in each zoo is too small for a viable project, so working in several zoos can increase the sample size; or perhaps because the project involves comparisons of different enclosure types or husbandry regimes. Tracking down the zoos in which a particular species is kept can be done by contacting ISIS (International Species Information System) through the ISIS website (www.isis.org). More information on ISIS can be found in Chapter 7 (Introduction to Research Using Zoo Records). For such multi-zoo studies you need to consider how many zoos need to be studied. Part of the answer to this is determined by your research question. Pure research questions, which aim to increase our knowledge beyond just the immediate group we are studying, require group to be the sample unit and thus a large sample size of zoos are needed to extrapolate results to the population as a whole. Applied questions are often only about one group, such as the effect of introducing a new member to that group, and thus data only need to be collected for that group and individual is the sample unit. They can also be extrapolated to the wider population if other groups are also studied. Remember that it is important to be realistic in your interpretation of data (e.g. if there are only five animals in your sample, then you can only make your conclusions about those five, and not for the wider population).

1.1.5. Finding out what's already known

When planning a project and contacting zoos, it is important to have sufficient background knowledge to inform your research. There are many zoo-specific sources of information that are useful starting points, and use of peer-reviewed literature is also essential.

Zoo associations

BIAZA: British and Irish Association of Zoos and Aquaria (www.biaza.org.uk)
The BIAZA website contains information on a range of topics such as animal care, education, legislation and conservation, plus links to other publications. The BIAZA Research Committee aims to promote the benefits of zoo research and to improve the quality of research in zoos in the UK and Ireland. The Research section of the website includes extensive advice for researchers, particularly where to find readily available information without contacting zoos. It is strongly recommended that anyone considering any form of zoo research should consult this website before starting and particularly before contacting any zoos.

EAZA: European Association of Zoos and Aquaria (www.eaza.net)
EAZA has a number of TAGs (Taxon Advisory Groups) which co-ordinate co-operative breeding programmes (EEPs and ESBs) within Europe, including the UK. The EAZA website will give you information on how this is done and for which species.

AZA: (American) Association of Zoos and Aquaria (www.aza.org).
Like EAZA, AZA has a number of TAGs which co-ordinate breeding programmes in North America. The AZA website contains lots of information on these breeding programmes and other aspects of animal care, conservation and education.

WAZA: World Association of Zoos and Aquaria (www.waza.org)
Although there are a small number of global studbooks (ISBs) this organisation's main role is not co-ordination of captive breeding, but rather raising standards of husbandry, education and conservation activity in zoos worldwide. It has published the World Zoo and Aquarium Conservation Strategy and has some useful conservation examples on its web site.

Other associations: there are many other regional zoo associations e.g. Australasia (Zoo and Aquarium Association, ZAA), Africa (African Association of Zoos and Aquaria, PAAZAB) and South-east Asia (South East Asian Zoos Association, SEAZA) which have varying levels of information available on their web sites.

Records routinely kept by zoos

Zoos keep many types of records on their animals. Records systems include ARKS (Animal Records Keeping System), SPARKS (Single Population Animal Records Keeping System), MedARKS (Medical Animal Records Keeping System) and ZIMS (Zoological Information Management System). The ISIS website (www.isis.org) is also a useful resource for information on zoo records. Please read Chapter 7 for more information about the types of records held in these systems, and how you can access information from zoo records.

Enclosure design and enrichment

Shape of Enrichment (www.enrichment.org): this is a quarterly publication on all matters related to environmental enrichment. The website gives information on how to obtain articles and also details and proceedings of the biennial International Conference on Environmental Enrichment.

Zoolex (www.zoolex.org): this website contains very detailed information, including size, materials, barriers, costs etc, on new exhibits at many zoos throughout the world. It also has an extensive bibliography of zoo design literature.

Zoo Design Symposia Proceedings (1-6): These symposia have been hosted at irregular intervals by Paignton Zoo Environmental Park. The proceedings include many papers on the design of new enclosures. Copies of the more recent proceedings can be obtained from Paignton Zoo (www.paigntonzoo.org.uk).

Other sources of information and suggested reading are given in the subject-specific chapters, and at the end of this handbook.

Peer-reviewed literature

Carrying out a thorough literature search on both the species and the research question is important when planning a project. Most academic organisations subscribe to at least one scientific electronic database. Commonly used databases include *Web of Science, Cambridge Scientific Abstracts, Biological Abstracts* and *Zoological Record*. These allow searches using appropriate keywords, which can include the scientific name of the animal, and they very quickly provide details on hundreds, or even thousands of scientific papers. Single keywords are generally of little use; the keyword “zoo”, for example, will yield thousands of hits, most of which are not relevant, simply because “zoo” is a component of lots of biological terms other than zoological gardens. Narrow down your search by using more than one keyword, along with the “and/or” and wildcard functions. Most of these databases give you access to the abstracts of scientific papers, and often they can be used alongside systems such as Athens and Science Direct, which allow subscribers to read the full text of papers.

If you don’t have access to any of these databases, a reasonable search can be achieved through Google Scholar™ (http://scholar.google.co.uk/), which is not as comprehensive as the subscribed databases, but which nevertheless gives you access to abstracts, and sometimes full text, of much of the literature. Chapter 4 lists scientific journals which regularly publish zoo research. Websites for these journals often have search functions, and may give access to the abstracts of relevant papers. An ordinary internet search should be avoided, because it will list all internet pages matching your search words, but most of those pages will be of unknown quality and accuracy. Pages from trusted organisations (such as those described above) are fine for background information, but most universities (if you are a student) will require you to use and refer to the scientific literature rather than web pages.

1.2. Designing the Research

Once you have decided which species you are going to study, and what your general research question is, then it is time to plan the project more precisely. This means specifying the research question or hypotheses to be tested in a clear, unambiguous way, identifying the methodology needed to answer the question and the variables which must be measured to give an answer; as well as ensuring that the research is ethical and legal. Finally, there are the practical considerations of contacting zoos, and getting approval and support.

1.2.1. Hypothesis testing and generating questions

Like any other scientific investigation, a zoo-based research project should be designed to answer a specific question. Again, as with other scientific investigations, a powerful way of doing this is to establish a research hypothesis, which can then be tested by the collection and analysis of appropriate data. The research hypothesis is effectively a "working theory" of what you think is happening; it is an informed theory because it should derive from knowledge that already exists, such as published literature, other people's or your own observations, and other data, for example, from a pilot study. What makes the hypothesis powerful is that you can use it to predict what your results are likely to be. If your results are indeed concordant with your predictions, then that supports the interpretation that your hypothesis is correct; if not, then you can look more closely at your study to see if there were shortcomings in your data collection or analysis which could account for the unexpected findings, or if not, then perhaps the original hypothesis can no longer be supported.

As an example from the field of behavioural research (see Chapter 5 for more details on observing behaviour), suppose that the zoo informs you that it is about to move its chimpanzee group into a new purpose-built naturalistic enclosure, and invites you to collect data on the animals' behaviour in order to monitor the move. You could just go and collect data, and see what happens; but you would then find it very difficult to interpret your results. Alternatively, you could ask the keepers, who might tell you that when they did a similar move with a baboon group the animals seemed to be more active and less aggressive in the new enclosure. A search of the literature might reveal previous studies on enclosure use which show that chimpanzees like to use elevated parts of their enclosure (e.g. Goff *et al*., 1994, Ross & Lukas, 2006). You may not find much in the literature on translocating chimpanzees, but casting your net more widely might reveal papers on moving gorillas to new enclosures (e.g. Lukas et al. 2003, Goerke et al. 1987). On the basis of all of this, you might then formulate the hypothesis that the new enclosure will provide more opportunities for the chimps to show naturalistic behaviours, and that this will be beneficial for their welfare. This then enables you to make specific predictions, such as that the animals will show greater use of higher positions in the enclosure, greater activity, less aggression, and fewer abnormal behaviours. Not only will this make it easier for you to interpret your findings, it also points you in the direction of what data to collect and how to analyse it.

In the example just given, you are effectively doing an experiment. A variable (enclosure type) is being changed, and your study then looks for changes in another variable (the animals' behaviour) which result from that change. In this case the change (or experimental manipulation) is being done by the zoo anyway, as part of its ongoing husbandry and management of the animals. Other similar "experiments" that the zoo might do include addition or removal of individual animals to and from established groups, addition of enrichment devices, and changes to diets. Investigating the effects of these on animals' behaviour and welfare can provide many very worthwhile research projects.

However, setting up a manipulation purely for research is generally unlikely to be approved by the zoo, and, indeed, may not be permissible under current legislation. For example invasive studies that require a Home Office Licence may be possible in a laboratory but will not be accepted in a zoo. For ethical reasons it is unlikely that you will be able to collect data that would require separating individual animals from a group (e.g. for individual feeding trials) or catching or anaesthetising the study animals (see Chapter 2 for more guidance on research ethics).

1.2.2. Variables and Measures

In our chimpanzee example, our **independent variable** (i.e. the variable we manipulate) is enclosure type, and our **dependent variables** (i.e. those we think are affected by the manipulation) are various behaviours. Even in a purely observational study where we do not manipulate any variables, we still think in terms of independent and dependent variables, mainly because it is usually cause-and-effect relationships that interest us. This needs to be justified, however, as part of the rationale of the project, and we should always be aware that if all we can show is some sort of association between our variables, this does not necessarily indicate a causal relationship. Any good textbook on scientific method will elaborate in more detail on this.

From the perspective of zoo research, it is worth pointing out that the identification of variables may not be straightforward and usually requires some thought. In the chimpanzee example we have happily referred to enclosure type as our independent variable, but if we think a bit more about this we will realise that this is not very specific. Perhaps what we really mean is enclosure size. Or enclosure complexity. These are clearly not the same thing, and we can think of them as two separate independent variables.

Similarly, we need to give some thought to what **measures** we are going to use to quantify our variables. One of our predictions in the chimpanzee example is that there will be fewer abnormal behaviours after the move to the new enclosure. Do we mean *all* abnormal behaviours, or just stereotypy or self-injury? And when we predict less aggression, do we mean fewer instances (frequency), or that the aggression doesn't last as long (duration), or even that the aggression that does occur is of a lower intensity? Defining our variables and measures is an important part of planning the research design, and a good methods textbook (e.g. Martin & Bateson, 2007), and if you are a student, advice from your supervisor, can help with this.

1.3. Planning the Research

1.3.1. Contacting Zoos: getting approval and support

Find out the registration procedure for conducting a project at the zoo you have chosen. This can often be done just by looking at the zoo's web site, or simply by a telephone enquiry to the zoo. If you are a student, start by contacting your supervisor who might have zoo registration forms or information leaflets. If there is no information available, contact the Zoo Research Coordinator. It is helpful, and often a requirement, to have a short, typed project proposal to submit to your supervisor and the zoo. Such a proposal should contain details of your project including: the rationale, proposed methods and timescale. Discuss with your supervisor and if necessary with the Zoo Research Coordinator how feasible your initial design will be to implement. The project needs to work around zoo regimes (e.g. animal feeding times, keepers' rota) and must avoid disrupting the animals or the keepers. Check the husbandry regime for your study animals and bear in mind that you may not be able to observe them at certain times (e.g. outside of zoo opening times or when they cannot be seen from public viewing areas). By handing in a preliminary proposal well before the start of your project, the zoo will have the opportunity to inform you about any possible problems.

It is important that you always keep to the arrangements that you made with zoo staff (e.g. for putting enrichment devices into enclosures) and ensure that you turn up on the days and at the times agreed. Be sure to inform the keepers and/or the Research Coordinator if you make changes to your research plans. Once your project has been approved by the zoo, you can start to identify your study animals and determine any group structure. The zoo may provide you with identification sheets or a copy of the taxon report for the species of your interest. The taxon report gives individual information on age, sex, parents, etc. It can be useful to check through any previous project reports that are available on these animals (ask the Research Coordinator or the Zoo Librarian). Previous reports may not only hold information on the individuals but often also contain enclosure maps. Determine whether identification of individuals is possible within your time constraints. It might be sufficient for your particular research question to subdivide the population by sex or age group.

If you are intending to work at, or to send questionnaires to, more than one zoo in the UK and Ireland, then it is important to get support first from the BIAZA Research Committee. The BIAZA web site gives details on how to do this, but broadly it requires sending an application form and a copy of the proposal to the Group, who, if they approve it, will supply you with a letter of support to send with your application to all of the zoos you are intending to work with. This can speed things up considerably, and also make it more likely that the zoos will accept your proposal. This can be particularly important if you are sending questionnaires, because effectively you are then asking the zoos to do some of the work for you!

1.4. Multi-zoo research

As mentioned above, it is possible to carry out research at more than one zoo, and this requires a bit more planning and organisation (Section 1.3.1). The following section gives more detail on types of research that can be carried out at more than one zoo, and considerations that should be made when planning such a study.

1.4.1. Why carry out multi-zoo research?

There are a number of advantages of conducting multi-zoo research:

- Data can be garnered from a large sample of animals (or people), and thus the results can be generalised to larger populations, in comparison with our limited ability to generalise data collected from just one group of animals (potentially a small group). Data from one group can only really tell us about those actual animals studied, whereas data from several groups can tell us something about the species as a whole.

- By collecting data on a large number of animals, it is possible to calculate what would be termed a ‘normal value’ for that species over various different measures. When establishing a ‘normal value’ it is also possible to then determine what variation exists around this ‘norm’, so we can determine what range of values exists for different measures (such as rates of particular behaviours, or physiological values).

- Data collected at several zoos can be used to answer different questions compared to those studies conducted at single sites. In one zoo, for example, it would not usually be possible to change the size of an enclosure, but because enclosure sizes vary across zoos this might provide a method of testing the impact of enclosure size (if there are not too many other confounding variables! See Section 1.4.3).

- The very fact that differences exist between zoos, which would otherwise be regarded as confounds, can be used as an opportunistic source of study. It might be possible to determine that differences in data collected between different zoos are associated with features which exist in some zoos and not in others. For example, the impact of different housing and husbandry regimes can be explored in terms of their effect on animal behaviour, fecundity, visitor satisfaction and many other types of dependent variables (i.e. the variables that you are asking questions about).

1.4.2. How might you conduct a multi-zoo study?

Multi-zoo studies can be achieved in at least two ways, where data are collected through either i) visits to multiple zoos by one or more researchers; or ii) making requests to zoos or holders of databases for information.

The types of data which can be collected using these two different approaches are outlined in Table 1.1.

Frequently conducted studies include:

i) **Visiting multiple zoos**:
- Opportunistic observations, for example of animal or visitor behaviour;
- Experiments, such as to test the efficacy of environmental enrichment, or undertake nutritional intake studies;
- Surveys of people, such as visitors or keepers.

ii) **Requesting information from multiple zoos**:
- Distributing questionnaires;
- Asking for biological samples (or other organic materials, for example food) (see Chapter 2);
- Requesting information from databases, usually information from multiple zoos held by one person (for example SPARKS) (see Chapter 7).
- Requesting copies of records held on file (for example from ARKS, MedARKS, or other details) (see Chapter 7).

But note that not all zoos will hold the same types of information on file and thus have them readily available. For example, information about housing and husbandry, such as enclosure sizes and diets, may not be kept electronically.

Table 1.1. Different ways to carry out multi-zoo studies.

Approach	Data type	Caution	Recommendations	Other relevant chapters in this handbook
Visit the zoo				
Single researcher Multiple researchers	Behaviour; survey people;	Lots of time Inter-observer reliability; Coordination between researchers	Clear standardised methods; train observers; test for inter-observer reliability	All chapters
Request data				
Questionnaires		Return rates can be low; speed of reply can be slow	Be specific Only ask for necessary data which can't be found elsewhere Provide easy reply route (i.e. stamp addressed envelopes; email; or phone them)	Chapter 11: Surveys and Questionnaires
Biological samples	Food samples; body parts and fluids thereafter	Life of sample (storage & packing) Legalities Collection, storage and transport of biological samples from zoo animals is an ethically and legally complex area		Chapter 2: Legal and Ethical issues in Zoo Research
Records	Life history;	Variation of data collection; different methods of data collection; potential time delay between data collection and interpretation		Chapter 7: Introduction to Research Using Zoo records
Databases		Standardised method of collecting data;	Some standard databases or records exist (e.g. ARKS); lots of data are available; Creation of databases can sometimes facilitate the analyses of multi-zoo datasets (cross-linking different types of data, education programmes, physical enclosure details)	Chapter 7: Introduction to Research Using Zoo records

1.4.3. Confounding variables in multi-zoo studies

Because different zoos differ from each other so much, many of the variables which affect whatever you are interested in recording will also differ between zoos, and therefore potentially become confounding variables.

No two zoos are the same (and even in the same zoo, no two enclosures are usually the same). Thus, we must recognise that there are inter- and intra-zoo differences in multi-zoo datasets, and that the confounding effects of these can potentially lead to problems with data analysis, if they are not overcome appropriately.

Some of these confounding variables include:

- A very heterogeneous range of animals. They will have different rearing histories, different life experiences, different temperaments, and so on.

- How does the climate, or time of year, affect the data set? In the British winter, for example, some animals might not have access to certain parts of their enclosure, like paddocks or moated areas (due to waterlogging or ice, respectively) – how does this affect your data, compared with other times of year? What about variations in ambient temperature and humidity at different times of year, or in different parts of the country or world, and how may these affect data?

- Zoo enclosure designs can differ greatly from each other (even within the same zoo), in terms of factors such as size, furnishings, substrate, off-show areas, etc. How do these variables affect the animals living in them? What about husbandry variables as well, for example, whether or not animals are allowed into off-show areas during the daytime, or if there are disruptions to data collection / animal behaviour caused by keeper routines (especially if the times of feeding, cleaning, and so on are somewhat unpredictable)

- Zoo animals are not only affected by the environment inside their enclosure, but also what is going on outside their perimeter fence. For example, are other species living in close proximity to them (within range of sight, sound, or smell?), and if so, does this have an effect (think, for example, of a predator and prey species living close to each other)? Or what about the effect of presence (or absence) of zoo visitors, on the animals? (See Chapter 9).

A final factor that may influence multi-zoo research is the amount of time animals in different enclosures will stay out of sight. If possible, pilot studies in each zoo would allow choice of zoo to be based on similarity of enclosure. However, in many situations time simply won’t allow that. At the least, commenting on the amount of time spent out of sight at each zoo would allow some sort of visual comparison.

1.4.4. Standardisation and inter-observer reliability (one researcher or multiple researchers)

Multi-zoo studies often involve the collection of data at the different zoos by different people. In this case, you should ensure that all researchers are agreed about what the observations and measures are that they are making, and then test statistically to ensure that they are all measuring the same things and their data are reliable. This can be done through **inter-observer reliability testing.** Details of different statistical measures for testing reliability can be found in statistical textbooks. It is also useful to carry out **intra-observer reliability testing** if only one person is collecting data on a project. A convenient way of doing these is as a training session for the observers before they go to the individual zoos. If observations are of behaviour this can be done using video recordings that can be revisited at a later date, rather than needing to co-ordinate two observers to be at the same enclosure at the same time. For behaviour studies it is worth remembering that use of the Observer package can make testing inter- observer reliability much easier. It may also be worthwhile investigating available freeware or shareware programmes.

1.4.5. Co-ordination and time management of multi-zoo studies

It is important to remember that different zoos will operate differently and have different requirements and facilities for research. Thus it is important to co-ordinate fully with whoever is your contact at each zoo. Timetabling is of high importance. Researchers should factor in 'lead time' – how long it's going to take to complete a zoo's application procedure / protocols before starting. We have already mentioned the need for inter-observer reliability testing and the problem of animals being out of sight. Both inter- observer reliability and assessing amount of time out of sight will increase the lead time before beginning a project and must be factored into the timetable. Also seasonality effects could have an impact on the timetabling. In with timetabling comes budgeting – can you (and other researchers if there are any) afford to travel to a range of different zoos? With both timetabling and budgeting it is important to be realistic. It will depend on how much time you have available as to how many zoos you can visit and this in turn will tie back into defining your research question.

The zoos must also be aware of your timetable, particularly completion dates. We would encourage researchers to maintain communication with the zoos after the data has been collected – there is often a period of time between the data collection and the completion on the project. Interim reports can help zoos feel included in the whole process.

Finally, it is worth planning for unexpected contingencies. For example, try to identify more animal groups than you need so that if something happens (animal deaths, animal transports, etc.) you have alternative groups to look at.

Box 1.1. Some multi-zoo case studies.

Here are some examples of previous research projects which have used a multi-zoo approach. These should help illustrate the range of studies that can be achieved this way. Other multi-zoo examples are also given throughout the subject-specific chapters of this handbook.

1) Survey using zoo records (Pickering, *et al*., 1992)
A survey was undertaken to determine whether flock size affected breeding success in flamingos. The researchers sought details of flock size and breeding data from between 1983 and 1989. To do this they collected completed surveys from known flamingo collections in Britain & Ireland. They found that very small flocks (N=4) will breed if given suitable conditions, but that larger flocks breed more frequently and tend to rear more chicks more successfully. They determined that the minimum flock sizes to ensure breeding were 40 birds for Chilean flamingos and 20 birds for Caribbean.

2) Survey using Studbook data (Lindburg, *et al*., 1989)
This study analysed the status of the captive lion-tailed macaque population using data from 461 animals that were recorded in the North American studbook. These data were used to evaluate the life history parameters of the animals, including:

- Mortality: 28% in the 1st year of life; ♂ > ♀
  - Attributable to rearing: hand-rearing (33.3%); mother-reared (11.5%)
- Age at 1st birth: ♀ 4.9 +/- 1.2 years; ♂ 6.6 +/- 3.6 year
- Interbirth interval: lactating ♀ (17.3 months)
- Seasonality of births: no evidence
- Reproductive output: ⬆ variable in ♂ > ♀, due to social groupings

3) Comparison using observations and published data (Melfi and Feistner, 2002)
In this study, data collected by observation of captive Sulawesi crested black macaques were compared with published data on the same species in the wild. The activity budgets of 3 wild troops of these macaques had been studied and the results published by O'Brien & Kinnaird (1997, *International Journal of Primatology* 18 (3): 321-351). The same methods were then used to study three captive macaque troops in zoos in the UK. The authors found that there were no significant differences between wild and captive activity budgets; age-sex class significantly affected behavioural expression; and differences between zoos significantly affected behavioural expression.

4) Questionnaire and Biological Samples (Shepherdson, *et al*., 2004)
This example is a large-scale study of stress response in zoo bears, using data from 22.26 individuals at 18 AZA zoos. Behavioural data were collected via keeper survey (at least 2 keepers/zoo), and physiological responses were gained from faecal samples, which were collected every 2 weeks for 12 months, and assayed for corticoids. The researchers found that bears which displayed stereotypies had different corticoid profiles compared to those which did not display stereotypies, in that non-stereotypic bears had greater corticoid peak and more variation in corticoid levels. The authors suggest that this implies that non-stereotypic bears are more reactive to their environment or find it acutely stressful.

Chapter 2

Legal and Ethical Issues in Zoo Research

Editor: Joanna Bishop

Contributing authors: Joanna Bishop, Andy Hartley, Geoff Hosey, Nic Masters, Vicky Melfi

This chapter introduces some of the legal and ethical considerations encountered in zoo and aquarium research. It can only be a guide and you should thoroughly investigate any legal and ethical aspects that may be relevant to your own research before you start your project. Zoo staff are unlikely to be experts in this field and equally unlikely to have the time to ensure you comply with legal requirements.

2.1. Ethical Aspects of Zoo Research

It is, of course, essential that your research is undertaken within an acceptable ethical framework and ensuring this is part of the research planning process to be addressed before the research starts. Even though your research is legal (see Section 2.2) you should bear in mind that it may not be considered ethical by the zoo(s) you wish to work with. If you are a student it is likely that your project will need approval from your institutional or departmental ethics committee, but even if it doesn't this committee can be a good source of advice in refining the ethical aspects of the research. Many zoos also have ethical committees or other ethical review processes for research proposals and you must allow adequate time for this to happen before you start research. If you are doing a multi-zoo project bear in mind that just because one zoo approves your proposal does not mean others will.

There are good published sources of advice on ethics that you can refer to when planning your project. If your project is behavioural, you should consult the ethical guidelines produced by the

Association for the Study of Animal Behaviour (ASAB), which are available online at http://asab.nottingham.ac.uk/ethics/guidelines.php. If your experimental subjects are human (e.g. as part of a survey), there are ethical guidelines produced by the British Psychological Society (BPS), and available online at http://www.bps.org.uk/the-society/code-of-conduct/code-of-conduct_home.cfm

2.1.1 Intellectual Property Rights

It is important to decide on the ownership of data, samples and any intellectual property arising from them (e.g. genetic sequences or commercial products) before starting a research project. Many universities consider that they have ownership of intellectual property or data generated by their staff or students, whereas some zoos may feel the data are properly theirs. Either way, this needs to be resolved at an early stage to prevent problems when it comes to publishing (see Chapter 4 for more details on publishing and authorship). For university projects it is useful for the zoo and university to come to an agreement for all projects, rather than individual projects. This will make it possible to build up archives and databases which can be used for meta-analysis by subsequent researchers, without the need to track down individual students or other researchers to get permissions.

In the majority of cases involving sample collection at zoos the zoo or aquarium will wish to retain ownership, if any sample remains after analysis. The logistics of this will need to be established before the study commences, and should be included in any contract drawn up between the researcher and the collection.

Many zoos will require researchers to sign research agreements in which details of ownership of data, samples and intellectual property may be stated. Remember that for multi-zoo studies, you need to abide by the research agreement of all zoos, and ownership must be decided for all zoos involved.

2.1.2. Health and Safety

When conducting your research you must make sure that you consider the Health and Safety implications of your research. You must comply with both the Health and Safety regulations of your University/research institution, and of the zoo/aquarium you are visiting. You may need to complete off-site safety assessments for your University and it is recommended that you carry out a risk assessment for your work, indeed for University students this is often a project requirement. Factors for consideration include: any physical requirements of your research such as entering enclosures or off-show areas (only with consent and/or supervision from zoo staff) or moving equipment; the conditions under which you will be observing (e.g. hot or cold weather conditions); personal requirements e.g. medical conditions; proximity to animals; disease transmission; other people such as zoo visitors; lone working; handling of biological samples (see later in this chapter); COSH requirements; travel; and other factors that are specific to the project or location. Make sure you read, and abide by any Health and Safety information provided to you by the zoo, and that you are aware of your nearest fire escape/assembly point when you are at the zoo.

Animal health and safety must also be considered, but this is generally covered in the ethical review of your project, as described in the rest of this chapter.

2.2. Legislation

The primary legislation regulating animal research in the UK is the Animals (Scientific Procedures) Act 1986, which regulates "any experimental or other scientific procedure applied to a protected animal which may have the effect of causing that animal pain, suffering, distress or lasting harm". The use of such procedures in research requires the authority of the Home Secretary via the issuing of a Home Office License encompassing the overall project aims and the specific regulated procedures including by whom and where they will be undertaken. The administration, training and research framework to obtain a Home Office Licence is considerable and would generally be beyond the scope of most zoos. Although collaborative work with other Licensed institutions is an option for some zoos we recommend that you do not propose a project that would require a Licence. The rest of this section is intended to clarify those areas where the zoo researcher may be unsure whether or not a proposed project would require a Licence. This information has been reviewed by a Home Office representative, but note that Home Office inspectors apply individual interpretation to the regulations so if in doubt please ask your local inspector.

2.2.1 Definitions

A *protected animal* is any living vertebrate, other than man. This includes mammals, birds and reptiles, from halfway through gestation or incubation periods, and fish and amphibians from the time at which they become capable of independent feeding. If following or during the course of a procedure performed for scientific purposes on an immature form, the animal reaches a stage of development at which it becomes a protected animal, it is treated as one. Similarly, if a procedure causes pain, suffering, distress or lasting harm to a foetus or immature form at or beyond the stage at which it becomes protected, it is regarded as a regulated procedure, irrespective of any effect on the parent animal. It should be noted that all cephalopods should also be considered as protected animals, though the Animals (Scientific Procedures) Act 1986 only currently includes *Octopus vulgaris*.

A *regulated procedure* is any experimental or other scientific procedure, which may have the effect of causing a protected animal pain, suffering, distress or lasting harm. These terms include death, disease, injury, physiological or psychological stress, significant discomfort or any disturbance to normal health, whether immediately or in the long term. This remains the case even if the result is mitigated or prevented by anaesthetics or other substance to sedate, restrain or dull perception, by prior decerebration or other procedure for rendering the animal insentient.

Death is defined as the permanent cessation of circulation or the destruction of the brain, which is not complete in decerebrated animals.

Ringing, tagging or marking of an animal or any other humane procedure for the sole purpose of enabling an animal to be identified for normal animal husbandry purposes is not a regulated procedure if it causes only momentary pain or distress and no lasting harm. Procedures carried out for the purposes of recognised veterinary, agricultural or animal husbandry practice are not regulated, if carried out for the benefit of the animal. For example, taking blood or other tissue samples for diagnosis and giving established medicines by injection are recognised veterinary procedures, if done for the benefit of the animal. Husbandry practices, which may cause pain, like castration, are not regulated procedures unless they form part of a scientific study.

Simply observing an animal in its normal situation is not a regulated procedure, however manipulation of its environment in order to observe changes in the animal may be a regulated procedure depending on the manipulation. Taking a situation that is legal under the Zoo Licensing Act and making (anticipated beneficial) changes that do not contravene the Zoo Licensing Act would not be regulated. Examples of such changes include substrate, furniture, diet, light regime, enrichment device addition, behavioural training and routine. However, making changes which it is anticipated might be detrimental to the animal in order to research the effect would be regulated. These might include manipulations that would increase the likelihood of aggressive contact or diets that would knowingly deprive an animal of appropriate nutrition.

2.2.2. Biological Samples

Biological samples include any body parts or tissues and their secretions. Many can be obtained from live animals (ante mortem) and some only from dead animals (post mortem)

2.2.2.1. Biological sample collection

Post mortem

Killing an animal in order to obtain samples for research would obviously be a regulated procedure. However acquiring samples opportunistically from animals that have died during the normal course of husbandry is not regulated. A post mortem examination is required following most zoo animal deaths (or at least a representative proportion in the case of a die-off in a group of con-specifics) and liaison with the vets or pathologist will usually be possible to obtain samples of tissues and organs. In the case of some animals, e.g. primates, such collection may be more difficult due to zoonotic risk (especially where diseases that are particularly harmful to humans are possible or suspected). Different zoos will have different guidelines over what samples can be provided, what samples they routinely keep and store from every post mortem and what sort of projects they will release these samples for.

Ante mortem

Most samples will require restraint of an animal and this in itself will usually be recognised as undue stress under Home Office regulations. The type of restraint constituting a regulated procedure, if used for a scientific purpose, includes chemical (e.g. anaesthesia) and metabolic cages. However, some material may be obtained opportunistically during health checks or other recognised husbandry procedures. It must be clear and defensible that the restraint is performed and the sample is to be taken for a genuine veterinary or husbandry purpose of direct benefit to the animal or its group. The owner’s informed consent must be obtained first.

Examples of such material include small amounts of blood taken when sampling is being done haematology and biochemistry tests anyway. It should be taken by the vet to avoid problems of responsibility and since this is deemed an invasive procedure (in other words for clinical purposes and under the auspices of the Veterinary Surgeons Act). The research objective for the blood must be clear; this affects how the blood sample is handled for storage, e.g. spun down to draw off serum and stored at - 80°C. The volume/type of sample should be commensurate with the veterinary purpose with only a small proportion, no more than 10% of the sample being taken for the veterinary purpose, being used for the secondary, research purpose. The sample collection must be taken at the time determined for the primary purpose (i.e. veterinary health check) and not changed to suit the secondary, research purpose and performed as a single blood-draw through the same venepuncture.

Non-invasive samples such as saliva or nail clippings are not regulated procedures if taken opportunistically when the animal is restrained for husbandry reasons. Hair (from a non-sensitive area of the body) and feathers (non-flight feathers) can be collected under the same opportunistic criteria, e.g. when sexing a bird or doing parentage analysis for managing breeding programmes, however feathers cannot be plucked purely for research purposes. Skin samples can be obtained by, for example, obtaining the plug when ear tagging, provided that ear tagging is the method of choice anyway and not used because this method would provide a source of skin which could be used for a scientific purpose. It should be noted that amphibians and fish can be very stressed on handling and there is the danger of damage to their thin skin and the introduction of infection (especially fungal) by taking skin swabs. Depending on the circumstances, the collection of semen via trans-rectal electro-stimulation and trans-rectal ultra-sonography may also be regulated, and thus require the appropriate authority.

Some non-invasive samples such as urine, feaces and saliva can be obtained without restraining the animal and are not regulated procedures. Positive reinforcement training can be used to assist in collection of these samples. The collection method can affect the quality and usefulness of the sample. So, for example, although urine and faeces can be collected relatively easily during routine husbandry, cross contamination of either one, chemicals used for cleaning, latency of collection after they have been voided and other factors will reduce the quality of the sample, and potentially impair further analyses of the samples.

f after reading this you still have any doubt about your proposed research, then you should contact the Home Office for guidance. There is a link on the Home Office website to the published guidance notes for the implementation of the act. The BIAZA Research Group is also happy to give advice and support. It is advisable to keep a record of all correspondence relating to ethical and legal considerations for possible future reference by possible publishers of consequential research reports.

2.2.2.2. Biological sample transport

Countries have their own regulations governing the packaging and transport of biological material in their domestic mail. International Postal Regulations regarding the postage of human and animal pathogens are very strict on account of the disease hazard they present. There are several organisations that set regulations controlling the international transfer of such material. These include the International Air Transport Association (IATA), International Civil Aviation Organisation (ICAO), United Nations Committee of Experts on the Transport of Dangerous Goods, the Universal Postal Union (UPU) and the World Health Organisation (WHO). It is common place to send microorganisms by post, as this is more convenient and less expensive than airfreight. However, many countries prohibit the movement of biological substances through their postal services. The International Bureau of the UPU in Berne publishes all import and export restrictions for biological materials by national postal services.

Different rules and regulations apply to the transport of biological samples according to their potential to cause disease, and these rules vary around the world. Essentially, all samples that are taken and sent for diagnostic purposes (in other words specific disease agents are not suspected or known) should be treated in the UK as "***Biological Substance Category B***". Such samples can be labelled as UN3373 and sent in the post as long as correctly packaged. Any sample known to harbour a disease agent that could cause disease in humans and/or animals must be labelled as "***Biological Substance Category A***" and can only be transported by a licensed shipper of such material.

Any researcher intending to transport biological samples is strongly recommended to refer directly to relevant legislation and regulations such as those provided by postal services and carriers. For example, useful links can be found on the International Air Transport (IATA) cargo web pages (http://www.iata.org/whatwedo/cargo/Pages/index.aspx).

As a general rule the packaging used must be strong enough to withstand the shocks normally encountered during transport, and constructed and closed in such a way as to prevent any loss of contents – by vibration, changes in temperature, humidity or pressure. The packaging must consist of three components: a primary receptacle; secondary packaging; and a rigid outer packaging. Primary receptacles must be packed in secondary packaging in such a way that they cannot break, be punctured or leak their contents. The secondary packaging must be secured in the rigid outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging. For more specific guidance on packaging requirements see the guidance documents provided by the carrier.

2.2.3. Summary of some key points of legislation

1. **The Animals (Scientific Procedures) Act 1986** requires that any "*Protected Animal"* (i.e. any vertebrate or *Octopus vulgaris*) be protected from any "*Regulated Procedure"* (i.e. any scientific or experimental procedure – any action undertaken for research alone) that "may have the effect of causing that animal pain, suffering, distress or lasting harm". "*Regulated Procedures"* require a Home Office Licence.

*In practice, this means that biological samples cannot be taken purely for the purposes of research in many zoo situations***.**

2. **The Veterinary Surgeons Act (1966 and subsequent amendments)** states that only veterinary surgeons can collect "*invasive*" samples, which are defined as those recovered from "entry into a body cavity"; this is deemed a *"Regulated Procedure"* and also requires a Home Office Licence.

For sampling to fall outside the definition of a "*Regulated Procedure*", the sampling must be primarily diagnostic (and with the owner's consent). In practice, this means any legal collection of "*invasive*" samples without a Home Office Licence must be conducted opportunistically, whilst "*non-invasive*" sampling (e.g. hair, urine, faeces) can be conducted without a Home Office Licence. The website of the Royal College of Veterinary Surgeons has a copy of the Act, and gives guidance on its interpretation (www.rcvs.org.uk).

3. The Convention on International Trade in Endangered Species of Flora and Fauna (CITES) requires that a licence be obtained to transport any "*body part*" (e.g. hair) but not "*by-products*" (e.g. urine) from an animal listed on CITES. The CITES website gives the text of the Convention (www.cites.org/eng/disc/text.php) and also an interpretation of how it works (www.cites.org/eng/disc/how.php).

2.3. Further Reading:

Some useful references and websites are listed here, however this is not an exhaustive list and we recommend that researchers do a thorough search for the relevant literature and legislation documents. You should bear in mind that such literature is often updated, particularly on webpages, so you must look for the most up to date version.

Legislation:

Animals (Scientific Procedures) Act 1986 Available on the Home Office website at: http://www.homeoffice.gov.uk/

Veterinary Surgeons Act (1966) Reed Elsevier (UK). Available on the website of the Royal College of Veterinary Surgeons at: http://www.rcvs.org.uk/home/

Websites:

Convention on International Trade in Endangered Species of Wild Fauna and Flora: http://www.cites.org

The International Air Transport Association: www.iata.org

Department for Environment Food and Rural Affairs: www.defra.gov.uk (particularly the pages on research and diagnostic samples: http://www.defra.gov.uk/animal-trade/imports-non-eu/iins/animal-by-products/research/abp-iin-30/)

Chester Zoo endocrinology webpages e.g. http://www.chesterzoo.org/conservation-and-research/research-disciplines/endocrinology-%20hormone-assessment/diagostic-services (links available to sample collection protocols)

Chapter 3

Analysing the Results

Editor: Amy Plowman

Contributing Authors: Amy Plowman, Graeme Ruxton, Nick Colegrave, Juergen Engel, Nicola Marples, Vicky Melfi, Stephanie Wehnelt, Sue Dow, Christine Caldwell, Sheila Pankhurst, Hannah Buchanan-Smith, Heidi Mitchell

3.1. Introduction

Despite a long history of fascinating, innovative and robust research carried out in zoos around the world (*e.g.* de Waal and van Roosemalen, 1979) zoo-based research is still regarded by some as inferior to laboratory or field-based studies. This is partly due to the misguided view that robust statistical analyses are often not possible due to many challenges frequently encountered in zoo-based research, such as small sample sizes, lack of independence of data points, and non-normal distributions. In published papers these challenges have been dealt with in many different, but more or less appropriate ways, making it difficult for novice researchers to know which techniques to use. In this chapter we provide advice on the most appropriate analytical methods for some common types of zoo research, mainly in biological fields, and show that robust analysis and conclusions are perfectly possible in zoo-based research.

3.2. Descriptives, graphs and charts

3.2.1. Descriptives

Field (2009) says that "rushing your analysis is like gulping down a bottle of wine: the outcome is messy and incoherent". Before considering statistical significance testing it is vital to get to know your data through data exploration, using descriptive statistics and graphical representation. Descriptive statistics are used to describe aspects of your data such as the mean, median or modal values and the total range of values using maximum/minimum, quartile ranges or standard deviations. This is important for screening data for obvious errors or outliers, checking basic assumptions (such as those for using parametric tests) and understanding the basic distribution of your data. Boxplots (or box-whisker diagrams) are often a very good way to have a first look at your data as they show the median, the interquartile range (middle 50% of scores), the top 25% range, the bottom 25% range and any individual scores deemed to be outliers. Thus they tell a lot about a distribution with one simple diagram. Many standard statistical textbooks give more detail on data exploration; if you use SPSS then Chapter 4 of Field (2009) is particularly good.

3.2.2. Graphs and charts

Graphical representation of your data is very useful during the data exploration phase, but it is also very important to illustrate the main results found during the significance testing phase (see Section 3.3). Good graphs are a vital part of almost any research report, scientific paper or conference presentation for the following reasons (Kelly *et al*., 2005): they can contain a large amount of information in a relatively small space, they allow rapid assimilation of the overall result, the same graph can be viewed at multiple levels of detail (e.g. overall impression or close-up and exact location of several adjacent points) and they can clearly show complex relationships among multivariate data (in two, three, four, or even more dimensions). However, graphs also have some disadvantages, especially if done badly: they take up a lot of space if showing only a few data points (important in a written report although usually not a problem in a presentation), they may misrepresent data, for example by plotting regularly spaced bars for irregular data intervals, a line may suggest interpolation between data points where none applies, and it can be hard to read off exact numeric values, especially if badly chosen axis scales are used. If exact numeric values are required, a table is best. Therefore, it is important to understand how to make the best use of graphs. Note that it may not be necessary to display all available data in your graph. The key requirement is that your graphs *honestly* and *accurately* represent the data you collected, clearly illustrate the key results you want to highlight and are clearly labelled and captioned. Kelly *et al.* (2005) give an excellent guide to producing good graphs.

3.3. Statistical significance testing

Your choice of statistical significance test depends on the precise question you are asking and the way you collected your data. Thus your tests should be planned during the experimental design stage (see Chapter 1). The final choice of test will also depend on whether your data are normally distributed (or can be transformed easily to a normal distribution). If this is the case we recommend using standard parametric tests as described in readily available text books. If this is not the case a key to choosing an appropriate test for common types of zoo-based research is given in Figure 3.1 below.

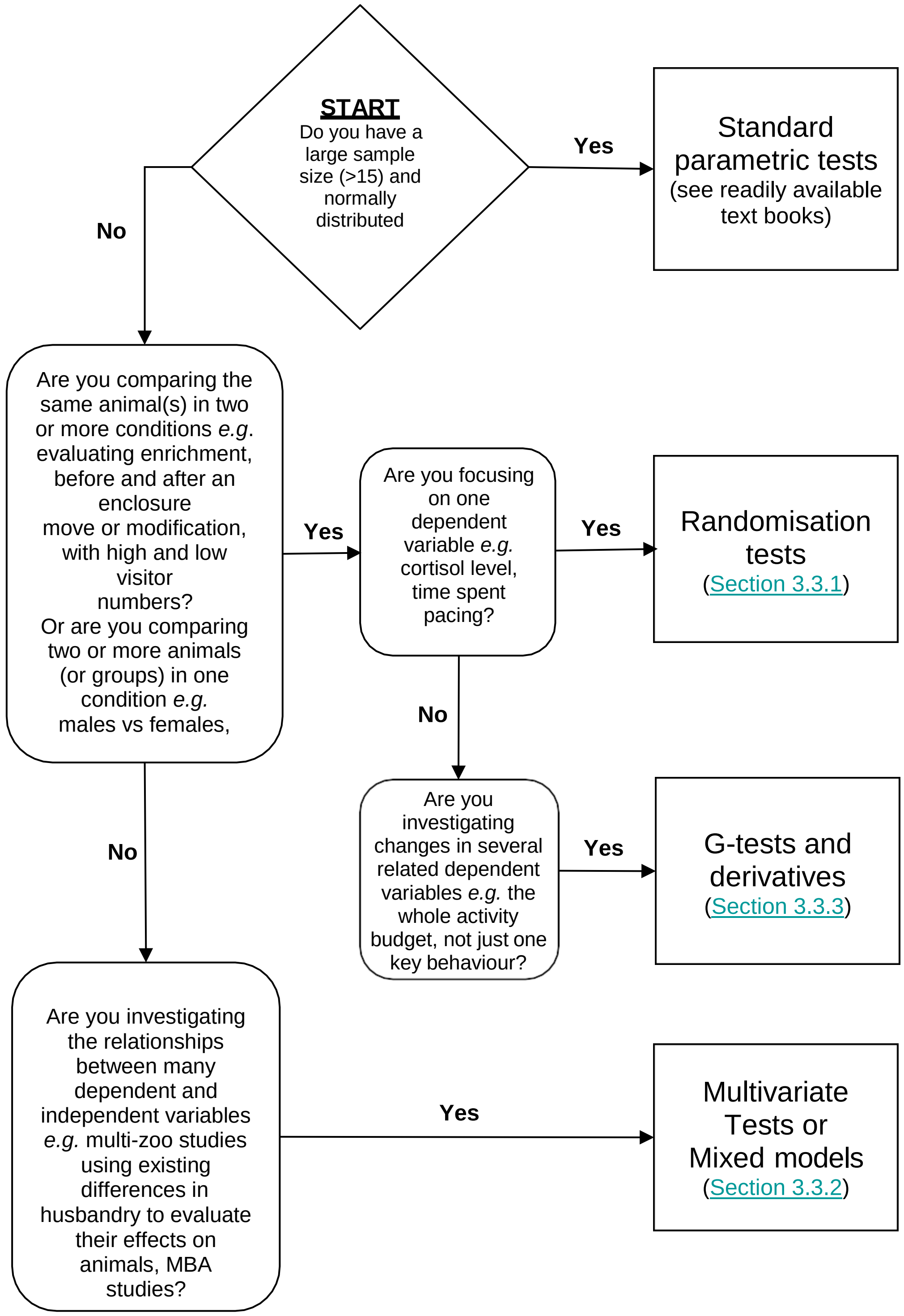

Figure 3.1: Key to choosing an appropriate test for common types of zoo-based research.

In the following sections we introduce some lesser known statistical tests that are useful in zoo-based research when data are not parametric.

3.3.1 Randomisation Tests

A common challenge in zoo-based studies is that, due to practical or ethical limitations, they are often based around a limited number of replicates. For example, zoos may be limited in the number of animals that are available to study, or the number of independent enclosures in which animals can be kept while being studied.

Small sample sizes present three different statistical challenges:

- With few data points it is difficult to decide with any confidence whether your data meet the assumptions required for a particular test, for example whether they are normally distributed.
- Small studies will generally have extremely low statistical power, and since the power of parametric tests declines rapidly as assumptions are violated, they may be extremely inefficient tools for extracting the maximum information from your data.
- Despite best intentions, it will often be difficult or impossible to design zoo studies with the idealised sampling regimes envisaged in statistical text books. Instead data will often be collected opportunistically, leading to obvious problems.

A common response to these challenges is to use non-parametric tests, such as Mann-Whitney, which make fewer assumptions about the data. However, and despite popular belief, such tests are not assumption free, and also frequently have low statistical power. In addition, it is difficult and sometimes impossible to use these tests for more complex designs including multiple factors or covariates. Randomisation tests provide a powerful alternative to overcome these challenges (Edgington, 1995; Mundry, 1999; Todman and Dugard, 2001).

Hypothesis testing generally relies on the production of a P value, that is the probability, assuming the null hypothesis is true, of obtaining a result equal to or more extreme than the one you actually got. If this P value is low (usually <0.05) we conclude that the null hypothesis is not true. Most well known statistical tests determine the P value using an assumed theoretical probability distribution for the test statistic (such as the standard normal distribution or the χ^2 distribution) which is why they tend to lose robustness if the actual distribution of your data does not match the assumed distribution. Randomisation tests do not make these assumptions because they generate the true sampling distribution directly through a large number of re-shufflings of your actual data points. Since they do not rely on an underlying assumed distribution, these tests are much more robust than parametric and non-parametric tests when the underlying distribution is unknown. They also have other advantages which make them particularly useful in a zoo setting, not least that they are flexible enough to allow for unexpected changes in data collection schedules due to unpredictable events that are a frequent hazard when working with animals. The main reason that these tests have not been more widely used is that they have only become a practical possibility relatively recently due to advances in computing power.

In theory, a randomisation test equivalent to any standard statistical test can be designed. Although more complex designs may need bespoke programming, appropriate randomisation tests for most types of study commonly carried out in zoos are available in a number of software packages. Furthermore, a randomisation procedure can be designed to specifically examine the study as it was actually carried out so non-standard designs and any peculiarities in sampling due to unpredictable events can be handled. They also have advantages over standard non-parametric tests because they use the original data values rather than ranks, so are more powerful and have no difficulty with handling tied data.

3.3.1.1 Basic principles of randomisation tests

We will demonstrate the basic principle common to all randomisation and exact tests using a simple example of testing for a significant difference between two means. This is the randomisation equivalent of a parametric Student's t-test or a non-parametric Mann-Whitney U-test for two independent samples A and B.

We wish to test the hypothesis that a novel nutritional regime increases the growth rate of penguin chicks in our penguin enclosure compared to the standard diet. We feed eight randomly chosen chicks the new diet (experimental group), and another eight the standard diet (control group), and we determine their change in weight over a set period. The first step in our analysis is to determine the average weight change for the experimental group and for the control group, let's say these are 15g and 12g respectively. Next we determine the difference in average weight change between the two groups by subtracting one from the other, in this case 3g. This is our actual observed difference in weight gain between the experimental and control groups. We now begin our randomisation procedure. Taking our 16 data points, we randomly assign eight to the experimental group and eight to the control group, giving us a random data set (but using the data actually obtained in the experiment). We then calculate the difference in weight gain in the same way as for the actual experimental data, and write it down. We then repeat this procedure to generate a second random data set, and a second difference. We continue this procedure (preferably using a computer) a large number of times. In all, there are 12870 different possible rearrangements of this data set but a subset of 1000 will be adequate. We can then estimate the probability of obtaining a difference between groups at least as great as the one observed in the experiment (the one-tailed P value) as the proportion of all the random data sets in which this was the case. Example 3.1 presents a detailed example with an even smaller sample size.

The same general procedure can be used to address more complex designs, although careful thought must always be given to the way in which the randomisation is done, and the interpretation that can be drawn from the P value. In the section that follows we will outline some of the most common designs that occur in zoo research, with guidelines on how to carry out an appropriate test. However, for more detailed discussion we would urge the reader to consult a specialised text such as the ones listed in Section 3.3.1 (e.g. Todman and Dugard, 2001).

Example 3.1.

