# Management of Captive Animals

## Global evidence for the effects of selected interventions

Coral S. Jonas, Lydia L. Timbrell, Fey Young, Silviu O. Petrovan,

Andrew E. Bowkett & Rebecca K. Smith

SYNOPSES OF CONSERVATION EVIDENCE SERIES

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**Katherine Whitehouse-Tedd**, Nottingham Trent University, UK
**Gwen Wirobski**, University of Veterinary Medicine Vienna, Austria

## About the authors

**Coral S. Jonas** works as an Education Manager at Dartmoor Zoo, UK and completed a Masters degree in Zoo Conservation Biology at the University of Plymouth in conjunction with Whitley Wildlife Conservation Trust, Paignton Zoo.

**Lydia L. Timbrell** completed a Masters degree in Zoo Conservation Biology at the University of Plymouth, in conjunction with Whitley Wildlife Conservation Trust, Paignton Zoo.

**Fey Young** completed a Masters degree in Zoo Conservation Biology at the University of Plymouth, in conjunction with Whitley Wildlife Conservation Trust, Paignton Zoo.

**Silviu O. Petrovan** is a Research Associate in the Department of Zoology, University of Cambridge, UK.

**Andrew E. Bowkett** is Field Conservation & Research Programmes Manager at the Whitley Wildlife Conservation Trust, based at Paignton Zoo, UK.

**Rebecca K. Smith** is a Senior Research Associate in the Department of Zoology, University of Cambridge, UK.

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# About this book

**The purpose of Conservation Evidence synopses**

| Conservation Evidence synopses **do** | Conservation Evidence synopses **do not** |
|---|---|
| • Bring together scientific evidence captured by the Conservation Evidence project (over 5,400 studies so far) on the effects of interventions to conserve biodiversity | • Include evidence on the basic ecology of species or habitats, or threats to them |
| • List all realistic interventions for the species group or habitat in question, regardless of how much evidence for their effects is available | • Make any attempt to weight or prioritize interventions according to their importance or the size of their effects |
| • Describe each piece of evidence, including methods, as clearly as possible, allowing readers to assess the quality of evidence | • Weight or numerically evaluate the evidence according to its quality |
| • Work in partnership with conservation practitioners, policymakers and scientists to develop the list of interventions and ensure we have covered the most important literature | • Provide recommendations for conservation problems, but instead provide scientific information to help with decision-making |

**Who is this synopsis for?**

If you are reading this, we hope you are someone who has to make decisions about how best to support or conserve biodiversity. You might be a zoo keeper, a conservationist in the public or private sector, a farmer, a campaigner, an advisor or consultant, a policymaker, a researcher or someone taking action to protect your own local wildlife. Our synopses summarize scientific evidence relevant to your conservation objectives and the actions you could take to achieve them.

We do not aim to make your decisions for you, but to support your decision-making by telling you what evidence there is (or isn't) about the effects that your planned actions could have.

When decisions have to be made with particularly important consequences, we recommend carrying out a systematic review, as the latter is likely to be more comprehensive than the summary of evidence presented here. Guidance on how to carry out systematic reviews can be found from the Centre for Evidence-Based Conservation at the University of Bangor (www.cebc.bangor.ac.uk).

**The Conservation Evidence project**

The Conservation Evidence project has four parts:

1) An online, **open access journal** *Conservation Evidence* that publishes new pieces of research on the effects of conservation management interventions. All our papers are written by, or in conjunction with, those who carried out the conservation work and include some monitoring of its effects.

2) An ever-expanding **database of summaries** of previously published scientific papers, reports, reviews or systematic reviews that document the effects of interventions.

**3) Synopses** of the evidence captured in parts one and two on particular species groups or habitats. Synopses bring together the evidence for each possible intervention. They are freely available online and available to purchase in printed book form.

4) ***What Works in Conservation*** is an assessment of the effectiveness of interventions by expert panels, based on the collated evidence for each intervention for each species group or habitat covered by our synopses.

These resources currently comprise over 5,400 pieces of evidence, all available in a searchable database on the website www.conservationevidence.com.

Alongside this project, the Centre for Evidence-Based Conservation (www.cebc.bangor.ac.uk) and the Collaboration for Environmental Evidence (www.environmentalevidence.org) carry out and compile systematic reviews of evidence on the effectiveness of particular conservation interventions. These systematic reviews are included on the Conservation Evidence database.
Of the 29 interventions to increase natural feeding behaviours in primates, 38 interventions to enhance breeding in amphibians in captivity and 29 to promote health and welfare in captive carnivores (Felids, Canids and Ursids) through feeding practices identified in this synopsis, none are the subject of specific systematic reviews.

**Scope of this synopsis**

This synopsis covers evidence for the effects of conservation interventions to increase natural feeding behaviours in primates, to achieve successful breeding of amphibians in captivity and to promote health and welfare in captive carnivores (Felids, Canids and Ursids) through feeding practices.

Evidence from all around the world in a captive setting is included. Any apparent bias towards evidence from some regions reflects the current biases in published research papers available to Conservation Evidence.

**Husbandry vs conservation of species**

This synopsis includes evidence from the substantial literature on husbandry of captive primates and carnivores and not from observations or interventions in wild groups. Observations of wild animals were not included unless human intervention was measured. It also includes evidence on captive amphibian husbandry, but only if the husbandry intervention is used to promote successful breeding. Observational or descriptive literature was not used unless human intervention was measured.

**How we decided which conservation interventions to include**

Lists of interventions for each topic were developed and agreed in partnership with Advisory Boards made up of international conservationists and academics with expertise in the conservation of each specific group of species. We have tried to include all actions that have been carried out or advised for each topic covered in this synopsis.

The list of interventions for each topic was organized into categories based on the type of intervention used:

- *Primate feeding* - food presentation, diet manipulation, feeding schedule and social group manipulation.
- *Amphibian breeding* - refining techniques using less threatened species, changing environmental conditions/ microclimate, changing enclosure design for spawning or egg laying sites, manipulating social conditions, changing the diet of adults and manipulating rearing conditions for young.
- *Carnivore feeding* - diet and feed type, food presentation and enrichment, feeding schedule and social feeding.

**How we reviewed the literature**

In addition to evidence already captured by the Conservation Evidence project, we have searched the following sources for evidence relating to captivite management:

*Primate feeding:*

- Two specialist primate journals, from their first publication to the end of 2015 (*American Journal of Primatology* and *International Journal of Primatology*)
- Six general zoo conservation, animal behaviour and animal welfare journals over the same time period (*Zoo Biology, Journal or Zoo and Aquarium Research, International Zoo Yearbook, Applied Animal Behaviour Science, Animal Welfare* and *Journal of Applied Animal Welfare Science*).

*Amphibian breeding:*

- Eighteen specialist amphibian journals had already been searched for the Amphibian Conservation synopsis (Smith & Sutherland 2014), from their first publication to the end of 2012, these searches were updated to May 2016 for additional relevant articles (*Acta Herpetologica, African Journal of Herpetology, Amphibian and Reptile Conservation, Amphibia-Reptilia, Applied Herpetology, Australasian Journal of Herpetology, Bulletin of the Herpetological Society of Japan, Contemporary Herpetology, Copeia, Current Herpetology, Herpetologica, Herpetological Bulletin, Herpetological Conservation and Biology, Herpetological Journal, Herpetological Monographs, Journal of Herpetology, Russian Journal of Herpetology and South American Journal of Herpetology)*. One additional specialist journal was also searched for this synopsis (*Salamandra*).
- Thirty general conservation journals had also been searched to the end of 2012 for the Amphibian Conservation synopsis (Smith & Sutherland 2014).
- A further four journals related to captive animal management were searched to May 2016 to add to the evidence for this synopsis (*Animal Welfare,*

*Journal of Zoo & Aquarium Research, Journal of Applied Animal Welfare Science* and *Zoo Biology*).