Data sampled (observations):

A	B
3	10
4	12
5	

Difference between the means: 4 – 11 = -7

All possible permutations:

A1	B1	A2	B2	A3	B3	A4	B4	A5	B5	A6	B6	A7	B7	A8	B8	A9	B9	A10	B10
3	10	3	5	3	5	3	4	3	4	4	3	4	3	5	3	4	3	3	4
4	12	4	12	4	10	5	12	5	10	5	12	5	10	10	4	10	5	10	5
5		10		12		10		12		10		12		12		12		12	

Mean of each sample:
4 11 5.67 8.5 6.33 7.5 6 8 6.67 7 6.33 7.5 7 6.5 9 3.5 8.67 4 8.33 4.5

Difference between the means of A and B:

-7	-2.83	-1.17	-2	-0.33	-1.17	0.5	5.5	4.67	3.83

The difference between the means of the two samples is equal or more extreme (in this case smaller) than the one calculated from the observations (*i.e.* -7) only once out of ten permutations. The one-tailed P value is 1/10 = 0.1

3.3.1.2 Uses of randomisation tests for single case and small sample sizes in a zoo setting

(i) AB designs

These are two-phase designs (baseline and treatment) in which one treatment, which cannot be easily repeated or withdrawn, is applied once to one individual (or group, if group is the sample unit). A realistic zoo example might be investigating the effects of moving to a new enclosure on the behaviour of an animal. In studies of this type the date on which the treatment is applied (the intervention date) should be determined randomly. Ideally you should do this using a truly random method of selecting the day from the range of those available. More likely the zoo will set the date due to its practical agenda. However, if the decision is based on practical zoo issues (*e.g.* when all the appropriate staff members are available) rather than animal issues (*e.g.* a particular point in an oestrous cycle/breeding season) then it is effectively random with respect to the animal so does not invalidate the results. Once the intervention date is decided the appropriate data are collected over a number of days before and after the intervention.

The difference between the daily mean value before the intervention and after the intervention of any variable measured can then be tested as in the example above by randomising the data and calculating the difference between the means.

Based on prior knowledge of the animal, and the day to day variation in the variable being measured, it is usually desirable to determine a minimum numbers of days of data collection before and after the intervention. For example, if there is a total of 60 days available for the study it may be determined that at least seven days data should be collected before and after the intervention. The intervention date would therefore be randomly allocated to any day between day 8 and day 53 inclusive. The re-randomisation procedure should follow the experimental procedure, so the re-randomised permutations should only include those on which the intervention date fell between days 8-53, and data collected on days 1-7 and 54-60 would be kept in the same position.

(ii) ABAB designs

These designs are when one treatment is applied repeatedly to one individual (or group, if group is the sample unit). A typical zoo example might be the repeated use of one single environmental enrichment device that can be provided and removed on a daily basis. In these studies treatment A (baseline) and treatment B (enrichment) days should be randomly assigned throughout the study period. Again as above if this cannot be allocated truly randomly then as long as the schedule is random with respect to the animal that is acceptable.

The difference between the treatment A and treatment B means of any variable measured is analysed exactly as in Example 3.1 above. However, in using such a design the researcher has to be aware that she/he might investigate some learning effects together with differences between the two conditions A and B.

(iii) ABCDABCD designs

These designs are similar to the one above but using multiple treatments applied repeatedly to one individual (or group, if group is the sample unit). For instance, multiple enrichment devices provided separately on a number of occasions. As above, the days on which each treatment is provided should ideally be allocated randomly throughout the study period.

The largest difference between any two means is calculated, followed by re-randomisation of all the data points across all treatments. If a significant result is obtained post-hoc tests on pairwise comparisons of treatments can be performed to find out which treatments really differ from one another. Logically consistent, these pairwise comparisons should be done by appropriate randomisation tests again. You should be careful not to inflate the chosen significance level by performing a number of tests with the same data (see Section 3.3.4.1 for possible solutions to this problem).

(iv) Any of the above designs for more than one individual (or group)

If you are not interested in the effects of the experimental manipulation on individuals separately then any of the above experimental designs can be applied to a small number (<15) of individuals (or groups, if groups are the replicates) and treatments should be applied in exactly the same way. However the analysis is slightly different since now we need a test analogous to a repeated measures test. Instead of calculating the difference between the treatment means the residual sum of squares (RSS) is calculated. The data are re-randomised across treatments but the same data points are kept within replicates (*i.e.* within the same individual or group). The RSS is recalculated and the P value based on the number of times the RSS is equal to or greater than that in the actual observed data.

In many cases you will be interested to know if a treatment had an effect on each individual in a group, for instance if it is expected that different age/sex classes may respond differently, and also in the overall result for the whole group (or when groups are the replicates, the effects on each group separately and an overall result for all groups). If this is the case, then separate randomisation tests for each individual can be performed as described above. Afterwards an agglutination test may be performed on those individual P values to generate an overall P value for the whole group. Several agglutination tests are described in the literature. One of the best known is Fisher's procedure which uses the χ^2 distribution to combine several P values from independent statistical tests of a general hypothesis to a single more powerful test of this hypothesis (see Sokal and Rohlf, 1994, p. 794). But there are other procedures which do not assume equal sample sizes and exact P values are also available.

(v) Opportunistic designs

These are studies that investigate the effects of uncontrollable events so the application of treatments cannot be truly randomised. A typical zoo example might be studies of the effects of large numbers of zoo visitors. Treatments in this case might be 'low visitors' and 'high visitors' (analogous to ABAB designs) or 'low visitors', 'medium visitors' and 'high visitors' (analogous to ABCABC designs). In these cases the analysis is carried out exactly as the analogous test above, but justification is needed in the discussion for the lack of planned random assignment of treatment applications. Similarly, interpretation will be limited as in any non-experimental study, with the potential that effects are being driven by uncontrolled variables. In many zoo cases this is not a major problem because the assignment of treatments will be effectively random with respect to the animal and the researcher, so should not produce confounding error. However, care must obviously be taken to ensure that other variables, such as time of day, are equalised or randomised across the treatment being studied, for example that not all low visitor observations occur in the morning and all high visitor observations occur in the afternoon.

(viii) Correlation / Regression

Sometimes the research question will not aim to look for differences between samples but instead at associations and dependencies between variables. For example you may be interested in the influence of a certain vitamin on the level of activity or the relationship between the number of group members and the number of social interactions. In this case special randomisation tests can be used to determine the error probability for a correlation coefficient or a regression analysis (described for example in Manly, 1997). In fact the published significance tables for Spearman's ρ are based on data permutation.

(ix) Choosing the right number of randomisations

The difference between exact (permutation) tests and randomisation tests is that exact tests use all possible rearrangements of the data whereas randomisation tests only use a subset. Randomisation has the advantage of saving computer time without much loss of precision compared with exact tests. For example, when comparing the means of two unrelated samples the total number of possible permutations exceeds 1 million with sample sizes of only 12. The number of randomisations is a trade-off between computer time and precision. Although 1000 randomised pseudosamples are commonly used several authors recommend using 5000 to 10000, especially if the desired significance level is smaller than 0.05 (see e.g. Onghena and May, 1995).

3.3.1.3 Limitations of randomisation tests

Having read this far, you may consider randomisation tests the ultimate procedure for statistical tests. However, although randomisation procedures provide powerful and useful tools that can be used in analysing typical zoo studies, these tests are not entirely assumption free (see Adams and Anthony, 1996; Todman and Dugard, 2001; Ewen *et al*, 2003). As with all statistical tests, researchers should be aware of their limitations, and use and interpret the tests accordingly.

(i) Limitations specific to randomisation tests

- Choice of test statistic

Most statistical textbooks dealing with randomisation tests use the same test statistic for the same type of test, such as the difference between the means of samples to test for differences of 'typical' values. Some test statistics have been found to be "equivalent test statistics". This means they will yield identical P values when using the same data; for example residual sum of squares RSS and the sum of observations in one sample have been found to be such equivalent test statistics.

Unfortunately not every test statistic gives the same result. There may be a significant difference between two samples when using one test statistic (e.g. difference between the mean values) and no difference at all when using another test statistic (e.g. difference between the median values). Considering a hypothesis with two independent samples a lot of different test statistics are conceivable, all of which could be used in a randomisation test: difference of means, difference of medians, difference of mean residuals, difference of sums, RSS, sum of observations in one sample. One of the great advantages of randomisation testing is it forces the researcher to think explicitly about the test statistic that is most appropriate in a given situation. There is no simple answer on the important issue of which test statistic to use and it is recommended that researchers consult a statistician. However, the selection is somewhat restricted because many reasonable test statistics (like the difference between medians) are not available in any software packages at the moment.

- Availability of randomisation test software

Although software is available for a large number of standard tests for many, usually more complicated, designs researchers will probably need to programme their own tests, which may be a daunting prospect. As stated previously, existing computer programmes only use very few test statistics.

(ii) Limitations in common with other tests

- Differences in variances

The difficulty of comparing the means of two samples that may come from populations with unequal variances has a long history under the name of the "Behrens-Fisher problem". When the hypothesis of interest concerns mean differences most parametric and non-parametric statistical tests (e.g. t-test or Mann-Whitney U test) assume equal variances in the populations from which the samples being compared are taken (Hayes, 2000; Kasuya, 2001). Randomisation tests also assume this because the null hypothesis is that the samples come from exactly the same source, which is not true if variances are not identical.

There is no simple, ideal solution to this problem. The first part of the problem is that it is very difficult to determine whether variances are equal or not in typical zoo studies with small samples. Randomisation tests can be designed to test for unequal variances (Manly, 1997) but with small samples their power will be very low. It many cases it may be reasonable to assume that variances are likely to be similar based on sound biological reasoning and inspection of the data. If this is not the case, there are several possible ways to deal with it, none of which are ideal:

- Manly (1995) examined six different randomisation tests to compare means with unequal variance. He found one test superior to all other tests under different conditions. Unfortunately, this test is not implemented in any of the common statistical software packages currently available and even this test does not remove the problem entirely.
- Researchers may use any available transformation to minimise the problem and then use randomisation tests in the knowledge that fewer of their assumptions are being violated. Of course, all P values obtained should be treated with caution and the results may be difficult to interpret.
- It may be possible to formulate the research hypothesis in a way that any difference between the samples is of interest (regardless of whether it is shape, variability, and/or location of the population distributions) in which case the problem no longer exists. For example a possible two-sided experimental/alternative hypothesis would not be:
- H1: Female and male elephant seals differ in the mean amount of time they spend with novel food objects,
  but rather:
  H1: Female and male elephant seals differ in the amount of time they spend with novel food objects. However, it is not possible to specify the type of difference (location, variability, skewness, kurtosis).
  Unfortunately, statistical software packages which will do such tests are not readily available at the moment.

- Autocorrelation of data

When repeated observations on the same subject(s) are done these data may be autocorrelated through time, meaning that the value of one data point might influence the value of the next, and therefore they are not independent of each other. This is especially true the shorter the time interval is between two consecutive observations. See Chapter 5 for advice on how to avoid autocorrelated data.

3.3.1.4 Presentation of results

Randomisation tests are still relatively uncommon in published papers in biological fields. It is, therefore, very important to provide adequate details of the test performed and suitable references. As a general rule, as for all other methods, you should provide enough information to allow repetition of the test by anyone reading the paper. For randomisation tests this should include what test statistic was calculated (*e.g.* difference between means, RSS), what data were re-sampled across what conditions and how many permutations were done, especially whether all possible re-arrangements were performed (exact test) or only a subset (randomisation test).

Example 3.2:

Here is an example of how we might report the results of a lion enrichment study in which the mean time spent feeding each day was compared with and without enrichment present: A randomisation test was performed on the difference between the daily mean time spent feeding on feeding enrichment and non-enrichment days. 5000 re-randomised pseudosamples were generated by randomising all the daily feeding times across both conditions. The difference between the daily mean feeding time on feeding enrichment and non-enrichment days was equal to or greater than the observed value (12.5) in 15 of the 5000 permutations (proportion = 0.003). Therefore, the observed difference in feeding time between enrichment and non-enrichment days is statistically significant (P<0.01; two-tailed).

3.3.1.5 Software for randomisation tests

We are aware of the fact that we will not be able to give a comprehensive list of all software packages which are available at the moment to run randomisation tests. We would just like to introduce some of the most prominent examples.

- SPSS includes an option for “Exact Test” with all its non-parametric tests. Selecting this option means that SPSS performs an exact permutation test instead of the analogous non-parametric test which uses an asymptotic distribution function. Another option labelled “Monte Carlo” enables the user to perform a randomisation test with a given number of permutations. However, the maximum number of permutations for the case at hand is not calculated, and the formula for the test statistic used is not given, and therefore it is difficult to report the statistical procedure comprehensively.
- StatXact is seemingly the most comprehensive package dedicated to randomisation tests at the moment. It carries out a variety of tests on one, two or k samples, contingency tables, and other situations using P values from either sampling or full permutation distributions.
- RT carries out one- and two-sample tests, ANOVA, regression, and several other tests, all by sampling randomisation distributions.
- PopTools is a free add-on for Microsoft Excel which enables a large number of randomisation tests to be performed. It can be downloaded from http://www.cse.csiro.au/poptools/
- Todman and Dugard provide a CD with their book (Todman and Dugard, 2001) containing macros for Microsoft Excel, Minitab, and SPSS to perform a range of randomisation tests. Unfortunately, exact tests are not possible even with small samples. Without having performed comprehensive tests, we do know one Excel macro from the CD which yields a wrong result under certain conditions.
- SsS is a statistical software package which is available from Zoolution. Among other tests it carries out different permutation and randomisation tests with two related or two independent samples. However, at the moment this package is only available in German.

3.3.2 Multivariate tests and mixed models

Some types of zoo research, especially multi-zoo studies (reviewed in Mellen, 1994), aim to test the effects of a large number of independent variables on animal biology, e.g. mortality (Carlstead, Mellen, *et al*., 1999), personality (Carlstead, Fraser *et al*., 1999), play behaviour (Spijkerman *et al*., 1996), activity level, (Perkins, 1992). To analyse the simultaneous effects of many variables often requires the use of complex statistical tests. The main challenge you will encounter with multivariate statistical analyses is choosing the appropriate test based on the distribution of your data and understanding how to carry out the test. This is often so challenging that researchers opt for simpler tests and therefore lose a lot of information from their complex data, providing only limited interpretation of the results. Another challenge, particularly of multi-zoo studies is lack of social independence as animals in the same enclosure influence each other and due to their shared environment are more likely to be similar to each other than to conspecifics in other zoos (see Section 3.4 for further information).

3.3.2.1 Choosing a multivariate test

Often your data will not fit the criterion required by parametric statistics, i.e. that the residuals of the data are normally distributed. In many cases this can be dealt with by using Generalised Linear Mixed Models (GLMM) or Generalised Estimating Equations (GEE, for repeated measures designs) both of which allow you to specify the distribution of the data. For these tests your data do not need to be normally distributed but you do need to know the distribution, or at least have a sound basis for assuming a particular distribution. These tests are very versatile and we recommend that you use them for any appropriate dataset involving multiple independent variables. Information on how to perform these tests are available in standard text books, Field (2009) is particularly user-friendly.

In some cases GLMM or GEE may not be appropriate to answer the research question. Unfortunately if this is the case there will usually be no non-parametric multivariate test available so the choice is to use a parametric multivariate test despite assumption violation or to use multiple univariate non-parametric options. The latter is not ideal as multiple statistical tests are generally not good practice (see Section 3.3.4.1) but also because interactions between your independent variables may not be detected or understood, leading to misinterpretation of the results. For example, single correlations between each dependent variable and the independent variable may all show a positive relationship. However, because the independent variables themselves may be correlated with each other the apparent relationship with the dependent variable may not actually exist. A multivariate technique, giving partial correlation coefficients for each variable once the effects of the others have been removed, will show the true effect of each variable (Martin and Bateson, 1993).

> *Example 3.3:*
>
> In a study by Wilson (1982) enclosure complexity appeared to have a greater influence than enclosure space on the behaviour of gorillas (*Gorilla gorilla)* and orang utans (*Pongo pygmaeus*) When Perkins (1992) replicated this study, enclosure space and complexity were found to be highly correlated and therefore considered to affect behaviour together as it was not possible to statistically untangle their separate effects. It appears that in the newer enclosures included in the later study complexity and size increased in parallel, whereas in older enclosures they did not.

Therefore, we recommend that you use the most appropriate multivariate test (Table 2.1) but ensure that you understand which assumptions are violated by your data, take all possible steps to minimise this violation (e.g. transform your data), explicitly state why the analysis was still appropriate and how robust it may be to the violations and interpret your results with appropriate caution.

Example 3.4:

The study by Perkins (1992) mentioned in Example 3.3 is a good example of evaluating and discussing statistical weaknesses. It included 29 orang utans in nine zoos and used seven independent variables in a step-wise multiple regression analysis. Prior to the analysis several steps were taken to ensure robustness. A power test was performed which indicated that 28 subjects gave a power level of 0.6 (i.e. 60% probability of detecting an effect if one existed, Cohen and Cohen, 1983). The distribution of the data was determined to be slightly biased towards values lower than the mean (Pearsons coefficient of skewness = 0.49), but multiple regression is robust to such deviations (Cohen and Cohen, 1983). An outlier was identified via four separate measures of residual analyses (Studentized residuals, hat diagonals, difference fitting and Cook's distance) and omitted from analysis (Afifi and Clark, 1984; Norusis, 1990).

Table 3.1: Choosing among statistical techniques (adapted from Tabachnick and Fidell, 1996, with kind permission of HarperCollins College Publishing).

Research question	Dependant variables(DVs)	Independent variables (IVs)	Covariates	Analytical tests	Goal of analysis
Degree of relationship	One, continuous	One, continuous		Bivariate regresson	Create linear combination of IVs to optimally predict DV
		Multiple, continuous	None	Multiple regression	
			Some	Sequential multiple regression	
	Multiple, continuous	Multiple, continuous		Canonical R	Maximally correlate a linear combination of DVs with a linear combination of IVs
	None	Multiple, discrete		Multiway frequency analysis	Create a log-linear combination of IVs to optimally predict category frequencies
Significance of group difference	One, continuous	One, discrete	None	One-way ANOVA or t-test	Determine reliability of mean group differences
			Some	One-way ANCOVA	
		Multiple, discrete	None	Factorial ANOVA	
		Multiple,	Some	Factorial ANCOVA	
	Multiple, continuous	One, discrete	None or some	Generalised linear mixed models	Create a linear combination of DVs to maximize mean group differences
		Multiple, discrete	None	Factorial MANOVA	
			Some	Factorial MANCOVA	
	One, continuous	Multiple, one discrete within-Ss		Generalised estimating equations	Create linear combinations of DVs to maximise group differences & differences between levels of within-subjects IVs
	Multiple, continuous/ commensurate	Multiple, continuous/ commensurate		Generalised estimating equations	
	Multiple, continuous	Multiple, one discrete within-Ss		Generalised estimating equations	

Table 3.1. Continued.

Research question	Dependant variables(DVs)	Independent variables (IVs)	Covariates	Analytical tests	Goal of analysis
Prediction of group membership	One, discrete	Multiple, continuous	None	One-way discriminant function	Create a linear combination of IVs to maximise group differences
			Some	Sequential one-way discriminant function	
		Multiple, discrete		Multi-way frequency analysis (logit)	Create a loglinear combination of IVs to optimally predict DV
		Multiple, continuous and/or discrete	None	Logistic regression	Create a linear combination of the log of the odds of being in one group
			Some	Sequential logistic regression	
	Multiple, discrete	Multiple, continuous	None	Factorial discriminant function	Create a linear combination of IVs to maximise group differences (DVs)
			Some	Sequential factorial discriminant function	
Structure	Multiple, continuous observed	Multiple, latent		Factor analysis (theoretical)	Create linear combinations of observed variables to represent latent variables
	Multiple, latent	Multiple, continuous observed		Principal components (empirical)	
	Multiple, continuous observed and/or latent	Multiple, continuous observed and/or latent		Structural equation modelling	Create linear combinations of observed and latent IVs to predict linear combinations of observed and latent DVs

3.3.2.2 Interpretation

Many of the tests given in Table 3.1 are based on correlations and as with all other tests based on correlation it is important not to assume that a significant correlation implies causation. For example, the results might show that animals in larger enclosures spend more time feeding. It is likely that large enclosures do not cause animals to feed more but that larger enclosures are correlated with some other factor such as complexity or more effective feeding enrichment which is actually the cause of the greater feeding time. It is recommended that analyses based on correlation be used as the basis for identifying possible causative factors and then planning studies in which these can be tested using manipulative experiments. In addition, the strength of a correlation (and any more complex tests based on correlation) is not necessarily reflected by its statistical significance, as the probability of finding a significant relationship increases with sample size. Sprinthall (1987) proposed an arbitrary set of definitions to interpret the strength of the correlation coefficient, which recognises that some significant correlations are so weak that they may be biologically meaningless. Thus even if you have found that an independent variable has a significant relationship with your dependent variable the actual strength of that relationship may be meaningless to the animal. This is another reason for directly testing the effect of that variable in manipulated experiments

Example 3.5:

Wielebnowski *et al*. (2002) investigated the causes of behavioural problems observed in captive clouded snow leopards (*Neofelis nebulosa*). Using multiple regression analyses they established that enclosure height, number of keepers per facility and the average hours that a primary keeper spent with each animal per week accounted for a substantial amount of observed variability in faecal glucocorticoid levels ($R^2 = 0.5$, $P < 0.001$). Subsequently Shepherdson, Carlstead and Wielebnowski (2004) set up an experiment to directly investigate the relationship between enclosure height and faecal glucocorticoid levels by providing additional perching of greater height in enclosures. Following the addition of higher perching faecal glucocorticoid levels declined suggesting that enclosure height is a causative factor in stress (as measured by faecal glucocorticoid levels) and that the strength of the relationship is biologically meaningful.

3.3.3 Analysing activity budgets with G-tests

If you have recorded an animal's activity budget, either by scan sampling or by focal animal sampling, you will have noted a number of mutually exclusive behaviours i.e. your animal cannot be doing behaviour A if it is doing behaviour B. The behaviours are therefore not independent of each other. Statistical tests which assume the activities to be independent are therefore not appropriate if you wish to investigate differences in complete activity budgets. This might include questions such as

1. How much time does the animal spend on different activities?
2. Is this the same as another individual?
3. Is this changed when I add an enrichment?
4. Is this the same as in the wild?
5. Given a longer day, which activity increases to use up the extra time?

These are all legitimate questions to ask but the problem is that the statistical test applied is usually a t-test/ANOVA or a Mann Whitney U-test/Kruskal-Wallis test between the amounts of each behaviour (Q1) or between individuals for a given behaviour (Q2) or situation (Qs 3, 4 and 5). This is not ideal because these tests assume the behaviours you are comparing are independent.

The problem is worst for questions like Q1 because you are directly comparing the values of two or more behaviours which are affecting each other's readings, so they really aren't independent at all. If your question is not "how does the suite of behaviours change" but instead "how does this specific behaviour change", then you are on firmer ground because your question is now not about the relative proportions of behaviours (which affect each other) but about how one single behaviour changes in two independent situations. In these cases we recommend using randomization tests (see Section 3.3.1)

For research questions involving suites of behaviours and how their relative proportions differ across individuals, or if there is no specific hypothesis about any particular behaviour, but instead a general hypothesis that behaviour will change under different conditions, you should use a categorical test such as Chi Squared, or, better, the related but more versatile G-test (Fowler *et al*., 1999). These tests are set up on the assumption that the readings are divided up amongst a number of categories, and they can't be in more than one category at once. This is clearly only appropriate for behaviours where the animals can't be doing more than one behaviour at once. We therefore strongly advise researchers to define an ethogram for the study such that it is not possible for an individual to perform more than one behaviour at the same time. This may seem difficult to do, but instead of having two categories of behaviour, such as "eat" and "run" which could be carried out simultaneously, you would have single category "eating while running", and distinguish that from "eating while sitting". If this makes your number of categories too large, then consider carefully whether your question actually requires separation of the two types of eating at all.

As these tests are based around asking about the probability of an animal (or animals) performing a particular behaviour, or being in a particular state, the tests cope well with different sample sizes in the experiment (for example if fewer observations have been made on one individual than on another). A major advantage of such tests over simple chi-square contingency tests is the possibility to extend to multiple factors. So imagine that you are interested in whether visitor numbers affect the way in which a chimp divides its time between sleeping, feeding and other activities. If you observe the chimp for periods with high, medium and low visitor numbers, and note which activity it is engaged in, a simple contingency test would allow us to examine whether the probability of engaging in the three different activities is independent of visitor number. However, such a study on a single individual might have limited generality. Now suppose we repeat the study on several chimps. Multi-way tests allow us to include the chimp identity as a second factor in our analysis, which allows us not only to test whether visitors have effects on behaviour, as in the previous study, but also whether any effects are consistent across animals.

Multi-way categorical tests are available on many statistics packages such as SPSS (where they are called Log-Linear models), and Minitab (where they are called nominal logistic regressions). This approach allows several different factors to be included in the analysis, and in the case of the logistic regression, also allows continuous variables to be included as predictors too. Whilst the details of these tests may differ slightly, they take the same basic approach, and make the same general assumptions. Essentially, these models examine whether the probability of observing any particular outcome, such as a particular behaviour, depends on the factors in the model, and any interactions between them. Thus, in the above study, suppose we have data on four chimps that tell us for each of a number of observations, the behaviour that the chimp was performing, and the visitor density outside the enclosure (measured categorically as low, medium or high). We wish to ask whether visitor number has any effect on the behaviour of the chimps.

Since we have multiple observations on several chimps, the first step in our analysis is to examine whether the behaviours of the individuals in the study change consistently in response to the visitor level. Statistically, this amounts to asking whether there is a significant interaction between individual and the number of visitors, or whether the effect of the number of visitors on the probability of observing an individual performing a particular behaviour is independent of the individual concerned. If no interaction is found (*i.e.* all chimps respond the same way to visitor numbers), it is then possible to ask general questions about how the behaviour of these animals' changes in response to the visitor number. On the other hand, if an interaction is found (*i.e.* visitor level is affecting the behaviour of the chimps, but in different ways for different chimps), then no general comments can be made about the effects of visitor level, but you have discovered that individuals differ in their response, an important finding in itself. To analyse this further you will have to analyse each chimp separately, and more detailed conclusions drawn will only apply to that individual.

3.3.3.1. Limitations

(i) Independence of observations

Often neglected requirements for the G-test (and the χ^2-test) are the following: the samples have to be random and the objects counted have to be independent. This latter requirement is fulfilled if every data point is sampled from a different individual. In studying time budgets this is obviously not possible, so the best thing we can do is to make sure that the data points are not autocorrelated (see Chapter 5). The requirement of randomness implies that your data should be representative, and hence data collection should not (for example) be triggered or prolonged by the expectation of getting particularly interesting results. That means you should not prolong a data collection period because the animals are being particularly interesting, or cancel or curtail data collection because they seem to be behaving in a less interesting way at that time.

(ii) Minimum number of observations

A well-known requirement for the G-test (and the χ^2-test) is that the total sample size has to be big enough that all expected frequencies exceed a certain value. This value differs from one publication to the other; a very conservative stipulation would be 5.0. Unfortunately, some of the behaviours may be very rarely observed, either across all individuals, or by a particular individual. These empty cells in the design make statistical inference difficult (how can you say much about a behaviour you rarely observe?). Furthermore, there is a real practical issue that the models will be hard to fit if cells have very low numbers of observations. The best solution to this is to design your study more carefully, based on initial observations, only including behaviours that are frequently seen, although of course, some rare behaviours may be important (such as mating) and you may need to include them. A solution of last resort may be to pool observations of behaviours that are rarely observed into a single category of rare behaviours, ideally based on some *a priori* rule. However, we would caution against arbitrary pooling of categories if possible. Also, if you are interested in the rare behaviours themselves, this may not be possible, and this part of your data set will not be suitable for analysis by means of categorical tests.

(iii) Problems of fitting the models.

Possibly the major practical problem facing the use of such tests is that the model fitting procedures used to carry out the tests are computationally intensive, and will often not converge for a particular data set. That is, the statistical software package will either fail to calculate your required statistics (coming back with an error message) or produce results with VERY wide confidence intervals. This will be particularly problematic with small data sets, data sets with several categories with low numbers of observations, and data sets with many factors. Whilst this may be viewed simply as an annoying computational problem, it is worth remembering that situations where algorithms have problems converging are exactly those where in reality robust statistical inference from the data is likely to be limited. In these situations, collapsing categories of behaviour (based on sensible biological criteria) may help. Alternatively redesigning your study to answer more focused questions, with fewer categories may be the only way forward.

3.3.4 Multiple test corrections

It is commonly known that performing multiple statistical tests on the same dataset is bad practice. It is less common to understand why and what to do about it. So why should we avoid multiple tests?

In statistical testing we take samples of wider populations and use these samples to make inferences about the populations of interest. It may be that sometimes, by chance, our samples mislead us. This chance can be reduced, by for example increased sample sizes, but can never be wholly eliminated. There are two potential types of error: Type I, where we reject the null hypothesis (of no effect) when there is in fact no real effect, and Type II where there is a real effect but we accept the null hypothesis. By convention, we adopt a significance level of 0.05, meaning that we consider P values less than 0.05 to be indicative of a real effect (so reject the null hypothesis), at this level we will be correct at least 95% of the time. However, we will be wrong (i.e. make a Type 1 error) 5% of the time. The more tests that are carried out, the more likely it is that, simply as a result of chance, a significant result is found. For example, if a researcher carried out a total of twenty tests, and the null hypothesis was in fact true in all cases, then on average one of those tests would nonetheless return a significant result. The likelihood of making Type I errors rapidly becomes unacceptably high, the more tests that are carried out. This is true irrespective of what tests are used (parametric, non-parametric, randomisation etc.)

García (2004) likens the process to buying lottery tickets, and states that, "In such purely random and independent events as the lottery, the probability of having a winning number depends directly on the number of tickets you have purchased. When one evaluates the outcome of a scientific work, attention must be given not only to the potential interest of the 'significant' outcomes, but also to the number of 'lottery tickets' the authors have bought. Those having many have a much higher chance of 'winning a lottery prize' than of getting a meaningful scientific result." (p662)

3.3.4.1 Solutions

The most common way to deal with this problem when carrying out multiple inferential statistical tests is simply to bury one's head in the sand and ignore it. Obviously, this increases the chance of making a Type I error, and therefore renders any conclusions less credible. However, the next most common way is to use a Bonferroni correction (see standard text books). Essentially, this correction reduces the significance level used in determining the statistical significance of P values in individual tests, making it more challenging to reject the null hypothesis in each case, and so reducing the risk of spuriously rejecting the null hypothesis. The stricter significance value is often set to maintain the *overall* Type I error rate at less than or equal to 5%. The problem with this approach is that it can increase the chance of a Type II error, i.e. failing to reject the null hypothesis when there is a real effect, to an unacceptably high level meaning you may fail to spot an interesting result. There are other methods which can partially overcome this disadvantage of the standard Bonferroni correction, but which still control for the overall error rate (*e.g.* see Shaffer, 1995). The simplest of these is probably the sequentially-rejective Bonferroni or Holm method (Rice, 1989).

However, sound scientific reasoning and better experimental design may be better solutions. Traditionally, the view has been that given the inevitable trade-off between Type I and Type II errors, it is worse to make Type I errors than Type II errors. However, there is no logical reason for this, and much will depend on the consequences of each type of error in a particular study. If the hypothesis being tested is that a certain food stuff will cause a painful disease in later years, which type of error would you prefer?

Moran (2003) provides a short but ruthless attack on the use of the sequential Bonferroni or other multiple test corrections in ecological studies. Neither Moran nor ourselves suggest that you should simply sweep the danger of Type I errors under the carpet. Rather, in your results section, you should present your absolute P values and accept or reject individual null hypotheses without consideration of other tests. It is in your discussion where you interpret the biological conclusions that stem from your results where you should address the danger of Type I errors. You should aim to convince the reader that your conclusions are unlikely to be based on Type I errors. This should be done on the basis of logic, scientific understanding of the likely mechanisms underlying results, effect sizes, experimental design and the pooling of information from a number of lines of evidence. Specifically, if you reject a null hypothesis, this is less likely to result from a Type I error if:

- the results can be reproduced in another study
- the significant results arose from a planned comparison
- the result can be interpreted logically on the basis of accepted understanding of the processes involved.

To give an example, imagine a study where you are seeking to explain between–day variation in activity patterns in the ten big cats held in a zoo. Each day, for three months, you record the percentage of time that each cat is active and thirty variables involving weather, husbandry, behaviour of animals in the same enclosure, vocalisations of nearby animals, and visitor effects. Imagine further that you then carry out a number of statistical tests, and find that the only variable that appears to affect activity patterns is the percentage of visitors stopping at that animals' enclosure that are wearing hats, the larger the fraction of hat wearers the less active the cats. We must now consider whether this statistically significant result is likely to reveal a real effect about captive cats' behaviour or whether it is due to a Type I error. It is likely that your intuition is telling you that this example is most likely to be a Type I error, because it seems relatively unlikely that either cats are influenced directly by hat wearing visitors or that the same factors that influence hat wearing in zoo visitors also affects activity patterns of cats. This is certainly what you should expect journal editors and reviewers to conclude unless you can mount a very persuasive argument otherwise.

Perhaps you do actually believe this correlation indicates a real effect. It is possible to suggest that the combination of weather variables that influences hat wearing in humans on a particular day does also affect activity in the cats (rain?). So you have the beginnings of a defence under point (3) above. In order to explore this further, you ought to go back and re-consider your results with respect to weather variables in the light of this hypothesized mechanism. In order to convince the readers that you have found a real effect, you must explain how your hypothesized mechanism (of weather as a confounding variable affecting both cats and hat wearing in humans) can be reconciled with your failure to detect any effects of weather variables on cats' activity.

Another way to convince the reader that your effect is a real one is to appeal to the repeatability argument (1) above. If you go out and repeat your monitoring (in the same or a different zoo) and get the same effect that only hat wearing by visitors (and none of the other 29 variables) is linked to cat behaviour, then this is compelling evidence that there is a real underlying effect. The chance of the same spurious result arising in the two cases is very low (< 0.0025, if the original αlevel was 0.05) and the most likely explanation is that a real effect is at work. Repeating the study also has the advantage that we now have a single and very specific planned comparison (only hat wearing is important), rather than the vague fishing expedition of the initial study. This allows a further defence under point (2)

Even more compelling would be to mount a defence based on point (2) above, but rather than repeating your correlational study, to do an experimental study where you manipulate hat wearing by visitors (and so reduce the chance of confounding variable interpretations based on say weather). If you still find an effect of hat wearing in this manipulative study, then this is compelling evidence that there really is a real effect at work.

In the above example the scientific reasoning solution has put you to a lot of extra work, but the underlying problem here is that you started off with a very loose experimental design where you were testing a very large number of null hypotheses simultaneously, in a study that was not tailored to critically addressing any one of these hypotheses. This is not how we'd recommend that you carry out a three-month study. Rather, we would recommend that you invest time in preparation: reading the literature, watching the animals, talking to the keepers, collecting some pilot data and thinking. At the end of this, you will have decided on what the most likely factors are in influencing activity patterns. That is, you'll have narrowed your scatter-gun list of thirty variables down to a number less than five. Now spend some time thinking of the best way (perhaps by manipulative experiments) to collect really definitive data to confirm or refute a small number of null hypotheses, and carry out the appropriate study. It may be that you went for the "wrong" variables and at the end of your three months, you cannot explain what drives daily variation in activity patterns. This is not a disaster, because your main data collection should have had very high statistical power to detect real effects, so you can conclude with considerable certainty that several factors (that seemed *a priori* to be highly plausible candidates) do not appear to influence your cats' activity. This is more valuable than the original experimental design that is likely to leave you uncertain of the effect of thirty variables, because you do not have strong statistical power to interrogate any one of them. As a side benefit from an improved experimental design, you find yourself doing much fewer statistical tests, and so Type I errors are less likely. Further, your improved design gives you a much better ability to discuss the plausibility of your conclusions in terms of the three criteria above.

3.4.3.3 Recommended best practice

There is no substitute for designing research in a clear and directed manner. Ideally, the researcher should be completely clear about the hypotheses they are testing, and there should be no need for 'fishing expeditions'. Our recommendation is that you design a well-focused experiment in which multiple testing is not needed.

However, sometimes a large number of related statistical tests will simply be unavoidable, so our recommendation is that you do not make multiple test corrections. However, this is not suggesting that you can cop-out of defending your interpretation of your results from accusations of Type I error. Rather, we are suggesting that your defence lies in careful experimental design, knowledge of biology and logical reasoning, rather than a statistical fix.

3.4 General issues in data collection and analysis

3.4.1 Social Independence

Most zoo animals are held in groups, or at least pairs, within an enclosure. Although group sizes at a zoo can be relatively high (*e.g.* flocks of birds, groups of small primates) the data sets taken from the individuals within a group cannot be regarded as strictly independent. This is mainly because one individual has the potential to influence others in the group. For example, when a chimpanzee spots a popular food item in the enclosure, this can also cause others to forage, or an aggressive encounter between two individuals can induce other group members to join in. In addition, when collecting data at several institutions there is very likely to be a 'group effect', such that due to their shared environment (husbandry, climate, diet) individuals in the same enclosure are likely to be more similar to each other than to those in other zoos.

Independence between data points is a requirement of most statistical tests. Strictly speaking, many non-zoo studies may also violate this assumption when, for example, fish are sampled from the same lake or primates of the same group are studied in a forest patch. Data independence can be regarded as a continuum between totally independent (*e.g.* individuals of the same species sampled in geographically separated areas) and very dependent (*e.g.* the same individual is sampled repeatedly over a short time period). Rather than dismissing any statistics, it should be identified on biological grounds where each case stands on this continuum, whether this is important for the research question and what the best available approach is that can be taken. In multi-variate tests, especially Generalised Linear Mixed Models (see Section 3.3.2), it is often possible to include group membership as a variable or co-variate and so overcome this problem.

3.4.2.1 Increasing group number

One possibility to avoid dependence of data is to increase the number of independent groups that are studied and use group as the sample unit rather than individual. In the case of zoo studies, this would mean visiting a sufficient number of zoos to meet the statistical requirements of the test chosen for the particular scientific question. In each individual zoo just one member of the group might be sampled or an average taken over several individuals. If only one individual per group is sampled, it is questionable that this animal represents the rest of its group (low external validity, Bart *et al.*, 1998). The group may consist of individuals of different age and sex classes and all individuals or a selection of individuals may need to be recorded (using a stratified sampling framework if necessary – *e.g.* to include equal numbers of males and females, juveniles and adults etc.). However, using a mean value may obscure effects on certain classes of individual and it is better to use a statistical test which will allow for group membership to be included.

3.4.2.2 Single group

There can be benefits to studying just one group, in one location. If the research question refers to only one particular group of zoo animals (*e.g.* access of group members to food; the effect of enrichment), general assumptions about a greater population are actually not necessary and groups at other zoos do not need to be sampled. In these cases, a high external validity of data is not required. For example, if one is interested in finding out whether individuals in a specific group are positively affected by a new enrichment, then it is not important to question whether the animals are independent or not. That animal A affects animal B's usage of the enrichment in such a way that both profit, will not affect the interpretation of the results; one is simply interested to know if all individuals benefit. The observation may need to be repeated to ensure the enrichment is still effective if one of the animals is removed, as he/she may have been the facilitator, but the immediate question of whether enrichment benefits this specific group with its current composition, has been answered; the aim was not to extrapolate the findings to the wider population. Statistical tests can be performed (by violating the assumption of independence), but it must be explained that the lack of social independence is unimportant for the reasons given above.

The best way to avoid strong dependence of data in behavioural studies is for animals to be observed at different times. In this case, scanning of the behaviour of the entire group at the same time should be avoided. Scanning can be replaced by focal animal sampling (Martin and Bateson, 1993) and by placing breaks between sampling. To identify the best sampling interval, a pilot study needs to be carried out to produce a histogram of bout length of behaviour categories (Martin and Bateson, 1993). The sample interval should well exceed the mean bout length of the behaviours studied (see section above on autocorrelation and temporal independence). Therefore, if individuals are used as replicates, focal sampling with randomly selected individuals should be used to increase the social independence of the data.

3.4.2 Interpretation

Although this is a very long chapter on statistics and the appropriate methods for conducting reliable significance tests, zoo and aquarium researchers should not become overly obsessed with P values. Much research conducted in zoos and aquariums will have implications for husbandry and management methods, therefore any action taken as a result of the research conclusions can have real effects on the animals, staff or visitors. Traditionally when using statistics we try to reduce the probability of making a Type I error (i.e. detecting an effect when there was none). Type II errors (i.e. failing to detect a real effect) are not regarded as being so serious. However, in many zoo situations a Type II error may be have more serious implications; for example, what if we found a trend suggesting some alteration to husbandry was stressful to the animals but it was not quite statistically significant. If we worry about a Type I error we would conclude there is no effect and carry on as before, possibly making a serious Type II error resulting in continued stress for the animals. Therefore it is important to consider biological significance along with internal and external validity of the results when making any recommendations about changes to current practices.

3.4.2.1. Statistical (internal) validity

Statistics are a powerful summary of trends in data and differences between sample groups, as they are less subjective than "eye-balling" raw-data or graphs. However, all statistical tests have constraints on their validity and these must be critically evaluated in the light of any potential assumption violations. This chapter should have given researchers ample guidance on how to perform robust statistical tests, even on data from single animals, thus ensuring statistical validity and should help to dispel some doubts about the quality of zoo-based research. However, the implications of the generality of any tendencies found, especially in single case or small sample studies, need to be discussed on a pragmatic basis (Ruxton and Colegrave, 2003).

3.4.2.2 Biological (external) validity

Although the tests described in Section 3.3 provide robust and valid means of statistically testing data even from single case and small sample studies this does not make up for poor external validity of the results. For example, in an enrichment study on one lion there was a statistically significant increase in feeding time on days when enrichment was provided compared with the days when it was not. If the experiment was designed and analysed correctly it is perfectly reasonable for you to conclude that the enrichment caused an increase in time spent feeding by that single lion. It is not reasonable to conclude that the enrichment causes an increase in feeding time in captive lions generally. However, it is reasonable to argue that, assuming this lion and its living conditions are not exceptional in any way, the enrichment could well be expected to have a similar affect on other captive lions and would be worthy of further testing.

3.4.2.3 Biological significance

In some investigations where we evaluate the effect of a 'treatment' we might find the resulting P value of the chosen probability test is slightly bigger than 5% so not statistically significant, but the result might still be interesting to discuss on a biological basis. Thus, it could be the case that all study animals showed the same tendency (*e.g.* an increase in foraging behaviour when a particular enrichment was presented) apart from one individual. The discussion could highlight that all individuals but one showed a positive response to the enrichment and the possible reasons why the one animal did not (*e.g.* the animal was very old and not often active at all). As the experiment might be time and resource consuming to repeat, the results might still be important to other researchers and, and more importantly to zoo managers, even if they did not reach statistical significance. Conversely you may find a statistically significant result but the actual size of the difference seen is not biologically significant. For example a particular enrichment may reduce the performance of a stereotypy from 8% of the time to 7.5% of the time – if there is relatively little sampling error this result could be significant statistically but in terms of making a real difference to the animal the enrichment is not very effective.

Chapter 4

Reporting and publishing the results

Editor: Joanna Bishop

Contributing authors: Joanna Bishop, Linda DaVolls, Anna Feistner, Fiona Fisken, Geoff Hosey, Sonya Hill, Charlotte Hosie, Nicola Marples, Heidi Mitchell, Sheila Pankhurst, Amy Plowman, Stephanie Sanderson, Angela Turner, Stephanie Wehnelt.

4.1. Disseminating useful information

It is a waste of time to do research that no-one ever hears about.

Any research, whether it is conducted in a zoo, in the wild or elsewhere, may teach the researcher something interesting about the animal or animals studied, or the best methodology to use to answer questions about those animals. It may be that the research reveals just how NOT to design such a study. However, something can be learned from almost every study and that knowledge is only useful to the researcher unless (and until) other people are told about it.

Zoo research that is not disseminated will not benefit anyone else and probably will not benefit the animals studied. Even if the researcher is in a position to put into practice what has been learned, other people may have to repeat the experiment or observations in order to learn the same information before they can develop or advance such studies. Making the knowledge obtained available to other people is a necessary part of any scientific research activity – not just zoo research – and publication is as important as any other part of a study.

Findings can be disseminated on a number of levels. For example, you can simply tell colleagues at your own zoo what you have found, or write a report on your findings, as is often a requirement for student projects. In addition, you could write an article outlining the findings for newsletters or websites, such as *The Shape of Enrichment, International Zoo News, Ratel, Zoo Research News (the BIAZA Research Newsletter)* or *LifeLines* (the BIAZA magazine). You could also go to a conference for zoo staff and researchers, and present a paper or poster.

Any of these methods of broadcasting results are extremely important for passing on the information to others who are in a position to benefit from it, and should be undertaken for the main results of every zoo-based study. An added advantage is that you may also benefit from talking to other researchers about their experiences. Sections 4.2 and 4.3 of the current chapter give guidelines for such dissemination of information in reports and at conferences.

However, these avenues for disseminating work may only pass on the information to a zoo-based audience in a fairly restricted geographical range. If findings are likely to be useful to a wider group, then data should be made available on a worldwide level. This is especially the case if the results could be useful to people who do not work in zoos, but who keep animals for other reasons or are interested in animal biology.

In order to reach this wider audience, there is really no better method than writing a paper for publication in one of the peer-reviewed journals. These are available online or as paper copies worldwide, and because articles go through a rigorous reviewing process, they are also considered trustworthy sources of information that make up the body of knowledge upon which future researched is based. Any new information that is reported upon may add to the archive of what is known about zoo animals and it will be available for everyone to access *ad libitum*. Section 4.4 of the current chapter gives guidelines on publishing your work.

4.2. The Project Report

For students and visiting researchers in particular, the first step in disseminating your results will often be to write a project report. The style of the project report will obviously depend on the requirements of your course and academic institution but it is essential that you produce at least one copy for the zoo. Check whether you should pass it to the zoo personally and to whom it should be addressed (usually to the Research Coordinator) or whether your academic institution collects it to pass on. Without a written report the knowledge gained from the research will not be available for zoo staff, which means your work will not benefit the animals. Be sure to agree on the ownership of any data collected with the Zoo Research Coordinator before you start your project. Some zoos set as a requirement that they are provided with an electronic copy of your data at the end of your project. Be sure to comply with any such requests to ensure valuable links and good relations between zoos and academic institutions are maintained.

Project reports must contain sufficient detail of methodology and analysis to a) enable another researcher to replicate the study, and b) enable other researchers to be able to make critiques of the study, and to be able to identify methodological or analytical features which could explain any differences that they find in their own research if they are using a similar methodology.

The report should state the background of the study and why it was carried out (the 'Introduction' section), in other words it should develop a rationale for the study. This should be done with appropriate reference to the scientific literature, and would normally show the development of a specific research question to be answered, or a hypothesis to be tested by the project. The report must include details of the methods used (the 'Methods' section), particularly when exactly the data were collected, how many hours of observations were collected and details of behavioural sampling methods and experimental design. Full details should also be given of the subjects, including information on the age, sex and housing of animals that were part of the study. State exactly what the results were (including any statistical details; the 'Results' section) and include a discussion of how the results can be interpreted, using knowledge of the theoretical background, and again with reference to the literature (the 'Discussion' section). It is often useful for the zoo if you add recommendations for husbandry procedures that result from your research.

4.3. Conference presentations

At conferences, well presented talks and posters will stand out and attract interest from other researchers. The following section gives advice on giving successful presentations.

Before you present your work at a conference you should inform the zoo at which you collected your data, of your intention to present.

4.3.1. Giving talks

4.3.1.1. Preparing your presentation

Plan the structure: Make sure your presentation has a clear structure that takes the reader through the 'storyline' of your research. You should plan the structure of your presentation before you start to produce your slides. It is often helpful to introduce your presentation and say what you are going to talk about, then give the content of your presentation, and then finish by recapping the main points. Some repetition or emphasis of points may be useful to ensure that the audience understands the key points of your presentation. Many research presentations follow a similar structure to a written research paper (e.g. introduction, methods, results and discussion etc.), but remember that a conference presentation should not be a spoken version of your detailed research paper; rather it is an opportunity for you to present some of the main findings and important points.

Know your audience: Try to find out some information about the audience and their level of knowledge. You should pitch your presentation so that it is clear and easy to understand. This might involve explaining particular terminology or techniques, but at the same time make sure you do not patronise your audience.

Time-keeping: Make sure you plan your presentation to fit within the allowed time-frame, with time available for questions at the end. Try to be realistic about the length of time that you need for each slide, and the overall presentation. As a general rule, a single slide would normally take about 1 minute to present, although some slides will obviously take more or less time depending on the content. Practising your presentation will help you to perfect your timing, although some people find that they speak more slowly when practising than at the actual presentation. If you are struggling to fit your presentation in to the available time, consider reducing the content. A short but clear presentation is preferable to rushing at the end, or skipping slides, as this may confuse your audience.

4.3.1.2. Format of PowerPoint slides

Slide content: Do not crowd your slides with lots of text or too much detail. This can be confusing for the audience, and if they are reading lots of text then they won't be listening to you! Short, concise points are better, which you can expand upon as you speak, and you can use these points as cues to what you are going to say.

Font: Use a sans serif font for clarity, such as Arial, Calibri, Tahoma or Verdana. Make sure your text is readable from a distance. As a guide, aim for 40 pt for titles, 32 pt for subtitles and 24 pt for content text.

Colour: Choose the colours for your slides carefully. You want your slides to look attractive; however your colour choice should be guided by practicalities. Choose a text colour which contrasts with your background colour in order to make the text clear to read, for example a dark colour on a pale background, or a bright colour on a dark background. Avoid combinations such as bright colours on white backgrounds and be careful when combining red and green as this may be difficult for audience members with red-green colour blindness. Colour can be used to highlight particular points, but over use of multiple colours can be distracting.

Figures and tables: Make sure that all figures, tables and diagrams are clear to read from a distance. Graphs should have clearly labelled axes in a large font and the graph itself should be clearly presented. This may mean re-drawing graphs for the purposes of the presentation, rather than copying graphs from written reports. You could consider highlighting particular parts of a graph on the slide if they are a focus of your presentation.