*Carnivore feeding:*

- Six general zoo conservation, animal behaviour and animal welfare journals over the same time period (*Zoo Biology, Journal or Zoo and Aquarium Research, International Zoo Yearbook, Applied Animal Behaviour Science, Animal Welfare* and *Journal of Applied Animal Welfare Science*).

Evidence published in other languages was included when it was identified.

The criteria for inclusion of studies in the Conservation Evidence database are as follows:

- There must have been an intervention carried out that conservationists would do.
- The effects of the intervention must have been monitored quantitatively.

These criteria exclude studies examining the effects of specific interventions without actually doing them. For example, predictive modelling studies, studies looking at providing gum and the effects on foraging primates without a control and not in captivity, and studies describing how amphibians breed (observational studies) were excluded. Such studies can suggest that an intervention could be effective, but do not provide direct evidence of a causal relationship between the intervention and the observed effect.

Altogether 25 studies for primate feeding, 18 studies for amphibian breeding and 42 studies for carnivore feeding were allocated to interventions they tested.

**How the evidence is summarized**

Within Conservation Evidence synopses, conservation interventions are grouped primarily according to the relevant direct threats, as defined in the IUCN Unified Classification of Direct Threats (http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme).

However some important interventions, such as *ex-situ* conservation can be used in response to many different threats, and it would not make sense to split studies up depending on the specific threat they were studying. These interventions are grouped, following the IUCN's Classification of Conservation Actions (http://www.iucnredlist.org/technical-documents/classification-schemes/conservation-actions-classification-scheme-ver2). The action we have separated out is: Species Management *Ex-situ* Conservation.

Normally, no intervention or piece of evidence is listed in more than one place, and when there is ambiguity about where a particular intervention should fall there is clear cross-referencing. Some studies describe the effects of multiple interventions. Where a study has not separated out the effects of different interventions, the study is included in the section on each intervention, but the fact that several interventions were used is made clear.

In the text of each section, studies are presented in chronological order, so the most recent evidence is presented at the end. The summary text at the start of each section groups studies according to their findings.

At the start of each chapter, a series of **key messages** provides a rapid overview of the evidence. These messages are condensed from the summary text for each intervention.

Background information is provided where we feel recent knowledge is required to interpret the evidence. This is presented separately and relevant references included in the reference list at the end of each background section.

The information in this synopsis is available in three ways:

- As a pdf to download from www.conservationevidence.com
- As text for individual interventions on the searchable database at www.conservationevidence.com.
    - As a chapter in the publication *What Works in Conservation*, which is available as a pdf and book (Sutherland *et al*. 2018: https://www.openbookpublishers.com/product/696)

**Terminology used to describe evidence**

Unlike systematic reviews of particular conservation questions, we do not quantitatively assess the evidence or weight it according to quality within synopses. However, to allow you to interpret evidence, we make the size and design of each trial we report clear. The table below defines the terms that we have used to do this.

The strongest evidence comes from randomized, replicated, controlled trials with paired-sites and before and after monitoring.

| Term | Meaning |
|---|---|
| **Site comparison** | A study that considers the effects of interventions by comparing sites that have historically had different interventions or levels of intervention. |
| **Replicated** | The intervention was repeated on more than one individual or site. In conservation and ecology, the number of replicates is much smaller than it would be for medical trials (when thousands of individuals are often tested). If the replicates are sites, pragmatism dictates that between five and ten replicates is a reasonable amount of replication, although more would be preferable. We provide the number of replicates wherever possible, and describe a replicated trial as 'small' if the number of replicates is small relative to similar studies of its kind. In the case of translocations or release of animals, replicates should be sites, not individuals. |
| **Controlled** | Individuals or sites treated with the intervention are compared with control individuals or sites not treated with the intervention. |

| | |
|---|---|
| **Paired sites** | Sites are considered in pairs, when one was treated with the intervention and the other was not. Pairs of sites are selected with similar environmental conditions, such as soil type or surrounding landscape. This approach aims to reduce environmental variation and make it easier to detect a true effect of the intervention. |
| **Randomized** | The intervention was allocated randomly to individuals or sites. This means that the initial condition of those given the intervention is less likely to bias the outcome. |
| **Before-and-after trial** | Monitoring of effects was carried out before and after the intervention was imposed. |
| **Review** | A conventional review of literature. Generally, these have not used an agreed search protocol or quantitative assessments of the evidence. |
| **Systematic review** | A systematic review follows an agreed set of methods for identifying studies and carrying out a formal 'meta-analysis'. It will weight or evaluate studies according to the strength of evidence they offer, based on the size of each study and the rigour of its design. All environmental systematic reviews are available at: www.environmentalevidence.org/index.htm |
| **Study** | If none of the above apply, for example a study looking at the number of people that were engaged in an awareness raising project. |

### Taxonomy

Taxonomy has not been updated but has followed that used in the original paper. Where possible, common names and Latin names are both given the first time each species is mentioned within each synopsis.

### Habitats

Within this synopsis, all interventions were carried out in captivity i.e. in 'artificial habitats.'

### Significant results

Throughout the synopsis we have quoted results from papers. Unless specifically stated, these results reflect statistical tests performed on the results.

### Multiple interventions

Some studies investigated several interventions at once. When the effects of different interventions are separated, then the results are discussed separately in the relevant sections. However, often the effects of multiple interventions cannot be separated. When this is the case, the study is included in the section on each intervention, but the fact that several interventions were used is made clear.

### Where no evidence was found

The phrase 'No evidence was captured for the effects of…..' written for some interventions indicates that we were unable to present any evidence for their effectiveness. This was because either no research was found during our searches testing these interventions, or previous work did not meet the criteria for this synopsis, i.e. interventions were not tested directly and quantitatively, or results may not have been reported or made publicly available. This does not mean that these interventions are not effective and should not be used, it simply highlights the need for robust monitoring and publishing of results in these areas to ensure that future conservation efforts will be appropriate and effective. A small number of articles could not be accessed by the authors and thus are not presented in this synopsis.

### How you can help to change conservation practice

If you know of relevant evidence that is not included in this synopsis, we invite you to contact us, via our website www.conservationevidence.com. If you have new, unpublished evidence, you can submit a paper to the *Conservation Evidence* journal http://conservationevidence.com/collection/view. We particularly welcome papers submitted by conservation practitioners.

# 1. Species management: Ex-Situ conservation – Increasing natural feeding behaviours in primates in captivity

## Author: Coral Jonas

**Background**

Primates in captivity may not be able to express certain behaviours, such as foraging for food, and may even lose them over time. It is a particular concern that they will lose the behaviours that are necessary for them to survive and find food in the wild. The loss of these innate behaviours could seriously reduce the conservation efforts made by zoos, where the possibility of eventually releasing primates back into their natural environment needs to be maintained. For example, the method that black and white ruffed lemurs, *Varecia cariegata cariegata*, use to gain much of their food in the wild, by suspending themselves upside down by their feet, and using their hands and mouths to gather up the food, was observed to be seriously reduced in captivity, because many were fed on the ground from dishes (Britt 1998). Therefore efforts should be made by zoos to encourage the use of natural feeding postures in captivity as shown by animals in the wild.

Britt, A. (1998) Encouraging natural feeding behaviour in captive bred black and white ruffed lemurs (*Varecia variegate v.*) *Zoo Biology*, 17, 379-92.