Animation: Use animation sparingly. Animation can be very effective for making a point, but if over-used, can become frustrating for the audience. Animation types such as 'Appear' or 'Fade' can be used to help the sequence of your points, but are slightly more subtle animations and are easier on the audience. Other, more obvious types of animation should be saved for when you want to draw particular attention to a point.

Consistency: Try to keep the formatting of your slides consistent throughout i.e. use the same theme, font style and general positioning of the content. This will help the audience to understand your slides.

General presentation: Overall, aim for clear and attractive slides. Images can help to make a slide interesting, but make sure that the presentation of your slides does not detract from your message.

4.3.1.3. Presentation style

Know your topic: Make sure you clearly understand your topic and presentation. This will help you to give a confident presentation. Don't be apologetic in your presentation, instead try to only present topics that you know and understand.

Practise: Make sure you are prepared and know your presentation, and are happy with the timing; this will also help you with any nerves! However, try not to learn a script; it is better to know your topic and speak about each slide, than to present a memorised script.

Presentation style: Speak clearly and try not to rush. Check whether the presentation room uses a microphone system, or whether you will need to project your voice to the back of the room. If you need notes to help you, consider using cue cards to jog your memory. However, do not use too many cue cards as it is easy to lose track of them and you will end up having to shuffle through cards in the middle of your presentation! Try to restrict notes to a few points and do not read from a script as this can become monotonous for the audience. You could use the points on your slides as cues, but equally do not read too much from the slides. Face the audience as much as possible and do not talk to the screen as the audience will struggle to hear you. Try to look at different members of the audience and if possible, make eye-contact. This will help to engage your audience. Do not be afraid to move and be dynamic in your presentation, for example gesturing or pointing to parts of the slide, but try to avoid any nervous movements such as pacing or playing with a pen or pointer.

Clarity: Aim to clearly present all of the technical parts of your presentation. It is important that your audience can understand what you did and why, and what the results and implications were. Remember that whilst you know all of the details of your research, this may be the first time that the audience have heard anything about it so you need to be clear. This is particularly important when describing your results. Take some time to clearly explain what your graphs and tables are showing. It will take the audience a few seconds to understand your graphs so if you plunge straight in to complicated details then you run the risk of leaving the audience behind whilst they try to make sense of your slides.

Animations: If you have used animations in your presentation, make sure you know where they are, and when you have to click. It can be frustrating to the audience if animations appear too early (which may spoil the point you are making), or too late (which may leave the audience waiting for the slide information to support what you are saying).

4.3.1.4. General points

Be prepared: Make sure you take a back up copy of your presentation with you on a memory stick, even if you have sent it in advance.

Video: If you have any video (or sound) clips imbedded in your presentation, you should save the video file in the folder containing your PowerPoint presentation and link the video to that file. You then need to take the whole folder with you to the conference. In order to play the video, PowerPoint follows the link to find the video file in its saved location, therefore if you only take the PowerPoint presentation with you, and not the video file then PowerPoint will not be able to find the video file and your video will not play.

Check that everything works: If you have chance before your presentation, check that your slides work properly on the presentation computer. This is your opportunity to check how to use the computer/pointer/microphone and to check that: all slides fit properly on the screen, the colours are projected correctly and that video and sound clips work.

4.3.1.5. Dealing with questions

Make sure you leave enough time at the end of your presentation for questions. Usually the person chairing your session will pick people in the audience to ask a question, but you may need to do this yourself. Make sure you listen carefully to the question and do not be afraid to repeat or check the question. This ensures that you are answering the right question and will also give you a little bit more time to think about your answer! Try to answer as informatively as possible, but if you do not know the answer, do not be afraid to say that it is an interesting point, but you would need to look into it further.

4.3.2. Poster preparation

Many of the guidance comments for preparing presentations (in Section 4.3.1 above) can also be applied to posters. It is important that conference posters are well designed in order to attract people to read them. Poster sessions at conferences can often be noisy and busy, and conference delegates will be walking around, talking to colleagues, and there may be food or drink to distract people. Therefore, for your poster to draw attention above all of this, you need to carefully plan the content and layout. Below is some advice for creating successful conference posters.

4.3.2.1. Content

A poster is a very useful tool for visually presenting some of the main, interesting points of your research. It should not be used as just another way to present your detailed research paper, or even to present your oral presentation. A poster should clearly convey the key points of your research as concisely as possible.

Try not to use too many words and avoid blocks of text. Bullet points are useful to present short, concise statements. As much as you might want to include all of the details of your research, in reality concise posters are more effective and memorable. Remember that there will usually be opportunity for you to stand next to your poster to tell people all of the extra details, and you can always include your contact details in case people have any questions for you.

You should aim to present your research in as few words as possible, but at the same time remember that your poster should also stand alone, and the content should be understandable just by reading the poster, since you will not be able to stand next to it all of the time. It is often useful to ask a friend or colleague to read your poster before you print it, to test that it portrays the correct message, and to identify any sections that could be made more concise.

A research poster will usually follow a similar structure to a paper (title, introduction, methods, results and discussion etc.), although this could be different depending on the message that you want to portray. An abstract is often helpful to tell people the main points of your research if they don’t have time to read the whole poster, as is a conclusion containing the important and memorable points.

Make sure you include your contact details and affiliations.

4.3.2.2. Design

Layout: It is important that your poster is quickly and easily accessible to the reader and that it is clear what the poster is about. Make sure you use clear and easy to follow sections, and that these sections flow across the poster in a logical order without jumping around the page. Conference delegates often do not read all of a poster in detail, and different people will want to focus on different aspects of your poster. It is therefore important that the different sections of your poster are easy to locate and understand.

It is often useful to design your poster so that the sections are visually separate, for example using boxes, or difference background colours etc. Think carefully about the alignment and presentation of your sections. If you are using a programme like Microsoft PowerPoint to design your poster you can set gridlines on the screen to help you align sections.

Do not crowd your poster with content. As discussed in Section 4.3.2.1. (above), it is better to have a clear and concise poster, as conference delegates are unlikely to read lots of detail. It is important to include some blank space. This ensures that your poster is not too full of information and it allows sections to be clearly separated.

Bullet points are useful for making concise points. Try to avoid large blocks of text and remember that it can sometimes be hard to read text that runs across the entire width of the page, particularly on large, or landscape posters.

Appearance: Aim for a poster that is visually attractive and eye-catching but is still clear to read and accessible. Your poster needs to look interesting to attract readers.

Colours: You should take similar considerations over colours as for a PowerPoint presentation (see Section 4.3.1.2). Consider contrasting and complementary colours and choose your background colour carefully. Whilst bright text on a dark background can work well on a projected PowerPoint presentation, such colour combinations can sometimes be hard to read when printed, so dark colours on a light background are often recommended for posters. Colours can be useful to highlight, or group points but be careful not to use too many different colours as this can become hard to read.

Font: You should use a clear, sans serif font, and make sure your poster is clear to read from a few metres away. As a guide, use around 72 pt for the title, 48 pt for section headings, and 24 pt for content text, but this may vary depending on the content, and any requirements of the conference organisers. Avoid using all capital letters as this can be hard to read.

Pictures and figures: Pictures can help to make a poster visually attractive. Aim to use pictures and photographs with a high resolution that are not too small. Make sure figures and tables are clear, with labelled axes and a caption or heading, so that readers can understand the figure without having to read the whole poster.

Size: Check with the conference organisers for the required poster size and whether there is space for portrait or landscape posters. Poster sizes are often either A1 or A0 in size and these can be created using programmes such as PowerPoint, by customising the page size:

A1: 118.9cm x 84.1cm
A0: 84.1cm x 59.4cm

4.3.2.3. Preparation and presentation

Proof-read: Make sure you proof read your poster and check for any spelling mistakes before you print, as this can be expensive to correct later.

Print: Check with your University for recommended printers as they may be able to print posters in-house, or know of a reasonably priced printer. You should also check the turn-around time of the printers, to make sure you leave enough time for printing before the conference.

Stand by your poster: At the conference, make sure you stand by your poster in the allocated poster session. This is your chance to tell an interested audience about your research so make the most of it! Be prepared to answer any questions that people have, or offer to talk people through your poster and give an overview of your research. It is often useful to have A4 copies of your poster available for people to take away.

4.4. Getting Zoo Research Published

The gold standard for scientific research is publication of your findings in a peer-reviewed journal – preferably one with a high "impact factor". Peer-reviewed journals (and impact factors) are discussed in more detail in Section 4.4.2, but for now, a quick definition of a peer-reviewed or refereed journal is, "*a scholarly or research publication in which the articles submitted for publication are evaluated by a group of individuals who are expert in the subject area*" (Keenan & Johnston, 2002).

These guidelines will help you to identify the steps you need to take to get your zoo research paper published.

4.4.1. Why publish zoo research in peer-reviewed journals?

4.4.1.1. Current status of zoo research

Zoo research is under-represented in the published literature. There is a lot of good research being carried out in zoos and aquariums that is not getting published. This may be for one of two reasons; either papers are being rejected by editors, or papers are not being submitted to journals in the first place (possibly owing to the perception that they will not be published).

It seems that there is a common perception that much research carried out in zoos is not a scientifically worthwhile activity. The most common reasons given for this are that animals in zoo environments are not "natural" and that robust statistical analyses are not possible. However, with recent developments in husbandry methods and naturalistic housing and social groupings, most modern zoos now provide an extremely useful research setting; bridging the gap between highly controlled, but often extremely unnatural, laboratory conditions and the much more natural but very difficult working conditions of the field. Small sample size is often a problem of zoo research which presents statistical challenges, but valid and robust statistical tests are possible for typical zoo datasets (see Chapter 3 – Analysing the Results).

Furthermore, the management of zoo animals, which formerly was based very much on keeper experience, is now moving much more towards an evidence-based approach, and the strongest evidence in science is that which is published in peer-reviewed journals. This is because the peer-review process itself ensures that the results presented in published work can be accepted with great confidence.

4.4.1.2. Enhancing the reputation of your zoo, and of zoos in general

Publication of good-quality research enhances the reputation not only of the researcher but also of the zoo or zoos in which the work was carried out. At a more fundamental level, zoos in the UK need to be active in research in order to qualify for a licence to hold animals (Zoo Licensing Act, 1981). Activity in research is most easily and unambiguously measured by published output from research projects. Publishing papers will add an organization to the fraternity of zoos which are undeniably research active.

In addition, the public are becoming increasingly aware of the need for research into conservation and management of captive populations. Journalists as well as academic researchers look at the contents of academic journals to uncover the latest science stories, and a zoo which is regularly quoted in the literature will be recognized as highly research active. Therefore, journalists will be much more likely to use that zoo as a source of information. Zoo staff may be invited to be experts on media programmes. The zoo would then develop a reputation as a “good zoo” in the eyes of the public, known for carrying out interesting research, promoting the conservation and welfare of their animals, and full of experts who understand the animals they care for.

Other research-active zoos and interested parties will also be reading the literature and be aware of which zoos are carrying out research that is good enough to be published. Thus, the worldwide reputation of the zoo as a modern, active organization will be hugely enhanced by published papers in journals.

4.4.1.3. Attracting funding

Funding for future research projects is dependent, to some extent, upon the results of previous research projects. Successful project supervisors and researchers are far more likely to get funding than those without a proven track record. However, “success” in this context really means presenting published information. The number and quality of papers produced by a researcher provides evidence of their track record. However successful you are, for example, at solving a behavioural problem or explaining the challenges of captive breeding in the animal studied, it does not count towards enhancing the ability to obtain further funding until the results are published in a reputable journal.

4.4.2. Where to Publish

4.4.2.1. How to choose the right journal

Before writing your paper, you should have an idea of where you are going to try and get it published. Choosing the right journal largely depends on why you are publishing it. For example if you are publishing a technical peer-reviewed report, you need to decide whether you want widespread publication for which you may need to publish in a less specialised journal, or whether you want to reach a specific target audience, in which case you need to publish in a more specialised journal. Alternatively, if you are publishing work aimed at journalists or the public, then you may want to aim for a glossy magazine, and research that is aimed specifically at zoo staff may need to be published in specific newsletters such as *Ratel* or *International Zoo News* (see Section 4.1). The rest of this section will focus on publishing in peer-reviewed journals.

Each journal has its own particular audience, topic and style. A good paper may be rejected because it is submitted to the wrong journal. There is a hierarchy of 'prestige' among journals, which to some extent is measured by impact factor (see Section 4.4.2.2 below). Submitting to very prestigious journals can sometimes mean a long wait before publication. However, you may get lucky and you will get good reviews and feedback, though it is helpful to be thick-skinned and not in a hurry. For topics where speed of publication is paramount (e.g. conservation), then time taken to turn around a paper will be a more important factor when choosing a journal.

The point of publishing research is to make it available to everyone. Therefore choosing a journal that makes at least the abstracts available to search engines such as Web of Knowledge, Zoological Record and Google Scholar should be a consideration.

By reading a few issues of the journal you think might be most appropriate for your paper, you will see the type of language and style previous authors have been successful with. This should guide you when preparing your paper for submission, as you may wish to 'tweak' your writing so that it fits with what you know the journal is likely to appreciate. However, this is unlikely to mean that the referees have no comments for you to consider!

Before submitting a paper to any journal, please read Section 4.4.4: Publication Ethics and Copyright.

4.4.2.2. Impact factors

The impact factor of a journal is effectively a measure of how often articles published in that journal are cited by other articles published collectively in all journals. In a sense, impact factors measure a journals "influence"; that is a journal's likelihood of being picked up and read by a greater number of scientists. If you want to reach a larger and perhaps broader audience, then higher impact factors are better. Indeed, this is what many researchers feel is the best strategy. However, it is worth revisiting your reasons for publishing when considering this. If you are targeting a specific audience with your findings, it may be better to select a specialty journal which by its very function, may also have lower impact factor. Clearly, decisions about which are the most appropriate journals for you to publish in should not be made purely on the impact factor.

Most journals have their own website, and the impact factor is usually given on the home page. For example, the website for *Animal Behaviour* gave an impact factor of 3.493 in 2012. The journal *Zoo Biology*, by comparison, had an impact factor of 0.84 in the same year (*Nature* scored 36.280!).

4.4.2.3. Which journals publish zoo research?

A wide range of journals publish research conducted in zoos, however for most journals, zoo-based research does not make up a high proportion of the published articles. The vast majority of zoo papers are published in *Zoo Biology*, (a journal concerned with the "reproduction, demographics, genetics, behaviour, medicine, husbandry, nutrition, conservation and all empirical aspects of the exhibition and maintenance of wild animals in wildlife parks, zoos, and aquariums"), *Applied Animal Behaviour Science*, the *Journal of Zoo and Wildlife Medicine* (a veterinary journal), and also in *Animal Welfare* and the *American Journal of Primatology*.

From 2013, the *Journal of Zoo and Aquarium Research (JZAR)* "will provide a forum for rapid publication of novel, peer-reviewed research papers, reviews, technical reports and evidence-based case studies" from zoos and aquariums (www.jzar.org).

Papers which commonly publish zoo-based research include:

Animal Behaviour
American Journal of Primatology
Animal Welfare
Anthrozoos
Applied Animal Behaviour Science
Behavioural Ecology
Behavioural Processes
Folia Primatologica
International Journal of Primatology
International Zoo Yearbook
Journal of the American Veterinary Association
Journal of Zoo and Wildlife Medicine
Journal of Veterinary Medical Science
Journal of Zoology
Oryx
Primates
Zoo Biology

Research can also be published in more specialist papers, as relevant to the subject area. Some examples may include:

Biological Conservation
Environmental Conservation
European Journal of Wildlife Research
Folia Zoologica
Journal of Mammalogy
Journal of Parasitology
Journal of Wildlife Diseases
Mammalia
New Scientist
Veterinary Microbiology
Veterinary Journal
Veterinary Parasitology

A more detailed list of journals publishing zoo-based research is available on the BIAZA website at: http://www.biaza.org.uk/uploads/Committees/RC/Research%20Guidelines/Publications4zoores.pdf

4.4.3. Presentation of your paper

The following section gives some general guidelines for publishing zoo research in peer-reviewed journals.

4.4.3.1. Know your audience

When presenting research keep in mind the audience for which you are writing. For readers of a general-interest journal, what is obvious to you may need explaining to them, especially if they are not specialists in your subject. Explain the details of, for example: dimensions, substrates and furnishings of enclosures; the methods used to gather data; the time period of data collection; and relevant information about the focal species (such as number, gender and age of animals studied). The more detailed the information in the article the more robust the results. Where possible use statistical tests to validate data if conclusions are going to be drawn from them.

If writing for a specialist journal (e.g. on enrichment) then it may not be necessary to explain specialist terms but, in general, it is better to start with full explanations and reduce the information if deemed necessary by referees.

The most basic rule is only use the words you need to use. The aim of publishing your work is to disseminate the details of your study, data, methods and conclusions. The more complicated the language the more difficult it is for readers to assimilate your work.

4.4.3.2. Use the "Instructions for Authors" provided by the publisher

Nearly all publishers supply comprehensive "instructions for authors", either on Web sites or on request. It is a really good idea to read these and follow the instructions. Such instructions may include details of how papers should be structured and any word limits that should be adhered to, as well as details of the required referencing style. The closer the style and layout of your article is to the required house style, the less work it will require and referees can concentrate on the contents of the study and not be distracted by presentation. It is also often useful to look over papers that have already been published in the journal of interest, for guidance on style and structure.

4.4.3.3. Authors and Acknowledgements

'Authors' are considered to be those who have contributed a substantial amount of the work described. This can be through significant contributions in planning the work, collecting and analysing the data or writing the paper. Others who have made only a small contribution should be credited in the acknowledgements. An exception to this may be a supervisor's name which tends to be put last. You must inform the zoo at which you carried out your research of your intention to publish, and your zoo supervisor, or any member of zoo staff with a substantial contribution to the project, should usually be named as a co-author. Indeed, this may be a requirement of any research agreements that you signed at the start of your research. You must also acknowledge any assistance that you received from the zoo in the acknowledgements section.

Guidelines on authorship are available from organisations such as the Council of Science Editors and the British Psychological Society. For example:
http://www.councilscienceeditors.org//i4a/pages/index.cfm?pageid=3355 or
http://www.bps.org.uk/system/files/images/statement_of_policy_on_authorship_credit.pdf

Other published guidelines on authorship can often be traced back to guidelines determined by the International Committee of Medical Journal Editors, widely adopted by the broader scientific publishing community. These are very comprehensive and provide the following criteria for authorship credit:

1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data
2) drafting the article or revising it critically for important intellectual content
3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

[From http://www.icmje.org/ethical_1author.html]

Where there are multiple authors, the lead author (the one who has done the most writing) should be first followed by authors either alphabetically or in descending order of work done. For large research groups, the head of the research group is frequently listed as the last author

4.4.3.4. Consistency and accuracy

Make sure your writing style is consistent throughout the article. For example, you should be consistent in your use of terminology, abbreviations and language. Check the instructions for authors for any specific requirements regarding abbreviations or language. Make sure that the content is accurate and that you include correct scientific names for species.

4.4.3.5. Photographs, figures and tables

Digital photographs should be >300 dpi to print well. Low-resolution photographs will be grainy and dull once printed. Provide copyright citations for images and obtain permission to use images from the copyright holder when necessary.

Use as little shading as possible in line art and remove “gridlines” on graphs. The simpler the figure the easier it is for readers to understand the information presented.

Ensure that column headings in tables are aligned with the entries below them.

Photographs, figures and tables should have legends that stand on their own as descriptions of the contents.

4.4.3.6. References

The reference list is very important. Providing a full list of articles/books/reports referred to during the course of your study demonstrates that you have researched your subject thoroughly. A good reference list also enables readers to find the background references to read for themselves. See Chapter 1 for guidance on finding literature.

Give as many details as possible in the references, following the style requested by the publisher. There are two main systems for citing references: Harvard (author names and dates) and Vancouver (numbered references) and journals often use their own house style.

Double check that all references cited in the text are given in the reference list and all the references in the list are mentioned in the text.

Referencing Style

Use the “instructions for authors” for guidance on the format of citations in the text. These instructions will advise you on: what information to use in the citation (usually the author, with the year in brackets), whether to list multiple citations in alphabetical or chronological order and when to use ‘*et al*.’ for multiple authors.

The “instructions for authors” will also give you guidance on how to structure your reference list. These instructions will usually give details of the required style for the different types of reference, such as journal articles, book chapters and conference proceedings etc. Detail will be given on the content required for these references, as well as the formatting required.

4.4.4. Publication Ethics and Copyright

Editors expect authors to be honest about the content of manuscripts submitted to their journals. Thus, results and other parts of the paper should not be fictitious or doctored, plagiarised (i.e. copied from another published or submitted work without attribution) or duplicated in another publication, whether already published or submitted for publication. The consequences of dishonesty can be severe; journal editors may bar dishonest authors from publishing in the journal again.

While there is no excuse for intentional fraud, there are some grey areas. For example, authors may sometimes be unaware that they are using other peoples’ ideas and results, perhaps from papers read some time ago. A good literature search is therefore essential when writing a paper, to ensure that all published material is properly attributed.

Authors may also be unaware of what counts as duplicate publishing. Most journals will only publish work that has not been published or submitted for publication elsewhere. In part this is to avoid infringement of copyright. In addition, part of the remit of high-quality international journals in particular is to publish original work, so editors do not want results that are already known to the scientific community. However, the definition of prior publication is not always clear to authors, and can vary between journals. The results are the main novel part of a paper and so prior publication of the results is likely to lead to a journal declining to publish the submitted manuscript. The criterion for prior publication is usually that the results have appeared in any journal, conference proceedings or a book that has an ISSN or ISBN.

Authors need to be particularly careful about abstracts that summarise the results and are published in conference proceedings. If these contain details of the data, with descriptions of methods, results and conclusions, they are likely to count as prior publication of the work. Similarly, data given in a book may well be seen by a journal editor as published and therefore not suitable for inclusion in a subsequent journal article. Authors who wish to publish the work elsewhere should check with the editors of conference proceedings before submitting an abstract; authors may have the option of not having an abstract published, or if they do to publish only a brief summary of the results without giving any data. Such a qualitative description of the results would not be seen as prior publication; nor would abstracts that are only handed out to conference delegates and are not published in conference proceedings. Guidelines on acceptable or unacceptable overlap between conference abstracts and manuscripts for publication may be available from journal editors. For example, a statement of policy and examples of overlap are available for the journal Animal Behaviour at: http://asab.nottingham.ac.uk/downloads/abstract_policy.pdf and http://asab.nottingham.ac.uk/downloads/abstract_examples.pdf

Authors are allowed to include some published material, such as a figure or part of a data set, in a paper submitted to a journal, but they should make it clear in the cover letter exactly what has been published and where, and the source of the material should be given in the manuscript. It will help the journal editors if they receive a copy of the published material when the new article is submitted so that they can see the extent of the overlap for themselves. For copyright material, authors will need to obtain a letter from the owners of the copyright (usually the publishers) granting permission to reproduce it. This is the case even if the published material is the author’s own work. For example, repeating a Methods section from one of your own published papers because your methods were the same is a breach of copyright. It would also be regarded as duplicate publishing by journal editors, as would submitting two papers with the same Methods section to two different journals.

If in doubt about the acceptability of material before submitting a paper to a journal, authors can always get in touch with the editor for advice. Editors are usually happy to help and would rather do so at an early stage when a problem can be rectified than have to reject an otherwise sound and interesting paper.

4.4.5. Relationships with editors and reviewers

4.4.5.1. Submission

Many journals use an online system for submission in which authors give all of the information required by the editors, such as contact details and affiliations. In addition, submitted papers should be accompanied by a cover letter to the editor (no more than a single side of A4).

The cover letter should:

- highlight the ways in which the paper falls within the journal’s Aims and Scope
- describe the relevance of the research to the zoo research community and how the paper furthers our knowledge in a particular field/subject area
- make the editor aware of the limitations of the study (e.g. be transparent about the sample size, explain that the statistical approach used is appropriate for the sample size)
- state that the research complies with BIAZA guidelines

- include a list of potential referees (with contact details)

Zoo researchers should make editors aware of the BIAZA Research Group as a source of potential referees. Editors seek publishing authors to review submissions and so it is important that the reference list includes other research published within the subject area. Editors should also be encouraged to look at the BIAZA Statistical Guidelines (Chapter 3), which describe the zoo-research community's approach to the issue of comparability and small sample sizes.

It may be advisable to mention if the paper has previously been rejected by another journal, as papers could be sent to the original reviewers. It is important to explain the changes made since rejection. When submitting to another journal re-format the manuscript to conform with the new journal's guidelines (and ensure that all reviewer comments are deleted).

Owing to high numbers of submissions, the majority of peer-reviewed journals will reject a number of papers prior to review. Oryx and Animal Conservation reject approximately 35% of papers prior to review. In most cases, the editor will indicate why the paper is considered unsuitable and an alternative journal may be suggested.

4.4.5.2. Peer review

Once a paper is considered suitable, it will be sent to two/three reviewers for comment. Editors may send the paper to a third referee if there is a strong disagreement between the first and second referees. Reviewers will be asked to rate the paper in a number of areas including:

- relevance to the journal
- significance to the area of research
- novelty
- appropriateness of methods
- clarity of writing and illustrations
- extent of interest beyond the particular species/habitat

The reviewers will also be required to make a recommendation for acceptance, minor revision, major revision, reject and resubmit, reject.

If a paper is rejected take the reviewer and editors' comments on board, rewrite the paper and submit to another journal. The peer-review process is a means by which papers are refined and improved, and it may take several submissions before the paper is finally accepted.

4.5. Further reading:

Butcher, J., Drake, C., Leach, M. (2006). Butcher's Copy-editing: The Cambridge Handbook for Editors, Copy-editors and Proofreaders (4th edn). Cambridge University Press, Cambridge.

Council of Science Editors. (2006). Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers (7th edn). Council of Science Editors in cooperation with the Rockefeller University Press, Reston, VA.

Ritter, R. M. (Ed.) (2000): The Oxford Dictionary for Writers & Editors (2nd edn). Oxford: Oxford University Press. ISBN: 0-19-866239-4.

Chapter 5

Projects involving behavioural observations of animals

Editors: Joanna Bishop, Sonya P. Hill

Contributing Authors: Joanna Bishop, Sonya P. Hill, Stephanie Wehnelt, Charlotte Hosie, Amy Plowman, Anna Feistner, Sue Dow, Juergen Engel and Heidi Mitchell

5.1. Introduction

Many zoo-based studies are animal behaviour related, and this is probably because this area is fundamentally interesting to many people. Animal behaviour projects tend to be particularly popular for student projects, with the added advantage being that behaviour can be measured relatively cheaply (Hill and Broom, 2009), without the need for expensive laboratory or other equipment. But, of course, there is far more to studying and interpreting animal behaviour than it just being "interesting and cheap!". A first step in conserving biodiversity is to understand the biology of the species. In zoos, behavioural research can further our understanding of how animals are affected by the captive environment, to aid breeding programmes, ensure high standards of animal husbandry and welfare, and to manage health and disease risk. Further knowledge and understanding of the behavioural ecology of many species have come from captive populations, due to these animals being difficult to study in their natural habitat (although caution must be exercised in interpreting behaviour in free-living versus captive animals).

Results from behavioural research can have applied and/or fundamental implications, and so there are many different reasons why animal behaviour is studied. Behaviour is the expression of an animal's response to a stimulus. If investigated and interpreted correctly, behaviour can tell us a lot about the individual's or species' requirements for resources (such as food or social partners), about preferences (e.g. food choice), about coping and adapting to challenges or changes in the environment, about cognitive capacity, and so on. Study of behaviour can involve investigations of different patterns of behaviour, including intensity, frequency or duration, and can investigate how an individual's behaviour changes over time.

There are some excellent published texts devoted to explaining the methods typically used in behavioural research and analysis (see Section 5.6), so we will not attempt to "reinvent the wheel" here by repeating those in detail. Instead, in this chapter we will discuss some of the pertinent issues that should be considered when carrying out behavioural research in zoos, and how to try to overcome the common pitfalls that you might face.

5.2. Types of Investigation in Zoos

As discussed in Chapter 1. It is not always possible to manipulate variables in zoo research, due to ethical, logistical and/or legal reasons. Therefore, many researchers use behavioural observation and take advantage of carrying out studies opportunistically, using variables that already exist in the zoo environment. For example, researchers may compare the behaviour of animals in different types of enclosure, either in the same zoo, or at multiple sites. Or they may look for the effect of time of day, or of visitor numbers, or of proximity to other enclosures containing other species, and so on. There are, however, occasions where manipulation of variables may be possible, or studies can also take advantage of changes that are already happening in the zoo. Such changes can include planned husbandry events like movement of animals between enclosures, addition or removal of animals to/from groups, and the provision of environmental enrichment. Observation of animal behaviour is therefore relevant to a range of study types. The current chapter gives some advice on methods and practicalities of observing behaviour. Please note, however, that as a common zoo research theme, other chapters of this handbook include advice relevant to behavioural observations and it is recommended that you read further into this topic. Chapters of particular interest may include chapters 1, 6, 9 and 13 ('Planning and Carrying out a Zoo-based Research Project', particularly the sections on multi-zoo studies, 'A Keeper's Guide to Evaluating Environmental Enrichment Efforts', 'Studies on the Effects of Human Visitors on Zoo Animal Behaviour' and 'Aquarium Based Research' respectively)

5.3. Measuring Behaviour

Once a basic knowledge of the species has been gained and the potential limitations of the methods are understood, the researcher must develop skills of measuring and interpreting behaviour. Behavioural research needs to be systematic, hypothesis-driven and incorporate appropriate controls in order to record accurate and reliable data (see Chapter 1 for advice on developing hypotheses). It's not simply a case of watching and writing down what an animal does (although descriptive data can be very useful in the preliminary stages of a study, to help refine the precise research questions to be investigated). Collecting behavioural data usually involves many hours of observations, either taken "live" or from video, and this demands patience, attention to detail and the full concentration of the researcher. Interpreting an animal's behaviour accurately requires an objective mind, and a good knowledge of the species – or of a similar species, if little is known to science about the one being studied. It can also involve a tolerance for standing still for long periods of time, in all weathers, watching animals that might not be "complying" with what the behavioural researcher secretly wishes them to be doing – for example, animals spending a lot of time being out of sight during an observation session, or doing something that isn't particularly "entertaining" to watch, like sleeping, when our time would pass much more quickly if we were observing them engaged in something like exciting social interactions!

Regardless of how exciting (or not) the observation session may be, our role as behavioural researchers is to record what the animal is doing accurately (and how often, for how long, etc.), and then interpret what that means, in terms of both statistical and biological significance – how, why, what, where and when is the animal doing (or not doing) what it does, and how does that relate to our research questions?

5.4 Some things to consider when carrying out behavioural research

Further to the general guidance given in Chapter 1, relating to planning your zoo-based project, there are some specific things to consider when planning and carrying out behavioural research in a zoo. Textbooks go into more detail about these kinds of issues, so you should ensure that you do thorough background reading before and during your project, to familiarise yourself with these.

5.4.1 Pilot studies

Once your research question(s) has been identified, it is important to first carry out a pilot study, in which you collect preliminary observations of the species (and preferably the precise individuals) you will be observing in your study. The pilot study enables you to practice and refine your methods, so that when you are eventually ready to start collecting your 'hard' data (i.e. usable data), you are confident it is accurate and appropriate. During the pilot study, you can learn to accurately identify each animal (if this is necessary for your research question) and accustom the animals to your presence at the enclosure. Or, if you notice that your presence seems to affect the animals' behaviour (which would subsequently affect your results and should be avoided), you can take measures to minimise the effects of this. For example, you could switch to carrying out video observations that do not involve your presence at the enclosure, or you could try standing further back from the enclosure and seeing if that removes the impact of observer presence on the animals.

The pilot study also enables you to practice the sampling and recording rules you will use to collect your hard data and to judge realistically how long the data collection for the whole project will take. At this time you can also refine the specific hypotheses and predictions that your hard data will enable you to test. It is important that you think about how you will analyse your data *before* you start your main data collection. This is so that you can collect the most suitable data for your analysis, which will allow you to answer your research question. The pilot study is an ideal time to try analysing some of your data to check that you are collecting everything that you need for your final analysis.

5.4.2. Choosing Sampling Methods

As we have already read in Chapter 1, exactly what data you should collect depends on your hypothesis or research question. The variables you measure should be chosen to best answer the research question. It is easy to get carried away and try to collect data on everything, so ensure you avoid taking unnecessary data by remaining focused on your hypothesis.

For example, instead of collecting general data on everything that each individual of a group does, you might focus specifically on where individuals are found in the enclosure and the behaviour shown there. If you are looking at the effect of enrichment devices (see Chapter 6 for more details on enrichment studies), you might focus on behavioural elements relevant to the device (e.g. foraging and feeding) or focus more broadly on changes in time budgets, but you would not necessarily need to collect data on details of, say, social behaviours, unless this was relevant to your research question (e.g. to ensure there was no adverse effects of enrichment resource competition on the animals).

5.4.3. Decide how, when and how often to record the data

Sampling rules enable you to specify which subjects to watch and when, whereas **recording rules** relate to how the behaviour is recorded. There are several different sampling and recording rules that are relevant to behavioural research. You might, for example, study one focal animal at a time, or select individuals out of sub-groups, or scan the behaviour of the whole group at the same time point. Sampling methods include: 'ad libitum', 'focal' and 'scan' behaviour sampling. Recording can be 'continuous' (i.e. recording everything the animals do during an observation period), 'instantaneous' (i.e. the record is made at a specific, pre-determined moment in time, e.g. every 5-mins, marked by the beep of a stopwatch), 'frequency', etc. Not all of these methods are suitable for every type of research question and there are advantages and disadvantages of each. So, you will need to learn about what different rules and measures there are, to enable you to determine which one(s) will best enable you to answer your specific research questions. Such detail can be found in other texts (e.g. see Altmann, 1974; Martin and Bateson, 2007). Choosing an appropriate sampling method is important in determining how your data can be analysed statistically, so read Chapter 3 of this Handbook before you start collecting data.

The number of observations you take will depend on how many animals are available to you, how many different experimental conditions you are planning to compare, the probability tests you have decided to use (if any) and how strong the effect of your independent variable is. You need to decide how long you will leave between sampling periods (among other factors, this will depend on the duration of the behaviours observed), and how you will avoid bias when choosing a subject to sample. It is important that issues such as these are considered carefully, to avoid generating errors in interpreting results and/or any statistical analyses. For example, if certain individuals are more active or visible than others in the group, there is the risk that their behaviour may be recorded repeatedly (and so be over-represented in the results), whereas really you need to obtain data from all individuals as evenly as possible. So, to avoid bias, pick subjects at random or in a set order, rather than picking the animal displaying the most interesting behaviour at that time. This might result in lots of behavioural categories like 'inactive' or 'out of sight' being recorded, but, even if this seems less interesting to you, the observer, these are still valid data and help make your data more robust.

One of the potential challenges with zoo-based research compared with, say, studying farm animal behaviour, is the number of animals available to you. Any one zoo typically has a relatively small number of individuals of a given species, so you should consider carefully the sample size that you need to answer your research question, or that is suitable for any statistical techniques that you plan to use. Multi-zoo studies may help to increase your sample size (see Section 1.4), although bear in mind other factors that may vary between multiple study sites, and how these may affect the way in which your data can be interpreted.

You should also determine how long your study should be in order to achieve your study's aims. How long this is depends on your research question. For some studies, the experimental manipulation is only expected to have a very short effect so observation times can be very short. For other studies, the intuitive answer is probably the longer the better (up to a point), because statistical power is improved by increasing the sample size (Martin and Bateson, 2007). Behavioural data can be time-consuming to collect, because you need to ensure you have a representative sample and enough data to analyse. If the data collection period is too short, your data may be biased by factors like poor weather, or any of the animals having “bad” days (e.g. temporary ill health). However, the time and effort involved in data collection means you have to weigh up the pros and cons of collecting more data; you may eventually get to the point where additional data does little or nothing to add to your conclusions.

You should also consider the issue of autocorrelation and temporal independence in your data. Most statistical tests require that data points are independent. If data are collected over time on the same animal (or group) then it is likely that the measurement at one point in time has been affected by the one previously. For example, the level of a reproductive hormone circulating in a female chimpanzee will not change instantaneously, and if two measurements of the hormone are made in quick succession, the value at the second measurement cannot be independent of the measurement taken at the first. This is equally true of behaviour. It is important that the chosen sampling regime minimises the likelihood of autocorrelation, thus ensuring temporal independence of the data points.

Time sampling, for example, has been criticised on the grounds of non-independence of samples. All time sampling methods are dependent on sampling at predetermined points in time to avoid biased results, and so determining the optimum sampling interval is crucial. Ideally, to avoid autocorrelation the sampling interval should exceed the maximum bout length, but this would not give a good approximation to a continuous record of behaviour and many short-to-medium duration behaviour categories would be undersampled. The optimum interval will depend on the mean bout length, mean inter-bout interval, size of group under study and the statistical analysis planned. It is very easy to generate large numbers of data points that are not independent of each other, e.g. for slow-moving animals that may have bouts of a particular behaviour that last for several minutes. Shorter intervals result in more accurate measurements as they approach continuous sampling, but sampling behaviour too frequently will result in runs of a particular behaviour that are not independent of each other as they are part of the same activity bout and thus inflating the sample size (Bernstein, 1991; Wirtz and Oldekop, 1991). Long inter-sample intervals require more observation time to collect appropriate volumes of data, during which time other confounding variables may change.

At least four procedures have been developed to determine optimal sample intervals.

- Bernstein (1991) proposed a calculation based on mean bout length and its standard deviation to avoid temporal autocorrelation. In practice, measuring mean bout length can be difficult and time consuming. This method will overcome autocorrelation but does not take account of discrepancies with a continuous record.

- Engel (1996) has produced a mathematical method that attempts to find an interval where data both reflect the accuracy of continuous sampling while avoiding autocorrelation.

- Martin and Bateson (2007) propose two solutions. The first involves a pilot study where a large sample of behaviour is recorded using continuous sampling. From this, scores are calculated for each behaviour as if they had been sampled using the proposed time sampling method at different intervals. The percentage difference between true score and each different interval score is calculated and compared. They suggest accepting an interval length that gives most behaviour patterns (e.g. 90%), but which yields small percentage differences (e.g. 10%) from the continuous sampling results. This method minimises discrepancies between continuous and time sampling, but does not address possible autocorrelation. It is also not always practical to use this solution, as the point of using time sampling may be because continuous recording is not possible or realistic to do in the first place.

- Martin and Bateson's (2007) second solution is very common in zoo studies. They suggest that individual sample points collected over short intervals within an observation session are not treated as statistically independent measures, but instead are used to give a single score for the whole session. For example, in a 40-minute period, which was divided into 2-minute sample intervals (i.e. 20 sample points), an animal was recorded as grooming for 6 of the sample points, then the score would be 6/20 = 0.3. It is the session scores, rather than the individual datapoints, that are then used for statistical analysis. In this case the interval between consecutive observation sessions should be at least the maximum bout length (determined in a pilot study) to ensure that session scores are independent. This method addresses both accuracy and autocorrelation, but it cannot be used for categorical analyses such as G-tests (see Section 3.3.3.) since the session scores are not counts. However, you can perform categorical analyses if you use counts of the number of sessions for which the scores (or count if all sessions are the same length) fall into certain categories, e.g. in the above example the number of sessions in which grooming was recorded 0-5 times, 6-10 times, 11-15 times and 16-20 times.

5.4.4. Dealing with "out of sight"

Another issue that behavioural researchers have to deal with is the animal being "out of sight" or not visible for parts of the study. This is particularly an issue in more naturalistic zoo enclosures, where the animals have many opportunities to get away from the observer's view, e.g. behind bushes, or in indoor off-show areas where the researcher may not be allowed access. When observing free-living animals, it is also the case that they can get out of sight of the observer, so it is not only a problem that zoo-based researchers have to deal with. If the animal is carrying out some activity while it is out of sight, such as grooming, you are underestimating the total amount of grooming as you can't see some of it happening. Unfortunately, this is just a fact of life so, if you really cannot arrange to see the animal (e.g. by video) in its hidden area, then you have to alter the question you are answering in order to make it realistic. For example, the question "What does the animal do between 2pm and 3pm" would become "What does the animal do *while visible* between 2pm and 3pm". You may find it useful to determine a specific rule for what you will do if your focal animal disappears from view in the middle of a recording session – if it matters to your research question, you need to decide whether to stop recording after a certain period of time and begin observing another individual, or whether you pursue it. If you are investigating the visibility of a species in the zoo, e.g. to contribute to our understanding of the zoo visitor experience, it will be important to your study that you know how long the animals are out of sight or visible for, so in this case "out of sight" becomes a kind of behavioural category in itself. It is also recommended that you record this "out of sight" category anyway, even if it is not directly part of your research question. This is because if the proportion of time the animal spends in that area changes suddenly after something like a particular husbandry event, e.g. provision of enrichment, it may tell you something about the animal's attitude to it.

5.4.5. Equipment

You should also use the pilot study to practice using the media you will use in collecting your data. Broadly speaking, there are five media typically used in behavioural research, some being more expensive or complex than others. Firstly, as part of the pilot study, you might use **descriptive verbal or written notes** to help you plan the collection of your hard data. Or, in your pilot and hard data collection, you might also collect data 'live' and record it onto **paper checksheets**, or use **video recordings** (see Chapter 9 for advice on using video recordings), or use **automatic recording devices** (e.g. pattern recognition devices), or **event recorders** (e.g. hand-held computers loaded with various behavioural data collection software; such software can be free or be purchased).

5.4.5.1. Checksheets

Many behavioural projects use paper checksheets as the medium to record data, although you should bear in mind any adverse weather conditions you might encounter and its effects on the use of paper outdoors – a clipboard with a waterproof cover may be a useful option, for example. You should take some time to carefully plan out the layout of your checksheet, as a sensible design can help you greatly whilst you are collecting the data, and when entering the data into a spreadsheet or statistical programme for analysis. Checksheet design will vary depending on the design of the project, the data collection method used and the preferences of the observer. However, as an example, it may be useful to use a table format with columns for the type of data to be entered (e.g. observed behaviour, location within the enclosure etc.) and rows for the observations (e.g. rows for each observation interval for instantaneous sampling). Make sure you include spaces on each checksheet to fill in the date and time of the observation session, and any other important factors that are relevant to the observation session, such as observation group or individual, weather conditions and temperature. It is also useful to include a "comments" column to give you some space to write any additional notes during an observation session, which may be relevant to your data (e.g. you could make a note if a feed occurred mid-way through your observation session, or if someone in zoo uniform walked past the enclosure etc.).

5.4.6. Categories of behaviour

Before collecting your data, you will need to decide which categories of behaviour you will be recording. To do this, you will need to determine which behaviours are relevant to your research question(s), and to drop any categories that are irrelevant. The fewer categories you have, the more reliable your data are likely to be, but be sure to collect as much robust data as you can, as you can always remove or pool categories later. To help you come up with the list of behaviours relevant to your study, you can use a published ethogram as a guide, if there is one for the species you are studying. An ethogram is a catalogue of the main types of behaviour that are typical in that species (regardless of study site), i.e. it shows the behaviour patterns that form the typical behavioural repertoire for that species. Ethograms are limited, in that not all animals behave in a "species-typical" way, and so during your pilot study you should determine which behaviour categories are relevant to your research questions and to your particular subjects; you may find your subjects do some behaviours that are quite different. Once you have compiled the detailed list of behaviours relevant to your research question(s), you should ensure each category is defined **clearly** and **objectively**, **without any ambiguity**, and should be **mutually exclusive**. Your definitions should be such that another researcher could use them and recognise the exact same behaviour. These behaviours will provide you with measurable units for which you can record frequencies, durations, latencies, or other unit. Preparation of the list of behaviour categories is an opportunity for you to decide which behaviours are necessary for your project, and which state (long duration) and event (short duration) behaviours you will observe. It is important that behavioural definitions are correct, and fixed at the start of the study to prevent problems of 'observer drift' as you become more familiar with the behaviour, so you should use your pilot study to help you perfect your methods. Literature research (see Chapter 1), previous studies on the same animals and pilot observations can help to identify and define these behaviours.

5.4.7. Enclosure use

In your study, it might be relevant to record information about the way in which animals use different parts of their enclosure. For example, you could study the amount of time animals spend in particular areas, or what behaviours they do in those areas. To do this you should visually split the enclosure into different areas and data collection can then take place using the methods outlined above.

Example: You could record the enclosure area in which your animal is located on each interval of observation, using instantaneous sampling.

In order to visually split the enclosure into areas you should draw a map of the enclosure and outline the perimeter of each of your chosen areas. Remember that it is unlikely that you will be able to mark the actual enclosure in any way, so make sure your map is as accurate as possible. It may help you to use specific enclosure features to position your imaginary boundaries between enclosure areas. If there are multiple observers involved in your study, ensure that everybody who will be collecting data can reliably determine the boundaries of the enclosure areas before commencing data collection. You can either split your enclosure into equally sized areas based on a 'grid' system, or you could split up the enclosure based on the distribution of resources. Bear in mind that if you use enclosure areas that are not equal in size, you will need to account for this in your analysis. Use of the modified spread of participation analysis may be useful for such analysis (see Plowman, 2003).

5.4.8. Inter-observer reliability

For studies involving multiple observers, inter-observer reliability should be tested to ensure that all observers are reliably recording behaviour. See Section 1.4.4 for further advice on inter-observer reliability. It is also useful to test intra-observer reliability, particularly for longer studies, to safeguard against observer drift (e.g. see Martin and Bateson, 2007).

5.5. The context of zoo data collection and confounding variables

Think carefully about the context of your data collection – what else might be going on at the same time and possibly affecting it? For example, some animals might reduce their activity when visitor numbers increase, but does this also coincide with higher temperatures due to better weather?

There are many confounding variables that are specific to the zoo environment, such as visitor and keeper presence. In addition to these regularly occurring variables, other events can take place (often without much notice) during the course of a study, such as animal moves, enclosure changes, diet changes, maintenance work and veterinary treatment, to name a few. It is important that you eliminate as many confounding variables as possible so that the only factor that affects your dependent variable is/are your independent variable/s. For example, you might only record data on days when visitor numbers at an enclosure are likely to be similar. You should also keep in mind contingencies should any other events take place. For example, you should plan a suitable sample size in case an animal is moved away, or you should timetable enough days of observation in case you have to discard any data. Any confounding variables that cannot be eliminated should be controlled for as far as possible and acknowledged and discussed in your project report.

5.6. Further Reading

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Chapter 6

A Keeper's Guide to Evaluating Environmental Enrichment Efforts

Editor: Sonya P.Hill

Contributing authors: Sonya P. Hill, Amy Plowman.

6.1. Why evaluate enrichment?

Zoos use environmental enrichment as part of good husbandry, to provide appropriate, stimulating environments for their animals, to contribute to the zoo visitor experience, and to help improve, and maintain, good animal welfare. Enrichment can be time consuming to provide, and so it is important for keepers to know that their limited time is not wasted on ineffective enrichment efforts. However, most importantly, since enrichment is provided to enhance the animals' welfare it is vital to know whether it is working and indeed to be sure that it is not having the opposite effect and having a negative impact on welfare. If it is not working, or if its impact is not yet known, it should not be called "enrichment" but more appropriately be called something like an "enrichment effort" (Hill and Broom, 2009).

Large-scale scientific evaluations of the impact of enrichment efforts on animal behaviour and welfare are important, but can be slow to achieve, because of the nature of behavioural studies (see Chapter 5). The time it takes to generate and interpret the empirical results can (rightly) lead to dissatisfaction among animal staff, can prevent new enrichment efforts being trialled in the meantime, and might ultimately mean that the animals are missing out in some way. Whilst empirical investigations should be carried out wherever possible, it is important that zoos also adopt a quicker, more manageable approach to evaluating enrichment efforts, which will be of more practical use. It is also important to assess the impact of one enrichment effort at a time, or else you cannot tease out which one had the impact (and some effort may cancel each other out, e.g. one might increase a behaviour, and the other might reduce it).

6.2. What are the aims of your enrichment efforts?

In developing an enrichment study, we first need to identify what we hope the enrichment effort will achieve. There can be several different aims, but often these relate to targeting a specific behaviour or behaviours, e.g. to provide opportunities for a wider range of natural behaviour, or to try to reduce the time spent performing a stereotypy (Box 6.1); enrichment efforts might also be used to encourage animals to use more areas within their enclosure.

Box 6.1: Examples of enrichment aims

• Reduce time spent pacing	Measurable
• Increase diversity of behaviour	Measurable
• Increase use of enclosure space	Measurable
• Increase adaptability	Not easily measurable
• Improve post-release survival	Not easily measurable
• Increase physical fitness	Not easily measurable
• Increase visitor dwell time at an enclosure	Measurable

Sometimes it is relatively easy to measure whether these aims have been achieved and sometimes it is not without specialist equipment, lab tests, a very carefully planned experiment or a very long-term study (i.e. years).

In order to assess whether your enrichment effort has achieved what you intended it to, you must be clear on what the aim was and that aim must be measurable.

6.2.1. What is measured?

Most enrichment evaluation is done by measuring the animals' behaviour with and without the enrichment effort. Physiological measures could also be taken (e.g. faecal glucocorticoids, see Chapter 8; or carcass characteristics at post mortem), but behaviour is often the most obvious indicator of an animal's response, and may be the useful measure to take. This guide focuses on evaluating enrichment efforts by comparing the animals' behaviour with and without enrichment, since it is assumed that most keepers will not have the opportunity to use other possible measures.

When an animal first has access to a new enrichment effort, either its behaviour will change in some way (e.g. in direct interaction with the device), or it will ignore it entirely (which is just as important for us to know). If the behaviour changes, it might be a reaction to the novelty of the enrichment efforts, and the effects could wear off relatively quickly, or the impact could be longer-lasting. Likewise, the enrichment being trialled might be explored later, even if the animal ignores it initially. There is no magic formula to predicting this, as each individual has the potential to react differently, but, by evaluating animals' reactions to an enrichment effort, we can determine whether we think it has been successful or not. We should not jump to an immediate conclusion as soon as the animal has access to a new enrichment effort, as it would be too easy to see animals using it and thinking that it is a success, or conversely not using it and thinking it has been unsuccessful. To be sure what the effect of the enrichment effort is, behaviour over the whole day should ideally be observed, systematically, on several days, with and without the new enrichment effort.

Please refer to Chapter 5 for more information on how to measure behaviour rigorously. When investigating the impact of a new enrichment effort, you may also find the "S.P.I.D.E.R." framework useful, created by Disney's Animal Programs (http://www.animalenrichment.org/spider/spider_framework.html). The S.P.I.D.E.R. acronym stands for each of the steps you should follow: Setting Goals, Planning, Implementing, Documenting, Evaluating and Readjusting. By following this framework, and the further guidance on their website, it helps you to complete each step in the process, from deciding on the aims (e.g. which behaviours you want to encourage the animals to do), right through to being able to evaluate and review your enrichment efforts and tweak them to make them more appropriate, based on your findings.

It is usually not feasible for keepers to devote a long period of time to collecting behavioural data during their working day, due to the other duties they are required to do. That said, keepers do pass by their animals' enclosures at various times throughout a typical day, and Canino and Powell (2010) have proposed a method for assessing effects of enrichment programmes, which should fit into keepers' work routines and produce useful data. Their method involves the keeper taking "multi-point scan sampling" at five pre-set times during the day, over a period of several months. Their data are not tested statistically, but instead are presented instead as the percentage of scans where the animal is seen doing particular behaviours (in their case, either when new enrichment items are being used, or when using previously-used items). The authors found that this multi-point scan sampling method, carried out throughout the zoo day, was a quick and effective method for keepers to contribute to the assessment of their enrichment programmes. Inevitably, there are limitations in this type of method, and it is likely to necessitate some loss of accuracy compared with the used of full-scale rigorous scientific methods, but sometimes there is no realistic alternative when the data are required as a management tool. Margulis and Westhus (2008) compared behaviour collected in 3 different ways, and found the 'multi-point scan sampling' approach was a useful management tool.

Box 6.2: Controlling other factors

If all your enrichment days are cold and wet and all your non-enrichment days are hot and sunny you any differences you find in behaviour could just be due to the weather not the enrichment.

Similarly if all your enrichment observations are done in the morning and all your non-enrichment in the afternoon then differences could be just due to time of day

Similarly if all your enrichment observations are done on individual A and all your non-enrichment observations on individual B then differences could be just due to individual animal differences.

You must keep all conditions as similar as possible so that the only difference between enrichment and non-enrichment days is the enrichment.

You should bear in mind the impact that you, the keeper-observer, may have on the animals' behaviour when you are at the enclosure recording your scan data. Is your presence influencing the animals' behaviour because they recognise you (or your uniform) and think you are going to feed them or interact with them, for example? You want to ensure that you are having as little impact on the animals' behaviour as possible, so that the data you are collecting is as representative of their typical behaviour (without you present) as it can be.

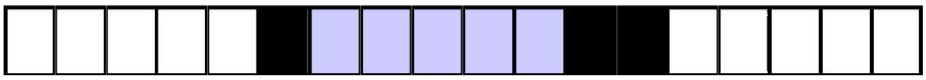
It is also important to make every effort to ensure that all other conditions on enrichment and non-enrichment days are kept as similar as possible (Box 6.2).

You won't be able to control some conditions, such as visitor numbers or the weather, and so you should try to randomise them so some enrichment days are busy and some quiet (with varying weather) and some non-enrichment days are busy and some quiet (with varying weather).

A randomised design (Box 6.3) is ideal as this allows you to be very flexible as to when you have study days, when you do not have time to have a study day or when you have days off. It is automatically randomised for factors such as weather and visitor numbers and allows statistical analysis by randomisation tests (the best tests for small numbers of animals). If a randomised design is not possible (e.g. because enrichment is too heavy to move in and out of enclosures every day) then a blocked design can be used, but it is important to include a post-enrichment block.

Box 6.3: Possible study designs

a) Blocks of 5 days pre-enrichment, 5 days enrichment and 5 days post-enrichment. Not recommended unless enrichment is not easily moved in and out of enclosure



b) Randomly assigned enrichment and non-enrichment days (5 each)

To be confident that any effects you see are caused by the enrichment you should really observe behaviour for a minimum of 5 days with and without enrichment. It is possible to have more than 1 person conducting the observations as long as you confirm that all observers recognise the same behaviour types. You would then record the behaviours (e.g. using one of the methods in Chapter 5 or the multi-point scans described above), and you should schedule your data collection times depending on what your routine allows.

Individual recognition

If you cannot identify individual animals and you cannot record your scan data on all animals at once, you must have a method for ensuring that you sample a random selection of individuals with and without enrichment. This can be any random method (such as 'eeni meeni mini mo', or 'the third animal from the right'), as long as it is not something that may bias the results (such as 'the animal nearest the enrichment resource' or 'the animal that looks most active'). If unrecognisable individuals go out of sight it is usually impossible to say that the same individual comes back into sight. It may be sensible to switch to another randomly chosen individual at that point.

Analyse your results

Ideally you should compare your data with and without enrichment using statistical tests. To do this you must use daily averages for the behaviour types of interest (Box 6.4). Once you have the daily averages you can apply statistical tests to compare these with and without enrichment.

Box 6.4. Calculating daily averages

State behaviour if using sessions

1. Calculate session scores for each state behaviour for each individual
   e.g. In a 30 minute session, the animal rested for 10 of the sample points, proportion of time resting = 10/30 = 0.33
2. Calculate the average of session scores for one day to get the daily score for each individual
3. Calculate the average daily score for all individuals

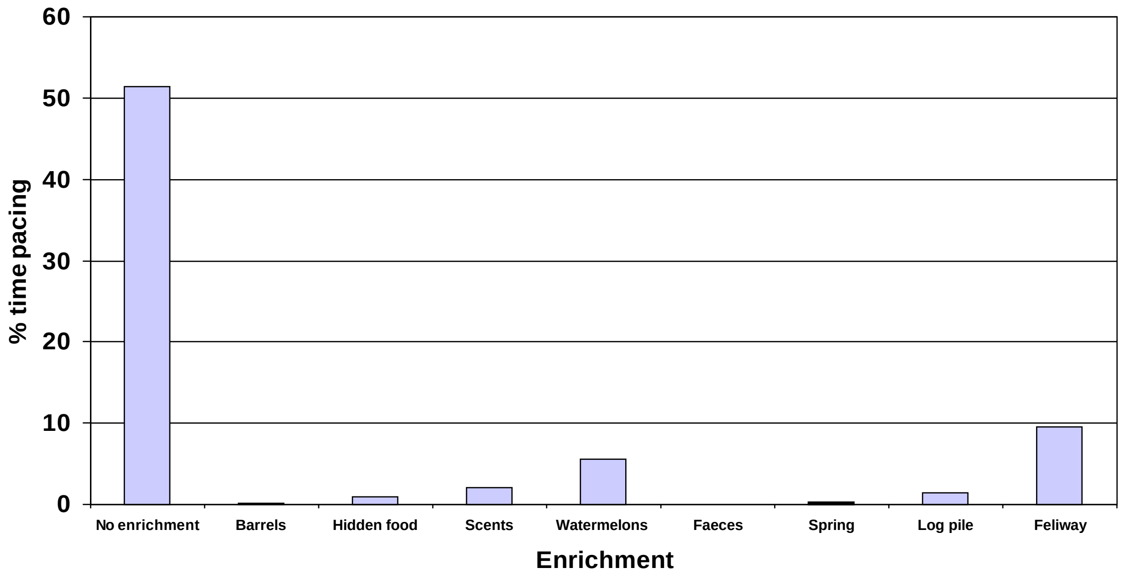
State behaviour if not using sessions

1. Calculate the daily score directly for each individual
   e.g. If you scored every half hour between 08:00 and 17:00 you would have 19 data points in total if 6 of those were resting the score for resting is 6/19=0.32
2. Calculate the average daily score for all individuals

Event behaviour

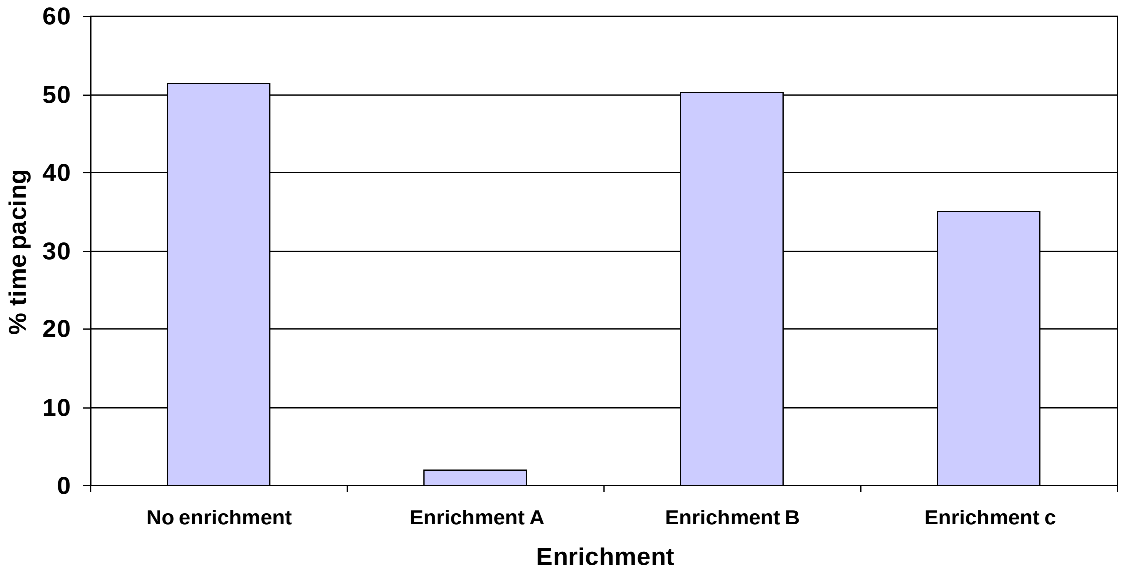
1. Calculate frequency of each event behaviour in each session for each individual
   e.g. Yawning was seen 8 times in a 30 minute session – Frequency = 4 per hour
2. Calculate the session average for each day for each individual
3. Calculate the daily average for all individuals

As a management tool, you can often make sensible biological decisions by looking at a graph of your results, without the need for carrying out statistical analyses, as these may not be possible without appropriate training, software, etc. Plot the overall average (average of the daily averages) values of the key behaviour types with and without enrichments as in the fictional example below.



This is the average time spent pacing by a pair of Sumatran tigers with no enrichment and with several types of enrichment. Even without statistics we can see that all the enrichment efforts made a substantial and real difference to the tigers.

Sometimes the difference is not so clear (as below)



In this fictional example enrichment A is clearly effective, enrichment B is clearly not, but what about enrichment C? It is better than nothing, but was it worth the cost in terms of money, time and effort? The decision about whether to use it again may depend on whether other more effective enrichment methods are available and how frequently these can be used before they become ineffective.

6.3. Useful Sources of Information

Canino, W. and Powell, D. (2010) Formal Behavioral Evaluation of Enrichment Programs on a Zookeeper's Schedule: A Case Study With a Polar Bear (*Ursus maritimas*) at the Bronx Zoo. *Zoo Biology*, 29, pp. 503-508.

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Excellent beginner's guide on how to observe and record behaviour

Chapter 7

Introduction to Research Using Zoo Records

Editor: Holly Farmer

Contributing authors: Andrea Fidgett, P. Kirsten Pullen, Dave Brunger, Alice Warren, Clare Collins, Holly Farmer, Jo Elliot, Dave Beeston, Zak Showell

Effective management of species in zoos and aquariums requires considerable knowledge of the biology of each species including reproduction, behaviour, group dynamics, husbandry, nutrition, medical needs and so forth. Scientific investigations are the basis for understanding the animals in a zoo and assessing the way they are cared for. Through careful observations and well-planned studies, much can be learned about, for example, reproductive and social behaviour, growth and development, basic nutrition and dietary preferences and interactions with the physical environment.

Fundamental research analysing animal records is conducted much less frequently, yet due to the limited sample sizes in 'living' collections, adding a historical perspective has the potential to test more robust hypotheses. Basic and advanced biological data on up to two million individual animals and 10,000 taxa have already been gathered and recorded in scientifically sound ways in zoos and aquaria and entered into custom-built, dedicated electronic databases. Such large data sets allow studies of reproductive patterns, infant or adult mortality, and many other components of species' life history, at both an institutional or population level.

It is advisable to read all sections of this chapter before beginning a project:

7.1. What do we mean by Zoo Records?

7.1.1. Types of information

Zoos initially stored records of animal arrivals, departures, births and deaths as notes in diaries, ledgers or stock books. At minimum, the information contained within these volumes formed a current stock list and record of 'transactions' for each collection. A later development was the use of cards for individual animals, to note their date of arrival, place of origin, sex and name and species. The cards were updated when the animal died or departed.

Currently in the UK, the Secretary of State's Standards for Modern Zoo Practice (SSSMZP) are designed to ensure that the welfare of animals in zoos is protected, that zoos are safe places for the public to visit and that zoos participate in appropriate conservation and public education measures. The Standards recommend best practice by which zoos are inspected and granted licenses by local authorities. Under Section 9 'Stock Records' all zoological collections in the UK are required to also keep and maintain records for all individually recognisable animals and groups.

The records should provide (wherever possible) the following information:

- identification and scientific name;
- origin (i.e. whether wild or captive-born, including identification of parents, where known, and previous location/s, if any);
- dates of entry into, and disposal from, the collection and to whom;
- date, or estimated date, of birth or hatching;
- sex (where known);
- any distinctive markings, including tattoos, freeze-brands, rings or microchips;
- clinical data, including details of and dates of any treatment given;
- behavioural and life history data;
- date of death and the result of any post-mortem examination and laboratory investigations;
- where an escape has taken place, or damage or injury has been caused to, or by, an animal to persons or property, the reason for such escape, damage or injury must be recorded and a summary of remedial measures taken to prevent recurrence should be provided;
- food and diets.

It is also compulsory that an annual stocklist of all animals must be kept which must be sent to the zoo's local authority and forms part of the zoo license inspection.

The SSMZP stipulates that records must be kept up to date, and must be available on site for six years. After this time, provision should be made to archive the records in a secure format on a long-term basis. This means that every collection in this country holding a zoo licence should have up to date, detailed animal records for both individuals and groups, available on site going back at least six years.

Recording details for individual animals such as tigers is relatively straight forward as animals can be identified from markings, tags, bands or transponder microchips, but it becomes more complicated for group-living animals such as leaf cutter ant colonies, which can be made up of up to 40,000 inhabitants. In this instance a 'colony' would be recorded; similarly for some invertebrate and fish species, a group record is kept.

Having information in one place can assist the management of an individual animal and also provides insight into a species in general. Depending on the species concerned, many different levels of data are collected at each institution. Furthermore this information is rarely managed independently, but forms the basis of managed conservation breeding programmes for species at a national, regional or international level.

7.1.2. Methods for recording/storing information

Data may be recorded by many zoo staff including keepers, curators, and veterinarians. The information they generate is often collated by one person, sometimes called a Record Keeper or Registrar, to a centralised records system. Note that for some collections, an alternative title may be 'ISIS Representative', for reasons which will become clear.

Originally all zoo records were paper-based using diaries, ledgers, stockbooks or index cards, which were stored in a secure place (e.g. a filing cabinet). Prior to the International Species Information System (ISIS) however, standards of record keeping were quite varied because there were no uniform record-keeping procedures among zoos.

Recognising that a system pooling all animal records in standardised format would enable more effective management of species, ISIS was founded in 1974 and continues to function as an international database to help zoos and aquariums accomplish long-term conservation management goals. To date (2013) there are currently 805 ISIS member institutions in 82 countries on six continents and the ISIS central database contains information on 2 million animals – almost 15,000 taxa/10,000 species – held in zoological institutions, plus some animals in the wild. ISIS members use the basic biologic information (age, sex, parentage, place of birth, circumstance of death, etc.) collected in the ISIS system to manage genetic and demographic programs for their animal collections.

Initially data was submitted on paper, using duplicate pads (i.e. including carbon paper); one copy was sent to ISIS while the zoo kept the other on file. Pads were also preformatted to standardise the information being submitted. From 1985 an alternative to paper forms became available, namely the suite of software programmes now most widely used for records management within the zoo community. These guidelines are strongly biased towards records maintained using the original programmes; ARKS, SPARKS, MedARKS and the new database programme ZIMS, therefore some explanation is necessary. These programmes are only available to members of ISIS, therefore not all zoos globally use these software.

7.1.2.1. ARKS - Animal Records Keeping System

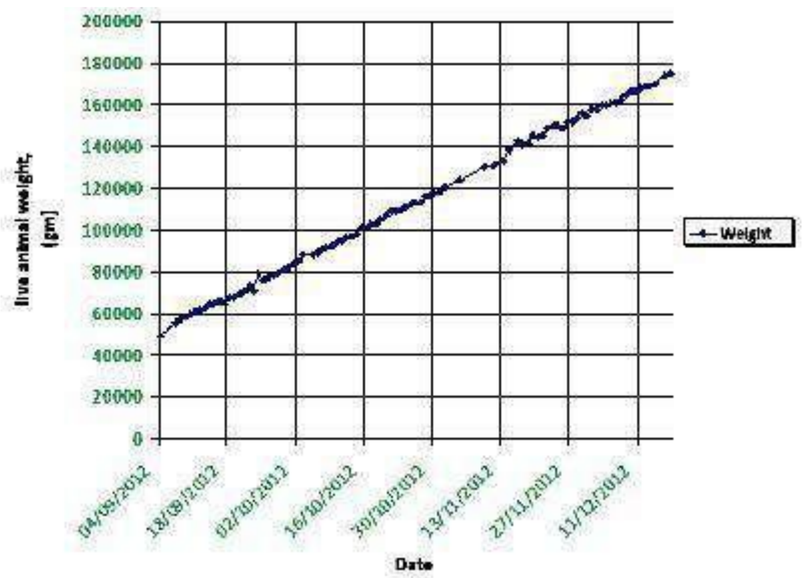
ARKS is a computer program used by institutions in the world to maintain animal records. All living specimens currently housed at an institution should be recorded (technically referred to as accessioned) and if historic data is available this may also be included in the ARKS database. The ARKS database is capable of rapidly retrieving data enabling an institution to review the overall management of an individual, group or species by using various reports as listed in Table 7.1 below:

Table 7.1: Selected ARKS reports

Report name	Explanation
SPECIMEN REPORT	Individual specimen or group record
TAXON REPORT	All specimens of a selected taxonomic group during a selected time span
TRANSACTION REPORT	The number of transfers in/out of collection, births and deaths for a selected taxonomic group
COLLECTION INVENTORY	The number of transactions (births, deaths, transfer into/out of institution for a selected taxonomic group.
ENCLOSURE REPORT	Details of specimens maintained in a specified enclosure at the institution
PEAK HOLDING	The maximum number of specimens held by the institution
CENSUS REPORT	The number of specimen at the beginning of each year of a selected species over a selected date span
WEIGHT AND LENGTH	Recorded weights/lengths for a selected specimen
Additional data can be obtained relating to reproduction and demographics:	
AGE PYRAMID	Age of current population of selected taxonomic group
RELATIONSHIP REPORT	Ancestors, siblings and descendants of selected specimen

ARKS records are often administrated by the registrar, record keeper, the curator or head keeper, designated as the ISIS Representative. You should contact individual zoos for information on who maintains their records. ARKS is currently in the process of being replaced by a new system, ZIMS (see below).

Example 7.1. Using records to monitor hand-rearing weight gain in a female giraffe



7.1.2.2. SPARKS - Single Population Animal Records Keeping System

EAZA have established 41 TAGs (Taxon Advisory Groups) (current to 2013)for the different species housed in zoos and aquariums whose main task is to develop Regional Collection Plans to decide which species should be kept in zoos, what level of management they require and what space is available in EAZA institutions. It is important to maintain healthy and self-sustaining populations of animals in zoo and as a result EAZA presently has two different levels of breeding programme; the European Endangered species Programme (EEP) and the European StudBook (ESB) (see Section 1.1.5 for more information). Studbooks were initiated as early as the 1930's (for a subspecies of European bison) and from 1965 studbooks became an integral part of managing endangered species living in zoos. Studbook keeping involves compiling genealogical and demographic data covering a species' history in captivity, typically across an entire region (e.g. Europe) rather than just at a national level. This includes numerically identifying individual specimens so as to record permanently data on their origins, parentage, date of birth, gender, locally-assigned identifiers and dates of transfers to another collection. Captive population sizes and fecundity rates vary across species, thus the job of maintaining a studbook ranges from simple and easy, to complex and challenging. SPARKS is DOS-based software used by hundreds of studbook keepers worldwide which supports studbook management and species analysis. Studbook co-ordinators also use PMx software to perform genetic and demographic management of studbooks populations. SPARKS will in time be amalgamated into the ZIMS application.

Examples of data that can be compiled in SPARKS reports are given in Table 7.2 below:

Table 7.2: Selected SPARKS reports

Report name	Explanation
LIVING ANIMALS	All individuals living at date criteria set (either during or at the end of a date span). Report can be ordered by studbook number or location
HISTORICAL	All individuals living or dead within the time the studbook data runs. Report can be ordered by studbook number or location
BIRTHS	Births over a selected date span
DEATHS	Deaths with death notes over a selected date span
TRANSFERS	Transfers between zoos over a selected date span
MORTALITY (Qx)	Age specific mortality. The average proportion of animals that are expected to die within an age-class
SURVIVORSHIP (Lx)	Age specific survivorship. The probability of a newborn surviving to the beginning of age class x
REPRODUCTION	Details youngest parents, oldest parents, interbirth intervals, birth seasonality etc for both sires and dams. Reports on reproduction history for individuals can also be generated
AGE REPORT	Details oldest animals (living and dead)
PEDIGREE CHARTS	Details the sires and dams of individuals back to the population founders
DESCENDANT LISTS	Details offspring of an individual through all known generations (living and dead)

SPARKS records are kept by the studbook keeper or studbook coordinator. Details of the European studbook programmes (EEPs / ESBs) can be found on the EAZA website (www.eaza.net), along with the studbook keeper / coordinator's information.

Example 7.2: Using records for evidence-based animal management

Although there is knowledge within zoos about husbandry practices for many species, much is anecdotal. Data within studbooks can be used to verify that knowledge, help to establish guidelines for good practice with species and increase our knowledge of factors influencing animal welfare.

Within the European studbook for the white faced saki monkey (*Pithecia pithecia*) one question concerning husbandry practices is regularly asked:

At what age should animals be removed from their natal group?

From anecdotal evidence, saki monkeys moved from their natal group before they reach sexual maturity (at about two years of age) may have a reduced lifespan. Using historical data in the studbook (held on SPARKS) it is possible to pull out when animals left their natal group and their age at death. In such a study, statistical analysis showed a positive relationship between the age an animal leaves the natal group and the age at which it dies, such that animals leaving below four years of age tend to die before reaching the age of 10 years.

Displaying this relationship graphically makes it easier for the studbook coordinator to demonstrate the rationale for why saki monkeys should stay within the natal group beyond the age of sexual maturity. Of course, individual recommendations must be considered in tandem with careful observations of social interactions of each group and the potential for interbreeding.

(Pullen, 2004)

7.1.2.3. MedARKS - Medical Animal Records Keeping System

MedARKS is DOS-based software which enables veterinary medical record keeping and collection management. Although part of the ISIS suite, its usage is far less common or consistent between collections and in many instances the software may be run alongside additional means of data recording, including paper records and other databases. Records will be for animals at that particular zoo only, both past and present although, on a regular basis (approximately every 6 months), all subscribers send their information to ISIS who compile this as a ‘Reference Values’ module for the software programme. A selection of reports are listed in Table 7.3 below – be aware that interpreting this data may require specialist knowledge.

Table 7.3: Selected MedARKS reports

Report name	Explanation
ANAESTHETIC USE & REGIMENS	Details physiological parameters (heart & respiratory rates), drugs and dosages, recovery and survivorship
BLOOD NORMALS	Range values for specimen or selected species
HISTORICAL DISEASE INVESTIGATION	All incidences of an aetiology or diagnostic term, e.g. air sacculitis and staphylococcus over a selected date span
PATHOLOGY REVIEW	Causes of death for a species or time span, highlighting disease trends
PARASITOLOGY	Frequency of testing, subsequent findings and treatment
WEIGHT REPORT	Can be for an individual or species, for a selected date span

Within individual zoos, Medarks records will usually be kept by the vet or vet nurse. Many zoos do not have a dedicated vet and in these cases, the records may be kept by the curator or added into the ARKS database. Contact the individual zoos to find out where the records are kept. MedARKS will soon be amalgamated into the ZIMS application.

Example 7.3: Using records to assess anaesthetic risk in great apes

Placing an animal under anaesthetic always carries an associated risk, particularly with exotic animals where details of their physiology may not be fully known. Zoos have many situations where anaesthetics are necessary for the treatment of their animals and therefore zoo records are an excellent source of information on the risks associated with anaesthetics.

Are the anaesthetic risks greater for great apes than domestic species?

Records on all anaesthetic procedures from 16 zoos with great apes were examined. Some of these records were stored on MedARKS, others were stored in paper records within the zoo. Variables recorded included outcome at seven days (whether alive, dead or euthanized) and the physiological and anaesthetic characteristics. Multivariate analysis was carried out on the 1182 records collected.

It transpired that peri-operative and anaesthetic related mortality risks are significantly higher for the great apes than for domestic species. Major risk factors for anaesthetic-related mortality include health status and extremes of age; the major risk periods are during recovery, immediately post-recovery and within 24 hours of the anaesthetic. These results provided important information for the veterinary care of great apes in zoos.

(Masters et al. 2005)

Example 7.4: Using records to look for sex ratio bias in avian breeding programmes

Recent studies have shown that egg sex ratios in many species of wild bird are not random. For example, in many species, sons are more 'expensive' (this may be measured in terms of energy, nutrition or developmental time) to produce than daughters. So, mothers may overproduce sons when they are in good condition, older and more experienced at rearing offspring, and/or when more food is available. The link between maternal condition and sex allocation shown in wild birds, suggests that captive breeding programmes in which individuals are kept on a high nutritional plane, are probably producing uneven sex ratios.

What factors influence the sex ratios of birds born within zoos?

Information available in studbooks and data on the life history in the wild and captivity, as well as diet and sexual size dimorphism, was compiled for 57 avian species. Overall the sex ratio across species was roughly 50%, but this ranged from 33 to 76% males. Such biases are likely to have important implications for the social dynamics and mating behaviour of captive populations. No significant correlations were found between sex ratio at hatching and any measured factors, not unsurprising since a third of these birds born in zoos were of unknown sex. Generalised linear models revealed that small, short-lived birds were less likely to be sexed, an observation likely explained by the perceived health risks associated with surgical sexing, the method most widely used by zoos at the time (feather sexing is now very common, but also expensive). The proportion of unsexed offspring was high in species with a high mortality rate during the first month, suggesting that few nestlings are sexed post-mortem. In conclusion, it was not possible to determine from the existing data whether captive dietary regimes differentially influence the production and survival of sons or daughters. Understanding of the effects exerted by captive conditions on species' management will remain limited if it is not possible to determine the sex of individuals.

(Riach et al., 2004)

7.1.2.4. ZIMS - Zoological Information Management System

Acknowledging that the original suite of records software was becoming increasingly out-dated, ISIS and the zoo community pledged to create a single online records system to combine the three existing programs and provide greater functionality and accessibility. Understandably this process has not been easy but since April 2012 increasing numbers of zoos and aquariums worldwide have been using ZIMS for their daily records keeping as a replacement for ARKS. All ISIS members will in time move to ZIMS and ARKS will no longer be used within zoos. ZIMS is a module-based application and currently the veterinary module is being developed which will go on to replace MedARKS. Once this has been deployed SPARKS will also be amalgamated. As ZIMS is online it holds all the global zoo data which was previously only available on the ISIS website i.e. species holdings which lists the species held in each ISIS member zoo; their sexes, births and deaths. It also has the great advantage of improving data quality as animals no longer have multiple records at multiple zoos but rather one record for life which is accessed at different time points by holders. ZIMS has retained all the report functions from ARKS and the ISIS website. A recently added report is the 'weight comparison report' which allows reports based on weights of animals across the global data at different ages (which can be filtered by sex). Global data can be accessed in the ZIMS application via 'Eye on ZIMS' for non-ZIMS and non-ISIS users. Requests for this access should be directed to ISIS.

7.1.2.5. ISIS - International Species Information System

The ISIS website provides details on the data held in ZIMS and therefore provides invaluable data to ISIS members, studbook keepers and species programme managers, who require information for co-operative breeding programmes and animal acquisition.

7.2. What types of questions could you ask?

Table 7.2 provides a quick summary of the types of information present in zoo record keeping systems. Although many zoos use the software programmes supplied by ISIS (i.e. ZIMS, ARKS, SPARKS etc), other population management programmes are in use. Also, direct access to electronic databases may well be limited. The number of 'users' within a zoo is usually restricted to ensure data quality and security, and also the programmes are not entirely intuitive and user-friendly. However, hard copies of database information will be held and these equivalents to databases are indicated wherever possible.

When access to databases is limited, record keepers may be willing to provide hard copies of information on request. Many zoos require students to fill out a research request from or similar, giving an outline of your study and what data is being requested. It is important to inform the record keeper fully of the search parameters necessary for the research (i.e. date criteria – within a date span, event type, location etc). Depending on accessibility of the information requested, a return rate of 50% should be expected. What might seem obvious information for zoo staff to have to hand, may not be so easy or be timely to pull out. If the key person is away, then the enquiry may not get answered, so polite reminders should be sent if no answer is received within three weeks.

It is essential that adequate preliminary work has been done to ensure questions are clear, sensible and answerable and DO NOT ask for information that could be easily obtained from published sources, websites etc. For more information on how to compose your query and tips on how to send it out, see Chapter 11: Surveys and Questionnaires. Please ensure that these guidelines have been read and as much information as possible has been gathered BEFORE questionnaires are sent out.

Table 7.2. What types of questions could you ask?

Data stored electronically in >	ARKS	ISIS	SPARKS	MedARKS	ZIMS
I am interested in:					
Single species	✓	✓	✓	✓	✓
Multiple species	✓	✓		✓	✓
Single institution	✓	✓	✓	✓	✓
Populations (multiple institutions)		✓	✓		✓
I want to know about:					
Genetic bloodlines (relatedness of individuals, kinship effects, descendants, pedigrees)	✓		✓		✓
Life history parameters (longevity, developmental stages, dispersal ages, sexual maturity)	✓		✓		✓
Fertility, fecundity & reproductive parameters (birth rates, interbirth interval, litter / clutch sizes, infant survivorship rate, breeding success)	✓		✓		✓
Mortality (mortality rates, infant mortality rates, causes of mortality)	✓		✓	✓	✓
Nutrition (feeding regimes, diet ingredients, nutritional content of diet)	✓				✓
Clinical data (health parameters, parasite checks, preventative health checks, anaesthetic procedures etc)	✓			✓	✓
Transport data (moves between zoos, number / distance involved, ages at time of moves)	✓		✓	✓	✓
Behavioural data (mating, aggression, care of eggs, lekking / nesting behaviour, rearing / infant care)	✓		✓		✓
Who holds the data?	Registrar / Isis Representative		Studbook keeper / coordinator	Vet	ISIS representative
Is there a hard copy equivalent	Arks records / daily diaries		Published studbook	Veterinary reports	Daily keeper diaries/reports, Published studbooks, Veterinary reports

Example 7.5: Using records to assess the effects of transportation of big cats

Part of the function of a studbook is to maintain genetic diversity within a population. To achieve this, animals may need to be transported from one zoo to another at appropriate points in their lives. The transportation and introduction of animals into new situations is assumed to be stressful but the potential effects of that stress have not been evaluated for the majority of species.

Is big cat reproductive success affected by age at transportation or by the number of transports in a lifetime?

Studbooks for Amur tigers and Amur leopards contained the data required to answer this question; age of animals at time of transports, number of times each animal was transported, number of offspring produced, number of offspring surviving to thirty days and to one year, lag time between transport and birth of consequent litter.

Using suitable statistical analyses, it was possible to conclude the number of transportation events in a lifetime did not affect reproductive success. Furthermore, as long as animals are sexually mature, the younger they are transported the more offspring they have and these offspring are more likely to survive.

(Mitchell and Nevison, 2006)

7.3. What are the limitations?

Although zoo records provide an excellent opportunity for detailed and extensive research both on an applied and a theoretical basis, due to the extensive nature of the datasets there are some constraints that need to be borne in mind. These can be considered as data consistency, cleanliness and continuity.

7.3.1. Data consistency and cleanliness

Consistency of the data is potentially the biggest issue. The chain of data from collection to input into the programme can in some situations be quite large and extended. In many institutions data is collected by the keeper and passed on through the head of section to the curator before being handed over to the records keeper. This can lead to opportunities for inconsistencies to be included.

In addition, the registrar or studbook keeper will often (in the case of transfers between zoos) receive information from both the sending and receiving zoo – this can lead to inconsistencies i.e. the animal may be recorded as leaving the donating zoo on the 1st September but may not arrive at the receiving zoo until the 2nd or 3rd September.

Part of the role of the studbook keeper and record keeper is to track down any of these inconsistencies and ensure that the data within the studbook/ARKS/ZIMS database is ‘clean’.

How to overcome data consistency limitations:

It is important to bear in mind that the data is only as good as the person inputting it. Originally, ISIS established a 'reconciliation' process to try and ensure that the data inputted to ARKS (through the institution) and SPARKS (through the studbook keeper) was consistent. The record keeper/studbook co-ordinator was able to log onto the member's area of the ISIS website and look for reconciliation values for their particular institution/studbook. These values (only visible to authorised personnel) gave them an indication of how 'clean' the data was i.e. ISIS' average reconciliation rate = 84%, above this and the data was very 'clean', below this and they had to be aware that there may have been some inconsistencies within the data set they were using. Now, in ZIMS, any discrepancies between records are highlighted on the specific animal's record. Business rules exist which specify which institutions data will be displayed on global views – i.e. birthdate is shown for the first institution, sex for the most recent. Registrars can then discuss inconsistencies and agree on any amendments to be made.

7.3.2. Data continuity

Although data sets can be extensive and can provide answers to many questions, in some circumstances there are potential confounds to the data e.g. where the studbook has collected data over a long period of time (some studbooks have data for over three decades). It is worth remembering that zoo practices, including husbandry and housing knowledge, have changed quite dramatically in those times. This could have a strong influence on parameters such as mortality rate and breeding success. When looking at these sorts of parameters it may be worth investigating longitudinal variation within the dataset.

How to overcome data continuity limitations:

Filtering the data will prove to be a necessary step for the student working with a database. Filter criteria will depend on the questions being asked and potential influences / confounds on that data. e.g. if looking at factors affecting the longevity of an animal, the student may want to filter data by decade to assess whether this has an impact (potentially due to housing or husbandry improvements) before pooling the data to ask the research question. The researcher should accept that in some circumstances, some data may need to be excluded from the analysis or treated with caution

Example 7.6: Using records to investigate 'purging' in zoo populations

It is generally agreed that inbreeding produces offspring which are less fit than their non-inbred counterparts. As a consequence, current captive breeding management plans aim to minimise inbreeding whenever possible. However, since the expression of deleterious genes is increased during inbreeding, selection against such genes is also increased meaning that, in theory, a population could perhaps be 'purged' of its mutations. Indeed, laboratory experiments have shown that, in some cases, intensive continued inbreeding can raise fitness levels.

Is purging occurring in zoo populations and, if so, how strong are the effects?

Whilst purging of detrimental genes might be thought to be advantageous to the conservation of threatened species, it must be remembered that purging will always result in a loss of genetic diversity. Genes that might be detrimental to species in captivity could actually prove to be advantageous in the wild. If purging was found to be occurring, it would be important to assess whether it was advantageous or detrimental to future reintroduction programmes and whether breeding programmes might need adjusting to incorporate its effects.

This PhD investigation would have been impossible without the vast wealth of studbook data and the co-operation of studbook holders. 136 populations which had experienced inbreeding over 2 or more generations were identified and contained sufficient known ancestry to be included in the analysis although permission was only granted to use 119 of these studbooks. Using regression models and meta-analyses, there was a significant trend of both inbreeding depression and purging across populations. However, the change in inbreeding depression due to purging averaged across the 119 populations was <1%, suggesting the fitness benefits of purging are rarely appreciable. The study therefore re-emphasises the necessity to avoid inbreeding in captive populations and shows that purging cannot be relied upon to remove deleterious genes from zoo populations.

(Boakes and Wang, 2006)

7.4. How can I access the data?

Throughout the BIAZA region there is a variation in the way records are maintained at each institution thereby restricting their research value. In conjunction with this, in order for records to be of value at institutional level there is a need for continuity in the way in which data is entered (e.g. if the same code is not used for specific data or if wording is not consistent then full data extraction cannot be successfully carried out). Often these issues only become evident when data quality/retrieval is carried out. BIAZA have a 'Records Group' who develop management procedures that guide best practice and create uniformity throughout the BIAZA region, thereby improving the quality of records overall for both archival and research purposes.

One of the greatest limitations with zoo research is that of small sample size. In many situations methodology and experimental design as well as statistical analyses need to be tailored to meet the specific requirements of small sample sizes and single case situations (re: stats research guidelines). However, zoo records research can give access to multi-institutional data (see Section 1.4) through the use of ZIMS, studbooks and SPARKS data sets. Although there are limitations that must be taken into consideration, using the records of a European breeding programme (either EEP or ESB) will dramatically increase the sample size of your research and allow access to a wider range of statistical tests.

Within the EEP there are 180 EEPs and 180 ESBs (current to the end of 2012 these numbers are continually updated) each of which are required to update data on a yearly basis. In some cases this can provide more than three decades of information which, at the most basic level of data extraction will include births, deaths and transfers between groups; at a more advanced level of data extraction will allow the analyses of pedigrees of known individuals.

Information on the co-ordinator / studbook keeper and which institution supports them can be found on the EAZA website (www.eaza.net), although contact details are not included.

Write to BIAZA or EAZA explaining why you want to make contact with a specific programme co-ordinator (see Section 7.6.2 for web addresses). They will forward your request on to the relevant person and it will be up to them to decide whether to follow-up. Your initial contact should give brief details of research objectives, what you require from the breeding programme and how you think the programme will benefit from participating.

Remember, adequate preliminary work must have been done to ensure questions are clear, sensible and answerable and you should NOT ask for information that could be easily obtained from published sources, websites etc. For more information on how to compose your query and tips on how to send it out, see Chapter 11: Surveys and Questionnaires.

7.5. Final considerations

7.5.1. Multi zoo research

For research that is done across a number of BIAZA collections, BRG suggests that you get support in the form of a letter from BIAZA (see Chapter 1). In order to get this support, please see the BRG home page (for web page see Section 7.6.2).

7.5.2. Acknowledgements

This is where researchers get the opportunity to thank all the people that facilitated or contributed to the research. Acknowledgements should be no longer than half a page and more in depth lists could be put in an appendix if need be.

7.5.3. Say thank you!!!!

By their nature, research projects involving zoo records may involve substantial input from zoo staff. Saying ‘Thank You’ to those who have taken time out of their already busy jobs to give information or data to a research project is very important and means you are more than likely to get the help you want.

7.6. Further reading and resources

This section contains a selection of relevant references (see also the main handbook reference list for those references cited in this chapter), but the main resources are online.

7.6.1. References

Faust, L.J. and Thompson, S.D. (2000) Birth sex ratio in captive mammals: Patterns, biases, and the implications for management and conservation. *Zoo Biology*, 19, pp. 11–25.

Flesness, N.R. (2003) International Species Information System (ISIS): over 25 years of compiling global animal data to facilitate collection and population management. *International Zoo Yearbook*, 38, pp. 53–61.

Shoemaker, A. and N. Flesness. (1995) Appendix 4: Records, Studbooks and ISIS Inventories. In: *Wild Mammals in Captivity: Principles and Techniques*. Kleiman, D.G., Allen, M.E., Thompson, K.V. and Lumpkin, S (Eds.). Chicago: University of Chicago Press.

7.6.2. Online Resources

ARKS	**Animal Records Keeping System software distributed by ISIS**	www.isis.org
BIAZA	**British and Irish Association of Zoos and Aquariums**	www.biaza.org.uk
EAZA	**European Association of Zoos and Aquaria**	www.eaza.net
EEP	**European Endangered species Programme**	www.eaza.net
ESB	**European StudBook**	www.eaza.net
ISIS	**International Species Information System**	www.isis.org
MedARKS	**Medical Animal Record Keeping System software distributed by ISIS**	www.isis.org
SSSMZP	**Secretary of State's Standards for Modern Zoo Practice**	http://www.defra.gov.uk/wildlife-countryside/gwd/zooprac/index.htm
SPARKS	**Single Population Animal Record Keeping System software distributed by ISIS**	www.isis.org
TAG	**Taxonomic Advisory Group**	www.eaza.net
WAZA	**World Association of Zoos Aquariums**	www.waza.org
ZIMS	**Zoological Information Management System**	www.zims.org

Chapter 8

Monitoring Adrenal Response in Zoo Animals as an Indicator of Welfare

Editor: Sonya Hill

Contributing authors: Sonya Hill, Tessa Smith

Introduction

This chapter, on the non-invasive monitoring of adrenal response in zoo animals, concentrates on the measurement and use of glucocorticoids as an indicator of welfare and is aimed at those relatively new to this field.

It is advisable to read all sections before beginning a project. It is also recommended that you read Chapter 2 on the legal and ethical considerations related to collecting biological samples.

8.1. Definition of animal welfare

It is important to have a clear definition of animal welfare in welfare-related studies, in legislation and in practical use, and to ensure the terminology we use is rigorously applied (Hill and Broom, 2009). There are many definitions of animal welfare in the scientific literature, but one that we find useful is by Broom (1986), who defines it as the state of an animal as regards its attempts to cope with its environment. This definition acknowledges that welfare is a measurable state of being and that it ranges from very poor to very good. It also introduces the concept of coping (which refers to the organism maintaining homeostasis and being able to tolerate a range of stimuli, including noxious ones). The definition of welfare also allows measurement separate from moral considerations, and refers to feelings as well as physical and psychological health (Broom, 2001). It is also important to emphasise that animal welfare relates to an individual, and thus welfare can differ between different members of a species, even if they have been exposed to the same conditions (Hosey et al., 2009).

For a wider introduction to measuring zoo animal welfare, please refer to Hill and Broom (2009).

8.2. The importance of monitoring welfare in zoo animals

The responsibilities of zoos include conservation (e.g. breeding / *in situ* support / reintroductions), education and science. As with any animal under human care, we have a legal and moral responsibility to ensure that zoo animals have good welfare and that we minimise any challenge to this. We should also maximise reproductive output and longevity, and conserve a species-typical behavioural repertoire that spans successive generations. Another reason that good welfare is important for zoo animals involved in captive breeding programmes is that poor welfare can be associated with impaired reproductive function. Furthermore, animals whose welfare is compromised may not exhibit their species-typical behavioural or physiological repertoire and thus provide poor educational models and compromise the validity of scientific research. By monitoring zoo (and other) animal welfare, we can aim for continuous improvement in our management of captive animals.

8.3. Selecting the indices to assess welfare

Exposure to a physical or psychological challenge produces a host of acute and chronic physiological changes, many of which can be used as indices for welfare if interpreted in context. Common components frequently used to assess welfare include changes in:

- Parameters of immune function (e.g. reduction in T and B lymphocytes)
- Cardiovascular output (e.g. heart rate or blood pressure)
- Darwinian fitness (e.g. reproductive output, longevity)
- Endocrine parameters (e.g. concentrations of adrenalin or cortisol)
- Behaviour (e.g. rates of scratching; stereotypies, inactivity, hyperaggression, etc.)

Which indicators of welfare are used in a study should depend on the species, the project's aims, the budget, time-scale, available labour and other practical limitations. As welfare is about an individual's response to a challenge, and as there are many different possible responses to many different stimuli, there is no universal indicator of welfare (Dawkins, 2001). So, it is preferable to use a combination of behavioural and physiological indicators when assessing welfare (Hill and Broom, 2009). The two most commonly-used indices when assessing welfare in zoo animals are behaviour and quantification of glucocorticoids. Since one of the major physiological changes in response to a challenge is increased activity in the hypothalamic-pituitary-adrenal (HPA) axis and concomitant increases in release of glucocorticoids, measurement of glucocorticoids can provide a useful indicator of welfare, when assessed in context (e.g. linking it to the associated behavioural response and/or reproductive output). That said, it is important to bear in mind that there can be an elevation in glucocorticoids due to beneficial behaviours (such as copulation), as well as situations in which welfare may be compromised, and so knowledge of the emotional state of the animal provides important information. These guidelines will focus on the use of glucocorticoids, as an indicator of animal welfare.

8.4. Confounding variables

The HPA axis is affected by a range of variables including: species, population, genetics, individual temperament, social status, reproductive condition, developmental history, age, health condition, social environment, social support, season, weather condition and time of day. The sensitivity of glucocorticoids to the latter potentially confounding variables is an important consideration that should be addressed when designing a study (see below).

8.5. Study formulation

As with any scientific study, the researcher should be clear at the outset about the exact goals and predictions of any individual study. Before planning a study one is advised to refer to Chapter 1 which provides an overview of how to plan a research project. Specific attention should be paid to the following experimental design issues, when using glucocorticoids as an indicator of animal welfare.

8.5.1. Dealing with individual variation

Due to large individual variability in glucocorticoids, the researcher is advised to:

- Incorporate an extended control (or baseline) period that covers a representative range of hormonal and husbandry events (e.g. if reproductively-cycling females are being used, the control period should span a full reproductive cycle). Experimental designs of the format ABA are advised.
- Maximise the number of available subjects, which may involve doing a multi-zoo study (see Chapter 1)
- Depending on your research question, a suggested statistical test is mixed models (incorporates random and fixed variables).
- Consider each change in glucocorticoids against its own baseline (e.g. use proportional change)
- Potential confounds such as those listed in Section 8.4 should be minimised or at least acknowledged. For example use subjects of the same sex and similar age, housing condition and social environment.
- Avoid using subjects that have recently (e.g. within a month) experienced a significant social or physical challenge, unless the aim is to conduct a biological validation of the assay, or to assess the impact of the challenge on adrenal response.
- Minimise disruption to the animal associated with sample collection:
    a) Use non-invasive sample collection techniques (e.g. faeces are commonly used)
    b) Collect from 'undisturbed' subjects in their natural social group and environment (e.g. avoid separation)
    c) Aim for predictable sample collection procedures using the same people and methods

8.5.2 Frequency and timing of sample collection

Ideally, collect samples at the same time each day (due to potential circadian rhythm). Ensure that you record the confounding variables associated with each sample, and the frequency of collection depends on your research question. Refer to the literature for guidance from similar studies.

8.6. Sample medium

The sample medium (e.g. urine or faeces) will depend on the criteria outlined in Section 8.5 and Table 8.1.

- In addition you should consider:
  a) the risks to the animal and researcher associated with collecting certain samples; please ensure appropriate Risk Assessments are carried out and PPE is worn
  b) if the cage design will facilitate sample collection (e.g. over-head passage ways facilitate the collection of urine and faeces if animals use the passageways)
  c) husbandry routine (e.g. will you be able to obtain access to the animals for sample collection when you need to? Or are the keepers given permission by their zoo to assist you in collecting samples for your project?)

8.6.1. Invasive versus non-invasive sampling

- Invasive methods will usually require a Home Office License (see Chapter 2) and therefore will not be acceptable or possible at most zoos. In addition, media collected in a non-invasive way such as urine, saliva and faeces are preferred over blood for the following reasons:
  a) Avoidance of pain and discomfort
  b) Procedures associated with blood collection often in themselves increase HPA activity and therefore mask or potentially augment changes in response to experimental treatment (e.g. capture, restraint, separation from social partners)
  c) Non-invasive samples can be collected more frequently and over a longer duration
  d) Home office license not required
  e) Non-specialist can collect sample (with instructions), so there is comparatively less personnel training required for collection of non-invasive samples
- Challenges associated with using non-invasive sample media include:
  a) Specialised validation required for hormone measurement, as non-invasive samples will be an indirect measurement of glucocorticoids circulating in the blood
  b) The animal and not the researcher has control over timing of sample production (although see Table 8.1.)
  c) Proper identification of the sample (i.e. which individual it has come from)
  d) Time lag associated with release of glucocorticoids into the blood and subsequent excretion into media (e.g. urine, faeces and saliva).
  e) Degradation of adrenal hormones if the sample cannot be stored appropriately (e.g. frozen) quickly after it has been voided by the animal.

Additional advantages and challenges associated with using samples collected non-invasively (urine, faeces and saliva) are presented in Table 8.1.

8.7. Sample collection

The following general points are among those that apply to the collection of all media:

- It is essential that advice and consent are obtained from all relevant personnel before commencing sample collection on: sample type, sample collection and storage protocol and frequency of sample collection. Examples of personnel whom you might need to consult include the zoo's research coordinator, veterinary staff, animal care staff, relevant higher management and, if you are based in an academic institution, the appropriate personnel there (e.g. course tutor, Head of Department, etc). The list is not exhaustive and researchers should be sensitive to the establishment and always go through the research coordinator of a zoo first.
- Sample contamination should be minimised by cleaning any substratum prior to use, and avoiding faecal samples contaminated with urine.
- Sample records are recommended that include the following information per sample (if it is available and required by project aim):
  a) Date and time of day that the sample was voided
  b) Date and time of sample collection and storage
  c) Animal identity
  d) Any known social or physical disturbance that may affect hormone levels
  e) General comments on sample condition, such as possible contamination by food, animal bedding etc.
- It is advisable to keep all samples on ice after collection and to freeze it at at least -20^{0}C within 2 hours of collection (other types of media, e.g. plasma samples, may require freezing at –70^{0}C, but as discussed earlier, this is not likely to be relevant to zoo-based research).
- Animal training can be used to improve sample collection, but this would require prior permission from, and the involvement of, relevant zoo personnel. Factors affecting the merits of a training regime include the philosophy of the animal collection, costs and benefits of the training, the species, social environment, cage design and diet.