## Key messages – Food Presentation

***Scatter food throughout enclosure***

Four studies, including one replicated study, in the USA, found that scattering food throughout enclosures increased overall activity, feeding and exploration and decreased abnormal behaviours and aggression.

***Hide food in containers (including boxes and bags)***

Three studies including two before-and-after studies in the USA and Ireland found that the addition of food in boxes, baskets or tubes increased activity levels in lemurs and foraging levels in gibbons.

***Present food in puzzle feeders***

Three studies including two before-and-after studies in the USA and UK found that presenting food in puzzle feeders, increased foraging behaviour, time spent feeding and tool use but also aggression.

***Present food which required the use (or modification) of tools***

No evidence was captured for the effects of presenting food which required the use (or modification) of tools.

***Present food in water (including dishes and ponds)***

One replicated, before-and-after study in the USA found that when exposed to water filled troughs, rhesus monkeys were more active and increased their use of tools.

***Present food frozen as ice***

Two studies in the USA and Ireland found that when frozen food was presented, feeding time increase and inactivity decreased.

***Present food dipped in food colouring***
One before-and-after study in the USA found that when food was presented after being dipped in food colouring, orangutans ate more and spent less time feeding.
***Present food items whole instead of processed***
One before-and-after study in the USA found that when food items were presented whole instead of chopped feeding time increased in macaques.
***Present feeds at different crowd levels***
One before-and-after study in the USA found that when smaller crowds were present foraging and object use in chimpanzees increased.
***Paint gum solutions on rough bark***
No evidence was captured for the effects of painting gum solutions on rough bark.
***Add gum solutions to drilled hollow feeders***
No evidence was captured for the effects of adding gum solution to drilled hollow feeders
***Plant natural food plants in enclosures***
One replicated, before-and-after study in the USA reported that chimpanzees spent more time foraging when provided with planted rye grass and scattered sunflower seeds compared to browse and grass added to the enclosure with their normal diet.
***Maximise both vertical and horizontal presentation locations***
One replicated, before-and-after study in the UK reported that when vertical and horizontal food locations were increased feeding time increased. One controlled study in the UK and Madagascar found that less time was spent feeding on provisioned food in the indoor enclosure when food was hung in trees in an outdoor enclosure.

## Key messages – Diet manipulation

***Formulate diet to reflect nutritional composition of wild foods (including removal of domestic fruits)***
Two replicated, before-and-after studies in the USA and UK found that when changing the diet to reflect nutritional compositions of wild foods, there was a decrease in regurgitation and reingestion, aggression and self-directed behaviours.
***Provide gum (including artificial gum)***
No evidence was captured for the effects of providing gum (including artificial gum)
***Provide nectar (including artificial nectar)***
No evidence was captured for the effects of providing nectar (including artificial nectar)
***Provide cut branches (browse)***
One replicated, before-and-after study in the Netherlands and Germany found that captive gorillas when presented with stinging nettles use the same processing skills as wild gorillas to forage.
***Provide live invertebrates***
One before-and-after study in the UK found that providing live invertebrates to captive loris increased foraging levels and reduced inactivity.
***Provide herbs or other plants for self-medication***
No evidence was captured for the effects of providing herbs or other plants for self-medication.

***Provide fresh produce***
One replicated, before-and-after study in the USA found that when fresh produce was offered feeding time increased and inactivity decreased in rhesus macaques.
***Modify ingredients/nutrient composition seasonally (not daily) to reflect natural variability***
No evidence was captured for the effects of modifying ingredients/nutritional composition seasonally (not daily) to reflect natural variability.

## Key messages – Feeding Schedule

***Change the number of feeds per day***
Two before-and-after studies in Japan and the USA found that changing the number of feeds per day increased time spent feeding in chimpanzees but also increased hair eating in baboons.
***Change feeding times***
One controlled study in the USA found that changing feeding times decreased inactivity and abnormal behaviours in chimpanzees.
***Provide food at natural (wild) feeding times***
No evidence was captured for the effects of providing food at natural (wild) feeding times.
***Provide access to food at all times (day and night)***
No evidence was captured for the effects of providing access to food at all times (day and night).
***Use of automated feeders***
No evidence was captured for the effects of the use of automated feeders.

## Key messages – Social group manipulation

***Feed individuals separately***
No evidence was captured for the effects of feeding individuals separately.
***Feed individuals in social groups***
One replicated, controlled study in the USA reported that the time monkeys spent completing an enrichment task increased in social groups than when feeding alone. One before-and-after study in Italy found that capuchins ate more unfamiliar foods during the first encounter in the presence of their groupmates.
***Feed individuals in subgroups***
No evidence was captured for the effects of feeding individuals in subgroups.

## Species Management: Food Presentation

### 1.1. Scatter food throughout enclosure

- Two replicated, before-and-after and one before-and-after study in the USA found that scattering food throughout the enclosure increased feeding and exploratory behaviours in rhesus macaques[4] and reduced abnormal behaviours in chimpanzees[1] and aggression lemurs[3].

- One before-and-after study in the USA[2] found that when food was scattered throughout the enclosure more foraging was seen and activity levels increased in gorillas.

**Background**

More natural foraging conditions can be stimulated by scattering food throughout enclosures and hiding food in materials covering the flooring (e.g. straw) instead of in bowls or trays. The aim is to increase the amount of time that primates are active in captivity.

A replicated, before-and-after study in 1991 in the USA (1) found that adding woodchips, into which sunflower seeds were scattered, in enclosures of rhesus macaques *Macaca mulatta* increased feeding and exploration activities compared to food thrown onto the bare floors. When food was thrown onto the bare floors, foraging time ranged between 1% and 7% of the total time in comparison to 35% when sunflower seeds were scattered in woodchips over the floor. Two groups of rhesus macaques (n=15) were housed continually in bare tiled enclosures with food thrown onto the floor. Woodchips and sunflower seeds were then scattered over the floors of both enclosures for four weeks. Each animal was observed for 10 minutes twice each week between 09:00 h and 10:00 h and between 13:00 h and 14:00 h over nine months.

A replicated, before-and-after study in 1996 in Atlanta, USA (2) found that adding straw, into which food was scattered, reduced abnormal behaviours in laboratory-housed chimpanzees *Pan troglodytes* compared to bare floors with food presented in one place. The average abnormal behaviour observations were reduced from 10% when food was presented in one place to 4% when it was scattered. Repeated eating of regurgitated food was reduced from 5% to 1% and other abnormal oral behaviours from 4% to 1%. Non-oral abnormal behaviours were not reduced. No evidence of habituation was found over nine weeks. Chimpanzees were housed in five enclosures in groups of two or three. Two cups of mixed seeds and nuts were scattered in the straw of each of the enclosures twice daily. For each chimpanzee (n = 13), behaviour was recorded every 15 seconds over five minutes/day. Observations were carried out on 60 occasions (total of five hours) over three months both during the control and when food was scattered.

A before-and-after study in 1994 in Georgia, USA (3) found that spreading food over a wider area reduced rates of aggression towards males from female ring tailed lemurs *Lemur catta* compared to when food was provided in a small area. When food was dispersed within a one metre radius, feeding males submitted to female aggression 42 times, within a two metre radius they submitted 38 times and within a four metre radius they submitted 19 times. Feed was scattered in a circular area to simulate a two dimensional food patch. Food patches with a radius of 1, 2, and 4m were created. All aggressive behaviours were recorded in nine adults and two infant lemurs from when the food was given to the end of the group's feeding session. Each radius length was tested eight times, during different feeding times.