Table 8.1.

Media	Advantages	Challenges
Urine	Most animals urinate upon wakening, facilitating collection and training opportunities Urine dilution can be corrected quantitatively Large quantities Sterile (minimal health hazards) Several animals use urine for scent marking thus increasing the frequency and supply One can generally collect at frequent intervals (e.g. hourly)	May soak into substratum Hard to collect in a field setting
Faeces	Large supply Several animals use faeces for scent marking thus increasing the supply Provides integrated index of HPA activity over an extended period of time (minimally affected by acute stress) Can be collected in a field setting Can be individually marked using dyes / foods	Hard to correct for sample consistency accurately (i.e. varying amounts of fibre) or water content Complex composition interferes with assay development Extended time lag Insensitive to acute challenge Lengthy and potentially costly extraction Extract for assay may be coloured which could interfere with EIAs Disease risk Some animals are coprophagic, which may affect sample collection / results.
Saliva	Minimal time lag No dilution factor required Good indicator of biologically active plasma "free" cortisol Good measure of acute challenge when collected within time lag period	Hormones may be present in small amounts Viscous consistency reduces manageability Hard to correct for sample consistency accurately Very situation dependent, thus strict consistency of sample method required when measuring daily totals Hard to obtain samples from dangerous animals

8.7.1. Animal training to assist sample collection

The advantages and challenges associated with training an animal to cooperate with sample collection are presented below:

- Advantages:
  a) Minimise disruption to the animal associated with the collection procedures
  b) Increase frequency at which samples are provided
  c) Increase opportunity to collect samples on demand
  d) Provide cognitive stimulation if training is enriching
  e) Increase positive human- animal interaction

- Challenges:
  a) Alter the natural behaviour of the animal with possible effects on social relationships within the group
  b) Encourage physiological processes at 'unnatural times'
  c) Present opportunities for conflict in a social group over potential rewards
  d) Potential negative effect if training stops

8.7.2 Important Considerations Specific to Sample Media

The above points apply to all sample media. You are advised to consult the literature on sample collection, storage requirements and preparation techniques since the methods vary with species and media, and some methods may be more suited to zoo-based studies or to those carried out in the field.

8.8. Analysis

- Some media, such as faeces, require processing or extraction in order to present the hormone in the correct form for analysis. You are strongly advised to consult the literature on these techniques as they vary significantly between species.
- Avoid repeated sample freeze / thaw cycles
- Aliquot large sample volumes into small workable amounts as determined by the assay
- There are two methods available for sample analysis as detailed in Table 8.2 (radio-immunoassay and enzyme-immunoassay). It is important that an assay should be immunologically and biologically validated for your species.
  a) Immunological validation is determined by demonstrating specificity, accuracy, precision and sensitivity (see Diamondus and Christopoulos, 1996). Specificity is defined as the extent to which the assay is free from interference from compounds not under study. It is tested by assessing the similarity of the inhibition curves produced by a serial dilution of the analyte and known standard solutions (i.e. parallelism). Accuracy refers to the extent to which the assay is quantitatively accurate. Typically you assess the degree to which varying amounts of known standard hormone preparation are recovered when added to various concentrations of a sample pool (accuracy is typically repeated for a high, medium and low media pool). Precision indicates the extent to which a set of measurements for single sample agree, both within the same assay and across different assays (usually referred to as coeffecient of variation). The sensitivity of the assay is the smallest amount of hormone that can be detected from an absence of hormone.

    b) Biological validation is ascertained by determining if an assay detects biologically meaningful changes in hormone levels (e.g. predicted increase in glucocorticoids in response to a psychological or physical challenge)

- The advantages and challenges of the two assay types are presented in Table 8.2

8.9. Interpretation of results

- Since HPA activation during exposure to a challenge is an evolved trait with adaptive value, one might assume that the response of the HPA axis would be predictable. However, activity in the HPA axis is modified by numerous psychological and physical factors, making it complex to predict the observed cortisol response. When predicting observed changes in glucocorticoids and subsequently interpreting these, you are advised to consider the range of potential modifiers, such as reproductive status, age, rearing history, current behaviour etc.
- Broadly speaking, exposure to challenge can have four outcomes:
    a) Increase in glucocorticoids(expressed as an absolute or relative increase)
    b) Reduction in glucocorticoids(expressed as an absolute or relative increase)
    c) No change in glucocorticoids
    d) Altered responsiveness of the HPA axis to subsequent challenge
- Hormone data might need transforming prior to statistical analysis to reduce heterogeneity of variance.

Table 8.2

Assay type	Advantages	Challenges
Radio-immunoassay	Ability to assay large number of samples at each go (200+) Detects small amounts of hormone Good inter-assay precision	Requires radioactive (RA) substances therefore: Premises must be licensed Extensive associated admin / record keeping Continual monitoring of premises and personnel required Potentially harmful effects of RA Radioactive waste requires specialised disposal Assay has many time-consuming stages Requires substantial lab / equipment space
Enzyme-immunoassay	Minimal health risks Non-harmful waste can be disposed of in conventional ways (NB disposal of sample media should still be handled appropriately, e.g. as clinical waste) Fast to conduct Assay is small and requires very little laboratory space Requires small amounts of media Can detect small amounts of hormone	Varied inter-assay precision

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Chapter 9

Studies on the Effects of Human Visitors on Zoo Animal Behaviour

Editor: Joanna Bishop

Contributing authors: Heidi Mitchell, Geoff Hosey

9.1. Introduction

Whilst the volume of research on visitor effects has increased in recent years, the effects of the presence of human visitors on the behaviour of zoo animals are still largely unknown. However, the presence of people in close proximity is likely to be a significant variable affecting animal behaviour. It should therefore be something that is relevant to anyone interested in undertaking scientific research projects on zoo animal behaviour as it may affect how their results can be interpreted.

Published research which aims to determine the effects of visitors on zoo animals has tended to focus on non-human primates, and there is a pressing need for us to know more about visitor effects on other mammals, birds, and the often under-studied reptiles, amphibians and fish (for example we are largely ignorant of the effect on reptiles and fish of people tapping on glass-fronted tanks). Furthermore, much has happened in zoos since the first zoo-visitor studies were published in the 1980s. Naturalistic cages, environmental enrichment, free-range exhibits, visitor experiences – all of these are a common part of the modern zoo, and it is not at all clear how they affect the animals' responses to human visitors. For those with more theoretical interests, the detailed analysis of inter-species interactions and communication is itself a current topic, and again one which has largely been unexplored outside of the primates.

Fundamentally, we need to know whether the presence of visitors has any implications for the well-being of zoo animals. Recent work has suggested that visitors may have either a positive, negative or neutral impact on zoo animals, and that there is a Human-Animal Relationship (HAR) (e.g. Hosey, 2000; Hosey 2008). Knowledge of such a relationship is of utmost importance since many zoos rely on the revenue generated by visitors to provide other aspects of animal care which are key to welfare, for instance, veterinary care and food. Therefore, zoos are particularly interested that clear, unambiguous results come from these studies, to inform best practice in maintaining captive animals.

These guidelines are designed to offer help in all aspects of visitor effects studies. The information contained here is divided into 6 parts; Initial practicalities, Different types of audience and audience conditions, Animal factors, Enclosure effects, Sampling techniques, References.

9.2. Initial Practicalities

9.2.1. Ethics

If you are carrying out observations on visitors then you need to consider any relevant human ethical procedures in addition to the relevant animal ethics. Make sure you check the human ethics policy of your university, and the ethics policy of the zoo at which you are carrying out your research (bear in mind that each zoo will have their own ethics policy). See Chapter 2 for more information on ethics.

9.2.2 Pilot Study

- It is highly recommended that you allow time at the beginning of your research schedule to carry out a pilot study. Heuristic observations will enable you to determine important factors that will influence your study methods, such as:
    - Typical visitor attendance patterns at the exhibit (quiet and busy times/days)
    - Typical visitor dwell times at the exhibit
    - Types of visitor behaviour that may be important
    - Types of animal behaviour that may be important
    - "Hotspot" areas where high concentrations of animal–visitor interactions take place. You may decide that your study only needs to take account of behaviour at these locations
    - Key individuals in large groups of animals that may be more important to observe than others. This will be dictated by your research question (Be careful that you do not engage in non-random selection of study animals unless it is justifiable in the context of your study).
- You can also use a pilot study to test your sampling procedures and ensure that they will enable you to effectively measure the variables in question and test your study hypotheses. An important aspect of this will be how often you record visitor behaviour. This will depend on factors such as the number of visitors and location and design of the exhibit (see below, Section 9.5).
- A pilot study will also allow the animals to become accustomed to your presence as an observer i.e. will allow for habituation. Although most zoo animals are used to many human observers some may still react to you and this reaction is likely to decrease over time. It is essential that this change occurs before your main data collection begins. It may be useful for you to record some measure of this process.

Example: You may find that the duration and frequency of vigilance in the direction of the observer declines throughout the pilot study, perhaps indicating that the animals are becoming less interested in the observer.

9.2.3 Tools and Equipment

- To carry out a visitor effects study you need to observe both animal and visitor behaviour. This creates practical problems such as how to observe both at the same time. Your university or college may be able to provide equipment to help overcome this problem. Most zoos will not be able to provide such equipment and should not be relied on to do so.

- A video camera could be used to record either the animals in the enclosure or the public viewing area. However, there may be difficulties in positioning a camera to record the whole area required, ensuring it is safe from animals and visitors and easily accessible to change tapes/memory cards and battery packs as necessary.

- Another possibility is to have two (or more) observers, one for the visitors and one for the animals. A problem with this technique is the simultaneous timing of events. Both observers must time their observations very accurately in order to later match their observations. It also should be noted that when using the two observer technique, the observers should avoid swapping between the animals and visitors. This would introduce the problem of inter-observer reliability and repeated trials would be needed to determine variation in data collected by the two observers.

- Other pieces of equipment which may be useful are a decibel recorder to measure visitor noise levels (ideally taken from within the animal enclosure), a clicker to count visitors and a Dictaphone to enable more rapid recording of quickly changing events. However you should consider the limitations of any equipment you use.

- If your study requires the use of other types of equipment to test a condition e.g. camouflage netting to obscure the view of the visitors to the animals, make sure you have full approval from the zoo to use this equipment in the context you intend.

- You may find that there are other ways of measuring some variables. For instance, noise levels may correlate well with total visitor numbers. You could also test if the number of visitors at your enclosure correlates reliably with the total number of visitors through the gate. If so you may be able to use daily gate admission numbers (which most zoos record anyway) as your measure of visitor pressure. You should ask permission to use these data prior to commencing your study, and should not rely on this as your only measure of visitor numbers.

- Visitor self-assessment could be an option you would like to explore. Questionnaires asking for information such as group size, age, clothing, items being carried, time spent at the exhibit, own behaviour and the animals' behaviour can be handed to the visitors to complete. This may help to reduce your work load during your observation periods. However, you should consider the reliability of this method and check whether it would be acceptable to the zoo at a very early stage of your project. Many zoos may prefer visitors not to be bothered in this way.

9.3. Different types of audiences and audience conditions

9.3.1 Different types of audience

- In addition to visitors, the observer will obviously be present at the enclosure, and there could be other categories of humans present (e.g. keepers or vets) who might influence the animals' behaviour. These guidelines are concerned with the effects of visitors but you should be aware of these other types of audience and attempt to keep their occurrences (timing, duration, numbers etc) as constant as possible throughout your study

9.3.1.1 Observer

- As mentioned in Section 9.2.2 above and in Chapter 5, measures can be taken to habituate the study animals to the observer's presence to reduce any impact on their behaviour.
- Even if complete habituation to the observer cannot be achieved this is not necessarily a problem, depending on your hypothesis. The observer is a constant presence throughout the study so comparisons between 'only observer present', 'low visitor numbers' and 'high visitor numbers' should still be reliable. A 'no humans present' condition cannot be achieved however, without a video camera (see Section 9.3.2).

9.3.1.2 Keepers and other staff

- Keeper presence and its potential effects on the animals' behaviour cannot be avoided since the cleaning and feeding routines must continue regardless of whether a study is in progress. However, it is advantageous that the routines remain as constant as possible and keeper presence occurs at the same time and for roughly the same duration every day throughout your study. Good communication with the zoo and a clear study schedule that you stick to will help in this respect.

Other non-routine events such as a veterinary visit or zoo maintenance staff working in the area may happen during your study. Again with good communication it may be possible for the zoo to arrange these events on days when you are not collecting data. However, in many cases this will not be possible and you will need to adjust for these events. You may have to decide to omit data from such sessions prior to analysis.

9.3.1.3 Visitors

- To understand the effect that visitors have on zoo animals, it will be necessary to collect data on the visitors themselves. In the simplest case this will be to measure visitor numbers. However, this is likely to overlook many complex factors influencing an animal's response to visitors.

- Visitor characteristics are very complex and there are many variables that could be measured:
  Examples:
  - Visitor numbers at enclosure (may be measured as daily gate numbers, see above)
  - Distance from enclosure
  - Distance from animals
  - Sex /age of visitor
  - Colour of clothing
  - Objects carried e.g. pushchairs, umbrellas

- Frequency of camera flashes
- Smells – any food being carried, perfume, hairspray etc

- Visitor behaviour is also very complex:

  Examples:
  - Noisy visitors
  - Quiet visitors
  - Groups / individuals who try to attract the attention of the animals, bouts of banging on glass etc.
  - Groups / individuals who ignore the animals
  - Eating around the enclosure
  - Offering food to the animals
  - Smoking around the enclosure
  - Groups / individuals who interact with the animals as part of an organised 'visitor experience'

- You may wish to record visitor behaviour using the same techniques you are using to record the behaviour of the animals. A pilot study (see above Section 9.2.2) is vital to determine which of these factors might be important to measure.

9.3.2. Audience Conditions

9.3.2.1 The "no audience" condition

- When carrying out a study into the effects of visitors on animal behaviour, it is generally assumed that visitor presence is a 'treatment' and that it is necessary to have different levels of visitor presence, ideally including a 'no visitor' baseline to which other levels can be compared. However, this can be difficult to achieve without confounding factors.
- A true 'no audience' condition is usually not possible due to the presence of the observer (see above, Section 9.2.2) however an 'observer only' or 'no visitor' condition can sometimes be achieved.

- Ideally the 'no visitor' condition should happen during the zoos normal opening hours since this avoids the confounding variable of time of day. This may be achieved opportunistically or through experimental manipulation:

  - Opportunistically – most zoos will have quiet and busy periods. By observing regularly over periods of fluctuating visitor numbers it may be possible to cover all conditions needed for your question (a pilot study will allow you to be more predictive about when quiet and busy times are likely to occur which may help to reduce the total observation time). One way of carrying out this type of study is to record the behaviour of the animals during quiet 'off peak' periods, when visitor numbers are predictably low, and comparing it to data collected during school holidays or weekends, when visitor numbers are expected to be high.

    Example: You could carry out observations in the week before and after Easter holidays for comparison with data collected during the Easter holidays.

    Limitations: There are many factors that affect visitor numbers – weather, time of day, season etc. There is a risk of misidentifying visitors as a causal factor in a behaviour change when it may actually be one of these confounding variables. Also, waiting for opportunistic sampling of various visitor conditions may take a long time to get sufficient repetitions of each condition.

- Experimental manipulation - restricting visitor access to an exhibit for short periods in a day could produce the 'no visitor' effect to which other visitor conditions could be compared. However, full consent and support from the zoo will be required for closing exhibits so you must check with the zoo at the early stages of your project planning. Many zoos will not be willing to close off exhibits even for a short time, particularly in the busy summer season.

  Limitations - by manipulating visitor conditions i.e. no visitors, many loud visitors, few quiet visitors, you may be creating an unrealistic situation, and therefore there is a risk of misinterpreting the animals' response to it.

- Another way of obtaining a 'no visitor' condition is to carry out observations outside public opening hours in the mornings or evenings, although you must get full consent from the zoo if you wish to do this. Whilst observing early/late would guarantee no visitors there are serious limitations with this method:

  Limitations Most animals have daily behaviour rhythms meaning that behaviour in the mornings and evenings is likely to differ to that in the main part of the day, irrespective of visitors. A similar problem may be encountered if trying to compare winter data to summer data due to changes in climate, day length, natural behaviour rhythms, visitor numbers and categories. Another argument against observations out of zoo opening hours is that the observer effect may increase at these times since the animals are not used to having humans around. Therefore the impact on their behaviour will be greater and thus your data will be more confounded.

9.4. Animal Factors

9.4.1 Behaviour

- When planning a project, the behaviours you record depend on the question you are asking. In the case of visitor effects studies your question should be as specific as possible with regard to what aspect(s) of visitor characteristics and animal behaviour are going to be studied.

- Broadly speaking the effects of zoo visitors may be negative, positive or neutral. It is crucial to identify carefully what types of behaviour demonstrate positive or negative effects and to support this with evidence from other published studies.

- You should, however, bear in mind the fact that these behavioural indicators may be species specific or specific to the individual. There is much literature available on behavioural indicators of welfare and we recommend that you research this topic before making interpretations of behaviour,

9.4.1.1 Positive Behavioural Indicators

- It should not be assumed that the effect of visitors on the animals' behaviour is always negative. Some studies have shown that visitor presence may induce positive behavioural changes, and so, may be described as enriching.

- If you want to investigate whether visitors have any positive effects on your study animal, you should try to be specific about what you consider these effects to be and these should be expressed in your study question.

Examples:

Play behaviour: This is a clear sign of good welfare as animals perform it if other conditions are good (e.g. if they are not stressed). However, it occurs mainly in young animals and therefore it may not be a very useful indicator for older animals.
Non-aggressive interactions: In circumstances where animals are housed with conspecifics, non-aggressive interactions between them, such as social grooming, may be essential to the physical and psychological well-being of the individuals. Care must be taken when interpreting these behaviours though, as in some species social grooming may also occur more frequently after periods of aggression as reconciliation.
Signs of interest in the visitors: Vigilance and other information gathering behaviours aimed at visitors, without signs of fear, aggression or begging, may indicate an enriching effect. For example, watching visitors play on a climbing frame may be interesting to animals.
Greeting behaviour: If shown in response to visitors could indicate that there is a benefit to the animal.
Natural behavioural repertoire: If the repertoire is more natural in visitor presence it could be argued to be enriching. This not only includes general activity budgets and a broad behavioural repertoire, but may include body postures, facial expressions and vocalisations.

9.4.1.2 Undesirable Behaviour

- Visitors may stimulate the expression of undesirable behaviours. If your study requires measurement of changes in undesirable behaviour there are a number of behavioural patterns that you could record.

Examples:

Stereotypies: Often defined as repetitive, non-functional behaviour, they can take many forms. N.B. as mentioned above, there is now a lot of literature available on the welfare implications of stereotypic behaviour (e.g. Mason & Latham, 2004) which should be consulted before any interpretation of stereotypic behaviour is made.
Locomotion / inactivity: Inappropriate levels of locomotion or inactivity can indicate a problem with welfare.
Vigilance: Repeatedly looking towards the visitors may indicate that the animal is not comfortable with the situation (care should be taken to define between this and vigilance towards the visitors that may indicate general interest).
Avoidance (hiding, turn back, cover, flee vertically): Actively avoiding the visitors may show a need to escape from this stimulus.
Infant shielding / clinging: Being over protective of youngsters, or constantly seeking reassurance from conspecifics can indicate fear.

Aggression: Tension caused by a stressful stimulus can result in increased intra-group aggression, or even aggression directed at humans.
Scent Marking: Inappropriately high levels of scent marking can indicate poor welfare in some species.
Urination / defecation (acute): If suddenly frightened, animals may urinate or defecate.
Self-directed behaviour: Increase in scratching in some species (e.g. primates) as well as other less frequent behaviours such as excessive grooming or self biting would be considered to be negative.
Fear vocalisations: may be emitted if suddenly frightened by the appearance of visitors.

Decrease in good welfare indicators such as natural behavioural repertoire would also be indicative of a negative impact of visitors.

9.4.1.3 Chronic or Acute?

When investigating whether a visitor effect is stressful it is important to identify whether your study animal is exhibiting signs of acute or chronic stress. Acute stress will be defined by short lived reactions to the stressor, for example, locomotion away from the stressor. Chronic stress will be defined by a response of longer duration; this may be long periods of inactivity following the stressor. **For more information** see Chapter 8: Monitoring Adrenal Response in Zoo Animals as an Indicator of Welfare

9.5. Enclosure effects

- There may be many aspects of the enclosure which affect the behaviour of the animals and the visitors.

  Example: Some visitors may bang on glass to get the attention of the animals, but this is not possible with an enclosure which is made up of wire fencing, therefore a different type of behaviour may be used to attract the animals' attention.

- The barrier type of the enclosure will determine the nature of many of the interactions between the visitors and the animals.

  Example: If studying the effect that noise has on the animals it is advisable to measure the noise levels inside the enclosure and relate them to those outside since some barriers such as glass windows can muffle the sound drastically.

- Details about significant parts of the enclosures in your study will be essential if you are carrying out a multi-zoo study (see Section 1.4 for more details on multi-zoo research).

- Many enclosure designs allow the animal to remove itself from public view. In the case of visitor effect studies it may be advantageous to look at this behaviour in more detail as the animal may be recoiling from the audience, or may only come into view when the public are present.

- Likewise the distance of the animal from the audience may also be of importance in your study. It might also be worth examining the percentage of the enclosure edge which is accessible to the visitors since this will affect the degree to which the animals can avoid the visitors.

- Effectively quantifying the many variables of an animal's enclosure may be very complex and time consuming and may not appear obvious at first, for example, recording the vertical dimension of an enclosure may be very important for some species (see Chapter 5 for more information on recording enclosure use).

  Example: You could consider visually dividing the enclosure into a 3D grid, and then recording the amount of usable space, refuge sites, food resources, shade etc. available in each one.

9.6. Sampling Techniques

- Your choice of sampling techniques depends very heavily on your study question, which should be as specific as possible (for details on constructing your study question and various sampling techniques refer to Chapters 1 and 5).

- One of the main questions in this type of research is how often to record visitor behaviour. A pilot study may help you to answer this question (see above Section 9.2.2). Your sampling intervals must ensure that you are not missing potentially important events, but you may find you do not need to record everything, and doing so may be wasting your observation time. A specific study question will help.

  Example: If asking what the animal's responses are to a certain visitor behaviour (e.g. eating around the enclosure) or characteristic (e.g. red clothing), you could continuously record the animal's behaviour for the duration that the visitor condition is present. This data could then be compared to data collected in sessions when the visitor condition is NOT present (using the same sampling technique), and controlling for other factors such as time of day, visitor density etc.

  Example: For the question "Are high visitor numbers related to aggression in the study animal" you may be able to correlate number of aggressive bouts per day with high numbers of visitors through the gate. It is important to note that this technique should be validated in a pilot study to ensure that daily gate admission numbers reflect visitor numbers at the enclosure.

  Example: If you were interested in finding a threshold of visitor numbers above which interactions with visitors and conspecifics become aggressive it would be wise to record visitor number at the enclosure as often as you record animal behaviour e.g. every minute. This would allow you to follow fluctuating visitor number with changing interactions with and between the animals. (N.B. This may create practical problems and the use of certain equipment may help, see Section 9.2.3)

- You may wish to record the number of visitors by counts, or by ranked categories (e.g. low, medium and high visitor densities). It is important to understand the limitations of each technique before making your choice as it will also dictate subsequent statistical analysis.

- It is important to bear in mind that a factor which may impact behaviour (such as zoo visitors) may continue to have effects even after it has been removed. Therefore it is advisable to include observations after the animals have been exposed to high levels of visitors in your experimental design.

  Example: Your study animals may respond to children banging on the glass, by immediately recoiling to the other side of the enclosure, therefore the reaction time may be quite short; but the animal may not return to the front of the enclosure for longer time periods so the duration of the response may be quite long. You should be sure that your observation periods are long enough to take account of this.

- It is a possibility that visitor effects might become obvious at night rather than just in the day, and therefore 24-hr studies might be an option. For example, high visitor densities may lead to disturbed sleep. Video analysis may be most appropriate to compare night-time activity following high and low visitor density days.

- Depending on your study question, it may be worth examining what initiates the visitor-animal interaction. Is it the animals or the visitors? What precedes the contact? The animal's behaviour prior to an interaction may tell you something about how the animal perceives that event.

  Example: Is the animal displaying signs of stress or frustration immediately before a visitor event, or does it appear relaxed and comfortable? Is the animal hiding from view or does it choose to be where the public can easily view it? Does the animal appear frightened or does it display signs of being interested in the visitors? You may be able to answer some of these questions by examining the animal's body posture (in all cases you should become as familiar with your study species and its natural behaviour prior to data collection).

9.7. Further Reading:

Blaney, E.C., and Wells, D.L. (2004) The influence of a camouflage net barrier on the behaviour, welfare and public perceptions of zoo-housed gorillas. *Animal Welfare*, 13 (2) pp. 111-118.

Chamove, A. S., Hosey, G. R., and Schaetzel, P. (1988) Visitors excite primates in zoos. *Zoo Biology,* 7 (4) pp. 359 – 369.

Cook. S., and Hosey, G. R. (1995) Interaction sequences between chimpanzees and human visitors at the zoo. *Zoo Biology,* 14 (5) pp. 431 – 440.

Hosey, G. R. (2000) Zoo animals and their human audiences: what is the visitor effect? *Animal Welfare,* 9 (4) pp. 343 – 357.

Hosey, G. R. (2008) A preliminary model of human-animal relationships in the zoo. *Applied Animal Behaviour Science,* 109, pp. 105-127

Hosey, G. R., and Druck, P. L. (1987) The influence of zoo visitors on the behaviour of captive primates. *Applied Animal Behaviour Science,* 18 (1) pp. 19 – 29.

Kreger, M. D. and Mench, J. A. (1995) Visitor-animal interactions at the zoo. *Anthrozoos*, 8, pp. 143-158.

Margulis, S., Hoyos, C., and Anderson, M. (2003). Effect of felid activity on zoo visitor interest. *Zoo Biology,* 22 (6) pp. 587-599.

Mitchell, G., Obradovich, S. D., Herring, F. H., Dowd, B., and Tromborg, C. (1991) Threats to observers, keepers, visitors, and others by zoo mangabeys (*Cercocebus galeritus chrysogaster*). *Primates,* 32 (4) pp. 515-522.

Mitchell, G., Tromborg, C. T., Kaufman, J., Bargabus, S., Simoni, R., and Geissler, V. (1992) More on the 'influence' of zoo visitors on the behaviour of captive primates. *Applied Animal Behaviour Science,* 35 (2) pp. 189-198.

Thompson, V.D. (1989) Behavioural response of 12 ungulate species in captivity to the presence of humans. *Zoo Biology*, 8 (3) pp. 275-297.

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Chapter 10

Behavioural Profiling

Editor: Kathy Baker

Contributing authors: Kathy Baker, Sheila Pankhurst, Olivia Walter.

Researchers studying animal behaviour have always been aware that individual animals may differ in their behaviour depending on variables such as age, sex, physical condition or past experience. An increasing body of evidence suggests that another factor responsible for consistent differences in behaviour is personality. In 1937 Meredith Crawford published one of the first articles in which the importance of personality in animals was identified. She proposed that laboratory housed chimpanzees (*Pan troglodytes*) showed significant individual differences in behaviour, "So different from that of every other animal, and so consistent with itself is the behaviour of each ape, that one cannot escape the conclusion that every chimpanzee must possess a distinct personality" (Crawford, 1937, p.79).

Behavioural profiling is an overarching term that can be used to describe a variety of different methods which attempt to measure individual differences in personality. The purpose of these guidelines is to consider some of the more popular behavioural profiling methods and examine the applications of this research in the zoo environment.

10.1. Introduction to behavioural profiling

10.1.1. What is animal personality?

The first thing that should be clarified is the definition of animal personality itself, i.e. what do researchers actually mean when they refer to animal personality? In the human personality literature there is no one definition of personality that is used consistently, however a broad definition suggests that personality can be described as those characteristics of individuals that describe and account for consistent patterns of thinking, feeling and behaving (Pervin *et al.*, 2004, p.6). While it is difficult to infer what animals are thinking or feeling it is widely accepted that human observers can reliably measure animal behaviour. Therefore most definitions of animal personality include references to 'individual differences in behaviour that are consistent across time and situation'.

Many terms other than personality are used to label these individual differences in behaviour and each term is used to refer to a slightly different construct. For example ecologists often use the terms behavioural styles or phenotypes and behavioural syndromes. Behavioural styles are suites of consistent behavioural responses observed in individuals, for example *boldness* and *aggression* are dependant variables used often in the study of fish species, and many authors are interested in correlations between the two traits across different contexts (Bell, 2005). Behavioural syndromes refer to population level correlations in suites of behaviours. Temperament is also a term used to refer to individual differences in behaviour and is often used interchangeably with personality

For the purpose of these guidelines the following definitions will be used.

Personality trait; a measurable aspect of an animal's behavioural repertoire, that when taken together represent the animals personality.

Personality; a combination of measurable behavioural traits that are consistent across time and situation.

10.1.2. The study of animal personality

The field of animal personality research is relatively recent compared to that of human personality research with a rise in research during the 1980s. In Gosling's (2001) review of the field he found published research on more than 60 species from cephlapods and insects to great apes. The sources of the articles covered a wide range of research disciplines from agriculture to psychology (Gosling, 2001). In his review Gosling identified the important findings in animal personality research to date, but also identified future research agendas for this field, such as researching personality from an evolutionary and ecological approach, and understanding the biological and genetic basis of personality. This review is now over a decade old and since its publication there have been many more advances in the study of animal personality.

Other recent reviews of interest include:

- Personality in nonhuman primates: A review and Evaluation of past research (Freeman and Gosling 2010)
- Comparative personality research: methodological approaches (Uher 2008)

- Are we barking up the right tree? Evaluating a comparative approach to personality (Gosling and Vazire, 2003).
- Animal personalities; consequences for ecology and evolution (Wolf and Weissing, 2012)

One argument against the study of animal personality is the risk of anthropomorphism i.e. projecting human characteristics onto animals. Implicit personality theories (IPT) of human raters have been identified as an issue in human personality research and as such should also be considered in animal personality research. IPT refers to raters' expectancies about the correlations of personality traits, i.e. people expect that talkative individuals will also be sociable. Gosling and Vazire (2002) suggest that personality does exist in animals, and is not simply a reflection of anthropomorphic projections, if personality assessments:

- *show strong levels of inter-observer agreement*
- *show evidence of validity in terms of predicting behaviours and real-world outcomes*
- *do not merely reflect the implicit theories of observers projected onto animals*

The issues of reliability and validity will be discussed later in these guidelines.

There has been a recent focus on addressing why personalities should occur in animals as animal personality is paradoxical from the point of view of behavioural ecology. Most modern ecological theory depends on the concept of optimality which implies that there should be one ideal set of adaptations to a given environment. Furthermore animals should consistently change their behaviour in response to changing environmental conditions to best increase their individual fitness (Dall *et al*., 2004). The idea of personality, however, implies that there are different evolutionarily stable personality types within a single species; if animals are said to have personalities then they should behave consistently at different times and in different situations.

Two recent reviews, Dingemanse and Wolf (2010) and Wolf and Weissing (2010), have proposed theoretical models for the understanding of animal personalities in terms of adaptive evolution. One example of which is fitness trade-offs between different personality types. Smith and Blumstein (2008) recently conducted a review of the potential fitness benefits of the personality dimensions, *boldness*, *exploration* and *aggression*. They conducted a meta-analysis of 31 articles covering a range of taxa. They found that boldness had a positive relationship with measures of reproductive success and a negative relationship with life span. They suggest that evolutionary mechanisms maintain variation in boldness due to fitness trade-offs: bold animals have greater reproductive success over shorter lifespans, while shy individuals have lower reproductive success but if they live longer, are able to breed for a prolonged period. Thus both behavioural types may have similar overall fitness benefits and could both be maintained within the population.

10.1.3. Benefits of personality research to zoos.

10.1.3.1. Research

Behavioural profiling of wild animals in zoos could provide interesting (and possibly useful) information on how humans perceive animals, for example:

- Observers are more reliable at rating the personality of mother reared chimpanzees compared to their hand-reared counterparts (Martin, 2005)

- Personality assessments of zoo housed-chimpanzees predicts their behaviour (Pederson *et al*., 2005)

This is perhaps the easiest use of behavioural profiling for zoos to justify - zoos are committed to research and data on human-animal interactions are certainly of academic interest.

A third question that could be added to the list above is whether or not animals respond differently to different humans - there is certainly some good evidence from studies of livestock that this is the case.

What is still not fully clear (but becoming clearer) is how zoos can make practical use of such information to inform their management decisions in areas such as welfare, or captive breeding.

10.1.3.2. Personality and health

Evidence from human personality research has established that individual differences in personality are important factors in disease resistance and/or progression (Cavigelli, 2005). A review by Cavigelli (2005) highlighted the importance of animal personality research, in understanding the relationship between human health and personality.

Probably the best examples demonstrating the relationship between personality and health in non-human primates are the series of studies by Capitanio *et al*. (summarised in Capitanio, 2011). Capitanio (2011) suggests that there are two main ways that personality can affect health. The first is behavioural, i.e. if you rate highly on a certain personality trait you may behave in a certain way that can influence your health. For example there is a positive correlation between Conscientiousness and longevity in humans; while the reason for the relationship is yet to be fully established, it is likely that highly Conscientious people take better care of themselves (Bogg and Roberts, 2004). The second is physical, and this relationship can be explained by two models. The *main-effects* model proposes that individuals rating 'high' or 'low' on a particular trait are actually 'built' differently in terms of their physiology, while the *interaction-model* proposes that traits influence the way animals adapt to stressful situations. Evidence for both models has been found by Capitanio *et al*., in their research on adult *M. mulatta*.

10.1.3.3. Personality and Welfare

Whether animals will adapt well in captivity is of concern, as coping strategies may affect an individual's welfare. By identifying potential stressors in captive environments animal management regimes can be adjusted accordingly to ensure that stressors are reduced or mitigated to a level which does not impair welfare. For example relationships have been found between the personality of zoo-housed black rhinos (*Diceros bicornis*) and zoo housing variables. Male rhinos' scores on a *fear* dimension correlated positively with the percentage of public access around the enclosure while femalerhinos' scores for a group of behaviours suggesting '*agitation*' correlated positively with the percentage of walls in the enclosure (Carlstead, Fraser, *et al*., 1999). While not establishing cause and effect these significant correlations could indicate that these particular environmental conditions are associated with differences in personality that could be indicative of reduced welfare.

Another zoo based study by Wielebnowski (1999) suggests that the human-animal relationship (HAR) may be important for the welfare of captive cheetahs (*Acinoynx jubatus*) as hand-reared individuals scored lower on 'Tense-Fearful' and 'Aggressive' personality components than those which were mother-reared. On the basis of this result it would not be suggested that generalised hand-rearing of individuals is a good management technique, as this would be counter-intuitive to individual welfare and conservation management efforts, both in terms of giving parents the opportunity to rear their own young and the developmental experience of the offspring. It may imply however, that developing a positive human-animal relationship (HAR) is important for individual welfare, and this can be done through methods other than hand-rearing (Hosey, 2008).

10.1.3.4. Personality and management

All captive animals are subject to management by human care-givers. An understanding of individual animals' personalities can enable better management of them. For example in primate laboratory situations individuals are often trained using positive reinforcement techniques, to facilitate husbandry and cooperation during experimental testing. Coleman *et al*. (2005) examined whether personality, in terms of response to a novel food object, correlated with the amount of time taken to train adult female *M. mulatta* to perform a simple task. Individuals were categorized as Exploratory, Moderate, or Inhibited personalities. Personality correlated with training success as over 75% of the highly scoring Exploratory or Moderate individuals were trained easily while only 22% of highly scoring Inhibited individuals performed the task.

In zoo situations one of the challenging management processes is the formation of social groups. Species that are held for conservation purposes are managed by a studbook keeper at a regional level. The studbook keeper will make recommendations about the movement and breeding of animals based on maintaining a genetically diverse and demographically balanced population and it is up to the individual zoo to manage the individuals within their institution requiring the introduction of individuals and formation of social groups. If the personality of an animal is known before it is recommended for a move, this information can be used to inform introductions (of animals) and consequential management. For example, all male groups are common in zoo housed western lowland gorillas (*Gorilla gorilla gorilla*), and it is recommended that these all-male groups should be formed with young males as this allows interactions to develop when there are relatively low levels of aggression (Stoinski, *et al*., 2004). Kuhar, *et al*., (2006) investigated the relationships between the Gorilla Behaviour Index (GBI; a rating tool originally used by Gold & Maple [1994]), and the age, housing, and behaviour, of 119 male gorillas at held at 38 AZA (American Association of Zoos and Aquaria) institutions. Behavioural data were collected on 25 males at 7 zoos. The authors found that animals rated low on the Understanding dimension of the GBI were more likely to engage in non-contact aggression behaviours. Therefore individuals scoring highly on this factor may be better candidates for the formation of all-male groups than their less Understanding con-specifics.

10.1.3.5. Personality and conservation

One of the main aims of modern zoos is to ensure that animal populations held in captivity are self-sustaining. This ensures that there is no need to take animals from the wild and that there are stable *ex-situ* populations of conservation dependant species should the opportunity to re-introduce occur. A major challenge in the management of zoo animals is achieving optimum environmental conditions to stimulate animals the opportunity to breed successfully. Some species of animal are notoriously difficult to breed in captivity, and researchers have used an understanding of animal personality to suggest possible explanations for this. In her study of zoo-housed cheetah (*Acinoynx jubtus*) Wielebnowski (1999) showed that reproduction success was significantly lower in individuals that scored higher on the personality dimension Tense-Fearful. Carlstead, Mellen *et al*. (1999) found that in zoo-housed black rhino (*Diceros bicornis*), males scoring highly on the Dominant dimension had lower reproductive success than males scoring lower on this dimension. The authors also found that the combination of the breeding pairs' personalities was also an important factor, the higher the female's Dominance score, relative to the males, the greater their breeding success.

So far there has only been one published study that looked directly at the effects of personality on reintroduction efforts. Bremner-Harrison *et al*. (2004) looked at the boldness of swift foxes (*Vulpes velox*) in captivity and related this to their survival in the wild, 6 months after re-introduction. They found that foxes that had been judged as 'bold' in captivity had lower survival rates than their 'shy' conspecifics.

10.2. Methodology

10.2.1. How can we measure differences in personality?

10.2.1.1. Trait ratings

The trait rating method requires observers who are familiar with the subjects to rate them on a personality trait, such as *aggressive* or *friendly*. Traits are usually accompanied by behavioural definitions in order to aid observers when assigning ratings to particular animals. The personality traits can be used for analysis in their own right but are often consolidated into underlying latent variables using data reduction techniques such as Factor Analysis (FA) or Principal Components Analysis (PCA). A classic example of this methodology, and one which many subsequent primate studies have been based on, is the work by Stevenson-Hinde and Zunz (1978) who developed the Madingley questionnaire. The authors generated a list of 33 trait items with behavioural definitions (e.g. *aggressive* was defined as, causes harm or potential harm, and *opportunistic* was defined as, seizes a chance as soon as it arises). Observers rated 48 rhesus macaques (*Macaca mulatta*) on each trait using a 7 point scale (1 = extreme antithesis to the item, 7 = extreme manifestation)

Pros: This method of collecting personality data is very efficient because it allows the observers to amalgamate their experience of an animal, perhaps across its lifetime, a task which would be quite difficult if basing ratings on behavioural observations of only a few weeks.

Cons: relies on the ability of the human observer to be able to accurately describe individuals based on their knowledge and experience of that particular animal. Therefore this method is thought to be more subjective than recorded observations of behaviour.

10.2.1.2. Behavioural coding (under natural conditions)

Observing animals under natural conditions and drawing inferences about individual personality based on their behavioural repertoire is one way that the behavioural coding method can be used. For example, Rouff *et al*. (2005) used data from behavioural observations in order to assess personality in 52 zoo housed lion-tailed macaques (*Macaca silenus*). They carried out a Principal Components Analysis (PCA) on this data and three personality dimensions emerged, i) extraversion-like behaviours, ii) agonistic behaviours, and iii) bold and cautious behaviours.

Pros: A relatively simple way of carrying out a personality study as the whole project is carried out by the researcher and is purely observational.

Cons: As with any behavioural study you will only get an idea of the animal's behaviour during the time period you are watching it. Your results may be biased by outside disturbances, or not reflect an animal's actual personality.

10.2.1.3. Behavioural coding (under experimental conditions)

The behavioural coding method is often used when exposing animals to experimental conditions and recording their behavioural responses. One area of personality research where this is most prevalent is in the investigation of the *bold/shy* continuum, a personality dimension that has been investigated across a wide range of taxa. For example Bremner-Harrison *et al.*(2004) judged bold swift foxes (*Vulpes velox*)as the individuals that, in the presence of a novel stimulus, left their dens more quickly, approached the stimulus more closely and showed more low-fear behaviours than their 'shy' conspecifics.

Pros: Extremely useful for studying one aspect of personality.

Cons: Difficult to carry out in the zoo situation.

10.2.1.4. Free choice profiling

The previous methods use behaviours or personality traits that have been previously defined by the investigator. Free choice profiling involves observers picking their own descriptive terms when describing an animal. This method has been used within the livestock industry, Wemelsfelder, Hunter, Mendl and Lawrence (2000) demonstrated that nine naïve observers on four separate occasions showed significant agreement when assessing pig temperament in this way.

Pros: Relatively simple to carry out, i.e. there is no need to generate a list of traits and a rating system before hand, as it is all generated by the observers.

Cons: A relatively new technique therefore there is no large body of evidence to support or reject its effectiveness. If using this technique it would be essential to include validation (see below).

10.2.2. Reliability and Validity

In order to be able to use any behavioural profiling method confidently, we must consider whether they stand up to certain criteria; i) that trait ratings show strong levels of inter-observer agreement and ii) they show evidence of validity in terms of being able to predict behaviour and real world outcomes

10.2.2.1. Reliability

Testing reliability should precede validity testing as a tool cannot, and should not, be used to measure anything if it is unreliable (Meagher, 2009). Within the field of personality research there are two main types of reliability testing, observer reliability which can be split into inter and intra-rater reliability, and test-retest reliability, which if using the same raters, could be considered to be type of intra-observer reliability.

Inter-rater reliability can be described as the extent to which two or more observers using the same rating instrument and rating the same set of animals agree on their ratings of traits/events. Inter-rater reliability is the most commonly used method to assess reliability of observer's ratings, however it is still an area which receives little attention. For example in Gosling's (2001) review of animal personality literature, of the 187 studies reviewed, only 21 reported inter-observer reliability for trait items. While reliability scores for individual traits may be highly variable e.g. Wielebnowski's (1999) study on zoo-housed cheetahs (*Acinonyx jubatus*) found reliabilities ranging from 0.48 (*playful*) to 0.82 (*active*), the mean reliabilities computed for these studies were relatively high; the mean inter-observer agreement was 0.52, a figure comparable with that of human personality research. In Gosling *et al's* (2003) comparison of human and domestic dog personality, the authors used parallel procedures and instruments and found that judgments of dogs were as accurate as those of humans.

Inter-observer agreement is obviously an important consideration when rating personality traits, and there may be factors that affect the outcome. Gosling (2001) suggests a number of things to take into consideration when looking at inter-observer agreement, raters acquaintance of the animal improving reliability, and communication between observers falsely inflating inter-observer agreement.

Intra-observer reliability is the extent to which a person's ratings of the same animals at two different time points correlate with one another. These measurements can obviously be influenced by changes in the animals' behaviour at two different time points, so the use of video recordings has been highlighted as an important method in assessing intra-observer reliability (Taylor and Mills 2006). If the same video recording of animal behaviour is presented to an observer at two different time points it would be expected that their assessments of the behaviour would be consistent. Video observations of behaviour are also beneficial as they control for any changes in the behaviour of the animal due to the presence of the observer (Diesel *et al*., 2008). If intra-observer reliability is found to be inadequate some authors propose that observers should be trained, through the use of video recordings, in order to maximise consistency and then only select those that are most reliable (Taylor and Mills, 2006; Murphy, 1998).

Retest reliability is the extent to which measurements of a particular trait can be reproduced when measured again at a different time period e.g. the likelihood that animal will behave in the same way when a test is repeated (Taylor and Mills, 2006). Within the animal personality research retest reliability has been assessed particularly when personality is assessed through experimental tests such as novel object testing. For example Fairbanks's (2001) study of 128 male vervet monkeys (*Cercopithecus aethiops sabaeus*) quantified individuals' responses to an intruder test (an unfamiliar male positioned at the edge of the home cage) in order to achieve an Impulsivity score for each individual. A sub-set of 70 males were subjected to the intruder test again between 2-9 weeks after the original test. The authors found that there was a high degree of consistency in individual Impulsivity scores recorded during the tests (average measures intraclass correlation coefficient ICC[3, k] = 0.83), suggesting that their Impulsivity score was measuring a consistent aspect of personality.

10.2.2.2. Validity

By assigning an animal with a 'personality' we should be able to know something about that animal; therefore we would expect to be able to use information about personality to predict behaviour and real world outcomes. Therefore if we can correlate personality ratings with some other variable it can validate our original personality assessment. With validity comes the problem of what is the ultimate measure of personality, within most animal studies two types of validity have been investigated, personality traits are either i) correlated with behaviour, and/or, ii) correlated with real world outcomes.

The first is assessed by correlating observer's trait ratings with, independently assessed, observed behaviours; this has been done in many studies covering a wide range of taxa. Lloyd *et al*., (2007) found several correlations between behavioural measures and personality components in horses, e.g. the 'dominance' component had significant positive correlations with herding and head threat behaviour and negative correlations with submissive, kicked, and groom-receiving behaviour. In a study on zoo-housed bachelor Gorilla (*Gorilla gorilla*) groups Kuhar *et al.* (2006) found that, high 'extroverts' are more likely to initiate and receive affiliative behaviours, while high 'dominant' individuals are more likely to initiate and less likely to receive displacement behaviours. These and other studies, often included correlates with behaviour as an aside to the main study, however some articles have solely investigated the personality-behaviour relationship. Pederson *et al* (2006) investigated the behaviour-personality relationship within zoo-housed chimpanzees (*Pan troglodytes*) and found that behaviours could be split into four contexts, and all were significantly correlated with one or more personality dimensions, agonistic, affinitive, submissive and public orientation.

As well as predicting behaviour, personality traits should be able to be used to predict real world outcomes such as stages in life history or physiological correlates. Fairbanks (2001) study on vervet monkeys (*Cercopithecus aethiops sabeus*) found that 'impulsivity' scores peaked at age 4, an age when vervet males typically emigrate from the natal group. Leblond and Reebs (2006), established that boldness (willingness to pass through a dark u shaped tube) in golden shiners (*Notemigonus crysoleucas*) correlated with the tendency to lead shoals. Byrne and Suomi (2002) investigated correlations between cortisol reactivity measures and subjectively judged personality ratings in juvenile tufted capuchins (*Cebus apella*). Personality traits, aggressive, confident, curious, effective, and opportunistic were all negatively correlated with cortisol reactivity levels, while apprehensive, fearful, insecure, submissive and tense were positively correlated with cortisol reactivity.

A commonly used method to test the ability of personality to predict real-world outcomes is to perform a novel test. This may involve introducing a novel object, sound, situation into an animal's environment and recording each individual's response. There has however, been some recent debate on the usefulness of novel object tests in assessing/validating personality (Carter *et al*., 2012).

10.3. Advice for Researchers

10.3.1. Study aims.

Ultimately the choice of methodology will be dictated by your study aims. A brief outline of each method has been given in the above sections with some useful references and it is up to you to research your chosen method further when you have established what your aims are.

What is the purpose of your project, are you interested in the subject of personality itself or do you want to see if personality affects another variable such as health, or do you want to know how variables such as enclosure type might affect personality traits. Below are some common aims of personality research accompanied by examples and references. These are not restricted to just zoo examples nor are they an exhaustive list, but are intended to be a starting point for further reading on each subject.

10.3.1.1. Personality

These are studies for which the intended aim is just to investigate the construct of personality in a particular species.

- Personality dimensions in spotted hyenas, Gosling (1998).
- Investigation of the five factor model in Chimpanzees, King and Figueredo (1997).
- Personality dimensions in free-ranging langurs, Konečná *et al*., (2008).

10.3.1.2. Personality traits

These studies look at one trait of personality such as the bold/shy continuum rather than investigating personality as a whole. They are often difficult to carry out in the zoo setting due to the experimental nature of the investigation therefore zoo examples are rare.

- In situ examination of boldness-shyness traits in the tropical poeciliid *Brachyraphis episcopi*, Brown, Jones and Braithwaite (2005).
- Investigation of the shyness-boldness traits in different contexts in the pumpkinseed fish, Coleman and Sloan-Wilson (1998).
- Behavioural trait assessment as a release criterion in a reintroduction programme for captive-bred swift fox (*Vulpes velox*), Bremmner-Harrison *et al* (2004).

10.3.1.3. Comparative studies (multi-species)

These are studies for which the focus is comparing the construct of personality across two or more species.

- A comparison of methods using two different species, the stumptail macque and the zebra finch (Figeuredo *et al*, 1995).
- Comparison of personality across the great apes (Uher *et al*., 2008).
- Comparison of personality structure across apes and rhesus macaques (Weiss *et al*., 2011).

10.3.1.4. Personality and captive environment

These studies focus on the impact that the captive environment may have on personality and/or the impact of personality for management issues.

- Comparison of chimpanzee personality structure in zoological parks and African sanctuaries (King, *et al*., 2005).
- The affects of age and housing on animal's scores on the Gorilla behaviour index (Kuhar *et al*, 2006).
- The influence of personality on training success of rhesus macaques (Coleman *et al*., 2005).

10.3.1.5. Personality and welfare

- Can chimpanzee happiness be estimated by human raters (King and Landau, 2003).
- Novel method for integrative animal welfare management in pigs (Wemelsfelder *et al*., 2002).