A before-and-after study in 2009 in the USA (4) found that when food was scattered throughout enclosures, western lowland gorillas *Gorilla gorilla gorilla* foraged more and increased their levels of activity compared to when food was offered in one area only. Foraging increased from five minutes (out of 30) when food was offered in one area to 11 minutes when food was thrown into the enclosure (a 'yard toss'), 15 minutes when food was scattered throughout the yard and 20 minutes when hay and forage filled feeders were suspended from a climbing structure. Inactivity was reduced from 23 minutes (out of 30) to 15 minutes in the yard toss, five minutes when food was scattered and four minutes when feeders were used. Behaviour of all six individuals housed in the same enclosure was observed every minute for 30 minutes before and after each treatment for five days.

(1) Byrne G.D. & Suomi S.J. (1991) Effects of woodchips and buried food on behaviour patterns and psychological well-being of captive rhesus monkeys. *American Journal of Primatology,* 23, 141-151
(2) Baker K. (1997) Straw and forage material ameliorate abnormal behaviours in adult chimpanzees. *Zoo Biology,* 16, 225-236.
(3) White F., Overdorff D., Keith-Lucas T., Rasmussen M., Kallam W & Forward Z. (2007) Female dominance and feeding priority in a prosimian primate: experimental manipulation of feeding competition. *American Journal of Primatology,* 69, 295-304.
(4) Ryan E. (2012) The effect of feeding enrichment methods on the behaviour of captive Western lowland gorillas. *Zoo Biology,* 31, 235-241.

## 1.2. Hide food in containers (including boxes and bags)

- Two before-and-after studies in the USA[1] and Ireland[2] found that the addition of browse to food in boxes, baskets or tubes increased activity levels and foraging behaviours in lemurs and gibbons.

**Background**

This intervention involves using boxes or bags to hide daily meals given to monkeys. This would normally be put in bowls or scattered within an enclosure, but instead it is put within a box or bag hidden within browse, with the aim of increasing foraging behaviours in individual monkeys.

A before-and-after study in 2005–2006 in the USA (1) found that adding browse to hide food in boxes significantly raised activity levels in ring tailed lemurs *Lemur catta* compared to when browse was presented on the floor of the enclosure along with food presented at a regular feeding station. Adding browse to food in boxes more than doubled (to 79%) the percentage of observation periods when at least one lemur was active. It also increased the percentage of active behaviours during that period from 4% of observations to 13% of observations. Spatially separating the four boxes reduced the amount of food that lemurs stole from other animals in the mixed enclosure by half (0.2% of observations to 0.1% of observations). The lemurs were presented with four treatments: food was added to four open boxes placed together and browse scattered on the floor of the enclosure; food scattered in the browse and added to boxes placed together; food added to boxes placed apart and browse scattered on the floor; food scattered in the browse and added to boxes placed apart. A group of eight lemurs, living in a mixed enclosure with hyrax and porcupine, were scanned every 60 seconds for two hours per day over six days and behaviours recorded. Boxes were given to the lemurs every day, with a different treatment given each day.

A before-and-after study in 2009 in Ireland (2) found that when food was presented in food filled baskets or presented in tubes foraging of moloch gibbons *Hylobates moloch* increased and time spent outside the indoor enclosure increased compared to when fresh fruit and vegetables were presented in one place. With the food filled baskets significantly more instances of being outside and number of times gibbons were seen foraging were recorded when food filled baskets were presented (times outside: 106; times foraging: 44) compared to when fruit and vegetables were offered alone (times outside: 96; times foraging: 20). With the tubes significantly more instances of being outside and the number of times gibbons were seen foraging were recorded when food filled tubes were presented (times outside: 112; times foraging: 43) compared to when fruit and vegetables were offered alone (times outside: 96; times foraging: 20). A group of gibbons was presented with food in baskets or tubes for five days, with three baskets or tubes suspended within the animal exhibit each time. The gibbons' behaviour was recorded every five minutes for five hours per day for 12 days.

(1) Dishman D.L., Thomson D.M. & Kamovsky N.J. (2009) Does simple feeding enrichment raise activity levels of captive ring tailed lemurs (*Lemur catta*)? *Applied Animal Behaviour Science,* 116, 88-95.

(2) Wells D.L. & Irwin R.M. (2009) The effect of feeding enrichment on the moloch gibbon (*Hylobates moloch*). *Journal of Applied Animal Welfare Science,* 12, 21-29.

### 1.3. Present food in puzzle feeders

- Two before-and-after studies in the USA[1] and the UK[2] found that the use of puzzle feeder's decreased food sharing, increased foraging behaviour, and the use of tools but also aggression.
- One replicated, before-and-after study in the USA[3] found that the use of puzzle feeders increased time spent feeding and less time inactive.

**Background**

Puzzle feeders are seen as a form of enrichment to make more natural and more rewarding ways to provide food to captive animals. The aim is to make the puzzle both mentally and physically challenging to increase their activity and foraging time.

A before-and-after study in 1992 in the UK (1) found that presenting food in puzzle feeders increased foraging behaviour and the use of tools in chimpanzees *Pan troglodytes*, gorillas *Gorilla gorilla gorilla*, and orangutans *Pongo pygmaeus pygmaeus* compared to food being placed in the enclosures. The percentage feed orientated behaviour, including tool use, increased from 2% when food was placed in the enclosure to 30% when a puzzle feeder was introduced to orangutans, 2% to 20% for gorillas and 3% to 30% for chimpanzees. An open-ended 3 m length of 15 cm diameter plastic drain pipe, attached horizontally to the outside of the enclosure weldmesh, was used with each group of apes (four gorillas, seven chimpanzees and two orangutans) studied for 12 two-hour trials with food placed into the enclosure and 12 two-hour trials with food in the feeder.

A replicated, before-and-after study in 1996 in the USA (2) found that when artificial turf foraging mats and acrylic puzzle feeders were provided separately to rhesus macaques *Macaca mulatta* more time was spent feeding and less time inactive than when pellets were fed. Using the artificial turf mats the time spent feeding increased from 14 minutes/hour when pellets were fed to 20 minutes/hour when artificial turf mats were provided. Inactivity was lower with artificial turf mats (two minutes/hour) than when just pellets were provided (five minutes/hour). When given the acrylic puzzle feeder's time spent feeding increased from 14 minutes/hour when pellets were fed to 18 minutes/hour when acrylic puzzle feeders were provided. Inactivity was lower with acrylic puzzle feeders (two minutes/hour) than when just pellets were provided (five minutes per hour). The individually housed monkeys (n=63) were presented with mats and puzzle feeders loaded with 20g of seeds or grain every 1.5 hours, during which 15 minutes of animal observations were conducted on all monkeys. Artificial turf mats and the acrylic puzzle feeders were presented to each monkey every weekday for six months, with control observations when just pellets were provided, conducted for six months between times when enrichment devices were given.

A before-and-after study in 1998 in the USA (3) found that the increased difficulty level of finding food distributed in puzzle feeders (but not the amount of food given) decreased food sharing and increased aggression in golden lion tamarins *Leontopithecus rosalia*. The mean frequency per minute of food sharing went from 0.25/minute with the simple task to 0.15/minute with the complex task. The mean frequency of aggressive behaviour went from 0.05/minute in the simple task to 0.15/minute in the complex task. A 15 hole puzzle box containing grapes behind sliding doors was installed for 40 days. Four, eight or twelve grapes were given and two difficulty levels established: the animal either had to reach into a tube to retrieve the grapes (simple) or rummage and rip away a barrier to retrieve the food (complex). Two observers recorded the monkeys (n=4) food transfer behaviours and aggression until all grapes were consumed.


(1) Gilloux I, Gurnell J. & Shepherdson D. (1992) An enrichment device for great apes. *Animal Welfare,* 1, 279-289.

(2) Schalpiro S. J., Suarez S.A., Porter L.M. & Bloomsmith M.A. (1995) The effects of different types of feeding enhancements on the behaviour of single-caged, yearling rhesus macaques. *Animal Welfare,* 5, 129-138.

(3) Rapaport L.G. (1998) Optimal foraging theory predicts effects of environmental enrichment in a group of adult golden lion tamarins. *Zoo Biology*, 17, 231-244.


## 1.4. Present food which requires the use (or modification) of tools

- No evidence was captured for the effects of presenting food which required the use (or modification) of tools.

**Background**

To be classified as a tool, an object must be held in the hand, foot or mouth by the primate and used to enable him or her to attain an immediate goal (Goodall, 1980), such as acquiring food.


Goodall J (1980) Tool-using in primates and other vertebrates. Advances in the study of behaviour. New York, Academic Press, 1980, Vol 3, 195-429


## 1.5. Present food in water (including dishes and ponds)

- One replicated, before-and-after study in the USA[1] found that when exposed to water-filled troughs, monkeys were more active and increased their use of tools.

**Background**
Food should be presented to primates in a way that encourages their natural food finding behaviour. Introducing new textures stimulates curiosity and interest, with the aim of increasing their activity and foraging behaviours as well as their use of tools to extract the food from the water.

A replicated, before-and-after study in 1993 in the USA (1) found that, when exposed to water-filled troughs, rhesus monkeys *Macaca mulatta* were more active compared to when troughs of water were absent. On average the number of times exploratory behaviour occurred increased from five, when there was no water trough present, to six when the trough was present, to four once the trough had been removed in two social groups of monkeys. Water was either standing or running and was contained in plastic troughs. For each of the two social groups, behavioural data was collected for 15 days every five minutes between the hours of 09:00 h and 10:00 h for each of the tests where no trough was present, where the trough was present with water and afterwards where no trough was present again.

(1) Parks K.A. & Novak M.A. (1993) Observations of increased activity and tool use in captive rhesus monkeys exposed to troughs of water. *American Journal of Primatology,* 29, 13-25.

## 1.6. Present food frozen in ice

- Two replicated and before-and-after studies in the USA[1] and Ireland[2], found that when food was presented in ice, more time was spend foraging and feeding and less time inactive.

**Background**
Whole pieces of food can be lightly frozen or frozen within juice and introduced to primates as a novelty. The food will be of an unexpected temperature and texture, which will change as the food warms slowly to ambient temperature. The aim is to increased activity levels and more natural foraging behaviours.

A replicated, before-and-after study in 1996 in the USA (1) found that when frozen juice was provided to rhesus macaques *Macaca mulatta* more time was spent feeding and less time inactive than when pellets were fed. Time spent feeding increased from 14 minutes/hour when pellets were fed to 24 minutes/hour when frozen juice was offered. Inactivity was lower with frozen juice (two minutes/hour) than when just pellets were provided (five minutes/hour). The monkeys (n=63) received frozen juice presented as a 100ml block of sugared drink mix; eight different flavours were used. The frozen juice was presented to individually housed macaques at intervals of 1.5 hours, during which 15 minutes of animal observations were conducted on all monkeys. Frozen juice was presented to each monkey every weekday for six months with control observations, when just pellets were provided, conducted over six months between times when enrichment devices were given.

A before-and-after study in 2009 in Ireland (2) found that when food was presented as frozen ice pops foraging of moloch gibbons *Hylobates moloch* increased and time spent outside the indoor enclosure increased compared to when fresh fruit and vegetables were presented in one place. Significantly more instances of being outside and number of times gibbons were seen foraging were recorded when food was presented in ice pops (times outside: 134; times foraging: 43) compared to when fruit and vegetables were offered alone (times outside: 96; times foraging: 20). A group of gibbons were presented with ice pops for five days, with three ice pops suspended within the animal exhibit each time. The gibbons' behaviour was recorded every five minutes for five hours per day for 12 days.

(1) Schapiro S. J., Suarez S.A., Porter L.M. & Bloomsmith M.A. (1995) The effects of different types of feeding enhancements on the behaviour of single-caged, yearling rhesus macaques. *Animal Welfare,* 5, 129-138.

(2) Wells D.L. & Irwin R.M. (2009) The effect of feeding enrichment on the moloch gibbon (*Hylobates moloch*). *Journal of Applied Animal Welfare Science,* 12, 21-29.

## 1.7. Present food dipped in food colouring

- One before-and-after study in the USA[1] found that when food was dipped in food colouring juvenile and adult orangutans ate more and took less time to consume it.

**Background**

Changing the colour of food items aims to make the food more interesting and appealing to the monkey, stimulating their curiosity and interest and therefore more natural feeding behaviours.

A before-and-after study in 1985 in the USA (1) found that when food was dipped in food colouring juvenile and adult orangutans *Pongo spp.* ate more and adults took less time to consume the food compared to when food was not coloured. The time adults spent feeding on chow decreased from 17 minutes when it was not coloured to 13 minutes when it was coloured. Juveniles averaged 1–2 pieces of plain chow daily, which increased to 3 coloured pieces, while the adults' daily consumption increased from 75 pieces to 84 once it was coloured. 'Purina' monkey chow was dipped in red, green, blue and orange food colouring and offered to three adults and two juveniles. Equal numbers of differently coloured pieces of chow were offered. Observations took place for 45 minutes/day for eight days with uncoloured chow, coloured chow and then uncoloured chow again.

(1) Barbiers R.B. (1985) Orangutans' colour preference for food items. *Zoo Biology,* 4, 287-290.

## 1.8. Present food items whole instead of processed

- One before-and-after study in the USA[1] found that when macaques were presented with whole foods instead of chopped foods the types of food, amount consumed and time spent feeding increased.

**Background**

Captive diets for many primate species consist of vegetables and fruit alongside pellets and browse. In captivity monkeys are primarily provided with chopped up vegetables and fruit, which is not what they would find in the wild. The aim of presenting vegetables whole instead of chopped is to increase the time spent foraging and manipulating the food before consumption.

A before-and-after study in 1989 in the USA (1) found that when lion-tailed macaques *Macaca silenus* were presented with whole foods instead of chopped foods the total amount of food consumed and the time spent feeding increased. The average macaque consumed 395g of whole food compared to 306g of chopped food per day. Time spend feeding was greater, as a result of increased consumption and processing requirements, when whole foods were offered. A group of 12 lion-tailed macaques was given fruits and vegetables whole or chopped on alternate days. Feeding data was collected by one observer between 09:00 h and 11:00 h, observing one animal's behaviour each day for two consecutive days for the chopped control and whole treatment totalling 40 days of observations.

(1) Smith A., Lindburg D.G. & Vehrencamp S. (1989) Effect of food preparation on feeding behaviour of lion-tailed macaques. *Zoo Biology*, 8, 57-65

## 1.9. Present feeds at different crowd levels

- One before-and-after study in the USA[1] found that when smaller crowds were present foraging and object use in chimpanzees increased.

**Background**

Crowd levels within zoos vary. At some zoos crowds could consist of up to hundreds of people at an enclosure at any one time. This can affect the behaviour of captive primates. The aim of presenting feeds at different crowd levels is to see when is best (high or low crowd levels) to give food to increase foraging time.