- Heritability of subjective well-being in chimpanzees (Weiss *et al.*, 2002).

10.3.1.6. Personality and conservation/reintroduction

- Predator discrimination and personality in Vancouver marmots (*Marmota vancouverensis*), Blumstein *et al.* (2006).
- Behavioural trait assessment as a release criterion in a reintroduction programme for captive-bred swift fox (*Vulpes velox*), Bremmner-Harrison *et al.* (2004).
- The effects of anti-predator training on the shyness-boldness trait in greater rheas (*Rhea americana*), deAzevedo and Young (2006).

10.3.2. Preparing a questionnaire

Depending on the species you decide to study you will probably need to carry out a multi-zoo study to achieve a good sample size. Therefore it is very likely that you will need to achieve some proportion of your data by means of a questionnaire, as you may not be able to visit all these zoos in person.

Details of questionnaire design are given in Chapter 11 and you should refer to this for general details about questionnaires. However the following issues are specific to using a questionnaire for personality research.

10.3.2.1. Provide background information and instructions.

As with all questionnaires it is important that you provide some sort of background to the study and a set of instructions for filling out the questionnaire. Instructions specific to personality questionnaires;

- People filling out the questionnaire should be able to individually identify and be familiar with the species in question, i.e. keepers, researchers, other individuals who spend a lot of time around the animals.
- You require at least two people to carry out the questionnaire (for inter-observer reliability analysis).
- It is best if these people do not carry out the questionnaire together

10.3.2.2. Personality traits.

One of the first things that your research will entail is generating a list of traits that will be assessed. Uher (2008) recently presented a review of the methods used to generate trait adjective lists in animal personality studies. She identified ten basic approaches that have been used by researchers. In turn these can be split into five broad methods based on their methodological approaches (Table 10.1). The differences in these methods are relatively subtle, for example King and Figueredo's (1997) study of chimpanzees (*Pan troglodytes*) sampled a range of trait adjectives which were derived in order to measure domains of the human Five Factor Model (FFM), so could be classed as either a 'top-down' approach or 'nomination' approach.

Table 10.1. Approaches for generating traits lists as proposed by Uher (2008).

Methodological approach	Outline
Nomination	Build on human concepts and theories of personality
Adaptive	Identify traits based on interactions between environment and personality variation
Bottom-up	Identify traits based on naturally evolved complex systems inherent to the species, such as behavioural or biological systems
Top-down	Apply trait dimensions found in one species and apply to another, looking for similarities/dissimilarities
Eclectic	Draw on trait dimensions and/or methodologies from various types of approaches

10.3.2.3. Types of questions and ratings.

You will probably want some information on the person filling out the questionnaire so if you decide later in your study that in-experienced raters are not reliable you can check this by using appropriate analysis. Some typical questions include, age, sex, experience with the study group, experience within zoos?

You should then have a list of all your personality traits (with their definitions) with space for the person to rate each **individual animal** on each **individual trait**. The type of rating scale you use is up to you, interval scales like the one in the example below are most common and usually range from 1-5 or 1-7 (Table 10.2).

Table 10.2. Example of a personality questionnaire.

Please rate these animals based on your knowledge and general impression of their behaviour. Please rate each item on a scale from 1 (the trait is not represented in the animal at all) to 7 (the trait is very strongly represented in the animal). Please do not discuss your answers with anyone else.

Personality Trait	Name of animal		
	Alvin	Simon	Theodore
Cautious Takes a long time to approach unfamiliar situations/objects.	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
Curious Is interested in changes in the environment.	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7
Dominant Has priority access to resources and can displace other animals.	1 2 3 4 5 6 7	1 2 3 4 5 6 7	1 2 3 4 5 6 7

10.3.2.4. Other variables

Depending on the aim of your study you may be interested in other variables that will need to be collected in the same questionnaire. These may include things such as enclosure design, enrichment, and breeding success. You should refer to Chapter 11 for further details, but the main point you need to take home is that you must be aware of your specific aims in order to construct a suitable questionnaire. There is no point in collecting lots of information that you do not need and equally you do not want to receive all your surveys only to find that you missed a vital question for your research.

10.3.3. Data analysis

10.3.3.1. General

As with most investigations calculation of descriptive statistics is usually the first step. This may include things such as;

- Response rates (all, by species, by area/country).
- General information on who filled out the survey.

10.3.3.2. Reliability

Some sort of reliability analysis is essential. Which method you use will depend on factors such as: your study aims, how many people filled out your questionnaire for each group of study animals, further data analysis.

Whichever method you use you should calculate inter-observer reliability for **each trait** for each **individual study group** (you can only assess reliability between people that are rating the same animals, i.e. the same zoo group). Once you have calculated these values there are two options for further analysis;

1. Disregard from further analysis any personality traits that fall below a minimum threshold value for inter-observer reliability (depends on the method of analyses but usually around 0.60, consult textbooks/peer-review articles for further information).
2. If the mean inter-observer reliability value is above the threshold conclude that general inter-observer reliability is good and therefore include all personality traits in further analysis.

10.3.3.3. Validity

The two main methods for assessing the validity of the personality assessments are behavioural observations and novel object tests. While it may not be feasible to use validation methods for all the animals in your study group due to the logistics of data collection you should try and at least obtain validity data for a sub set of your population.

10.3.3.4. Factor analysis

Many studies of primate personality aim to explore personality structure using factor analytic techniques. Normally this is either exploratory factor analysis (FA) or principal component analysis (PCA) (discussed below, 2.4.2). The purpose of both FA and PCA is to explain the shared variance of a set of measured variables using the fewest latent variables

This type of analysis benefits researchers in two ways: i) it enables descriptions of the relationship between the original set of variables and ii) scores for the new latent variables can be derived from the original observed scores. Scores for latent variables can then be used in various subsequent analysis (Velicer and Jackson, 1990).

See Fabrigar *et al*. (1999) and Budaev (2010) for further details of factor analysis.

10.3.3.5. Other relationships

Depending on the aims of your study you may wish to analyse the relationship between personality and other variables such as disease incidence or breeding success. You can look at the relationship between your dependant variable (i.e. breeding success) with either your personality dimensions (factors) or individual personality traits. Either way the analysis will be the same and will require some form of correlation or regression technique.

10.4. Further Reading

10.4.1. Personality resources

API: The Animal Personality Institute (www.animalpersonality.org)

Founded in 2004, is an interdisciplinary organization for bringing together research scientists whose work contributes to understanding personality and temperament in non-human animals. It is dedicated to building a stronger and integrative study of animal personality.

Primate Personality Net: (www.primate-personality.net)

Primate Personality Net is a new research group at the Humboldt University Berlin, Germany, dedicated to the scientific investigation of personality differences and their influences onto social relationships in primate species.

Chapter 11

Surveys and Questionnaires

Editor: Andrew Moss

Contributing Authors: Amy Plowman, Geoff Hosey, Miranda Stevenson and Kat Whitehouse-Tedd.

Questionnaires can be a good way to gather a lot of data from many zoos in a relatively short period of time. However, all too often questionnaires are ambiguous, too long, do not appear relevant to the problem or are not easy to complete. Sometimes a questionnaire or survey may not be an appropriate method to achieve the research goal or the information requested is already compiled and easily obtainable from a single source without the need to ask many different zoos. Zoo staff receive a very large number of questionnaires and are extremely busy people, so researchers who seem rude (many do!) or ungrateful, and questionnaires that appear irrelevant, poorly researched, ambiguous or overlong are very likely to be ignored.

This chapter is designed to help researchers produce better questionnaires and to help zoos set up systems to make responding to questionnaires quicker and easier. This chapter is intended to cover those sorts of questionnaire that ask for factual information; if you wish to find out about people's attitudes, beliefs or personality, then we recommend that you seek the initial guidance of a specialist (for example from a psychologist), as particular skills and techniques are needed for this kind of investigation. See also Chapter 10 on behavioural profiling. We hope this chapter will help make zoo research through questionnaires more productive and useful for all concerned.

Advice For Researchers

11.1. Before you Start

11.1.1. Is a questionnaire the best way to achieve the aims of your research?

Although questionnaires can be very useful in some instances they are simply not appropriate for some research questions. They are generally most suitable for accessing information that the zoo will already have recorded somewhere, so detailed information on aspects such as rates of behaviour and activity budgets, for example, are not likely to be available.

Housing and enrichment

Questionnaires conducted in a limited timeframe are not really appropriate for general surveys of housing, husbandry and use of enrichment. For instance, "An investigation into how BIAZA zoos use enrichment" is far too vague and thus not feasible. A more specific research question is much more reasonable, such as "What types of enrichment are used for elephants in BIAZA zoos".

Behaviour

Many aspects of behaviour, e.g. detailed activity budgets, require extensive observations over long time periods and could not be investigated by questionnaire. Researchers and their supervisors should be very cautious about using questionnaires to study zoo animal behaviour, as there are limited occasions where it will be appropriate. These may include investigating the occurrence of rare behaviours that would require weeks of observation to see, and where general rather than detailed data are required. Questionnaires may also be suitable to assess the prevalence of unusual behaviour such as self-injurious behaviour, stereotypic behaviour or visitor induced behaviours. There may also be a role in investigating seasonal behaviour if data collection can only happen at one time of year. Often it is more appropriate to investigate other features of behaviour rather than the behaviour itself, such as how particular kinds of behaviour were dealt with by the zoo, how they started, etc. Remember, quantitative data on rates of behaviour are unlikely to have been recorded by the zoo.

Breeding and reproduction

If there is a captive breeding programme for a species (see Chapters 1 and 7) then it is likely that all the data you want is already compiled by the studbook keeper. Because of this, zoos will be reluctant to provide this information again, and anyway it will be quicker for you to contact the studbook keeper and ask for access to the data, rather than collect it all again.

Some feasible research questions that can be tackled using studbook data are:

- What is the birth sex ratio?
- How has the population size and age/sex structure changed over time?
- What are the inter-birth intervals?
- Does age of first birth or inter-birth interval affect future breeding?
- Do some individuals have better breeding records than others?

Veterinary

First consider if your question is worth answering and whether it has been answered already in the literature. A major issue is what population will be available to you (size/accessibility/age) and will it be adequate for this type of study? If you are investigating a rare disease you will need a very large population, possibly over successive generations, to collate enough data to make worthwhile conclusions. Consider whether it is worth GOING to the zoo/vet surgery to ask the questions in person or to take part in sampling, but it is essential to appreciate you would be impacting on the working time of others and this may be of increased importance when dealing with private practice veterinary surgeons.

Conservation

General questionnaires about zoos' conservation activities are extremely problematic due to highly variable definitions, attitudes, methods of storing information, calculating budgets and classification of projects across zoos. EAZA has attempted to survey its member zoos on this subject with little success, and it is unlikely that a student project with limited time will fare better. If you are interested in this area you should perhaps consider investigating a small number of zoos in detail, rather than trying to survey a large number, or limiting your survey to particular taxa.

11.1.2. Background research

Many questionnaires sent to zoos often indicate very little knowledge about the subject on the part of the researcher. Extensive background research using individual zoo websites, BIAZA, EAZA and WAZA websites (see Section 1.1.5.) will help you. As with any other scientific investigation, make sure you research the literature thoroughly, using appropriate search engines such as ISI Web of Knowledge™, Cambridge Scientific Abstracts or Google™ Scholar, as described in Section 1.1.5.

> *Example 11.1.*
>
> Suppose you want to find out about enclosure sizes for giraffes. Some research should be done on what is the typical design of a giraffe enclosure, and about how giraffes are kept in British zoos. You should find out from this that giraffe enclosures usually consist of indoor heated housing, an outside yard or 'hard standing' area and a grass paddock. Therefore you will need to ask for the size and presence of each of these component parts. If you had not done the initial research then you would not know this, and might then ask wrong or ambiguous questions.

Information on which species and how many of them zoos hold is also available from BIAZA and ISIS (see Section 1.1.5). This will allow you to avoid sending questionnaires to zoos which do not have the animals you are researching.

Enclosure Design and Enrichment

As well as the sites listed above, ZooLex, Shape of Enrichment and various zoo design symposia proceedings (see Section 1.1.5) are good places to look for information. Websites of specific zoos often include information on enrichment that may be adequate for your purposes.

Breeding and reproduction

It is vital to find out if your species is the subject of a captive breeding programme (see Chapters 1 and 7) and to make sure you understand how these programmes operate before approaching anyone. You will probably only need to contact each individual zoo if there is no co-operative programme.

Conservation

Questionnaires in this area very often reflect a poor understanding of what conservation can be. Prior research should include investigating each target zoo (through their website) to find out what they consider to be conservation and to make sure you keep your questions relevant. Do not simply ask for information 'about your conservation projects'

11.2. Planning the Study

11.2.1. Defining the Research Aim

Just as with any other type of research project the first step is to precisely define its aim and identify what hypotheses are to be tested, or which research questions are to be asked. It is vital to know exactly what you want to find out before even starting to think about the questionnaire content. Also, again as with any other research project you should be clear why this research is interesting/important/useful and who the beneficiaries might be. A thorough survey of the literature, as mentioned in Section 11.1.2, will help you to do this.

Generally, in a project with limited time it is not possible to compile and analyse a large amount of information about how things are done in a way, for example, that would be suitable for writing husbandry guidelines. It is also not really feasible to try to make miniature studbooks, and this sort of project should normally be avoided. It is much better to attempt to test a particular hypothesis, for example that zoo giraffe enclosures have become significantly larger in the last 10 years. If you are doing an undergraduate project, make sure you know how much time you are expected to put into this, as this will guide you as to whether you are being too ambitious, or not ambitious enough.

Do not use your questionnaire as a 'fishing expedition' to help you to design a project, you need to clarify your research question first. If you are in doubt as to which of several specific hypotheses would be most appropriate to test first, then it might be helpful to arrange to speak to a relevant zoo person. They will probably have a good idea of the most productive angle of investigation. It is often useful to talk at the planning stage to someone who has zoo knowledge/experience, and many zoos now have appropriate people; however, if you are an undergraduate student, you should discuss this with your supervisor first.

Examples of **good**, well focused research aims for survey or questionnaire would be:

- Assessing the effectiveness of carcass feeding for felids across British zoos: this is a good aim because it focuses on a specific technique and a specific group of animals. It is also an aim which is achievable, and you would know when you had achieved it.
- Determining the occurrence of self-injurious behaviour in primates: here again we have an achievable aim, and again it is focused on a particular behaviour and a specific taxon.

- Identifying what enclosure and enrichment features are essential for parrots: Again, the aim is clearly defined and focuses on the specific requirements of a particular taxon.

- Evaluating knowledge and/or attitude change in relation to an education programme. Knowledge and attitudes can be fairly easy to measure using a questionnaire, and a pre- and post-test would allow for any change to be assessed in each participant.

Examples of **poor** research aims would be:

- Identifying the contribution of zoos to animal conservation: although this sounds like a worthwhile aim, it is so wide ranging and vague that it is unlikely to be achievable within an ordinary research project.

- Surveying the use of environmental enrichment in zoos: here again the aim is vague, and it would not be clear whether the aim had ever been achieved.

- Assessing change in environmentally responsible behaviour (e.g. recycling) in visitors. This relies wholly on accurate and truthful self-reporting from the participant, which is not always the case. Particularly in cases like this, where one particular outcome is much more socially desirable than another.

Note that in the examples given above of good research aims the aims as stated are clear and achievable, but further thought is needed as to how those aims will be achieved. For example, in the carcass feeding study you would have to think carefully about how you would judge "effectiveness". Research questions that could help achieve that aim might be "has the introduction of carcass feeding been associated with better dental health?" or "has parasite burden of carnivores changed since carcass feeding was introduced?" So the next step is clearly to identify the variables you need to measure in order to achieve your aim.

11.2.2. Variables and Measures

As with any other research project, think carefully about what variables you need to collect data on to fulfil your aims, and what measures you are going to use to get those data. Dependent variables appropriate for questionnaire-based research might include numbers of live births, ages at death, incidences of stereotypies, and so on. Independent variables might include cage sizes, husbandry methods and sex of the animal. In all these cases it is important to define your measures. The giraffe example in Example 11.1, for instance, shows that a simple variable of enclosure size is inadequate to get meaningful data because most giraffe enclosures have several different parts. What measures of these would be appropriate? In the case of the giraffe, area (m^2) might suffice, but if you were asking for information on, say, red panda enclosures, then some measure of vertical space would also be appropriate.

It will be necessary to define the terms you are using in many cases. Terms like "abnormal behaviour", "rearing condition", "naturalistic enclosure" and many others can mean different things to different people. The person filling in the questionnaire has to be clear what you are asking, so you also have to be clear.

For most hypotheses, you will need to define your target population, e.g. giraffes in BIAZA member zoos, zoos in England, EAZA member zoos. You will also need to set a time limit. You might want to ask about the current situation e.g. how many giraffes do you currently have and how many currently perform oral stereotypies. Or it might be more useful to ask e.g. how many giraffes have you held over the last 5 years and how many have displayed oral stereotypies over that period. Remember again that many questions, especially regarding numbers, genders, ages and origins of animals can be avoided by asking the zoo for a copy of the relevant ARKs records (see Chapter 7). You will know this if you do your background research (see Section 11.1.2)

At this stage you should also plan your methods of data analysis (see Section 11.6). A common error is to complete the data collection before deciding on how the data will be analysed. It is important to plan your method of analysis and statistical tests that you will use before designing the questionnaire. Among other things this will give you a better idea of how much detail you will need. For example, do you need to know the exact size of an enclosure or will it be adequate to ask which of three or four size classes it falls into? The latter will be much simpler for the respondent.

11.3. Designing your Questionnaire

11.3.1. General points

The questionnaire should be simple in layout, easy to read and as short as possible, anything that takes longer than 5 minutes is unlikely to be favourably received so make sure all questions are relevant and necessary to test your hypotheses. Be realistic in the time you tell respondents your questionnaire will take; this needs to be determined during the pilot study (see Section 11.4.1) , ask pilot respondents to time themselves. In addition to a cover letter include an introductory paragraph on the questionnaire to explain the purpose and aims of the research. This is important because the person filling in the questionnaire may not be the same one who receives it with a covering letter. Ensure that all instructions are clear and state exactly what you want people to do. Make the flow through the questionnaire logical and simple and keep section skipping to a minimum (e.g. if ‘no’ go to 12). The language used must be appropriate to your target audience and terms that may be ambiguous or unclear should be defined. Tables with an answer space or checkboxes make responding much easier. Ease of completing the questionnaire will be affected by how much information respondents will know versus having to find out (e.g. medical records that won’t be readily available to keepers versus daily feed data or faecal consistency data that won’t be readily available to vets, etc). Give respondents as much warning as possible about the type of information required – e.g. “before starting this survey, please ensure you have the following information to hand: studbook number, date of birth, feed records, medical records”….etc.

11.3.2. Questions

Questions should be:

- simple and clear
- unambiguous e.g. never use a double negative in fact better not to use negatives at all
- straightforward to answer
- relevant, concise and efficient

You should avoid:

- leading questions i.e. questions that suggest the answer
- double questions i.e. two questions in one

Examples of problem questions

Example 1:
Have your Hyacinth Macaws bred this year and do you think they will breed next year?

Problem:
This has two questions in one, so if the respondent simply answers 'yes', are they answering 'yes' to the first question, the second, or both? The second part of the question is asking someone's opinion on potential future breeding success; can the person answering the questionnaire provide a valid answer?

Example 2:
Why do you think zoos are important conservation organisations? Tick all that apply:
a) Education
b) Research
c) In-situ conservation
d) Zoo breeding
e) All of the above

Problem:
The question makes the assumption that the respondent actually does think that zoos are important conservation organisations. By providing a multiple choice list, you have to be sure that all possible answers are covered (what if a respondent thought that the most important zoo function was to be a 'fun day out'? How would they respond to this question?). Giving the option 'all of the above' is incredibly leading.

Example 3:
How often have you seen affiliative behaviour between any two of your animals over the past week?

Problem:
Asking questions about animal behaviour can be problematic. This question is an empirical one. That is, a question essentially needing data to answer. Does your respondent have these data or are they giving their opinion based on anecdotal observations? If it is the latter, then this is not a direct measurement of behaviour and should not be reported as such. Even worse, what if one respondent uses data to answer, but another anecdotal observations?

The validity of your questionnaire in answering the research question is a very important factor in its design. However you should also remember to stick to the most pertinent and relevant questions. Zoo staff get a lot of questionnaires to fill in, they will put the very long, very complex ones to the bottom of the pile, where they may well remain!

11.3.3. Types of question

There are basically two types of question: *open* and *closed*. Closed questions ask for a score or measurement, a selection of a yes/no answer, so effectively you are providing a set of answers for the respondent to choose from. They are usually quick to complete and easy to analyse, but they do not give the respondent the opportunity to give an answer that you have not already thought of. Open questions allow the respondent to answer with anything they want, and are usually used for assessing views or opinions. These are much more difficult to analyse than closed questions, and often you have to think of ways of collapsing the answers into groups in order to analyse them. However, they can be valuable in getting information that you hadn't anticipated. Going back to the giraffe (Example 11.1) example, a closed question would be something like "What size was your giraffe indoor housing in 2006?", whereas an open question might be "What, in your opinion, is the optimal design for a giraffe house?"

It should be obvious from this example that the closed question will provide quantitative data that are relatively straight forward to answer, whereas the open question will provide qualitative data (opinions in this case) which will be more difficult to analyse. In general it is best to try and stick to closed questions and make it clear what you are looking for. Keep open questions to a minimum and make sure that you can use the answers. Often you can turn an open question into a closed one by giving a number of options; for example, you could turn "what do you think are the most important things for good welfare in giraffes?" into:

Rank the following in order of importance for good giraffe welfare:

- abrasive substrate
- correct diet
- quality of hay
- provision of browse

Both open and closed questions have their uses, and you should choose which are the most appropriate according to the sort of data you need, which should be clear to you if you have defined your variables and measures. The table below summarises the advantages and disadvantages of these two kinds of question:

Table 11.1: Summary of the advantages and disadvantages of closed and open questions

	Advantages	Disadvantages
Closed	Standardised Quick to do Easy to score	Suggest answers May be ambiguous May omit responses
Open	Wider responses Novel responses	Longer to do Difficult to analyse

The more specific you are with your questions the more meaningful the answers will be and, more importantly, the easier to analyse. If you have a series of options as an answer to a question make it clear if you expect all of them to be answered or only one, i.e. make it clear if the categories are mutually exclusive or not. If you want a ranked response make this VERY clear, and what the rank numbers mean. Ditto with rated responses.

Example 11.2.

In the giraffe example (Example 11.1), pick two years, say 1996 and 2006. Ask for the size of the indoor housing (in sq metres), hardstand and paddock space in 1996 and 2006. To make it clearer, define what you mean by the three categories of enclosure. You may also wish to know how many giraffes were in the zoo at that time. Therefore make it more specific – ask for June 1996 and June 2006. Be realistic, most zoos should know what their giraffe enclosure was like 10 years ago – but if you go back 50 years you are less likely to get answers, especially reliable

Make sure the answer will make sense. For example, the question: "*Are your zebra used for breeding at all*?" is a closed question, but what sense can you make from the answer? You don't say what you mean by breeding (part of a managed programme, do the young survive?), and you don't give a time span, so the zoo that is breeding them now will answer 'yes', but the zoo that last bred them 10 years ago will also answer 'yes'. Also, to a North American zoo 'breeding' means mating, so take care that your meaning is clear and not ambiguous.

Another example: "*Have you successfully managed to breed zebra*?" is totally meaningless unless you:

- state the species of zebra
- say what you mean by successful (are there any survival criteria?)
- give a time span (e.g. in the last five years).

If you ask: *"Have you had any problems with the birth of any foals you have bred*?" the *yes/no* answer will be pretty meaningless here as the zoos that are the most prolific breeders are bound to have had some birth problems – so unless you are listing potential problems and asking for numbers or percentages the *yes* or *no* answer will not really tell you anything meaningful or of significance.

Avoid words that make questions leading or directional e.g. words like "better" or "improved" or "poor" used when asking about animal performance (e.g. breeding, faecal consistency, disease state, etc.) whilst on a particular diet. Be objective and use alternative words such as "change" or "different". Leading words may limit the response you get e.g. respondents may be unwilling to say performance has been poor but may say that performance has changed since diet changed.

11.3.4. Putting your questions together

Make the order of questions logical. For example, your questions might be:

1. how many capuchins do you currently house per enclosure?
2. what species of capuchin do you keep?

But it would be much better to ask what species of capuchin the zoo keeps, and then ask them to fill in separate forms for each species. Avoid asking general questions for several species, as they may be managed very differently by the zoo.

Try to structure questions to avoid conflicting or missing data in the responses and decide what you will do if this still happens. For example if a respondent answers “yes” to a question about whether a dietary supplement is used, but then fails to provide details of the supplement. Or if a respondent says “no” to the use of carcass meat but then describes the diet as including carcass meat in a separate question.

11.3.5. Advice for some typical questions

Housing and enrichment

If your questionnaire aims to find out something about zoo enclosures you should ask a series of precise questions. Do not just have the one question: “Describe your enclosure”. You should be aware that enclosures may have indoor and outdoor areas and a variety of subdivisions within these, e.g. small holding areas, sleeping stalls, hardstand, grass paddocks, etc.

If you are asking about enclosure size, remember that the height as well as the length and width of the enclosure is important for many species. Specify the units of measurement required and whether you want dimensions or floor area or volume. It may also be important to know animal user space, off show areas, proximity to public, access to surrounding enclosures, size of public area, age of enclosure, and how many animals would be expected to occupy the enclosure.

If asking about enclosure furnishing, remember that this may be permanent or temporary, and that information on the provision of certain items such as number and location of nest boxes may be very important. Enclosure topography (e.g. the presence of hills in middle of the enclosure) may also be an important aspect of housing to ask questions about.

Enclosure barriers are the subject of many questions, and if you are going to use closed questions for this it is important to give respondents the maximum number of possible replies – haha, water-filled moats, dry moats, traditional chain link, electric fencing, glass/acrylic, invisible/climatic barriers, natural (i.e. a tree for arboreal species), roofed, bars, walls.

To determine the effects of enclosure on some other variable, you may also need to ask about materials and substrates, and possibly construction costs. Also, if and how the climatic conditions of the enclosure are controlled. Additionally, the age of the enclosure and whether it was designed specifically for the current species or recently modified to allow change of use may also be pertinent questions.

If your questionnaire is about environmental enrichment, then it really is important to define it! You must have a working definition of what you regard as enrichment, so that it is clear to the person filling in the questionnaire. What is considered enrichment for one zoo may be just routine husbandry or good enclosure design for another. Defining different categories of enrichment (such as permanent or temporary enclosure features, food presentation methods, temporary objects added to the enclosure) will help. You may also want to define categories with respect to what they are intended to do, e.g. sensory, manipulative, feeding.

It may be important to ask what the objectives or goals of enrichment are, and what the frequency of enrichment is for each category you have defined.

If you are asking for an assessment of how well enrichment has worked, it is important to ask what the assessment is based on e.g. keeper notes, a full scientific study.

Conservation

Questionnaires in this area have some major terminology problems, and because of this you must define exactly what you mean. For instance, a common starter question is: “Do you participate in any conservation projects?”. In this case you must define what you mean by “conservation project”; does it, for example, include being part of a co-operative captive breeding programme even if no reintroductions are planned for the foreseeable future? You must also define ‘participate’; does it include donating £100 to an orphan chimp sanctuary in Uganda, does it mean active staff involvement in some way, or does it include research projects carried out in the field?

Questionnaires on conservation often contain what appear to be ‘filler’ questions. These bear no obvious relevance to the research aim, but can be quite specific and hard to answer, so should be omitted. For example, if you are asking about a species that is being bred for a potential reintroduction programme, why ask what is the precise enclosure size?

The conservation work of zoos varies to such an extent that it will be hard to keep your questions relevant to each zoo and it is strongly advisable to keep your research to a very narrow focus or small number of zoos e.g. those with annual turnover greater than £6m or annual visitor numbers less than 150,000.

Breeding and reproduction

Questions in this area tend to be very vague, such as: “Please let me know all about your breeding programmes”. What exactly is being asked here? Do you want to know which species have breeding programmes, or who they are co-ordinated by, or how successful breeding has been?

If you want to find out about the level of breeding success you must define what you mean by “success”, for example it could be the number of offspring which survived to 30 days, or the number of offspring which survived and successfully bred themselves.

Many questions about breeding success can be answered simply by examination of the ARKs taxon report and/or studbook data (see Chapter 7). Therefore it may be easier to ask the zoo and/or studbook keeper to provide you with those reports. These should give you some basic information such as sex, age, parents, date of birth, place of birth, number of births in x time, age of first/ last birth, interbirth interval, and so on.

This information may then give you pointers for specific questions on factors affecting breeding success. Successful breeding can be broken down into several components:

- Keeping male and female animals of breeding age together (many zoos do not/cannot do this for various reasons at various times)
- Mating behaviour (or AI techniques)
- Conception and pregnancy or fertilisation and hibernation

- Birth/hatching
- Infant survival

If you are trying to discover reasons for lack of breeding you will need to ask relevant questions about all these components.

Studbooks may give you information on housing and husbandry and appropriate social groups, but you may then need a questionnaire to investigate further topics like opportunities for breeding. For example, an animal may have only bred once in 6 years, but on further investigation you might find that it has only been housed with a member of the opposite sex for 1 of those years.

Veterinary

Veterinary surveys are often accompanied by requests for samples, such as blood or urine. Within the UK only opportunistic sampling will be permitted at most zoos, it is advisable to seek authoritative guidance before embarking of any sample collection. You must ensure that you include handling and storage protocols, especially health and safety guidelines. You will also normally need to provide any containers and chemicals and pay for postage of samples. Zoos will probably want to know how samples will be used, who owns the data, and guarantee of feedback. Sample ownership should remain with the collection. You should also be mindful that stores of historical samples may be limited and would probably have been retained by the zoos for their own future diagnostic or monitoring purposes.

Behaviour

As mentioned in Section 11.3.2, asking questions about animal behaviour can be problematic. You should not use questionnaires to ask people to give an opinion on behaviour, where empirical data would be more appropriate. If questions on behaviour are necessary, then there are certain common problems which should be avoided as described below. You should also read Chapter 10 (Behaviour Profiling) as this may offer a more appropriate method for obtaining data on behaviour.

As with other subject areas, do not ask vague questions such as “Are your animals stressed and bored?”. If you are asking whether a certain behaviour, for instance aggressive chasing, occurs some respondents may be reluctant just to say “Yes” if it only occurs very rarely. It may be better to ask for responses on a sliding scale of e.g. 1-5, NEVER- EVERY DAY. This will also give you better information. See Aitken (1969) for help with the design of response scales. Always specify a time frame – within past week/month/year/five years - when asking if a behaviour has been observed

Some behaviours are reactions to a keeper and may not be representative of the wider behaviour of the animal. They may even be an atypical response to a particular keeper and not to other people, so it may be important to ask information about the respondent, such as “How long have you worked with this animal?”, “Are you the only keeper that looks after this animal?”, “How often do you work with this animal?”, “Have you heard of this animal performing this behaviour, even if you have never seen it yourself?”

Some examples of questions which are ambiguous and not easy to answer are: Do your animals like each other? Are your animals aggressive? Does your dominant animal always take the enrichment item? Do any of your animals show abnormal behaviours? Do you have a problem with stereotypies.

11.4. Before You Send It Out

11.4.1. Road-testing your survey

It is vital that all your questions are unambiguous and mean the same to all your respondents as they do to you. You will only know this if you trial the questionnaire on a small set of people initially. The first person to test it on is your academic supervisor (if you have one), but asking your friends can also be useful, even if they do not understand some of the technical terms. Next, if you have a local zoo, ask for an appointment to see an appropriate staff member and work through your questionnaire with them. Once you have tested your questionnaire at this level a pilot study on a small number of zoos is still advisable before sending your questionnaire to all zoos you wish to include. This will ensure that your questions are as clear and simple as possible, and so will save time in the long run and ultimately lead to a greater response rate and better data.

11.4.2. Gaining support

Given that zoos receive a large number of questionnaires, it is a good idea to get support for your study from the BIAZA research group (see Contacting Zoos section in Chapter 1) and/or the chairperson of the relevant EAZA TAG (Taxon Advisory Group). In particular, any questions about controversial or sensitive issues (veterinary research is likely to include these) are extremely unlikely to be answered if you have not got this support. Some zoos do not have their own vet and use an external veterinary practice which will hold all their vet data, it is essential that you obtain the zoo's permission before approaching the vet practice. Gaining the appropriate support will indicate to zoos that you are a *bonafide* researcher, that your research is valuable and that you are using appropriate methods.

11.4.3. Ethical approval

Most zoos will have an ethical review process to which all research proposals must be submitted. Most students will also need to obtain ethical approval from their university/college. With questionnaires this is unlikely to be a problem unless you are asking for personal information about zoo staff or visitors. However, these processes can take time so you will need to allow for this.

11.4.4. Generating goodwill and instilling confidence

It is important that whoever receives your survey feels well disposed towards you! Make sure you include a polite cover letter explaining who you are and why you are doing the research. Preferably also include a note from your supervisor and your letters of support (see above Section 11.4.2). Often zoos receive similar questionnaires from several students at the same institution. This is very likely to annoy them and it is important that your supervisor is co-ordinating this and not allowing too many questionnaires to be sent out at any time. If your survey involves sensitive information (e.g. many veterinary surveys), stress that all information will be treated confidentially and that all respondents and zoos will be assured anonymity. Explain how the data will be used and where the results will be reported, and ensure that all respondents receive at least a summary of your main results and conclusions.

When zoos share their data they want to know that those they share it with are capable of understanding them correctly. This is particularly true of complex veterinary data, and the zoo vet will be very concerned that someone involved in the project has the ability to interpret the data correctly.

11.5. Maximising Responses

11.5.1. How many responses do you need?

You need to have a large enough, representative sample to allow you analyse your results using inferential statistics, however for some questions this may never be possible. For example, if there are only 3 zoos in Britain that hold the species you are interested in you will only be able to use descriptive statistics to describe parameters of that species in Britain. You could increase your sample size by extending your population of interest to European zoos, and possibly also American zoos rather than just British zoos, but make sure your sampling method is statistically valid.

You should also remember that you will never get 100% response to your questionnaire, and in fact it may well be a lot less than that. The clearer and easier your questionnaire is the better your response rate will be, but do not be surprised if it is as low as 20%. If you get a 60% response rate you will be doing extremely well. Giving respondents some type of motivation can be a useful strategy (e.g. a prize draw) and a guaranteed acknowledgement and copy of the final report should always be offered and you should always check if respondents wish to be anonymous of acknowledged by name.

If you are attempting a multi-country survey you need to consider if language is going to be a barrier. Is it possible to translate your survey? If not how will this be accounted for in terms of interpretation of questions and/or potentially low representation of non-English speaking facilities in the respondent list?

11.5.2. What are the best media to present your questionnaire?

Paper questionnaire by post

This is the traditional method and is still relatively easy for most zoos but you MUST include an SAE to make replying as easy as possible. There is a possibility that the person who opens your letter is not the best person to reply, so it may be worthwhile asking them to forward it to the person they consider most appropriate. In this case it is possible that by the time it reaches the right person your cover letter (see Section 11.4.4), questionnaire and SAE may get separated, so make sure they are securely attached together.

Email

You can probably send your questionnaire to most zoos by email and many of them will find this the easiest method to respond to. There is less risk that your cover letter and questionnaire will get separated within the zoo and it will be easier for the zoo to get it to the right person to complete. If the relevant person does not have regular computer access it can be printed out for them to complete. In case this is the situation you should always include your postal address and offer to send an SAE if required.

Web-based

Online surveys are becoming more common and have many advantages, mainly that they are very easy for the respondents to complete. There are websites that help you to construct questionnaires based on ready made formats (see below) and it is also possible to link web-based questionnaires to databases so that data compilation is done automatically. Other advantages are that it is possible to correct errors or missing data as the respondent completes the questionnaires by not allowing progress until acceptable responses are given to each question. Zoos are often concerned about sustainability issues and online surveys will reduce the amount of paper used. BUT it is absolutely vital to make sure it works perfectly before you go live! It may also be necessary to protect your site so that only the target participants can access it.

An online questionnaire may not be the easiest option for some potential participants. Web browsers may not be capable of dealing with your online questionnaire and some zoo staff, such as many keepers, may not have daily access to computers. Even if you use a web-based questionnaire you will still need to contact your target participants, probably by email, with a good cover letter asking them to take part (see Section 11.4.4). It is a good idea to attach your questionnaire to this email so that participants have a choice of response methods and can select the one that is easiest for them.

Some examples of online survey websites are listed here (although that does not mean that we necessarily recommend them) and there are many others. Some sites are free and some require a fee, although many that charge will give you a free trial which will probably allow enough responses for a student project.

http://web-online-surveys.com/
http://www.surveymonkey.com
http://freeonlinesurveys.com/

In person

Visiting each zoo and asking the questions by interview is probably the best way to ensure a response and may be the best option in certain cases involving lots of complex areas. However there are many disadvantages, the most obvious being that you probably don’t have enough time or money to visit enough zoos. In addition, with face to face questioning it is very easy to introduce a bias and lead respondents to answers you are anticipating.

11.5.3. Timeframe

You must provide a reasonable time frame for participants to complete your questionnaire and this should probably be at least 3 months. It is extremely likely that within this time you will need to remind people. Most people will not be offended by one or two polite reminders and it is a good idea to include your questionnaire again so they do not have to search for the original. On the other hand most people will be intensely annoyed and not inclined to respond if you are rude and/or have unrealistic deadlines for responses.

11.5.4. Who will be responding?

In larger zoos there may be several people with different responsibilities who will need to provide answers to different parts of your questionnaire. This is often the case with questionnaires about 'Conservation', which is a multidisciplinary subject and might involve staff in Education, Fundraising, Research etc. as well as keepers. If possible try to avoid designing a questionnaire that will need to go through more than one zoo department. This can be difficult to organise for the zoo and increases the chances that your questionnaire will be 'lost'. In terms of obtaining valid answers to your questionnaire, you have to be confident that the person responding has the necessary knowledge and/or expertise to provide you with the information you seek.

11.6. Analysis

There is nothing particularly different about analysing the data from questionnaires in comparison with data from other kinds of investigation. As with all research projects, clear identification of research hypotheses and questions leads you in the direction of the appropriate statistical tests.

As always, calculation of descriptive statistics is the usual first step. What these are depends upon the questions you are asking, but often they comprise summarised data on the characteristics of your dependent variables. In the case of the giraffe example they would probably be mean values, with standard errors, for the areas of different enclosure components across all the zoos who replied to your questionnaire. As part of your descriptives you should also report on relevant characteristics of your respondents, such as what proportion of your questionnaires were returned, as well as such things as, perhaps, how many came from UK compared to American zoos, or how many include current data compared to how many report historical (e.g. more than 5 years old) data.

Selection of appropriate methods for significance testing is driven by your research questions, but must also, as with all scientific analyses, take account of the amount of data you have, and whether it is parametric or non-parametric. Research projects in zoos often end up with small sample sizes, and advice on dealing with this situation is available in Chapter 3. Apart from this, you should choose an appropriate statistical test just as you would with any other data set. In many cases questionnaires are designed to look for associations between variables, so tests like correlation and χ^2 would be chosen. The best advice is to plan the data analysis as soon as you have identified your research question or hypothesis.

Back to the giraffe example

Let's go back to the example of giraffe enclosure sizes, which we have used throughout these guidelines. In Section 11.1.2 we decided we wanted to find out about giraffe enclosure sizes, and by Section 11.2.1 we had narrowed that down to a hypothesis that giraffe enclosures had become significantly larger in the last ten years. Our data for this, then, would be enclosure sizes for 1996 and 2006, and because we were very specific in our hypothesis and our measures, we know that a very simple calculation of mean enclosure size for each of those years would start to answer our question. Then a significance test (in this case ANOVA) will tell us if the difference is significant. But we also (Section 11.3.3) wanted to find out people's views on optimal enclosure features for giraffe welfare. Our initial question ("what do you think are the most important things for good welfare in giraffes?") was open, and would have been difficult to analyse. We closed the question by offering four features and asking respondents to put them in rank order. At the planning stage we can think about how we will deal with the answers. Do we want to see how well the respondents agree with each other, in which case correlation by ranks may be appropriate? Or do we just want to find out what is deemed overall to be most important and least important, in which case we just count up the scores for each category. The point is that complicated analysis is not necessary; the analysis should answer the questions that you are asking.

Use of data from zoo records

If you have obtained data from records, e.g. from studbooks for breeding questions, you must acknowledge that they are unlikely to be 100% accurate in all cases. If possible try to estimate the likely error involved.

11.7. Providing Feedback

On completion of your research it is common courtesy and extremely important to thank all your participants and provide them with a report or a summary of your research. Hardcopies would be expensive to send to all participants but it should be no effort to email a pdf version to each zoo or even to post it on a website and let all participants know that it is available to download. In addition, if you have received support from the BIAZA Research Group or TAG chairs they should also receive a report.

Research is only of value if its results are made known to people, and there are several ways of doing this with zoo research. You should seriously consider presenting or publishing your findings as described in Chapter 4.

Advice For Zoos

11.8. How can Zoos Deal Efficiently with Questionnaires?

Zoos can receive a lot of questionnaires, often all at the same time of year, and attempting to deal with them all efficiently and in a way which is useful to the researcher can seem like a major imposition on time and resources. Nevertheless, the best of them can produce very worthwhile results, both for zoos and for the researchers. Even those that are not going to produce ground-breaking publishable research can be worth making an effort for, as a good, positive response from the zoo can have wider long term beneficial effects, such as enhancing the reputation of the zoo and building relationships with Higher Education Institutes (HEIs). What can zoos do to make the process easier?

11.8.1. Keeping track of the questionnaires

It is very easy, particularly in a large zoo, for things to get lost. An issue here may be about how dealing with questionnaires can be centralised, and how their progress can be tracked, particularly if the response involves more than one person. It may be helpful to designate one member of staff to deal with and coordinate research enquiries (who this is will be variable and may include administrator, education officer, curator). This person could be identified on the zoo web site, and on any information that goes to HEIs. It may also be useful to create a database of questionnaires received to record when each came in, from whom and where, decision on whether or not to respond, who completed it within the zoo (did someone else need to authorise it), and what the deadline is. A copy should be kept of the response, and the copy of the final report from the student. Questionnaires take time to fill in, but keeping a record of how many questionnaires are submitted and responded to is useful for managers to estimate how much time is spent on them, and is evidence of contributing to research for Zoo Licensing purposes.

11.8.2. Who fills the questionnaire in?

Several different people in the zoo may be the most appropriate, depending upon the information that is being sought. Who in the zoo has the remit, and indeed the authority, to fill questionnaires in on behalf of the zoo? If filling in questionnaires is part of the keepers' jobs, are they aware of that? If one person is designated to deal with all questionnaires, as suggested above, this person should be responsible for deciding who is the most appropriate person to complete each one and ensuring they do. Zoos may need to improve internal communication to explain why various staff should comply with completing questionnaires.

11.8.3. Which questionnaires should zoos fill in?

It may be that the zoo really is unable to fill in all of the questionnaires it receives. Should it attempt to prioritise them, and if so on what basis? What are the zoo's professional obligations with regard to responding to questionnaires? Are there issues of courtesy? If possible it helps to make and maintain contact with supervisors in the HEIs from which you receive many questionnaires. This communication leads to fewer, better questionnaires since the supervisors have better knowledge of how zoos work. If you are not sure whether a questionnaire is worthwhile ensure that the researcher has asked for BIAZA research group, EEP or TAG support.

11.8.4 Judgments about the quality of questionnaires

Many questionnaires may be perceived as of poor quality, perhaps because they are vague and poorly designed. There may be a repetition of surveys, with several researchers asking similar things, possibly because they are based on a class assignment given to a whole cohort of students. Again communication with supervisors at HEIs will help improve the quality of questionnaires and reduce the numbers of inappropriate and unwelcome surveys. It may even be possible to have direct influence on the subject of questionnaires if you let supervisors know questions of relevance to the zoo. Zoos should all include a direct link to the Research section of the BIAZA web site on their own web sites as there is a wealth of advice there to help improve all types of zoo research.

11.9. Further reading

Aitken, R.C. (1969) Measurement of feelings using visual analogue scales. Proceedings of the *Royal Society of Medicine*, 62 pp 989-993.

Burgess, T.F. (2001) A general introduction to the design of questionnaires for survey research. Available at http://www.cavehill.uwi.edu/cermes/SocMonPub/Workshop_trainin_%20resources/Recommended_reading/Questionnaire%20design%20and%20analysis/Burgess_2001_Survey_design.pdf

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Labaw, P.J. (1981) *Advanced Questionnaire Design*. Cambridge, Mass: Abt Books.

Oppenheim, A.N. (1992). *Questionnaire Design, Interviewing and Attitude Measurement*. London and New York: Continuum.

Sudman, S and Bradburn, N.M. (1982) *Asking Questions: a Practical Guide to Questionnaire Design*. San Francisco: Jossey-Bass.

Willis, G.B. (1994). *Cognitive Interviewing and Questionnaire Design: A Training Manual* Washington: US Dept. of Health and Human Services.

Chapter 12

Nutrition and Diet Evaluation

Editors: Andrea L. Fidgett and Amy Plowman

Contributing authors: Andrea L. Fidgett, Amy Plowman and Kat Whitehouse-Tedd

12.1. What do we mean by Nutrition and Diet Evaluation?

Diet is the usual food of an animal, nutrition is the process by which a living organism assimilates that food and uses it for growth and for replacement of tissues. One of the challenges which zoological institutions face when housing and maintaining exotic animals is providing them with a diet adequate for achieving optimal nutrition.

There are a number of factors that need to be taken into account when designing diets. These include: knowledge of the animal's diet composition and foraging behaviour in the wild, knowledge of the functional anatomy of its digestive tract, knowledge of dental morphology and adaptation to the natural diet, knowledge of the bases on which animals select particular food items, and an understanding of the implications that foraging and diet selection have for social behaviour. This is a tall order, given our lack of knowledge about many exotic species. It is compounded by the fact that even when these factors are known, devising suitable substitutes for a wild diet is hard. For example, we may know that 75% of a wild primate's diet is fruit, yet feeding 75% fruit in captivity may provide a very different intake in terms of nutrients, since fruits produced commercially for consumption by humans (and thus readily available as components of zoo diets) are so different from wild fruits (see O'Brien et al., 1998). Deficiencies in diet presentation may result in obesity, stereotypies, or increased aggression. It is clear that an understanding of the nutrition of exotic animals is vital to their well-being and our ability to maintain them successfully in captivity, yet animal nutrition is a new and relatively unexplored field. Part of the problem is a lack of facilities in zoological institutions and a lack of expertise. There is thus a strong need to develop nutritional studies and departments in zoological institutions.

Research on nutrition is carried out both as a problem-solving exercise (in relation to ill-health or apparent infertility) and as part of ongoing programmes to improve animal husbandry. Studies carried out to solve problems tend to focus on assessing nutrient quality of the diet, whereas the husbandry work is more oriented to food presentation methods and behavioural and environmental enrichment involving food, and feeding and foraging techniques. The aims are to enhance nutritional quality and palatability and to stimulate natural foraging behaviour. The emphasis is on non-invasive research methods. The ideal situation for diet evaluation would be to monitor the intake of individual animals. However, for the evaluation to be valid it is essential that the animals are behaving normally and separating animals for the purpose of a study would likely cause stress, which in turn may affect appetite and hence intake. Thus animals should not be separated from their usual social groupings, normal routines disturbed as little as possible, and most assessments can be done indirectly through weighing food remains or analysing faeces.

12.2. Dietary evaluation

Food and water as basic needs of zoo animals and the Secretary of State's Standards of Modern Zoo Practice (SSSMZP) places strong emphasis on the importance of the frequency of feeds and the nutritional balance of diets provided. The standards state that "Food should be presented in a manner and frequency commensurate with the natural behaviour of the species, as well as its nutritional requirements, which may vary according to season." This statement is expanded to a series of more specific recommendations, including "A record of all diets must be maintained."

For many zoos, diets are recorded on kitchen notice boards, a practical solution to keep all keepers informed and easily allow for changes as species or numbers of animals change. However useful, they only form a starting point since they often lack sufficient detail about the feeds and quantities used, data which is necessary for properly evaluating the efficacy of a diet. Even if the feeds and quantities are described in detail this can differ considerably from what individual animals actually consume (Box 12.1).

a) Diet evaluation by weighing feeds and remains

The most commonly used means of diet evaluation involves weighing feeds and remains. Also termed an intake study, it is relatively simple but can be time-consuming. The purpose is to determine the actual amount of food fed to and consumed by an animal or group of animals in an enclosure. For the reasons given above consumed diets differ from provided diets, an intake study can provide considerably more information than simply looking at the provided diet and thus allow for a more accurate assessment of the diet than simply evaluation the diet offered. **There is a step-by-step guide to conducting an intake study in** **Appendix I**

Box 12.1. Why diets consumed may differ from diets offered

- Food preferences of individuals leading to selection of preferred items and discarding of others
- Social group/hierarchy
  - Dominant individuals may take certain items or prevent subordinates from taking items. Dominants will have a choice of items whereas subordinates may have no choice.
  - Individuals at various lifestages (pregnant/lactating/growing) may consume different quantities.
  - Individuals of varying size may consume different quantities.
  - All food may be eaten, but it may be difficult to determine which individuals have eaten what.
- Live food
  - Difficult to tell what has been eaten as live food may escape or hide, and animals may not eat food immediately.
  - Nutritional benefits of gut-loading live foods are diminished if prey are not eaten immediately
  - Differing abilities of animals to catch live prey will affect intake, particularly for group-housed animals with competition for food.
- Mixed species exhibits
  - In mixed species exhibits with different dietary requirements, individuals of one species may consume food intended for the other species.
  - Dominant individuals/species may take control of the food supply and prevent food choice for other individuals/species.
- Food presentation and storage
  - Method of presentation may affect the amount of food reaching individuals e.g. need to account for top and bottom feeders in a fish exhibit when feeding from the top of the tank.
  - Supplements: how to measure how much of the supplement is consumed i.e. which individuals get the supplements, do supplements stay in the bucket or end up on the ground, how much supplement dust remains on live food by the time it is eaten?
  - Pre-prepared food mixes can settle when stored therefore feeds may become unbalanced and quantities of food types unknown.
  - Environmental conditions in some enclosures, especially for reptiles, can make food degrade quickly if not eaten immediately
- Other food available in enclosure
  - What is the contribution to the diet from grazing/browse/visitor feeds?
- Demands of visitor experiences
  - Contribution to diet from visitor feeds, considering seasonal variation in visitor numbers.
  - Different individuals come forward for visitor feeds therefore food may not be distributed evenly.
  - Food restriction of everyday items in order to encourage animal ‘attendance’ at visitor feeds (where more palatable food may be fed)
- Individual differences in appetite
- Training
  - Rewards given as part of training may not be accounted for as part of the overall diet.
- Pests
  - What proportion of diet is eaten by pests rather than zoo animals?
  - Pests may eat the top layer of food which often contains the supplement.
- Big groups
  - Can you be sure that each individual has the correct diet/monitor the intake of each individual?