A before-and-after study in 1993 at Los Angeles, USA (1) found that foraging time and object use among chimpanzees *Pan troglodytes* presented with new food and environmental enrichment was greater with small viewing crowds than with large ones. Foraging increased from 0% of the overall time observed with old enrichment and low crowd levels to 29% when there were high crowd levels and new enrichment and up to 33% when new enrichment was given with low crowds. Object use was increased from 3% with low crowds and old enrichment to4% and 5% when new enrichment was given with high crowds and low crowd levels respectively. Researchers filmed the group of chimpanzees for three hours between 12:00 h and 15:00 h with one of four treatments each day: large crowd, new enrichment; large crowd, old enrichment; small crowd, new enrichment; small crowd, old enrichment and low crowd, no enrichment. The group of 11 chimpanzees were given access to the enrichment, browse, ice blocks and stuffed sacks at 12:00 h. Repeated observations were made over five weeks. Scan sampling methods were used to record the behaviour of each chimpanzee every 60 seconds.

(1) Wood W. (1998) Interactions among environmental enrichment, viewing crowds, and zoo chimpanzees (*Pan troglodytes). Zoo Biology,* 17, 211-230.

## 1.10. Paint gum solutions on rough bark

- No evidence was captured for the effects of painting gum solutions on rough bark.

**Background**
Some small primates, such as marmosets, eat gum in the wild by gouging in tree branches and eat the gum that accumulates. In captivity we can provide artificial gum in hollow feeders or painted on rough bark., The aim is to encourage foraging behaviour and promote feeding behaviours seen in the wild.

## 1.11. Add gum solutions to drilled hollow feeders

- No evidence was captured for the effects of adding gum solutions to drilled hollow feeders.

**Background**
See background 1.10 'Paint gum solutions on rough bark.'

## 1.12. Provide live vegetation in planters for foraging

- One replicated, before-and-after study in the USA[1] reported that chimpanzees spent more time foraging when provided with planted rye grass and scattered sunflower seeds compared to browse and grass added to the enclosure with their normal diet.

**Background**
Ground cover is important in enclosure design for primates in captivity. This is especially so for species which forage at ground level. Live plant cover aims to encourage natural food items, such as insects, into the enclosures to increase foraging levels.

A replicated, before-and-after study in 1991 in the USA (1) reported that chimpanzees *Pan troglodytes,* provided with a foraging device containing planted rye grass and scattered sunflower seeds, spent more time foraging, compared to a feeder containing just grass, with browse added to the enclosure as their normal diet throughout, although no statistical tests were carried out. Chimpanzees foraging behaviour increased from an average of 2% when the feeder contained grass to 12% when the container contained grass with sunflower seeds. PVC pipe cut in half lengthwise and planted with rye grass seed was attached to the outside of six different enclosures containing two or four out of the 14 chimpanzees observed in the study. All chimpanzees in all six enclosures were then given sunflower seeds added to the grass. Behavioural observations over 54 hours were conducted under the two conditions: grass container alone and grass container with scattered sunflower seeds.

(1) Lambeth S. & Bloomfield M. A. (1994) A grass foraging device for captive chimpanzees (*Pan troglodytes*). *Animal Welfare,* 3, 13-24.

## 1.13. Maximise both horizontal and vertical food presentation locations

- One controlled study in the UK and Madagascar[1] found that when food was hung in trees in an outdoor enclosure, less time was spent feeding on food in the indoor enclosure.

- One replicated, before-and-after study in the UK[2] reported that monkeys spent longer feeding in bowls positioned at the top of an enclosure than in bowls positioned on the floor.

**Background**

In the wild, many primates avoid foraging on the ground if they have the option. This is to decrease the risk of predation. The aim of maximising horizontal and vertical presentation of food is to increase more natural foraging behaviour.

A controlled study in 1994 in the UK and Madagascar (1) found that black and white ruffed lemurs V*arecia variegate variegata* fed for less time on provisioned food indoors and more time on natural vegetation in their outdoor island exhibit than when caged. The percentage of time spent feeding reduced from 89% when caged to 61% on the island, but 39% of the time was spent feeding on natural vegetation rather than provisioned food on the island. The variety of locations also encouraged them to exhibit similar use of feeding postures (96% of feeding time) and support postures (20% of ground feeding) to that observed in wild lemurs (75% and 25% respectively). On the island, chopped food was suspended from trees; in the cage, it was placed on a mesh roof rather than on shelves. Four captive lemurs, were observed in a cage environment from March to June and on an island from June to September over 24 days (192 hours of observation). As a comparison, observations of a wild population were collected for a focal group of five lemurs every two minutes for 600 hours, to record individual behaviour and posture.

A replicated, before-and-after study in 2002 in Scotland, UK (2) reported that the amount of time spent feeding in bowls positioned at the top of the enclosure was longer than in bowls positioned on the floor in common marmosets *Callithrix jacchus*, although no statistical tests were carried out. The marmosets spent more time stationed in the top half of the enclosure than in the bottom half (overall 79% vs 21%) with 59g of food eaten at the top bowl compared to 19g from the bottom bowl. Eight marmosets were housed in pairs in four cages. During the experiment, bowls were placed at different heights, either: on the floor, at the top; or on the floor and at the top. Each pair was observed for 15 minutes per day over nine days for each condition after the food bowls had been presented.

(1) Britt A. (1998) Encouraging natural feeding behaviour in captive-bred black and white ruffed lemurs (*Varecia variegata variegata*). *Zoo Biology*, 17, 5, 379-392.

(2) Buchanan-Smith H.M., Shand C. & Morris K. (2002) Cage use and feeding height preferences of captive common marmosets (*Callithrix j. jacchus*) in two-tier cages. *Journal of Applied Animal Welfare Science,* 5, 139-149.

# Species Management: Diet Manipulation

## 1.14. Formulate diet to reflect nutritional composition of wild foods (including removal of domestic fruits)

- One before-and-after study in the USA[1] found that replacing milk with fruit juice in gorilla diets led to a decrease in regurgitation and reingestion.

- One replicated, before-and-after study in the UK[2] found that when lemurs were fed a fruit-free diet aggression and self-directed behaviour were lower.

**Background:**
Many primates are classed as fruit eaters, and so are provided with a diet with a high proportion of domestic fruits in captivity. Studies have shown this can lead to obesity, diabetes and dental disease (Plowman *et al* 2014). Domestic fruits have now been replaced with vegetable diets in many zoos, with subsequent reductions in obesity and dental conditions (Plowman *et al* 2014). The aim of this intervention is to reduce abnormal feeding behaviours, social tension and aggression within primates.

Plowman, A. (2013) Diet review and change for monkeys at Paignton Zoo Environmental Park. *Journal of Zoo and Aquarium Research*, 1, 73-77.

A before-and-after trial in 1999 in the USA (1) found that replacing milk with fruit juice in gorilla *Gorilla gorilla gorilla* diets led to a significant decrease in voluntary regurgitation and reingestion of food or fluid from stomach to mouth and an increase in feeding behaviour. Regurgitation and reingestion were reduced from 11% of the time when milk was given to 8% of the time when fruit juice was offered instead of milk. In addition, the consumption of hay doubled from 10% to 22% of the time in the evening meal. The study used a withdrawal design method, in which 10 days of feeding non-fat powdered cow's milk was followed by a treatment period of 10 days when milk was replaced with apple, orange or pineapple juice followed by a return to feeding milk for 10 days. Seven males and 12 females were studied for five minute sessions during a one hour period during the evening meal.

A replicated, before-and-after, site comparison study in 2012-2013 in Cornwall and Devon, UK (2) found that when fed a domestic fruit free diet, aggression and self-directed behaviour in four species of lemurs, were significantly lower. Self-directed behaviour reduced from an average rate of 0.28 times/minute when fed fruit to 0.1 times/minute when no fruit was provided. Aggression reduced from an average of 1% of time when fed fruit to 0.1% of time when no fruit was provided. There was no significant effect of diet on foraging and auto grooming. Four species of lemur (17 individuals *Varecia variegata*, *Varecia rubra*, *Lemur catta*, *Eulemur coronatus*) were observed for 35 days at 15 sessions of 20 minutes at Paignton Zoo, during May–July 2012, and 30 sessions of 20 minutes at Newquay Zoo, during May–July 2013. The transition to a vegetable and leaf-eater pellet diet, which excluded fruit, was implemented over the course of a week. Observations were carried out before and after the diet change.

(1) Lukas K., Hamor G., Bloomsmith M., Horton & Maple T. (1999) Removing milk from captive gorilla diets: The impact on regurgitation and reingestion (R/R) and other behaviours. *Zoo Biology,* 18, 515-528.
(2) Britt S., Cowlard K., Baker K., Plowman A. (2015) Aggression and self-directed behaviour of captive lemurs (*lemur catta, Varecia variegata, V. rubra and Eulemur coronatus*) is reduced by feeding fruit-free diets. *Journal of Zoo and Aquarium Research,* 3, 15.

### 1.15. Provide gum (including artificial gum)

- No evidence was captured for the effects of providing gum (including artificial gum).

**Background**
See background 1.10 'Paint gum solution on rough bark.'

### 1.16. Provide nectar (including artificial nectar)

- No evidence was captured for the effects of providing nectar (including artificial nectar).

**Background**
Nectar forms a significant component of the wild diets of many species of primate (Sussman 1978, Cabana et al. 2014). In captivity providing nectar offers primates the opportunity to manipulate their food with their feed and hands to extract the nectar, expecially if given in line with an enrichment device.

Sussman R. (1978). Nectar-feeding by prosimians and its evolutionary and ecological implications. In: Chivers D, Joysey K, editors. Recent Advances in Promatology. New York, NY, USA: Academic Press; pp. 119–126.
Cabana F., Plowman A. (2014) Pygmy slow loris *Nycticebus pygmaeus* – natural diet replication in captivity. *Endangered Species Research*, 23, 197-204.

### 1.17. Provide cut branches (browse)

- One replicated, before-and-after study in the Netherlands and Germany[1] found that when presented with stinging nettles captive gorillas used the same processing skills as wild gorillas to forage.

**Background**
Many primate species are leaf eaters which spend significant amounts of time browsing in the wild. In captivity, pellet and produce-based diets are supplemented with cut branches from trees and shrubs referred to as browse. Browse is an important dietary component but also aims to increase time spent feeding and promote natural foraging behaviours.

A replicated, before-and-after study in 2006 at three zoos in the Netherlands and Germany (1) found that western lowland gorillas *Gorilla gorilla gorilla,* presented with stinging nettles *Urtica dioica*, used the same processing skills as wild mountain gorillas, *Gorilla beringei beringei.* Eight of nine captive western lowland gorillas gathered and processed leaves before eating them (just as wild mountain gorillas do). A captive gorilla with no experience of nettles gathered and processed leaves 32% of the time and omitted the process stage 59% of the time. As a comparison, captive gorillas given willow plant *Salix sp*., omitted the process stage 79% of the time and 17% of the time ate the leaves as soon as the willow was obtained. Leaves were offered either inside or as a scatter feed outside. Sixty-eight hours of video contained 296 behavioural sequences of nettle feeding from nine gorillas and 176 behavioural sequences of willow feeding from 11 gorillas.

(1) Tennie C., Hedwig D., Call J. & Tomasello M. (2008) An experimental study of nettle feeding in captive gorillas. *American Journal of Primatology,* 70, 584-593.

## 1.18. Provide live invertebrates

- One before-and-after study in the UK[1] found that when provided with live insect prey inactivity reduced and foraging increased in captive loris to levels seen in wild loris.

**Background**

Releasing live insects or other invertebrates into enclosures aims to encourage more natural foraging behaviour in primates which hunt live prey in the wild. Live insects attract the attention of the primate, encouraging natural behaviours such as chasing, stalking and pouncing.

A before-and-after study in 2015 in the UK (1) found that when live insect prey were provided to captive loris *Loris lydekkerianus nordicus* inactivity was reduced and foraging increased to levels seen in wild lorises. Average inactivity time reduced from an average of 46% to 29% (wild loris averaged 43%) and average foraging time increased from 9% to 24% (wild loris averaged 27%). In addition, a significant increase in postures used in foraging in the wild and a wider behavioural repertoire was seen by recording positional behaviours. Observational data was collected over five consecutive days at five-minute intervals over six hours/day for each of the five animals for each of three conditions: pre-enrichment (usual diet); enrichment (usual diet plus live insects); and post-enrichment (usual diet). Approximately 200 crickets were scattered into an indoor enclosure at 10:00 h over the five days of the enrichment condition (approximately 40 each day) in addition to their normal diet.

(1) Williams E., Cabana F & Nekaris K.A.I. (2015) Improving diet and activity of insectivorous primates in captivity: Naturalizing the diet of Northern Ceylon gray slender loris, *Loris lydekkerianus nordicus*. *Zoo Biology,* 34, 473-482.

## 1.19. Provide herbs or other plants for self-medication

- No evidence was captured for the effects of providing herbs or other plants for self-medication.

**Background**

This behaviour; known as zoopharmacognosy, typically involves ingestion or topical application of plants, soils or insects in order to treat and prevent diseases. One of the first documented cases of self-medication was observed in wild chimpanzees (Wrangham 1983), where they were observed folding and swallowing *Aspilia spp* leaves without chewing them. These leaves have a rough and bristly surface which it is suggested helped the chimpanzees to expell parasitic worms from their digestive systems. Providing potentially medicinal plants as browse or growing in herb gardens may allow primates to self-medicate in captivity.

Wrangham RW., and Nishida T (1983) Aspilia spp. leaves: A puzzle in the feeding behaviour of wild chimpanzees. *Primates,* 24, 276–282.

## 1.20. Provide fresh produce

- One replicated, before-and-after study in the USA[1] found that when fresh produce was offered instead of pellet feed more time was spent feeding and less time inactive in rhesus macaques.

**Background**

Different species of primates show considerable variation in their natural diets. For example marmosets will eat gum as well as fruit, flowers, insects and other small animals and Macaques will eat both plants and meat. It is important that the diet of primates in captivity should be designed to reflect the appropriate natural diet, using fresh produce, of the species, giving them the opportunity to search and manipulate their food as they would in the wild.

A replicated, before-and-after study in 1996 in the USA (1) found that when fresh produce (fruit and vegetables) were provided to rhesus macaques *Macaca mulatta* more time was spent feeding and less time inactive than when pellets were fed. Time spent feeding increased from 14 minutes/hour when pellets were fed to 27 minutes/hour when fresh produce was offered. Inactivity was lower with fresh produce (two minutes/hour) than when just pellets were provided (five minutes/hour). A portion of 125g of fresh produce was offered (60% fruit and 40% vegetables) for six months, with the varieties of fruit and vegetables (n=40) offered rotated weekly. The fresh produce was presented in feeding devices to 63 individually housed macaques at intervals of 1.5 hours, during which 15 minutes of animal observations were conducted on all monkeys. Fresh food was presented to each monkey every weekday for six months with control observations when just pellets were provided conducted over the same six months between times when enrichment devices were given.

(1) Schalpiro S. J., Suarez S.A., Porter L.M. & Bloomsmith M.A. (1995) The effects of different types of feeding enhancements on the behaviour of single-caged, yearling rhesus macaques. *Animal Welfare,* 5, 129-138.