Intake studies are run over a period of time, during which all the food items offered to the study animals are weighed at the time of preparation. All food remaining at the end of a feeding period is carefully collected and also weighed. Some means of adjusting for changes in moisture content of the uneaten food should be used, and this is achieved either by drying feed and remains samples to a constant weight in a drying oven or through the use of a dummy or control dish to assess changes in moisture content in the actual the climatic conditions experienced. Although the first method is more accurate, it can often prove impractical for large volumes of food. Adjusted weights of remains, subtracted from original weights fed, produce an estimate of feed intake. These values are then averaged over the length of the diet trial. The nutritional composition of the diet is typically estimated from data on the nutrient composition of individual items in the diet and is therefore only as accurate as the data upon which the estimate is based. Hence from weighing feeds and collection of remains and the use of food tables/diet management software it is possible to arrive at an estimate of nutrient composition. Care must be taken to ensure the correct units are used, particularly when the use of a conversion factor is required. Comparisons between the intakes of different groups are best made on the basis of total group body weight, rather than by the number of animals in each group.

The data from intake studies provide estimates of what is fed, and more importantly, what is being consumed by the group so may reveal whether nutritional inadequacies apparent in the diet consumed are due to the animals' choice of food items, or because the diet provided is inadequate. A simple index of preference is also useful, whereby food items are categorised into low, neutral or high palatability according to the percentage eaten. Some food types make it very difficult to weigh leftovers such as live feed which might escape capture and remain hidden in the enclosure or mixed liquid feeds such as anteater broth where it is impossible to separate out the component feed types from leftovers. For live feeds it is often easier to record intake if animals are hand fed (although this may not give the same hunting opportunities so not be a true measure of normal intake).

For animals living in group situations the intake study method will rarely give useful information on individual differences in dietary intake due to many of the factors in Box 12.1. In most cases individual intake can only be reliably evaluated using detailed and usually time-consuming behavioural observations of feeding (see below).

b) Diet evaluation by focal animal observations

To calculate individual feed intake through direct observation it is necessary to know the average piece size of each food type and then to count how many pieces of each food are consumed. This is relatively straightforward for some species e.g. macaques that are fed discrete food items, such as pellets and pieces of fruit or vegetables, that can be easily weighed (e.g. see Plowman, 2009). However, for other animals that are provided with large or non-discrete items, such as browse or exudates, or have access to paddock grazing/browsing in their enclosure it is necessary to estimate bite size. For browsers average bite size can be determined by providing a known-weight of browse, counting the number of bites taken then removing the browse and re-weighing. This is likely to affected by type of browse, relative leaf/twig ratio (e.g. Shipley et al., 1999) and individual animal (especially size) so average bite size needs to be determined over a range of these conditions and preferably for each individual animal if feasible. For grazers bite size determination is also affected by sward type and structure (e.g. Burlinson et al., 1991) and ideally should be estimated in a similar fashion for browsers (above) using a sward sample in a movable container. If this is not feasible it can be estimated by the hand-plucking method (e.g. Vries, 1995). For sloppy or liquid feeds a similar method can be used to estimate intake rate per unit time - a known weight of food can be provided and then removed and reweighed following a measured amount of time spent feeding by an individual. Determining whether an individual has eaten certain food types can be done by marking that food with indigestible markers such as food dye or edible glitter which will appear in the faeces

This type of study provides much more detailed information about individual food preferences and consumption than can normally be obtained by an intake study (a. above). However it is extremely time-consuming; observing only one animal per feeding session will require a long period of time over which to establish a reliable data set if group size is large. Nevertheless it is important to consider this type of diet evaluation in situations where there may be strong social effects in group-living species, leading to monopolisation of palatable food items by one or a small number of dominant individuals. It has been suggested that at public feeds members of the public could be used to observe individual feeding choices or to monitor food lost to pests. This is potentially a useful way to collect information much more quickly but it is likely to be much less accurate than a single trained observer. Attempts to ensure equal access of all individuals to all food types include feeding groups more food than is actually required (this can lead to dominant individuals becoming overweight), feeding only one food type per meal, providing food in more than one location and even co-operative feeding training. However, short of separating animals at feeding times it is extremely difficult to guarantee that every animal will obtain its 'fair' share.

Evaluation by this method also allows the determination of the contribution of enclosure vegetation to the diet. Zoo enclosures are being increasingly more naturalistic and contain more vegetation than in the past. Some of this vegetation may have been chosen deliberately because it is palatable to the animals and some deliberately because it is not palatable. If animals eat significant amounts of enclosure vegetation this will have an impact of the nutrient composition of their diet and also needs to be taken into account. There is no way to do this other than by focal animal observation and even so it is still difficult to reliably estimate weight of food eaten due to variation in bite size (see above)

c) Diet evaluation by survey and review

This technique is increasingly used by EEPs/TAGs to gather diet data for a single species across a range of zoos. If done well it can provide good information on nutrient contents of diets which can be compared with breeding and health records across institutions. Given the lack of data on wild diets for many exotic species, surveying current feeding practice **and** evaluating the results against how well animals perform when fed these diets is often the only way to determine dietary recommendations (see Section 12.5 below and also Wright *et al.*, 2011). However, this method has major limitations in that the quality of data returned is usually not good enough to do anything with at all. This is largely due to poor survey methods which do not make it clear what information is required; it is imperative each question provides useful and usable data, but to provide the level of information needed requires quite a lot of effort on the part of the respondent.

To be of any real use diet survey respondents must give the weight of each individual food item offered to the animal/group of animals each day over a period of time. This period should be long enough to incorporate all the regular variations in the daily diet – typically a week in many zoos. Other information required includes the numbers, sexes and ages of animals in the group and their weights if known. Also consider whether frequency of feeding is a factor of interest. Do you need to know when in the day the animal was fed or just how many meals/day it had? The manufacturer and product name of any manufactured feeds must be included. To ensure that all this information is received the surveyor must ensure that the questionnaire they send out is very precise, clear and easy to complete. Anyone considering this method is strongly encouraged to read Chapter 11 on

Questionnaires for further advice on how to conduct research by questionnaire. An example of a good diet survey questionnaire is included in Appendix II.

Even if a large number of institutions respond to a survey with excellent information it must be remembered that, unlike the above methods, this technique only tells us the diet actually offered – not what is actually consumed. As previously stated, these can differ quite substantially but it is unrealistic to expect many zoos to provide accurate actual consumption data. The best way to acquire these data from many zoos is to actually visit them and collect it yourself.

More complex studies, such as epidemiological (i.e. to determine dietary risk factors for certain health issues) can also be achieved by surveys but data collection may need to be more strategic. For example, case-control studies may be preferable in which case a lot of background data collection is required to match cases (e.g. those suffering a particular health condition or non-breeders) with controls (e.g. those without the health condition or breeders). In such a study you must be clear how you will define a "case". Definitions may vary by zoo depending on level of veterinary vigilance, access to diagnostic tools, observation and recording of clinical symptoms. If using a cross-section design, you will need to decide how to you control for influence of facility, geographic region etc. and also our time period, will you only ask for *current* status or status within past week/month? If the diet has changed recently prior to the survey being completed are you more interested in the previous diet (which may be more likely to be associated with the animal's current medical status) or the recent diet which may not yet have had sufficient time to influence animal condition/health. Working with an epidemiologist and/or biostatistician from the start will provide confidence in data collection strategy and subsequent analysis. Epidemiological analysis of risk factors is not as simple as correlation analysis.

Diet surveys can be very long and detailed so it is very important to know what you are going to do with your data and think carefully how much detail you actually need to do this. Will you be able to assign categories for analysis post-hoc (e.g. if you get a list of ingredients will you be able to assign all potential diets to a category?) What about diets with a mix of categories across the week/year? What criteria will you use for categorisation? Do you need to know what species of animal is used as meat or species of plant is used as browse? Or is it enough to know carcass versus chunk meat or browse versus pellet? Is food preparation or storage important for the study aim? If not, exclude it. If it is, then it may be preferable to provide categories of responses rather than an open question to enable ease of analysis and avoid overly complex answers or conversely overly brief/simplistic answers. How much detail is required about the individual animal? Do you want exact date of birth or its age? Don't allow both to be used as this will complicate data analysis (e.g. DOB will give an exact age such as 2.86 years, versus age will give a rounded age such as 2 years). If you are intending to compare diets among groups of animals (e.g. diseased vs. healthy), is there anyway of conducting a power analysis to predict how many responses you need, or post-hoc how powerful your sample size was? Also think about which animals you are interested in, do you want data on all individuals of one species in a collection or a "representative" generic diet for the species? This will influence how many surveys need to be completed per zoo. Zoos with large collections of your species of interest may skew results if you ask for all individuals in a collection to be represented by a separate survey, so you may want to pre-determine a maximum number of responses per zoo. If so, how will the zoo be told to decide which individuals to include in their responses? Random selection based on studbook numbers or birth date? Spread of ages? Sexes? Etc. If different surveys are required for individual animals can you pre-complete some of the data (e.g. Institute details and animal details from studbook information) so that the respondent isn't repeating his/herself each survey.

Often it may be helpful to avoid bias to be quite generic about the aims of the project and avoid telling respondents the exact hypotheses. Even a project title such as " A study to determine the influence of diet on breeding success" may bias the way they respond or the animals that they select for inclusion (despite instructions about randomisation). For example, respondents may try to 'help' the study by only providing data on animals that have bred due to a misguided perception that this will be more useful in answering the study question than information on animals that haven't bred (and likewise for diseased versus non-diseased animals). It may also focus the respondent's mind on particular details at the expense of other details (which may still be relevant). As a way of testing for bias in the sample it may also be a good idea to include a question on an unrelated topic. For example, in a study on GI disease and diet, a question on non-GI disease was included. If the distribution of answers to this unrelated question differed between animals that were diagnosed with and without GI disease then bias would be suspected in the sample selection. However, if the distribution was the same then this provides evidence that the two groups are similarly random in terms of variables unrelated to the study aims.

12.3. Feed composition and nutrient calculation tools

Having recorded the ingredients in a diet, along with quantities fed and consumed, this data must then be combined with information about the nutrient composition of each ingredient to provide a nutritional summary of the diet. This can be relatively straightforward but there are a number of issues you need to consider. If the animal receives a different list of ingredients each day of the week or has a starve day how will you calculate the typical daily intake for the animal? You could use the daily average across the week by combining all ingredients for 7 days and dividing by 7. Or you could work on a proportional basis. You may also need to factor seasonal changes (in ingredients, quantities, sources, etc) into this?

Various food tables are available in print and online, from which the relevant nutrient values can be extracted and copied into a spreadsheet to perform the calculations.

Table 12.1. Common zoo feed categories and sources of composition information.

	Fruit, Veg, Nuts, Seeds	Forage	Whole prey	Prepared feeds/ supplements
AZA's Nutrition Advisory Group website		✓[1]	✓[2]	
Feedipedia		✓		
Foragers Source		✓		
Manufacturer's Guaranteed Analysis				✓
McCance & Widdowsons Composition of Foods	✓		✓[3]	
Peer-reviewed literature	✓	✓		
USDA Nutrient Database	✓		✓[3]	
ZOOTRITION™	✓	✓	✓	✓

[1]See NAG Technical Paper 6: Hay and pellet ratios: considerations in feeding ungulates. [2]See USDA Publication "Nutrient composition of whole vertebrate prey (excluding fish) fed in zoos". [3]Contains data for meat, fish and shellfish products as used for human consumption (e.g. fish fillets, rather than whole fish).

Food composition tables or databases (FCDBs) are resources providing detailed information on the nutritional composition of foods, usually from a particular country. Originally, these resources existed only in printed form, with the oldest tables dating back to the early 1800s. Nowadays, a trend towards electronically available FCDBs can be observed. They can hold large amounts of data and allow easy access to and manipulation of data. More recently, many European FCDBs have become available online on the Internet, a move influenced by EuroFIR within Europe.

FCDBs usually contain information on a wide range of components, including:

- Energy
- Macronutrients (e.g. protein, carbohydrate, fat) and their components (e.g. sugars, starch, fatty acids)
- Minerals (e.g. calcium, iron, sodium)
- Vitamins

In addition, some FCDBs have values for individual amino acids and/or vitamin fractions (e.g. individual carotenoids, such as lycopene and lutein).

See http://www.eurofir.net/food_information/food_composition_databases for a list of FCDBs in Europe.

In the UK the Royal Society of Chemistry publishes McCance and Widdowson's The composition of foods (2002), now in its 6th Summary Edition. This is a revised and updated set of official UK food tables, covering more than 1100 different types of food. The nutrient coverage of this edition has been extended to include selenium, manganese, iodine and fatty acids. The text also covers proximates, vitamins, inorganics and lists scientific names for foods.

The United States Department of Agriculture (USDA) are responsible for compiling equivalent food tables in North America and their Nutrient Database is available to search online. The web address is for the home page of their Agriculture Research Service Nutrient Data Laboratory, which has other useful information in addition to the Nutrient Database link. (www.nal.usda.gov/fnic/foodcomp/)

Feedipedia (www.feedipedia.org) is an open access information system on animal feed resources that provides information on nature, occurrence, chemical composition, nutritional value and safe use of nearly 1400 worldwide livestock feeds. As a world-wide compendium of up-to-date information on feed resources, it covers feeds mainly available for feeding livestock in tropical, subtropical and Mediterranean regions but also includes common feeds used in temperate countries.

Diet management software packages (e.g. Zootrition) offer a comprehensive electronic database of feedstuffs with the facility to compare nutritional content of specific food items and calculate overall nutritional composition of diets.

Features of zoo diet management software:

- Database containing thousands of feedstuffs with published nutrient values, many unique to zoo and wildlife species.

- Comprehensive library of nutrient recommendations for US domestic and zoo species. The latter are largely based on AZA Species Survival Plan and Taxon Advisory Group nutrient specifications, though recommendations for several species are from international husbandry guidelines.
- Only data from peer-reviewed sources is considered for inclusion in the ‘locked’ global database

When using either published tables or software programmes as nutrient calculation tools, be aware the final nutrient values are calculated, not measured. In all instances, missing data (indicated by a dash ‘-‘) does not indicate the nutrient is not present. It simply means the specific content for the feed was not assayed and there is no available data. Thus totals for a diet or combination of feeds should be indicated as minimum calculated values only.

- Even if published data on composition is available for feeds, the nutrients consumed may be different.

Box 12.2. Software in development:

In 2010, members of the EAZA Nutrition Group and AZA’s Nutrition Advisory Group were invited to discuss if and how we might work together to design and build the next generation software. Key questions to address were: who are the main contributors to what and how animals get fed, what information do they need to feed their animals effectively, and what can be done to optimize animal feeding in zoos? A partnership has been forged with Format International to develop and design a bespoke software application. Based in UK, Format International designs technical software for the food and feed industry worldwide and specializes in formulation software, recipe design, management and auditing. Working together through a series of meetings and workshops, our vision is a zoo diet management system for all those concerned with feeding our animals well, encompassing features associated with feeding, formulation, inventory management and auditing. The software application Fauna is undergoing testing and is due for release later in 2013.

Why feed composition might vary from published values

- The nutrient value of food can be changed by the way it is processed, cooked and stored.
- A variety of things can happen during the growing, harvesting, storage and preparing of food that can affect its nutritional content. Processes that expose foods to high levels of heat, light or oxygen cause the greatest nutrient loss.
- Food composition values for items eaten by humans and livestock are estimations based on a chemical analysis of food samples carried out in a number of analytical laboratories, with data from other sources.
- By their nature, the composition of all feeds which are cultivated (fruits, vegetables, forages and browse etc.) vary due to location grown, season cropped, cultivar or variety.
- Some vitamins are more stable (less affected by processing) than others. Water-soluble vitamins (B-group and C) are more unstable than fat-soluble vitamins (K, A, D and E) during food processing and storage.
- The composition of whole prey items (both vertebrate and invertebrate) is influenced by the diet which they were fed/reared on.

Some ingredients are not listed in databases and others, particularly supplements, may be brand-specific in composition and this information may or may not be available to you, relying on website information for brands is not reliable. You need to decide if using substitutes for some ingredients is good enough or if you will need to acquire true values by direct analysis of the ingredients.

12.4. Chemical analysis of foodstuffs and faeces

Chemical analysis of faeces and foodstuffs is a non-invasive technique because it does not involve the animal directly. Most zoos do not have the facilities to do these analyses so must rely on external collaborations. Chemical analysis of foodstuffs is important for food types which do not appear in existing databases or which might be very variable depending on region, storage, growth conditions etc. Chemical analysis of faeces allows the determination of the apparent digestibility of nutrients from the diet. It is often important to analyse the digestibility for species for which it is difficult to provide food in its natural form.

The chemical analysis of a food gives us values for the total amounts of particular nutrients that are present. Commonly, when a food is to be analyzed, it is first treated with a strong acid, or an enzyme, that breaks up complex materials into simpler, soluble products. This mimics digestion, which is essentially the breaking down of feed items – or rather their chemical constituents (carbohydrates, fats, proteins etc.) - in the body, into a form that can be absorbed by the blood stream. Digestion involves physical, chemical and enzymatic processes all along the gastrointestinal tract, and thus digestibility can be considered a measure how well this works. There may be other chemicals in food items which are not nutrients (e.g. tannins); these may be toxic or reduce the consumption or digestion of those nutrients which are present.

However, it is important to differentiate between chemical analysis of feed or faeces, and how much a digestive system will be able to extract in a form that can be absorbed into the bloodstream. The term 'digestibility' may be used to designate this, but depending on the nutrient in question, the term 'bioavailability' may be more appropriate and the final measure of bioavailability must therefore be some overall response of the subject to a test dose of the food in question. Specialist expertise may be required to assist with the interpretation of the data generated.

12.5. Dietary standards and recommendations

Having collected data on what is being fed, how much and then calculating or measuring the nutritional content, the final consideration is which standards to use for assessing the adequacy of a diet.

A preferable situation is when comparisons can be made between captive diets and data collected from animals in the wild. The majority of field studies of feeding ecology have tended to focus on the proportion of time that animals spend feeding on different food types, rather than their actual intake rates because the latter are often very difficult or impossible to obtain. In addition, it is only rarely that food samples are collected and analysed for actual nutrient content. Sterling (1993) was able to collect these data for aye-ayes, mainly because of the low dietary diversity of the species in the wild.

In the absence of data from the wild dietary standards can sometimes be developed from captive situations where the animals are healthy and breed well. Often well-studied nearest-relative domestic species are used as nutritional models for exotic species. However this is not always a good technique since taxonomic closeness does not always confer similar foraging habits and nutritional requirements.

National Academies web page *(www.nationalacademies.org)*

The National Research Council Committee on Animal Nutrition produces a series of reports on the 'Nutrient Requirements of Domestic Animals'. This includes publications on Laboratory animals, poultry, swine, cats, horses, non-human primates etc. To find out more about these publications look on the National Academies web page (www.nationalacademies.org/). Use SEARCH, and look for 'Nutrient Requirements of Domestic Animals: Report Series' in 'The National Academies Only'.

AZA Nutrition Advisory Group *(www.nagonline.net)*

AZA formed a scientific advisory group, the Nutrition Advisory Group (NAG), giving the discipline, and/or practice of nutrition appropriate recognition. The NAG facilitates improved communication and coordination among nutritionists and those requiring nutrition information (i.e., AZA management groups, AZA member institutions). The website is excellent, featuring information and papers freely available to download for all. Nutrition Chapters from published Husbandry Manuals: many Taxon Advisory Groups (TAGs) or Species Survival Plans (SSPs) publish manuals of information on their species of interest. The website lists those that have provided information to the NAG about their husbandry manuals. Links allow the user to view or download the chapter for use. NAG also produce an ongoing series of technical papers:

1 - Hay Quality Evaluation
2 - Vitamin D and Ultraviolet Radiation: meeting lighting needs for captive animals
3 - Feeding captive insectivorous animals: nutritional aspects of insects as food.
4 - Elephants: nutrition and dietary husbandry
5 - Feeding captive piscivorous animals: nutritional aspects of fish as food.
6 - Hay and pellet ratios: considerations in feeding ungulates
7 - Leaf-eating primates: nutrition and dietary husbandry
8 - Assessment of nutritional status of captive and free-ranging animals
9 - Micronesian kingfishers: nutrition and dietary husbandry
10 - Quality control of feedstuffs: nutrient analyses
11 - Asian small-clawed otters: nutrition and dietary husbandry
12 - Penguins: Nutrition and Dietary Husbandry
13 - Callitrichids: Nutrition and Dietary Husbandry

EAZA Nutrition Group *(www.eaza.net)*

EAZA is the European Association of Zoos and Aquariums and also has a Nutrition Group (ENG), with an online library of materials located on the main EAZA website. Fewer Husbandry Guidelines have been written nonetheless materials from numerous Nutrition Conferences are freely available to download.

12.6. Diet implementation and monitoring

This chapter has so far focused on diet evaluation in terms of nutrient quality. However, devising a diet of optimum nutrient content is of little benefit if the animals do not eat it and thus presentation and palatability are also important considerations. They must also be monitored to ensure that all individuals have equal, or near equal, access to food resources, and excessive selection of preferred items or monopolisation of food does not occur. Finally, since finding and consuming food may occupy a substantial proportion of daily activity in the wild, simply providing a good diet, well distributed to ensure equal access to all, may still not be sufficient if the animals consume their daily energy intake in an extremely short time period. Thus behavioural and environmental enrichment is an extremely important component of providing captive exotics with appropriate nutrition.

Monitoring effects of diet changes

- **Weight and body condition scoring (BCS)**

  The ability to regularly weigh animals without causing stress is a huge advantage when monitoring diet effectiveness. Many animals can be trained to position themselves on weighing scales or scales can be built into sections of the enclosure where animals have to pass frequently eg tunnels between indoor and outdoor sections. If these are not possible an alternative to regular weighing is body condition scoring. Body condition scoring is based on palpation and/or visual examination of the fat and muscle over various body parts, rating the body condition of an animal against a standard scale usually ranging from emaciated to obese. When an animal is losing condition, the fat reserves are mobilized and muscle wasting sets in to supply the required energy demand. Body scores have been used in the domestic hoofed stock industry for many years. In recent years body condition scores have been used in a wide variety of captive animals, as a means to improve animal care excellence. When assessing an animal's body condition, several factors should be taken into consideration. Among these are the animal's body weight, frame size, general health status, and body condition score. For example, two different animals of the same species may have different body weights and frame sizes, but have the same body condition score (i.e., same degree of thinness or fatness). If a reliable standardised method is used BCS can actually be a better way of monitoring diet efficacy than body weight alone as it more easily allows for difference in animal size , e.g. the nutritional concerns of an adult man weighing 80kg would be very different depending on whether he was 165cm tall or 200cm tall. Standardised BCS ratings have been developed for an increasing number of species using information from the wild. Important points to consider are:

  - BCS is best is done by a consistent small team rather than single person, especially a different person, on each scoring occasion.
  - Standardised scales need to be agreed and tested for repeatability, vets will often have existing scales for many animals.
  - Scoring should be done regularly and photographs each time can be a big help
  - Descriptions and pictures of free-living conspecifics can be used for comparison and often provide a better indication of "normal" than just using captive animals
  - Long-term monitoring is needed, including pre- and post- diet change as changes in body condition may be slow
  - BCS can be more difficult when the silhouette, shape of the animal and visibility of skeletal features are masked, with thick fur, winter pelt or feathers but scoring systems are being developed for a much wider range of animals

  - Body condition will vary with lifestage (e.g. pregnancy and lactation) and variation according to season (winter/summer) may also be observed, so collecting data over time may be necessary to determine 'normal' or expected fluctuations

- **Health indicators**

  - Several indicators that are relatively easy to observe or obtain samples for tests can be used to assess health and any changes with respect to diet change
  - Coat, feather, shell (eg terrestrial molluscs) or skin condition
  - Faecal consistency
  - Teeth condition
  - Growth rate
  - Size of adults (especially invertebrates eg Fregate beetles)
  - Fecundity (again especially invertebrates eg Partula snails)
  - Fungus condition/growth in leaf-cutter ants
  - Heat detectors and infra-red cameras can give non-invasive indicators of condition insome species
  - Urine tests for dietary analysis (eg ketones)

  More invasive veterinary checks may also be necessary in some cases and of course veterinary records and PM reports can be used to investigate many factors related to diet quality.

- **Behavioural indicators**

  Behaviour can be affected by diet content and also scheduling and presentation methods and it is useful to collect pre and post diet change behavioural data to monitor this. For instance higher protein and/or sugar levels in diets may lead to higher levels of aggression in groups but other behaviour changes such as monopolisation of preferred items, lethargy (common if overfed), foraging and feeding time, use of species specific feeding behaviour, feeding anticipation may also be important to monitor.

- **Visitor effects**

  Visitors can have significant effects on dietary intake in several ways such as deliberate or accidental feeding of animals or through scheduled public experiences and feeds. Planned public feeding should be monitored and controlled and the keeper-provisioned diet reduced accordingly. Sometimes visitor perceptions of feeding, such as the use of whole carcasses, may influence the diet and it may be important to involve the education/interpretation team to prevent this.

- **Keeper effects**

  Unless a zoo has a central kitchen preparing all feeds every day it is keepers who actually have most influence over what food is provided to the animals. There are many reasons why this may not always be exactly what it says on the diet sheet.

  - Keeper drift is a very common phenomenon – keepers may deviate slightly each day from the diet sheet for many reasons, such as an ingredient isn't available so they replace it with something similar. This is usually not a problem unless it leads to a permanent change or if the "similar" food item is not in fact similar. Ongoing change most often happens with quantities; unless keepers weigh ingredients every day or use measured scoops/cups there is a tendency to gradually increase the amounts provided over time leading to overfeeding and ultimately obesity.

- Changes to quantities of feed given to groups to reflect group size or life stage changes may not always be calculated correctly or at the right time
- Keepers can make 'knee jerk' reactions to new information without proper evaluation or more commonly do not include reliable new information from publications or TAGs into diets
- Keeper perceptions of what animals like or need can sometimes lead to well-meant but incorrect deviations from the diet sheet. This may be related to advice on human nutrition such as "5 a day" or the need to reduce salt and fat intake.
- Keepers must ensure that enrichment and training foods are included in the overall diet formulation and not additional to it. These foods should also be made as appropriate as possible whilst still maintaining the animals' motivation to take part in training.

Documentation

The importance of maintaining good written accounts of all diet changes and any effects (health, behavioural etc.) of these cannot be over emphasised.

There is a gap within the current suite of software provided by International Species Information System (ISIS); the facility to record diet notes is currently available within both the Animal Records Keeping System (ARKS) and its successor, the Zoological Information Management System (ZIMS). However as a free text box, it can completed with varying attention to detail. Bespoke software designed for the zoo community to help with diet evaluation could also ensure diet information is stored in a rigorous, standardised format, and be used for diet formulation, permitting the exchange of true 'diet' data – the nutrients that are being offered and consumed in specific quantities, not just a list of the food ingredients involved

Descriptions of feeding practice are required for legislative and accreditation purposes. In the UK, the Secretary of State's Standards for Modern Zoo Practice (SSSMZP) are designed to ensure that the welfare of animals in zoos is protected, that zoos are safe places for the public to visit and that zoos participate in appropriate conservation and public education measures. The Standards recommend best practice by which zoos are inspected and granted licenses by local authorities. Under Section 2 'Provision of Food & Water' there are 13 statements pertaining to feeding, including hygienic aspects of food provision, opportunity for natural behaviours, feeding methods must safe for animals and staff, legal aspects of feeding live prey and specialist advice in all aspects of nutrition must obtained and followed.

Feeding practice might include **diet sheets**, instructions on what and how to feed each species / specimens and **diet records** i.e. descriptions of what was actually offered and consumed.

Recording and cataloguing feeding practices and the outcome of adjustments, along with the means for systematic retrieval of said records at some later point in time, would represent a significant advance in the evaluation and dissemination of effective feeding practice. Equally reporting these in publications or at conferences and workshops etc. is also vital. Sharing knowledge about best feeding practice can improve global management of living collections, with anecdotal descriptions replaced by meticulous observation, documentation and analysis.

12.7. Further reading and other resources

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www.feedipedia.org (Feedipedia: An on-line encyclopedia of animal feeds)
www.foragerssource.org (The Foragers Source)

www.eurofir.net/eurofir_knowledge/european_databases (Food composition databases)

Appendix I. Conducting an Intake Study

Ideally the study involves 5 consecutive days of measuring individual diet components both offered and remaining. To approximate a normal diet schedule, collection during the 5 day study period should ideally be performed by the primary keepers for the animals.

- All feed items must be weighed individually before feeding.
- Items may be mixed after they have been weighed.
- A desiccation dish must be set up separately from the main feed, to measure the amount of water lost to the air. This dish should be placed in an area as similar as possible to the exhibit being evaluated but with no animal access.
- All items must be separated after animals have been fed. Each item must again be weighed separately and recorded. If this is not possible due to items being mixed into a mixed 'porridge' the total amount remaining should be weighed and the amounts of each food type remaining estimated from the relative amounts used to make the mixture.
- Remaining food amounts need to be adjusted using desiccation factors measured from the desiccation dish.

Recording Feed Intake

- Prepare a list of feeds that are included in each animal's diet and note the number of animals in the exhibit.
- Locate and become familiar with the scale that will be used.
- Weigh each item of the diet separately.
- Record individual feed weights for each dish, or the total if the food is being scattered. Be sure to note any supplements. If an in-house mix of ingredients is prepared, the components and recipe will be necessary for full evaluation. Also note ingredient types, brands, and any other specific information available.
- Feed animal.
- After "normal" amount of feeding time (i.e., animal is fed in AM and PM), retrieve feed dishes and/or collect all food items remaining in the exhibit.
- Separate all feed items from each other.
- Weigh each item individually (including discarded items) and record on intake sheet.
- Continue this procedure for 5 consecutive days.

Desiccation/Absorption Dish

The purpose is to estimate the amount of moisture lost to (or gained from) the environment of the exhibit while the food sits out. Select a site for this dish that is as similar to the exhibit being measured as possible. Try to select an area that will minimize the amount of loss to rodents and insects.

- Weigh all items into the dish and then leave it out for the same amount of time as the main diet.
- When feed dishes are collected, the desiccation dish can also be evaluated.
- Follow the same procedure as above for measurements; record data on a daily desiccation record sheet.

Calculations

Sample calculations are for one item only. It will be necessary to repeat for each ingredient, on each day of the trial. Numbers in **BOLD** are measured, all others are calculated using the instructions below:

	A	B	C	D	E	F	G	H
All wts in grams	*Offered*	*Remains*	*Desiccation Dish* *Start*	*End*	*Difference*	*Correction Factor (as %)*	*Corrected Remains*	*Eaten*
APPLE	**821**	**54**	**200**	**190**	10	0.05	57	764

- Calculate desiccation *correction factor* (F) by first calculating the difference in weight [C-D = E] in the dessication dish, then expressing this as a percentage of the initial weight [E/C = F]. In the example above, the correction factor would be reported as 5%, but for calculations in the spreadsheet is expressed as a fraction (0.05).
- Apply correction factor to feed remains then add to feed remains to calculate *corrected remains* [(BxF)+B = G].
- Use corrected remains to calculate how much was *eaten* [A-G = H]. Since original measurements were in whole numbers, it is appropriate to round values accordingly.
- Use columns A and H to describe diets in terms of food quantities offered and eaten. Estimate nutrients offered and consumed using food tables or diet management software.

Suggested formats for data recording sheets…

Data recording sheet for intake trial

Intake Trial No.:	*Day:*		*Date:*		*Keeper:*	
Times of food presentation:	*AM:*	*PM:*	*/collection*	*AM:*	*PM:*	
	Dish					
Food Item	1	2	3	4	5	6
Total Weight						

Data recording sheet for correcting wet weight remains from desiccation dish

Food Item	A	B	Water Loss C	Water Loss %

All weights are in grams, unless stated otherwise

A - fresh weight of food given at feeding time

B - wet weight of food remains at time of collecting food

C - weight difference (A - B)

% - Percentage (C / A)*100

Appendix II

FEEDING PROGRAMME at YOUR ZOO

1. Approximately how long has the diet described been in use?

....

2. Have animals been well maintained while on this diet? (e.g. have the animals bred? Any recurring health problems that maybe diet-related? Any concerns for species in general even if not observed in the collection here? Give brief details).

....

....

3. Instructions for food preparation (e.g. is fruit chopped (finely/coarsely?) first thing or done the night before and stored in fridge)

....

....

4. Are diets being eaten by pest birds, rodents and insects? (Give indication of how much might be eaten by pests and whether a significant problem?)

....

....

5. Are there opportunities for 'paddock' feeding? (Do the animals consume plants/insects in their enclosure which are not fed directly?)

....

....

6. Are amounts altered on a seasonal basis? (Brief description changes for breeding diets; conditioning diets; diets for lactating females; medical considerations etc.)

....

....

7. Notes and Remarks: (Include such data as how food is presented; how well food is accepted; which are 'favourite' items; is there a 'pecking order' for access to food? Any other comments that don't apply elsewhere)

....

....

8. Reference Material (Details of most important references relevant to wild or captive diets, including international husbandry manuals if used)

....

....

9. If the diet is the same on daily basis indicate below using D ; if some items are not fed daily please complete for full week. Continue on separate sheets if necessary

How many animals are fed from the diet described below? (males.females.juveniles).

	Weight of feed item offered (grams)* and time of day (e.g. am/lunch/pm)						
Feed Item**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**

*** Please provide weights (grams) wherever possible. If not possible please use units e.g. 3 carrots, 4 mice, 2 bales**

**** For any manufactured feeds please provide exact manufacturer and product names**

Chapter 13

Aquarium Based Research

Editors: Deborah Cracknell, Graham Hill

Contributing authors: Graham Hill, Ashley Sharp

Just like zoos, aquariums provide researchers with opportunities to access a vast range of species that they could not maintain in experimental cultures or feasibly study in the wild. Many of the methods and principles of aquarium-based research are the same as those for zoo-based research and readers should refer to other relevant chapters of this handbook. However, due to the nature of many aquatic exhibits, e.g. very large numbers of individuals and multiple taxa in each tank, they provide a unique set of challenges and opportunities that do not occur in most terrestrial exhibits.

This chapter is designed to help researchers understand the aquarium-specific challenges and opportunities, and encourage more research into under-represented areas in aquarium husbandry and knowledge.

13.1. Identifying individual animals for research

Aquariums often house many individuals of the same species, often within a single exhibit. It is important to be able to identify individuals for much experimental research, including behavioural, genetic and life history studies, and also for husbandry record keeping, tracking parentage and/or origin, managing studbooks and maximising genetic variation.

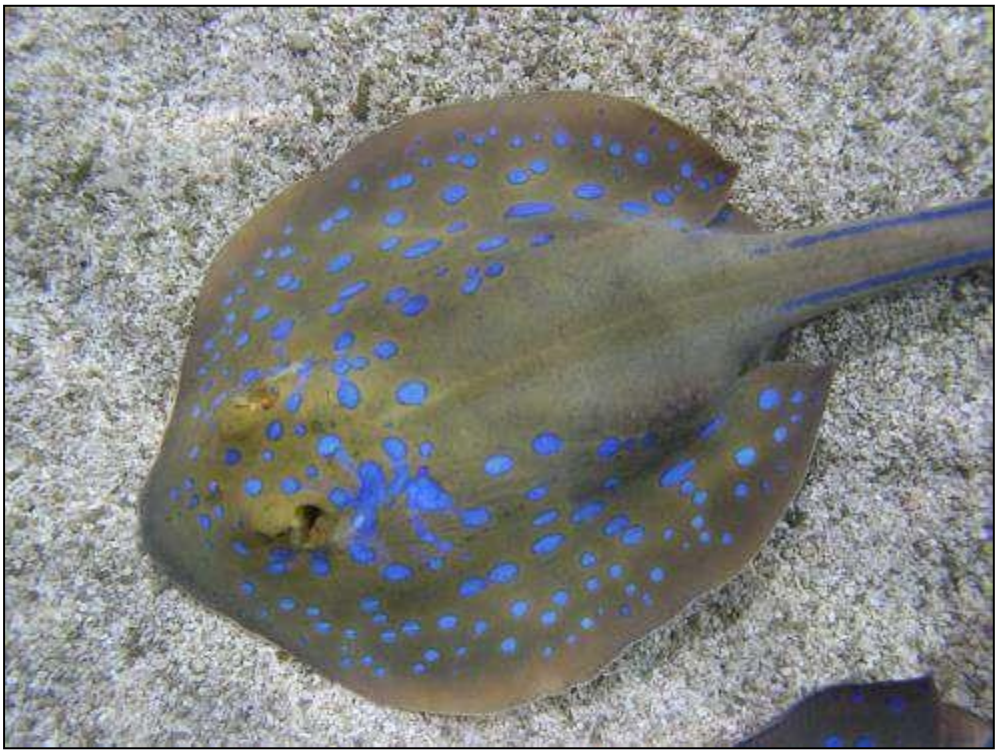
Identifying individuals in aquarium exhibits can often be very difficult, both for vertebrate and invertebrate species, as morphological differences between individuals are often very small. Even keeping track of one individual within a single observation session can be extremely challenging. Fish pose some particular difficulties as they move in a three dimensional medium (in contrast to many terrestrial animals) often in large shoals. Tracking individuals within a shoal is difficult because a shoal works on the principle of all individuals being morphologically similar to avoid being singled out by predators. Equally, coordinated movements of a shoal make tracking difficult.

13.1.1 Other challenges for identification

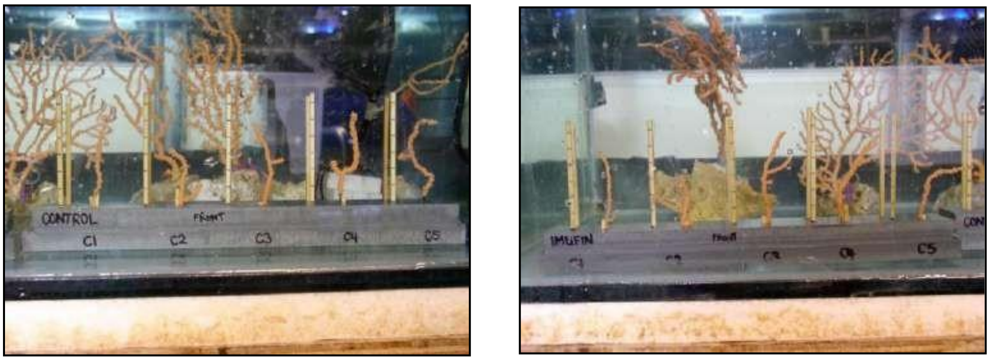
- Many species do not have obvious morphological sex differences. However, behavioural differences may help to discriminate between genders (e.g. males chasing females, displaying aggression etc.)
- Individuals can change colour over time (e.g. seahorses)
- The complex, three dimensional envelope of an aquarium often leads to individuals hiding
- Nocturnal species hide during the day
- Many reef fish live within the reef structure itself, retreating into structure if threatened
- Cryptic species are difficult to spot!
- Sessile invertebrates present a different set of challenges
- Exposure to different environmental parameters may lead to plasticity of appearance (e.g. photosynthetic corals change colour depending on light intensity)
- Growth forms of many species change over time
- Encrusting growth forms may cover identifying tags or marks etc
- Even deciding what is an individual can be difficult for some invertebrates (e.g. corals). Asexual reproduction through either deliberate ‘fragging’ or budding of new colonies from parent is common
- Pelagic larval phases lead to settling of daughter colonies (i.e. some brooding species of coral)
- Identification needs to follow daughter colonies when removed from the parent colony

13.1.2 Practical ways to differentiate between individuals

- Regular photographic record keeping may help track changes over time and help to track individuals as they change over time
- Tagging allows easy identification and is commonly used for larger animals, such as elasmobranchs, but there are limitations:
    - Concern that tags/identifiers may be visually obtrusive to display animals
    - Tags may be large in relation to animal (e.g. PIT tags are too large for small fish)
- If done purely for research purposes there may be Home Office licence issues with tagging procedures (see Chapter 2). This should be checked before carrying out any marking
- Tags/marks may not last long enough in water, salt water is a particularly aggressive medium and the glue for attaching tags fails after a while. This may not be a problem if marking is only required for a relatively short term study period
- Tags may damage animals, in particular corals are sensitive to tissue damage and seahorse mortality is increased with elastomer marking
- Fin clipping has been traditionally used but may also have welfare issues and fins may regrow over the clip



Bluespotted ribbontail stingray (Taeniura lymma) – Unique spot pattern identification BSR002f, The Deep – Graham Hill



Setup and labelling of pink sea fan fragments in growth experiments. The Deep - Kimberley Grundy (student, Hull University)

- Tagging involving removal of mucus layer of fish risks infection and there can be handling stress when carrying out any form of marking
- Visual tracking software is available for tracking movement of individuals which can help with following one individual within a session, especially for large bodied animals. However:
    - It is still difficult within large groups
    - It requires a relatively large expense initially to purchase equipment
    - If the individual goes out of sight the system may not correctly identify it on reappearance
- Keeper/aquarist knowledge
- Keepers may sometimes be aware of small identifying characteristics or can identify individuals based on behavioural differences.

PIT tag implant of Atlantic big-eye (Priacanthus arenatus), The Deep – Graham Hill

13.2. Behavioural research in multi-taxa aquatic exhibits

Most aquarium exhibits are multi-species assemblages, sometimes based around a particular biotype or habitat. Whilst this makes for some exciting display possibilities and opportunities for interesting research, designing experiments to assess the behaviour of individuals or cohorts within this environment can be difficult.

13.2.1. Opportunities for behavioural research

Multi-species aquarium exhibits present many opportunities for basic and applied behavioural research. There are several husbandry issues that would benefit from better information about how the different species behave but there are also many more fundamental questions about species relationships and comparative behaviour that can be done much more easily in an aquarium than in the wild.

Husbandry related research areas:

1. Distribution of species in the exhibit, use of space, effects of different times of day or husbandry routines e.g. do they predict feeding events? (Example: Effects of predictability of feeding schedule on the behaviour of *Monodactylus argenteus* in a public aquarium, Major & Gee, 2011, UFAW scholarship).

2. Intra- and inter-species interactions are clearly important and baseline data on when and where these happen are fundamental to most other research questions. It is likely these should centre around competitive points e.g. food, nest sites etc. It should be remembered in any research that intra-specific interactions, especially aggression, may be just as significant as inter-specific (Example:

Passive control of fish to fish aggression by alteration of the algae feeding regime in the Coral Lagoon at The Deep: Gilbert, 2006, Honours project).

3. Although single species aquarium exhibits are rare there may be opportunities to compare behaviour of the same species in single species and multi-species exhibits e.g. in quarantine tanks.

4. Research into how to carry out introductions of new individuals and species would be very useful e.g. the effects of time of day, method (small cages within tank etc.), age at introduction, or disrupting territories of established fish before introduction etc. (Example: The introduction of bluestreak cleaner wrasse (*Labroides dimiatus)* to an aquarium display. Harrison, 2008, Honours project).

5. Compatibility studies of various species would also be very beneficial, ultimately leading to development of predictive models that use life history, ecology and maybe niche separation to predict whether certain species are likely to be compatible, and what husbandry methods can be used to ensure they are. It should be noted that it is not usually possible to intentionally introduce animals in order to look at compatibility, but there are avenues to look at experiences already observed; for example, behavioural observations, or desktop study of records using data from multiple aquariums. The complexity of interactions and combination of species kept together varies between facilities, and the lack of definitions for those interactions/behaviours can make such study very difficult. More work is needed to define and interpret behaviours in fish.

6. Feeding each species appropriately in a mixed exhibit is difficult as they will all have access to food meant for other species. For instance many herbivorous fish actually prefer to eat the animal based food provided for carnivorous fish leading to serious health problems. Investigations into feeding methods (e.g. washing powder bags), location and timing of feeds to encourage each species to only eat the food that is meant for that species are needed (Examples: Food preferences in the bonnethead shark, *Sphyrna tiburo*, the blue-spotted ribbontail stingray, *Taeniura lymma*, and the bluespot stingray, *Dasyatis kuhlii.* Do prey sex pheromones attract predators? Wilcox 2008, Honours project; Food preferences, olfactory attractants and foraging behaviour of the blue-spotted ribbontail ray *Taeniura lymma* (Forsskål, 1775) and the blue-spotted stingray *Dasyatis kuhlii* (Müller and Henle, 1841) Wilcox, 2009, MSc. Project.

7. Research into visitor effects is likely to prove interesting, there are likely to be species differences in reactions and sensitivity. It is not clear what measures would be taken but changes in space use may be a good indicator.

8. Assessing fish welfare through behaviour is not obviously as feasible as it might be for many mammals or birds. However, there is quite a large body of academic research into assessing stress in fish by chemical methods which can be done by sampling tank water. Seawater chemistry very often interferes with standard analytical methods for water sample analysis. Therefore methodology development, rigorous standards testing, confirmation of detection without interferences etc. make this a very expensive type of project, but it none-the-less provides unique opportunities for study. Ideally, behaviour and chemistry should be monitored before and after any significant husbandry event or change. Over time and through collaboration of several aquariums we may be able to establish reliable behavioural indicators and criteria for optimal fish species welfare.

Basic behavioural and psychological research:

1. Many aquarium exhibits are created with natural species assemblages that would be found together in the wild. Basic investigations into space use in an exhibit can provide useful information about whether some species actively avoid each other, tolerate each other or actively maintain close contact with each other. This may indicate natural relationships found in the wild.

2. Inter-species interactions in such tanks may also give us information on competitive or mutualistic interactions that would be much harder to investigate in the wild (Example: Torus formation in horse eye jacks (*Caranx latus*). Sidoli, 2007, Honours project).

3. Even in exhibits which are not natural species assemblages research into inter-species interactions would be interesting e.g. in investigating whether some mutualisms can develop even between species that would not normally meet, some species becoming 'ecological surrogates' perhaps.

4. Multi-species exhibits provide an ideal opportunity to compare learning and cognition across species e.g. discrimination tests, social learning etc. This may have applied uses too. For instance, some juvenile angel fish learn different behaviour depending on which other species they are reared with (Gómez-Laplaza, 2009). This could be used to manipulate behaviour as adults to make them more compatible with others (Example: Positive feedback conditioning to visual targets with the grey reef shark *Carcharhinus amblyrhynchos* - A technique to segregate species for target feeding, The Deep, 2005). This has been instrumental in ensuring regular feeding, vitamin supplementation and/or treatments are maintained.

5. Many fish occupy vastly different habitats at different stages of their life cycle and, for instance, as adults do not even encounter juveniles of their own species. In aquariums they are mostly maintained in the same exhibit throughout their lives allowing investigation of the effects of age on behaviour and interactions e.g. at what age do 'normal' intra- and inter-specific relationships start, does this vary depending on other species in the exhibit etc.?

13.2.1. Challenges of behavioural research

Many of the problems which are likely to be encountered by behavioural researchers in multi-species aquarium exhibits are similar to those in other situations and there are good frameworks that can be adopted. For instance, in the agricultural sector, as well as zoos, many of the principles of introducing two groups of the same species together will be similar to introducing two different fish species to each other, and it won't be necessary to invent methods from scratch. Some of the main challenges likely to arise are:

1. When attempting research into environmental enrichment or the effects of training on one species, interference from another species may make it hard to assess. One species may completely dominate any item or training session meaning it is impossible to assess the effect on another species. This may be partially overcome by using multiple enrichment items or perhaps different times of day if peak activity of target species differs.

2. If it is not possible to distinguish individuals (see Section 13.1) there will be difficulty ensuring adequate sampling of the whole group, especially for species that do not shoal. Ensuring that all individuals are more or less equally included in the sampling can be achieved by using more sessions

spread throughout the possible observation times in a day, multiple observation points and good baseline knowledge of the overall space use of the species in the exhibit.

3. Assessing space use is slightly more difficult because of the vertical stratification. However, 3D zoning of an enclosure is routinely done for flying and climbing mammals and can be carried out for aquarium exhibit studies (Example: The behaviour of sand tiger sharks, *Carcharias taurus,* in captive environments, Harris, 2002, MSc. project).

4. Many aquarium exhibits are very large and will have a lot of out of sight areas. Again, good background data on the typical amounts of time a species is in view, many sessions spread over the day and lots of viewpoints will help minimise this.

5. There is less general understanding of fish behaviour than perhaps mammals or birds so it may be difficult to assess what a behavioural change actually means. Extensive literature research and precise documentation of accompanying events will help to overcome this (Example: Deep meaningful conversations: Sound production and associated behaviour in a species of damselfish *Dascyllus aruanus* (Pomacentridae). Britton, 2009, MSc.).

The need for baseline data as a starting point for any research cannot be over-emphasised – this includes distribution and space use of species in exhibits, interactions around main sources of competition (e.g. food sites etc.) and responses to husbandry events and changes.

13.3. Experimental design for life history analysis

Much of the life history data for many of the species maintained within aquatic collections is either missing or poorly understood. Additionally, much of what is known is anecdotal and is based around isolated reports from single individuals. This is especially true of species not commercially important either as food or for the ornamental trade. Consequently much of the basic knowledge and understanding of aspects such as social structure, spawning cues, age at maturation etc. is lacking.

Aquarium-based research can play an important role in gathering missing life history data from animals held within their collections. This is especially important for the many species held in collections that are vulnerable to extinction (e.g. Charco Palma pupfish) or are extinct in the wild. Learning about these species requires an experimental approach to inform breeding protocols and husbandry regimes and may also provide valuable information on restricted ranges (e.g. *Cyprinodon longidorsalis*), and data to prevent overexploitation. However, often we have small population sizes within aquaria and so it is difficult to extract meaningful data, particularly without risking the wellbeing of the population.

13.3.1 Life history data - an experimental approach

Obtaining meaningful data is often difficult as individuals collected for public aquaria are often of unknown origin. It is crucial to obtain as much information as possible on the provenance of animals entering collection both for management and research purposes.

Keeping small groups, same sex groups or even single individuals of large species (e.g. sharks, rays etc) in aquaria mean that life history data is difficult or impossible to gather. However, there are opportunities for institutions to collaborate to exchange animals to increase group size (e.g. blue spot stingray and angel sharks) and in some cases small group size is not necessarily a problem:

- Many species form pairs, or are territorial and will not tolerate conspecifics except to mate
- Individuals within a small group may be easier to identify, sex, and make behavioural observations
- Small groups provide opportunities for small scale husbandry studies with focused aims (ideal for students).

For many species so little information is available on their life history that it is impossible to know where to start. However, more may be known about similar species and this may provide a starting point for the study species. Basic information that should be relatively easy to acquire through accurate record keeping and data collection includes:

- Morphological sex differences (e.g. live bearers often have obvious external sex organs)
- Predominant mating systems
- Brooding strategies
- Characteristics of young (e.g. precocious, long planktonic stage etc.)
- Signs of display, aggression, courtship, nesting etc.
- Females becoming obviously gravid

Once these basic observations have been obtained they can be used for more rigorous hypothesis generation and testing.

It should be remembered when studying life history traits in captive environments that the environment may affect those traits. This could be via:

- Lack of seasonality (e.g. constant temperature, lighting photoperiod, food availability)
- Lack of opportunity to make habitat choices (e.g. unsuitable substrates for spawning, migratory restrictions)
- Lack of mate choice
- Inappropriate age structure of the population
- Chronic stressors affecting behaviour

To evaluate whether any of these factors are causing significant effects on life history it is vital to gain as much background information on wild ecology (e.g. spawning cues, habitat preferences etc.) of the study subjects or very similar species. Also, it is important to find any information on whether similar species are known to be affected by captivity in particular ways. This could help to identify possible husbandry techniques e.g. testing if a sudden temperature change will trigger spawning.

Basic life history research offers a huge potential for projects and even small scale projects are valuable for gathering basic information. Aquarium collections have a crucial role to play in creating refuges for endangered species. Life history research underpins the success of future rescue operations.

13.4. Experimental design for multi-facility research

Aquarium-based research offers a great opportunity for collaboration between institutions as many of the species held can be reproduced, distributed and housed in identical conditions across multiple sites. However, with these opportunities also come pitfalls such as ensuring that experimental design is consistent, husbandry regimes are identical and that support staff and aquarists are well briefed on the experimental protocol.

There are different kinds of multi-zoo study (see also Section 1.4):

- Collaborative working on the same species facilitating larger sample size
- Each institution looking at different aspect of a species biology and compiling results
- Each institution using a common methodology to investigate similar species

Although it is always beneficial to replicate research across institutions wherever possible it is important to:

- Ensure consistent methodology at each site
- Ensure that all environmental parameters (temperature, light etc.) are measured and regularly monitored
- Maintain detailed records of all events occurring in the study period
- Conduct inter-observer reliability tests if multiple observers are collecting data
- Examples:
  a) Biology and husbandry of *Eunicella verrucosa* final project report for Natural England (2004-2008), Jones *et al.* (2008).
  b) Coral Zoo – a European led programme over 5 years with a mix of academic, commercial and aquarium institutions on a very large scale.

Potential problems arise because no two exhibits are identical in terms of the physical or chemical environment e.g. lighting, water chemistry, diet, tank mates, feeding regimes and visitor effects (numbers, noisiness, flash photography etc). Off-show facilities can offer a more controllable environment but even in these situations it is important to regularly monitor all these variables. Variation in all these factors means that it is difficult to identify an effect of an experimental treatment due to variation in data caused by these other differences. Analyses can be performed on relative changes between treatments to minimise the effect of this variation.

13.5. Research to inform genetic programme management

In contrast to other vertebrate species, which often have well managed national and international collection plans and management strategies, most of the animals held within aquatic collections are managed on a rather more *ad hoc* basis. The process of collaboration between institutions to manage the genetic diversity of their collections is in its infancy for many species. This process is particularly important for endangered species where founder populations are often small and few collections maintain the species. However, good genetic management is important in maintaining a healthy population for all species in captivity. Some species are currently not restricted and new stock is easily obtainable but may become more difficult in the future. In view of this, all species need to be well managed so we do not become reliant on a small founder population.

Potential researchers in this area should bear in mind that the current level of understanding of many aquatic species is behind that of mammals and other taxonomic groups on such things as social structures, breeding strategies and optimal group sizes. Also, record keeping is often not as standardised as within most zoos. Most aquariums are not using ARKs (or ZIMS) so there may only be minimal information available about individuals or groups of animals.

Despite these difficulties there are good opportunities for student/small scale research projects to assist in this area. Compiling census information for many species is a huge undertaking even just in the UK and could be combined with research projects. Given the paucity of current knowledge it is vital to disseminate any information gained and there are many species badly in need of species champions to push forward understanding and improve management.

13.5.1 Research questions of importance to genetic management

1. Why maintain genetic diversity?

- Does it increase disease resistance, general health etc.?
- What is a suitable size of founder population?

2. What taxa are most important for genetic management?

- Species particularly susceptible to inbreeding problems
- Endangered species, species which are likely to become endangered - what are these (conservation status research)?

3. What problems arise from genetic drift or artificial selection in captivity?

- There is evidence from Lake Victorian cichlids that suggests rapid phenotypic adaption occurs in captivity (Fiumera *et al.*, 2001). This can result in phenotypic differences from wild and captive types. Is this caused by genotypic change or is it plasticity?
- Are individuals losing predator avoidance strategies in a predator-free environment?
- What other phenotypic and genotypic characters are affected?
- Are there significant domestication issues as have been found in many zoo animals? For example: skull morphology in tigers (e.g. Duckler, 1998), captive diet/food presentation preferences and adaptations

4. Issues of parenthood

- Difficult to trace parenthood in large populations – can we do non-invasive genetics to determine pedigrees? Do we need to?
- Depending on breeding systems, all or only some individuals may be reproducing. What are the effective population sizes? These may be very small relative to the actual population size.

5. Breeding strategies

- What are the implications in adding new individuals to a group?
- Aggression/mortality/reduction in breeding success?

6. Founder population choices and problems

- Should we take individuals from the wild from a single location, or varied populations?
- Are there taxonomic uncertainties that need to be resolved before we can being a captive breeding programme?
- In many instances there is insufficient time to make an informed choice. What strategy should we adopt until questions have been answered?

7. Factors to consider when exchanging animals between collections

- How can we ensure social structure remains intact?
- Can we accurately determine genetic history of individuals – are they related, what is the provenance (these are often unknown, especially when acquiring animals from a dealer)?

13.6. Recommended reading:

Anderson, R.C. & Wood, J.B. (2001) Enrichment for Giant Pacific Octopuses: Happy as a Clam? *Journal of Applied Animal Welfare Science*, 4 (2) pp. 157-168.

Bentivenga, F., Costello, J.H. & D'Ambra, I. (2001) An example of collaborative research at the Naples Aquarium: feeding mechanisms of the scyphomedusa (*Phyllorhiza punctata* Ledenfeld, 1884). *Bulletin de l'Institut Océanographique*, 20.

Brown, M.R., Crear, B.J., Dunstan, G.A., Kolkovski, S., Nelson, M.A., Nichols, P.D., Ritar, A.J., Smith, E.G. & Thomas, C.W. (2004) Nutritional and bacterial profiles of juvenile *Artemia* fed different enrichments and during starvation. *Aquaculture*, 239, pp. 351 – 373.

Dhont, J. and Van Stappen, G. (2003). Biology, Tank Production and Nutritional Value of Artemia. In *Live Feeds in Marine Aquaculture*: 104-105. StØttrup, J.G. & McEvoy, L.A. (Eds.) Oxford: Blackwell Publishing.

Faleiro, F., Narciso, L., Vicente, L. (2008) Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? *Aquaculture.* 282, pp. 33-40.

Lague, M. & Reebs, S.G. (2000) Food-anticipatory activity of groups of golden shiners during both day and night. *Canadian Journal of Zoology*, 78, pp. 886-889.

Lin, Q. Lin, J. & Zhang, D. (2008) Breeding and juvenile culture of the lined seahorse, *Hippocampus erectus* Perry, 1810. *Aquaculture*, 277, pp. 287-292.

Lin, Q. Zhang, D. & Lin, J. (2009) Effects of light intensity, stocking density, feeding frequency and salinity on the growth of sub-adult seahorses *Hippocampus erectus* Perry, 1810. *Aquaculture*, 292, pp. 111-116

Chapter 14

Ecological Research Methods for Native Species on Zoo Sites

Editors: Tracey Hamston and Amy Plowman

Contributing authors: Jen Nightingale, Rebecca Perry, Katie Macdonald, Sarah Bird

Zoo and aquarium grounds are often wild oases in largely urban or intensive agricultural environments so can be important refuges for native species. Many BIAZA member sites are home to locally, nationally or even globally rare species. See the BIAZA web pages http://www.biaza.org.uk/campaigns/bioblitz-2013/ and click on Native and Introduced Fauna found Wild on BIAZA Collection Sites to download a species list from member sites.

Considerate management of zoo grounds can therefore make significant contributions to the conservation of native species; provide opportunities to enhance the visitor experience and to engage the public with local conservation. Zoos can also contribute to national databases for many species and increase UK wide understanding of species distributions and how these may be changing. In order to do these things it is vital to know what species are on the site, where they are and what impact management decisions may have on them.

This chapter is intended for people with little or no experience of ecology. It introduces basic ecological techniques that can be used to document the occurrence of native species on zoo sites and monitor their populations in a way that is useful for management and wider conservation networks. It does not attempt to duplicate the extensive literature on ecological methods but we do point to sources of further information and help where appropriate. Key texts and case studies are enclosed in a text box and full references are at the end of the chapter.

14.1. Introduction

14.1.1. Why do native species research?

There are many reasons for and potential benefits of conducting research on native species in zoo grounds

Figure 14.1: Alison Smith, Community Scientist, OPAL, at Paignton Zoo Environmental Park Bioblitz.

- From a mission point of view it is as important to conserve the local species around us as it is those species we hold in our collections. Research and monitoring is an important part of how we can do this.
- Research into what species are present on site, where they are there, what resources they need, etc. is necessary for developing zoo management practices that allow them to persist on site.
- It is important to monitor all nature reserves, including zoo sites, as even well documented sites still provide new and exciting biological records. For example several new county records and one first UK record were recorded in the 2013 BIAZA Bioblitz.
- Native species research can enhance our understanding of these species and their ecology and contribute to their conservation on a wider scale.
- Research allows zoos to contribute to important regional and national databases of biological records, as well as species lists produced by BIAZA collections.
- Native species research can improve staff enthusiasm, public engagement and student learning. It can be used to enhance training and educational sessions. Recording and identification activities can enthuse and inspire people. Zoos provide an excellent environment for these activities.
- Research into native species on site provides good PR opportunities, can raise the profile of the zoo and widens the publics' perspective about what we do.

14.1.2. What are the costs of native species research?

Generally the costs of ecological research are low compared with other branches of biology and mostly arise from the time taken to collect data, equipment is generally low-tech and relatively inexpensive. There may be costs involved in training courses for staff, for instance in identification methods, in time needed to attend training and then collect data and in time needed to ensure legal compliance. Working with some native species requires an appropriate licence under the Wildlife and Countryside Act and the Conservation of Habitats and Species Regulations and this can be a time consuming process. Sometimes students or volunteers can be used to collect data which saves time but potentially requires a small cost in "thank you perks".

Often costs can be reduced to an absolute minimum by forming partnerships with local special interest groups.

Examples of local partnerships: Beale Park co-operates with several county groups such as bat, moth and mammal and allows them to use a building within the Park for meetings and training etc. In return they do periodic surveys and help at their BioBlitz. Kent Bat Group funded, erected and monitor bat boxes in partnership with Wildwood Trust.

14.2. How to start: Planning and carrying out ecological research in the zoo

Ecological research carried out on zoo sites will generally not be subject to those challenges and restrictions encountered in research on animal collections discussed elsewhere in this handbook. It can usually be planned and carried out in the same way as one would collect ecological data elsewhere. Therefore, we have focussed on a few key pointers here and referred the reader to standard text books where appropriate.

Where to start with native species research

Good research should have clear aims and a successful project can come from simple beginnings. As with much ecological research, this may start with some general observations or anecdotal sightings. These may be from zoo staff or visitors who have a personal interest. However, to produce data which has real use to either the zoo and/or the wider community, it is worth considering the following:

- The expertise and interest of the researcher.
- Are there particular questions that relate to future development on the zoo site? This may be part of an environmental audit or impact assessment.
- Research on under-studied species and habitats can make a vital contribution to the collective knowledge.
- Ecological research on commonly occurring species can still be used to evaluate a particular type of management e.g. grass cutting regimes, wildflower planting, coppicing.
- Zoos can conduct research to help with local ‘problems’ e.g. invasive species.
- Native species research can focus on under-studied topics for which grants may be available.
- Native species research can build on what has already been researched on site to gather trends and long term data sets. These are useful for both the site management and national data sets.
- Good communication channels, links and working relationships with other organisations can often prompt native species research focus, so zoos can approach such organisations for help and vice versa. Collaboration with universities, conservation organisations and local groups can provide expertise and academic support.
- Native species research can start from local and national biodiversity action plans and priority species. This often comes out of the above relationships with external organisations.

The research planning process is important and many stages depend on each other. Figure 14.2 shows a suggested route you might take. Each step is explored below.

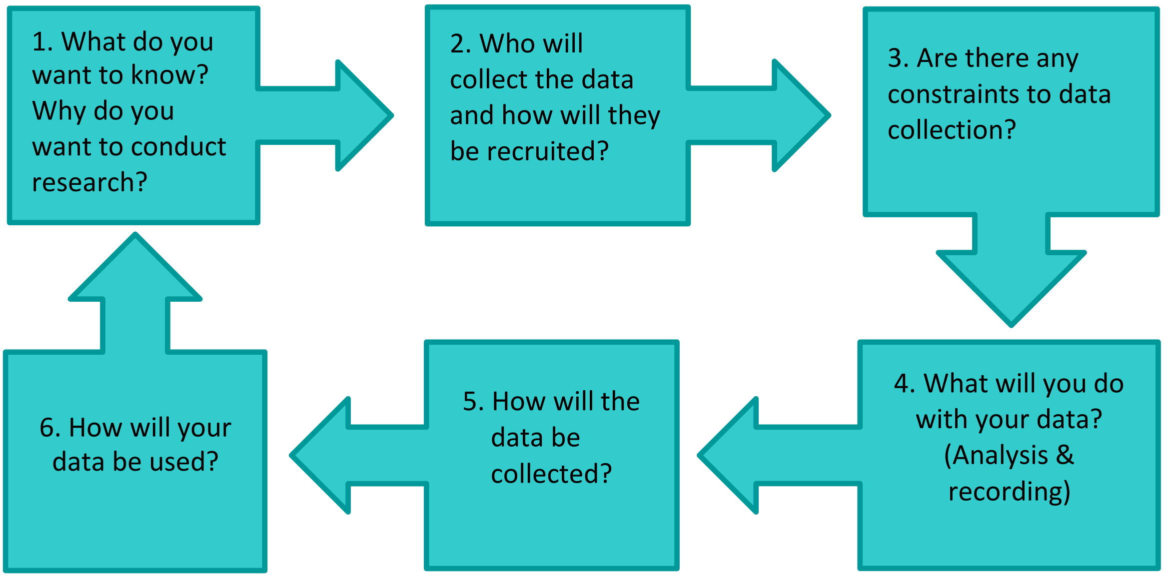


Figure 14.2: Process for planning a research project

14.2.1. Research questions

Before collecting any data you should be clear what you are collecting data for. You should have a hypothesis or a question you are hoping to answer. This may be as simple as 'What bird species are found in my Zoo?' or something more specific such as 'What is the effect of visitor numbers on blue tit breeding success?'

Both of these questions are valid. An answer to the first may contribute to national records and start to build a picture of your site, but with a little more focus an answer to the second question may be obtained. This would then give you real data on which you can base management decisions, as well as producing something with potential to publish. It could also form the basis of a research project across multiple zoos.

The best subjects for your hypothesis are those that you know something about – a species you know well, a site you are familiar with or an activity you have been involved with. Hypotheses can be extracted from scientific literature or policies or plans e.g. a biodiversity action plan.

For information on how to formulate a research question and generate a hypothesis see section 1.2.1 Hypothesis testing and generating questions.

There are also many standard text books on this subject such as Gilbert et al. (2011).

14.2.2. How to find help to conduct surveys and research.

Who collects the data?

Many zoos will not have the staff time for dedicated data collection. However, some data can be collected as part of daily routines e.g. bat droppings noted during cleaning. Generally research will be carried out by:

- Students
  - Conducting placement positions through universities (students may have to conduct a certain number of hours of placement work)
  - Needing BSc or MSc dissertation projects. In this instance, zoos may have to wait for students to come to them but it helps to have a list of projects that you want done.
  - From colleges offering practical methods training
- Volunteers - can be useful if they have a particular interest or expertise in a certain area.
- Experts - vital as many zoos will not have expert staff with such in depth knowledge
- Members of the public participating in events such as the Bioblitz
- Local conservation groups – will often conduct research for zoos in return for meeting spaces
- Keepers/gardeners etc. – perfect for collecting data that involves minimal time input

Students are a potentially massive source of manpower. Zoos should make links with universities and colleges and be more proactive in approaching them and advertise their research priorities via priority lists. It is a challenge to get universities to think of zoos as a location for dissertation studies, even more so when such studies involve native species. Make sure you keep copies of the raw data collected and any final reports.

14.2.3. Constraints: Health and safety, risk assessment, legal requirements

Once the participants in the research have been identified it is important to consider factors such as:

Accuracy of identification by untrained data collectors – quality control is needed with an expert to check recordings. If the ground work is conducted to train the data collectors first, via identification sheets and training sessions, then a lot can be gained from using volunteers.

Health and Safety issues surrounding equipment use, hazards on site, wildlife diseases, particular health issues of the researcher, lone working etc. A risk assessment should be carried out to evaluate the hazards and reduce the risks both to the researcher and the study species. There should be a standard risk assessment form used by the Zoo, or when a student is conducting research, the University. Some examples of wildlife diseases to be aware of are:

- Lymes disease carried by ticks; there is a symptom checklist at http://www.nhs.uk
- Chytrid is a fungal disease that affects amphibians. There are risks of disease transmission between wild and captive amphibians.
- Salmonella from a variety of animals; birds and reptiles. Nitrile gloves should be supplied for handling reptiles to prevent disease transmission
- Weil’s disease carried by small mammals. There is a risk of transmission to humans if bitten or scratched.

Legal requirements when working with native wildlife can be quite restrictive, requiring specific licences in order to undertake certain activities or prohibiting some activities altogether. The relevant legislation varies according to which country or region you are working:

ENGLAND Read this section for general information, but for more information, use the links to the relevant organisations below depending on where you work.

- At designated sites such as SSSI's (England, Scotland & Wales) there will be a list of prohibited actions which should be consulted. If in doubt contact the local office for Natural England.
- Wildlife law is complex but you should be aware that some species are protected and licences are required to carry out activities affecting them, this may include surveying. The main acts that cover this are the Wildlife and Countryside Act (WACA as amended http://www.legislation.gov.uk/ukpga/1981/69/contents) and the Countryside and Rights of Way Act (CROW http://www.legislation.gov.uk/ukpga/2000/37/contents). The Conservation of Habitats and Species Regulations 2010 (http://www.legislation.gov.uk/uksi/2010/490/contents/made) covers European protected species.
- Some small mammal species are protected by law (e.g. shrews in the UK) and you must obtain a licence from the relevant organisation if you set traps with the intention of trapping any species of shrew. Please ensure you are aware of and meet the requirements of any relevant laws in the country in which you are trapping.
- Breeding birds are protected Under Section 1 of the WACA (as amended), wild birds are protected from being killed, injured or captured, while their nests and eggs are protected from being damaged, destroyed or taken, but nests can be monitored without a licence for non schedule 1 species. Schedule one species need a licence with a valid reason. For information see the NE fact sheet at http://www.naturalengland.org.uk/Images/BreedingBirds_tcm6-21703.pdf. It is generally recommended that hedge cutting and tree works be suspended between 1st March and 31st July for this reason. Surveys should not generally be restricted by this legislation as they will not be carrying out any of these activities.
  If you are in any doubt about wildlife legislation in England contact Natural England; they are the organisation that issues licences. Applications are made via their websites at http://www.naturalengland.org.uk/ourwork/regulation/wildlife/licences/applicationforms.aspx.

SCOTLAND

The organisation responsible for the above in Scotland is the Scottish Natural Heritage;

http://www.snh.gov.uk/protecting-scotlands-nature/species-licensing/european-species-licensing/

WALES

The organisation responsible for the above in Wales is Natural Resources Wales;

http://naturalresourceswales.gov.uk/apply-buy-report/apply-buy-grid/protected-species-licensing/european-protected-species-licensing/plant-licencing/?lang=en#.Utz32NLFLvg

NORTHERN IRELAND

Site designations:

- At designated sites such as ASSI's there will be a list of prohibited actions which should be consulted. If in doubt contact the local office for Northern Ireland Environment Agency.
- Information about this and licensing can be found on the Northern Ireland Environment Agency website who are responsible for the implementation of the Wildlife (Northern Ireland) Order 1985 as amended by the Wildlife (Amendment) (Northern Ireland) Order 1995 and the Wildlife and Natural Environment (Northern Ireland) Act 2011 and The Zoo Licensing Regulations (Northern Ireland) 2003.
- The Wildlife (Northern Ireland) Order 1985 (as amended) is equivalent to Part I of the Wildlife and Countryside Act 1981 in England and Wales and the Wildlife Act 1976 as amended by the Wildlife (Amendment) Act 2000 in the Republic of Ireland. http://www.doeni.gov.uk/niea/biodiversity/wildlife_management_and_licensing.htm

REPUBLIC OF IRELAND

- The organisation responsible for the above in the Republic of Ireland is the National Parks and Wildlife Service http://www.npws.ie/licences/disturbance/

14.2.4. How to collect data

14.2.4.1. Standard methods and national schemes

There are different levels of data collection. You may be using data collected by a university researcher, school groups or the general public (citizen science). This can be very variable and sometimes limited in its usefulness. By following a standard approach you may be able to compare data from other sites, collected in the same way. There are many standard survey methods, some examples of national recording schemes that operate using a standardised approach are shown in Table 14.2. Also see the biological record centres website for links to numerous recording schemes http://www.brc.ac.uk/recording_schemes.asp. Note that these may just be about recording rather than conducting any in depth surveys.

Fig 14.3: Invertebrate sampling at Slapton Ley NNR

If you wish to compare data collected by other people you should ensure the data were collected in exactly the same way. Some methods are more subjective than others and it has been shown that variation between observers is a source of bias (Rich & Woodruff, 1992). This is explored in more detail in Chapter 1.

Using a standard method means you can contribute to national datasets. Table 14.2 is a guide to some of the surveys run by various naturalist and conservation groups. They can also be useful sources of methodological information and adapted for your own use. They have been grouped according to taxon group.

The Open University project guide by Chalmers and Parker (1986) is an excellent source of information that covers everything from planning, data analysis and fieldwork techniques. A real 'must have'. Available through the Natural History Book Shop http://www.nhbs.com/

Practical Field Ecology: A Project Guide by Wheater, Bell and Cook (2011) is a more recent publication.

For a general text book guide on what types of methods are used to survey for different groups see Sutherland (2006).

For an excellent checklist for general survey work see http://www.cieem.net/general-survey-advice .

Table 14.1: National recording schemes and volunteer surveys

Group or species	Recording or survey scheme	Useful links	Organisation	Other information (from website)
PLANTS				
Flowering plants	Wildflower Count	Survey form http://www.plantlife.org.uk/things_to_do/wildflowers_count/	Plantlife	Wildflowers Count is the UK's only annual national wild plant survey. Every year you can help keep track of some our most common wildflowers.
Trees	Urban tree survey	http://www.nhm.ac.uk/nature-online/british-natural-history/urban-tree-survey/index.html	Natural History Museum	Help to find out what species are growing where in the UK.
Seaweed	Big Seaweed Search	http://www.nhm.ac.uk/nature-online/british-natural-history/seaweeds-survey/index.html	Natural History Museum	Take a walk along the coast and help monitor the effects of climate change and invasive species on the UK's seaweeds.
AQUATICS				
Plants and invertebrates	PondNet	http://www.freshwaterhabitats.org.uk/projects/pondnet/	The Freshwater Habitats Trust	A volunteer survey to collect information about pond quality and pond species, including uncommon plants and animals.
Marine recording	Sea Watch	http://www.seawatchfoundation.org.uk/recording-and-submitting-sightings/	Sea Watch Foundation	Sea Watch is a national marine environmental charity working to improve the conservation of whales, dolphins and porpoises in the seas around Britain and Ireland.
	Seasearch	http://www.seasearch.org.uk/recording.htm	Seasearch	Seasearch is a project for volunteer sports divers who want to help protect the marine environment around the coasts of Britain and Ireland.

TERRESTRIAL INVERTEBRATES				
Butterflies	Butterfly monitoring scheme	http://www.ukbms.org/	Butterfly Conservation	The UKBMS mission is to assess the status and trends of UK butterfly populations for conservation, research and quality of life.
Butterflies	Wider Countryside Butterfly Survey	http://www.bto.org/volunteer-surveys/bbs/research-conservation/butterflies	BTO, Butterfly Conservation & CEH	The Wider Countryside Butterfly Survey is the main scheme for monitoring population changes of the UK's common and widespread butterflies.
Moths	Moths count	http://www.mothscount.org/text/27/national_moth_recording_scheme.html	Butterfly Conservation	Moths Count aims to encourage interest in moths throughout the UK and to run the National Moth Recording Scheme.
Moths	Moth night	http://www.mothnight.info/www/	Atropos and Butterfly Conservation	The annual celebration of moths and moth recording, takes place on specific days each year. See website for the next dates.
Bees, ants and wasps	A variety of recording schemes	http://www.bwars.com/	Bees Wasps & Ants Recording Society	BWARS is the national society dedicated to studying and recording bees, wasps & ants (aculeate Hymenoptera) in Britain & Ireland.
MAMMALS				
Small mammals: including hedgehogs, voles & woodmice	Owl Pellet Survey Footprint Tunnel Survey Mini Mammal Monitoring (MMM)	http://www.mammal.org.uk/surveys	The Mammal Society	A list of all the Mammal Society volunteer mammal surveys you can participate in with links for uploading your data. Also training events and resources.
Dormice (licence required for many survey activities)	National Dormouse Monitoring Programme	http://www.ptes.org/dormousemonitoring/	PTES people's trust for endangered species	Have you seen a dormouse, found a dormouse nest or have a found a hazel nut that's been chewed by a dormouse? Please enter these details in the National Dormouse Database.

British mammals	National Mammal Atlas Project (NMAP)	http://www.mammal.org.uk/nmap	The Mammal Society	The National Mammal Atlas Project (NMAP) aims to produce the first atlas in over 20 years to present vital new baseline distribution data
Bats	National Bat Monitoring Programme Surveys	http://nbmp.bats.org.uk/Surveys.aspx	Bat Conservation Trust	The National Bat Monitoring Programme surveys are carefully designed so that anybody can take part in monitoring these fascinating but easily overlooked mammals.
BIRDS				
Birds	The Nest Record Scheme	http://www.bto.org/volunteer-surveys/nrs	BTO (British Trust for Ornithology)	The Nest Record Scheme (NRS) gathers vital information on the breeding success of Britain's birds by asking volunteers to find and follow the progress of individual birds' nests.
Birds	Garden BirdWatch (GBW)	http://www.bto.org/volunteer-surveys/gbw	BTO	Garden BirdWatch monitors the changing fortunes of birds and other garden wildlife through its network of 'citizen scientists'.
Birds	BirdTrack	http://www.bto.org/volunteer-surveys/birdtrack/about	BTO, RSPB & other partners	The scheme is year-round, and ongoing, with Important results produced by BirdTrack: mapping migration timings and monitoring scarce birds.
REPTILES & AMPHIBIANS				
All reptiles & amphibians	National Amphibian & Reptile Recording Scheme	http://www.narrs.org.uk/	Amphibian and Reptile Conservation (ARC) in partnership with ARG UK and many other partners.	The National Amphibian and Reptile Recording Scheme (NARRS) Is a widespread amphibian and reptile survey that began in 2007.
All reptiles & amphibians	Record pool – general recording	http://www.arguk.org/recording	ARG(UK) Amphibian and Reptile Groups of the UK	Have you seen or surveyed reptiles or amphibians in the UK? Put them in the Record Pool. Find out what species you saw using downloadable species Identification Guides

Box 14.1:
CASE STUDY: **Which Environmental Variables and Habitat Characteristics Best Predict the Presence or Absence of Common British Reptile Species Under an Artificial Refuge? Implications for Surveying and Monitoring. VICTORIA BULLER WWCT / Paignton Zoo**

Three sites were surveyed for reptiles using corrugated tin sheets of approximately 60cm square. These act as refugia and attract basking reptiles. 25 refugia were placed at 6m intervals on each site and any reptiles were recorded three times a week at randomly selected times of day. Other data collected included under tin temperature, air temperature, humidity and cloud cover. The surrounding vegetation was also recorded using a point quadrat.

Generalized Linear Mixed Models and Spearman's Rank Correlation were used to analyse the data.

The study found that below refuge temperature and survey date were key predictors of presence, but that interactions between these and less well-recognised variables, such as cloud cover and vegetation height, also influenced reptile behaviour.

<u>Some of the findings in this study conflict with recommendations made in commonly used UK reptile survey guidelines and so raise questions about the application of current protocols to consultancy and conservation surveys. Following existing guidelines may result in inaccurate population estimates and may miss the presence of some species altogether. This study suggests that guidelines could be expanded to include other factors that may help to maximise survey success.</u>

Box 14.2:
CASE STUDY: **Great Crested newt monitoring at Dudley Zoo Amphibian Pools**

Great crested newts (*Triturus cristatus*) were introduced under licence to former ornamental fish ponds at the centre of the zoo site in a converted castle moat following preparations including the removal of fish and the establishment of locally sourced aquatic plants.

Monitoring of breeding adults is conducted from March to early May with 15 minute torchlight surveys of each pool to assess which pools are used and the state of the population.

Egg surveys can be conducted either by removal of weed or (more successfully) by placing strips of cut black bin-liners in the pool attached to a weight and with a cork float to help retrieve them. The females often favour plastic over living plants for egg laying and fold the white eggs into the plastic. This practice requires licencing and more preparation and staff time than the methods above and below which prove adequate at Dudley Zoo and so has not been used since the time of introducing the population.

Monitoring of larvae is conducted from June to August by visual searches for larvae (day or night) at the pool edge.

<u>The population has survived for 17 years and offers potential for night-time safaris, hitherto unexploited but very popular with zoo-workers! The zoo population was used by a local wildlife group (harvesting eggs on plastic strips) to re-populated new sites.</u>

14.2.4.2. Equipment

Much ecological equipment is very simple and inexpensive such as quadrats of various sizes but there are some useful items that can be much more expensive such as traps for small mammals, moth traps and remote recording devices such as movement trigger cameras and radio collars. Some species, mainly Red Data Book listed and some specific groups (e.g. bats) need a licence to work with and mark. Invasive tagging (micro-chips, also known as transponders, or PIT tags) needs to be done by an approved person.

Box 14.3:

Field Equipment Suppliers

http://www.nhbs.com/ - NHBS; where you can find a range of equipment you need for fieldwork, conservation, research, and working with wildlife.

https://www.efe-gbnets.com/ - Fieldwork equipment for professional workers, consultants, research staff & universities

http://www.wildcareshop.com - For any product relating to ecology, conservation or park management.

http://www.watdon.co.uk - Watkins and Doncaster, the naturalists. Established 1874. Over 130 years specialising in the supply and manufacture of equipment for the study of the Natural Sciences.
e-mail: sales@watdon.co.uk or phone 01580 753133
Fax +44 (0)1580 754054

http://blx1.bto.org/btos/ringingSales.htmlx - A source of selected field equipment for use in bird ringing but rules and scales useful in other activities. Scales in particular weigh down to 0.1g.

ALS - Anglian Lepidopterist Supplies - Station Road, Hindolveston, Norfolk NR20 5DE, tel. 01263 862068

http://www.bats.org.uk/pages/bat_detectors.html A review of bat detectors and links to suppliers
http://www.bats.org.uk/pages/manufacturers_and_suppliers.html

BIAZA members top tips!

Data loggers - Record temperature and humidity, can be left out for a long period of time. USB versions are available that can directly download data to computer.

Camera traps - Infra-red Bushnell HD video and photographs, £160 for medium range camera recommended.

Longworth traps (small mammal traps) - Expensive, cheaper if you buy in bulk and direct from the manufacturer. An alternative is to make one from a plastic tube (approx. £20 each), but these can leak or they may have issues with condensation which may be a welfare concern for trapped animals.

Mark-recapture for badgers - red hair spray can look like a wound one week on, so it's recommended to use blue, green or purple dye. Badgers do not like the sound of aerosol cans (sound like a hissing badger). Could try non-toxic paint but it is difficult to 'dab' a badger. Clipping under the guard hairs and spraying there so it reaches the skin avoids loss of colour due to grooming.

Mark-recapture for bats - Bats groom off non-toxic paint so can use same agricultural paint used on badgers.

Mark-recapture for other small mammals - Fur clipping for small mammals- clip guard hairs to reveal underfur.

Long term markers - microchips and radio collars.

Trap labels - Tips for labelling traps; Use small laminated piece of paper (including zoo logo, what is in the trap, venomous species warning if needed and tin code if related to a website/GPS).

14.2.4.3. Some Training, Guidance and Resources

Mammals

See http://jncc.defra.gov.uk/pdf/Small%20Mammal%20Monitoring%202006.pdf for a review of trapping methods for the different species.

Ponds

There are a couple of practices that all pond surveyors need to follow to ensure we protect, rather than damage, the ponds we study. Please familiarise yourself and rigorously apply the two protocols detailed in the document from the Freshwater Habitats Trust: Biosecurity Information for PondNet. This can be accessed via http://www.freshwaterhabitats.org.uk/projects/pondnet/survey-packs/
Key message: Check, Clean & Dry your kit to prevent the transfer of organisms between ponds.

Figure 14.4: Check, Clean and Dry campaign, Environment Agency

Pond dipping guide for teachers KS1 & 2
http://www.rbkc.gov.uk/pdf/pond_pack_2010.pdf.

All

Field Studies Council run many natural history courses for identification and survey methods
http://www.field-studies-council.org/individuals-and-families/natural-history.aspx
The OPAL website has many simple identification guides suitable for public participation type events.
http://www.opalexplorenature.org/Identification

14.2.5. Managing data, analysis and biological records

14.2.5.1. Data analysis

We do not attempt here to give you a complete guide to statistical methods for ecologists as there are many standard text books on the subject (see references). A good one is Dytham (2010) which is available online here:
http://sunsetridgemsbiology.wikispaces.com/file/view/Choosing+and+Using+Statistics.pdf

Another good online resource with a lot of information plus instructions on how to carry out various tests is: http://www.dataanalytics.org.uk/index.htm

Some general tips

At the beginning of your project think about what data you might collect and create a data sheet in Excel or similar program. At this stage inputting some 'dummy' data and plotting some graphs is very useful. This can help you to work out which statistical test you will want to use and make sure you collect appropriate data. **Make sure you know what tests you will be using BEFORE you start data collection.** It might be worth conducting a short pilot study to test your methods and generate some data to play with before starting in earnest. If the subject area or site is new to you this will enable any unforeseen problems to be dealt with before you start your research. When planning your analysis ask yourself 'am I looking for differences, links /correlations or associations. Are my data likely to be normally distributed or not (parametric or non-parametric)?'

Ecological research often concerns measures such as:

Diversity The biodiversity index is a non-parametric tool used to describe the relationship between species number and abundance. There are a number of different indices. Commonly used are:

- Simpson's diversity index (D) is often used to quantify the biodiversity of a habitat. It takes into account both species richness and evenness. Richness is the number of species per sample. Evenness is the relative abundance of the different species. D measures the probability that two individuals randomly selected from a sample will be the same species.
- The Shannon diversity index (*H*) is another index that is commonly used to characterize species diversity in a community. Like Simpson's index, Shannon's index accounts for both abundance and evenness of the species present.

An advantage of these indices is that they are simple to calculate in the field. However, an easy Excel addin is available from http://www.reading.ac.uk/ssc/n/software/diversity/Diversity.html this enables you to calculate different diversity measures on your data set. Also see the references on this web page.

Abundance is a measure of how many individuals are in an area. It can be measured in a number of ways:

- Percentage cover – the proportion of each quadrat occupied by the species (vegetation)
- Population density – the number of individuals per quadrat or other fixed area (vegetation & animal data)

- Species frequency – the proportion of quadrats with the species in it (vegetation & animal data).

Vegetation quadrat data can also be used to indicate environmental factors using Ellenberg values. These are environmental values calculated for many plant species including L- light, F- moisture, R – pH, N – nitrogen and S - salt or salinity. Therefore, plants can be used as indicators of their environment and changes over time can be investigated. To read more go to http://www.ceh.ac.uk/products/publications/untitled.html and download the list for the UK plant species. There is also an open source software package called MAVIS developed by CEH, available here http://www.ceh.ac.uk/products/software/cehsoftware-mavis.htm. This software will automatically calculate Ellenberg values from quadrat data as well as giving information about the NVC (National Vegetation Classification) community to which the data most closely matches.

14.2.5.2. Data management and biological records

Biological records

Wildlife observations, collected as biological records over many decades (or even centuries) give invaluable information on the histories of species. Their changing distributions and abundance build a picture and help to shape our conservation efforts. There are many national schemes for collecting biological records for different taxon groups.

What is a biological record

All research projects that include observations on native, naturally occurring wildlife, contain biological records. It is vital that the records taken from your research are made available to the site manager and local and national record centres. This may mean rearranging data to make a comprehensive list. A full, useful biological record contains a minimum of FOUR pieces of information:

- WHO - Full name of the person who made the observation. If the identity of the species was verified by a second person, this is also included.
- WHAT – Full species name. The common name may also be added but these may vary and are not reliable.
- WHERE – A meaningful site name. This can either just be the name of the zoo, but if you have a large site you may want to be more specific e.g. picnic lawn, Paignton Zoo (remember in a zoo, areas change over time so names may not stay consistent). A grid reference will be needed too and this will help overcome problems of changing names. See here for information on grid references http://www.ordnancesurvey.co.uk/resources/maps-and-geographic-resources/the-national-grid.html. You might consider using a site centroid grid reference. This is useful if you are collecting observations over an area and don't need a separate grid reference for each. An example might be a meadow species list. A grid reference is taken in the centre and all the records are attached to that. In this case a six figure grid reference is the longest used. Many people use GPS to generate grid references or latitude and longitude. Be aware of the degree of error shown on the unit and adjust the length of the grid reference accordingly, e.g. a 10 figure grid ref is reporting to the nearest 1m, so if you have an accuracy of ± 7m you should only report 8 figures (nearest 10m).
- WHEN – A full date in the format 05 May 1996. This avoids ambiguity with other systems.
- Other information: You may also have information about survey methods, abundance scores, sex, behaviour etc. These can all be listed alongside your record.

Managing your records

All data should be stored electronically and backed up regularly. A simple Excel spreadsheet is most often used but if you do a lot of recording and want something that will generate reports and has a search facility then there is software specifically designed for managing this type of record. Information about these can be found here http://www.nbn.org.uk/Tools-Resources/Recording-Resources/Mapping/Recording-software.aspx. There are also web based tools for online recording and you can also build your own; see Indicia http://www.indicia.org.uk/ for an open source wildlife recording toolkit.

Where to send your records

There are many national recording schemes you may wish to contribute to. Some have standard methodologies for specific surveys (see Table 14.1). In addition BIAZA also collects records of wildlife found on zoo sites. These can be sent directly to **the BIAZA office**.

A good place to start is your **Local Biological Records Centre.** They will usually have a standard format to submit records but will accept most file types. A species list on Excel can be imported directly, provided it has all the required information. The Local Biological Records Centre is also a good place to discover what records have already been made for your site. Often visitors, staff or volunteers with a particular interest may have submitted records to the records centre but not to anyone in the zoo. Records Centres are usually hosted and run by either the local Wildlife Trust (these are usually county focussed – see information below) or by the relevant county council.

Records centres (Biological Records Centre) provide a local facility for the storage, validation and usage of biological data under the National Biodiversity Network (NBN) project (see links below). Data can be requested for specific locations and will be provided free of charge so long as you're not profiting from the data e.g. a consultant who might then sell data on to a client. The easiest ways to find the right record centre is to Google your county and "biological records centre" or if you're in Wales this link takes you to a list http://www.lrcwales.org.uk/. Your local records centre will also be able to help you understand your site's data and will be able to put you in contact with relevant specialist and groups recording data in your area.

The National Biodiversity Network

The National Biodiversity Network (NBN) is taking the data, which until recently have been held by many different organisations and the individuals who collect them in a variety of formats, from computer databases to handwritten record cards, and compiling them into one location where they can make as much of the information as possible available to everyone who needs it. One of the principal means of collation and interpretation of this data is the network of local records centres and at the national level, the Biological Records Centre that collates and interprets data from national recording schemes.

One way it does this is through an innovative website known as the NBN Gateway. The NBN Gateway quite simply acts as a "data warehouse" for biodiversity information, which can be quickly and easily accessed to understand the distribution of particular species in the UK. Individual records, covering plants, mammals, birds and invertebrates, are stored on the NBN Gateway and these can then be displayed on a map of the UK in a number of different ways. The data held in this way are often not available for general enquires at a very detailed scale. It does help identifying the species that occur more generally in your area and could be used to help decide which species or groups might be important to start recording in a geographic context. http://www.nbn.org.uk/Home.aspx

Locations for data uploading

- **iSpot** **-** Free website, add records and photos, experts to help ID, iSpot app for android phones
- **iRecord** - Free website, managing and sharing records
- **National Biodiversity Website** - NBN gateway, sharing and accessing data, submit to iRecord
- **BTO Nest Record Scheme** - to determine trends in breeding performance
- **BTO Ringing Scheme** - Record a rung bird, information on the movements and longevity of individual birds

Recording in Ireland - Two very good websites to refer to about biodiversity recording and what taxa are currently of interest in Ireland are: http://www.biology.ie/ http://www.biodiversityireland.ie/

14.3. Translating results into effective site management

Once you have done some research on native species on site and have good data for what occurs where and perhaps even what environmental factors are important etc., how can you ensure that this information is used by site managers. Often in a zoo or aquarium the research people are not management people and they may not even have a direct line to the management team. Some zoos do have a range of procedures in place to ensure that site management benefits native species while many do not and consideration of native species on site depends on individual champions.

14.3.1. What formal processes currently exist and do they work?

Site Biodiversity Action Plans (BAP)

A small number of zoos either already have a site BAP in place or are planning to produce one. This is a comprehensive document identifying important native species or habitats on site, detailing a monitoring plan for them and recommending management actions to promote them. They can be achieved in-house (see Hambly and Marshall [2014] for a useful guide) and as a written document should allow all staff to know what should and should not be done in certain areas of the site. Any zoos where national BAP species are present or which encompass SSSI areas should probably consider writing a site BAP or similar plan for site management for native species. Co-operation with external organisations such as Natural England or the local (usually county) BAP group is recommended.

If there is no official national status (e.g. SSSI) already it may help to encourage organisation wide protection for the site or parts of it if an official status can be obtained. This could open up avenues of external funding and expertise to help manage the area, but will probably also limit future development plans.

Site master plans

Some zoos do have a formal process within their master planning systems for considering the impact on native species on site when making plans for major developments such as demolishing old buildings or constructing new ones. These include making assessments of native species on site, such as important bird or bat nesting sites, before buildings are demolished and sometimes creating new habitats to replace the ones that will be lost. However, it seems that zoos rarely formally monitor native species during or after constructing new areas or buildings.

Native species groups or Green Teams

A successful scheme in several zoos has been the creation of cross-department groups of staff with a particular interest in native species. These groups meet, or at least communicate, on a regular basis and drive pro-native species related behaviours, such as encouraging staff to record any sightings. Information can be shared and used to inform all departments about important native species areas. Staff who have a direct impact on the site, such as gardeners and maintenance/development workers, can then be made more aware of areas where particular protocols have to be followed or where some activities should not happen at all.

Why do systems sometimes fail?

Even with the above systems in place appropriate management of native species is sometimes still not implemented. This largely seems to be due to communication issues which are generally worse in larger organisations where more staff members are likely to be involved. Native species issues and site management may cross over several different departments and there is rarely just one person responsible for ensuring a positive impact of site management on native species.

The overall ethos of the zoo also seems important and it is key that all senior managers are fully committed to the principle of site management to benefit native species in order to ensure that all employees and volunteers are motivated to achieve this. Without the full support of managers it often falls to one or two members of staff to be enthusiastic about native species and it can be hard to engage others due to high workloads and sometimes an attitude that the exotic species in the collection are a higher priority. Management can make consideration of native species part of the staff selection and/or induction process to help overcome this. They can also help with potential conflicts such as what can be used for browse and where it can be cut or helping to find the appropriate balance between garden plantings to be aesthetically pleasing or to be of maximum value for native species.

14.3.2. Achieving management buy-in for native species

Given that management support for native species is crucial how can this be achieved? For any zoo a potential starting point for demonstrating the value of native species conservation based activities may be the development of a small initial project. This should be planned well and evaluated at various stages to provide evidence of any beneficial outcomes in terms of conservation, education or financial. This can then be used to encourage more and bigger native species projects.

14.3.2.1. Attracting visitors

Native species on site can attract both general members of the public and specific educational groups. If you can show that they contribute to increasing general income streams and thus have economic value projects are much more likely to be supported. Native species projects have the potential to draw in people who are not traditional zoo-goers, but perhaps more likely to be supporters of their local Wildlife Trust or the RSPB. However, there is a perception that visitors do not want to see native species in captive enclosures but rather to see them living free in the grounds.

- Interest in native species is increasing as indicated by and possibly because of the success of programmes such as Spring and Autumn Watch.
- Similar methods to develop fantastic visitor experiences can be used on zoo sites e.g.
    - At one zoo, a nest box camera in a blue tit box streamed on a zoo website received 16000 hits.

  - At another zoo, a badger hide with views into the sett is used for organised badger watching events in the evening and gives the zoo an additional income stream.
- Many zoos have hosted BioBlitz events involving the public in surveying for a variety of species. These are very popular, and even if not part of a BioBlitz, visitors are keen to have opportunities to be involved in bat surveys, fungi walks (especially to find and cook edible varieties), moth nights, bird watching with experts, tracking for field sign of deer/otters etc. and will pay for special events to do these.

Figure 14.5: Moth trapping at Beale Park

- Corporate (or other group) events to build bird or bat boxes or make insect hotels etc. have also been successful , as have public box building sessions, in some collections.

Native species can also be very appealing to various education groups and can be used to increase these numbers too. Zoo sites rich in native species can be used for practical ecology teaching to groups of students or for individual student research projects. In addition universities and colleges can provide a rolling quota of students who can monitor key areas or species during their learning experience.

- One zoo has worked with the University of Reading to study hedgehog populations on site using mark recapture
- Students have done projects to become familiar with iSpot
- A-level students have learnt vegetation sampling methods
- Wildlife gardens and ponds are very popular with school groups and children's clubs
- University groups have learnt mist netting techniques and provide data to the zoo for annual monitoring

14.3.2.2. Achieving mission and recognition

All zoos have a legal requirement to achieve conservation and most zoos strongly emphasise this in their mission statements and strategies. Conservation of native species is often a very cost effective way of achieving this aim. Providing bird boxes or a wildlife pond or an area of wildflowers to benefit pollinators is vastly cheaper, feasible for all zoos with little expertise or resources and often more effective than supporting projects overseas. Even bigger and more complicated native species projects could still be the most cost effective way for many zoos to achieve their conservation mission.

It is vital that all zoos promote the work they are doing to protect and conserve native species as it demonstrates to other zoo directors and the wider community that there is a 'collective consciousness' about native species issues. Many of our visitors to UK zoos can have very little direct impact on exotic wildlife populations but their behaviour can and does impact native species. Zoos can encourage their visitors to make this impact positive. There are some high profile native species conservation issues about which some of the public are already well informed e.g. declines in UK bee numbers. Zoos can have a real impact focusing on these issues, where many people can easily make a difference such as simply by choosing the right plants for their gardens.

Zoos should make every effort to disseminate information about native species projects. Scientific publications such as JZAR are good to hit academically or like-minded people within the industry. However the zoo-related grey literature may hit a greater range of zoo staff and is also important. Most importantly we need to let the public know what we are doing through popular magazines, newspapers and online social media so the profile of native species work by zoos is raised with all audiences.

14.4. Further information from BIAZA

The BIAZA Native Species Working Group falls under the Field Programmes Committee. The group holds an annual meeting at a different member collection each year. These are open to all and usually last two days. It also produces resources such as How to Run a BioBlitz. Information about the group can be found here http://www.biaza.org.uk/conservation/native-species/. For BIAZA members this includes details of the Chair, Vice-Chair and Steering Committee who can be contacted for any enquiries.

14.5. Further Reading

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