## 1.21. Modify ingredients/nutrient composition seasonally (not daily) to reflect natural variability

- No evidence was captured for the effects of modifying ingredients/nutrient composition seasonally (not daily) to reflect natural variability.

**Background**

Primates display a good variation of behavioural flexibility, allowing them to adjust to changes in food availability in the wild. This is critical for the survival of leaf eating and fruit eating primates that live in small forests, where food resources are lower than in large and continuous forests. In captivity it is possible to adjust their diet accordingly by giving seasonal leaves, fruit and vegetables with the aim of promoting activity levels.

## Species Management: Feeding Schedule

### 1.22. Change the number of feeds per day

- Two before-and-after studies in Japan[1] and the USA[2] found that when the number of feeds per day were increased the amount of time spent feeding increased in chimpanzees, but hair eating also increased in baboons.

**Background**
Anticipating the arrival of food can lead to stress behaviours in captive animals. Changing the number of feeds per day reduces the predictability of feeds within the day with the aim of increasing the amount of time animals spend foraging for foods by decreasing stress behaviours.

A before-and-after study in 1999 in Japan (1) found that when food was given twice a day instead of once a day to a group of chimpanzees *Pan troglodytes* the amount of time spent feeding increased. On average, feeding time contributed to 7% of the behaviours expressed when one feed was offered per day and 24% when two feeds were offered per day. A group of five chimpanzees was observed for 15 days over five one hour time periods when all food was provided in the afternoon at 15:00 h and when one feed was given between 10:00 h and 11:00 h and a second feed at 15:00 h.

A before-and-after study in 2012 in the USA (2) found that increasing feeds from one to two per day increased hair-eating in baboons *Papio hamadryas sp*. When one feed was provided, hair eating was seen during 1% of the observations, increasing to 3% with two feeds. Eleven baboons, housed as a social group, were offered their standard feed of 5 kg of monkey diet in the afternoon, which was then split into two, 2 kg feeds, given morning and afternoon. Each monkey was observed over six months and two hours of data were collected on each animal.

(1) Morimura N. & Ueno Y. (1999) Influences on the feeding behaviour of three mammals in the Maruyama Zoo: Bears, elephants and chimpanzees. *Journal of Applied Animal Welfare Science,* 2, 169-186.
(2) Nevill C.H. & Lutz C.K. (2015) The effect of a feeding schedule change and the provision of forage material on hair eating in a group of captive baboons (*Papio hamadryas sp*). *Journal of Applied Animal Welfare Science,* 18, 319-331.

### 1.23. Change feeding times

- One replicated, controlled study in the USA[1] found that when chimpanzees were fed on unpredictable schedules inactivity decreased.

**Background**
Anticipating the arrival of food can lead to stress behaviours in captive animals. Changing the times when feeds are given to captive primates throughout the day reduces the predictability of feeds with the aim of increasing the amount of time animals spend foraging for foods by decreasing stress behaviours.

A replicated, controlled study in 1995 in the USA (1) found that inactive behaviour was lower in chimpanzees *Pan troglodytes* fed on unpredictable schedules than for those fed on predictable schedules, but abnormal behaviour were similar. Inactivity was reduced from 35% of time when predictable feeding times were maintained to 27% of time when predictable feeding times were given. Abnormal behaviours did not change significantly from when feeding times were predictable (1%) to when they were unpredictable (0.2%). Two groups of chimpanzees were fed on a predictable schedule and two groups were fed on a more unpredictable schedule. A total of 30 chimpanzees were observed within the four groups. Four meals of fresh produce were given daily and observational data were collected at two times each day, during the pre-feeding period and feeding schedules were unchanged, between 09:30 h and 10:00 h for 30 minutes for a total of 100 hours.

(1) Bloomsmith M & Lambeth S.P. (1995) Effects of predictable versus unpredictable feeding schedules on chimpanzee behaviour. *Applied Animal Behaviour Science,* 44, 65-74.

## 1.24. Provide food at natural (wild) feeding times

- No evidence was captured for the effects of providing food at natural (wild) feeding times.

**Background**

This intervention is specifically for nocturnal and diurnal primates. Feeding them during daylight hours is not suitable and therefore provisions must be put in place in captivity to allow them to express natural feeding behaviours at night or if preferred at dawn and dusk.

## 1.25. Provide access to food at all times (day and night)

- No evidence was captured for the effects of providing access to food at all times (day and night).

**Background**

Different species of primates show huge variation in their natural diets with some wild animals spending a considerable amount of time foraging for food (up to 70%), this could be during the day or at night. In captivity food needs to be offered in a manner and frequency appropriate to the species, to provide variety, with the aim of promoting the foraging and social feeding behaviour seen in the wild.

## 1.26. Use of automated feeders

- No evidence was captured for the effects of using an automated feeder.

**Background**

Anticipating the arrival of food can lead to stress behaviours in captive animals Automated feeders work by dispensing fresh food in pre-measured quantities, at timed intervals throughout the day with the aim of increasing time spent searching for, extracting and consuming food.

# Species Management: Social Group Manipulation

## 1.27. Feed individuals separately

- No evidence was captured for the effects of feeding individuals separately

**Background**
Some primate species lead solitary largely lives in the wild but are normally housed in pairs or social groups in captivity where they may experience feeding competition. In captivity, training, feeding devices or enclosure modifications may allow group-housed primates to forage individually.

## 1.28. Feed individuals in social groups

- One replicated, controlled study in the USA[1] reported that an enrichment task took less time to complete when monkeys were in social groups than when feeding alone.
- One before-and-after study in Italy[2] found that in the presence of their groupmates monkeys ate more unfamiliar foods during the first encounter.

**Background**
Many primate species feed in social groups in the wild but may be housed individually in captivity either routinely (e.g. in some laboratory contexts) or temporarily (e.g. for medical treatment). Many primate species feed in social groups in the wild but may be housed individually in captivity either routinely (e.g. in some laboratory contexts) or temporarily (e.g. for medical treatment). Time spent feeding and foraging as a group may be important for reinforcing social structures (Brennan & Anderson, 1998) and for the development of natural feeding behaviours in young animals (Lefebvre 1995). Captive primates can be fed in social groups or given visual access to animals in other enclosures, with the aim of allowing natural group dynamics and social learning of feeding behaviours.

Brennan, J & Anderson, J.R. (1998) Varying responses to feeding competition in a group of rhesus monkeys (*Macaca mulatta*). *Primates*, 29, 353-360.
Lefebvre, L. (1995) Culturally-transmitted feeding behaviour in primates: Evidence for accelerating learning rates. *Primates*, 36, 227-239.

A before-and-after study in 1998 in Italy (1) found that capuchins *Cebus apella* ate more unfamiliar foods when they first encountered them if they were in the presence of their groupmates than if they encountered them when alone. The average number of food samples the capuchins ate averaged six as individuals, but 15 in a social condition. After the first encounter, consumption of the unfamiliar foods became equivalent to when they encountered the food alone. Capuchins were presented with eight novel food types in one of two conditions: individual and social. Each animal received four food types individually and another four with groupmates.

A replicated, controlled study in 2009 in the USA (2) reported that completion of an enrichment food task by capuchin monkeys *Cebus apella* took less time in social situations than when feeding alone, although no statistical tests were carried out. Monkeys with another nearby completed the trial three times faster (100 seconds) than solo monkeys (373 seconds). In each of two groups, 12 of 24 monkeys were randomly assigned to the solo condition and 12 to the social condition. One monkey was in the left side of the test chamber, and either the right remained empty (solo condition), or contained a second monkey with a cup of cereal and peanut butter (social condition). Each left-hand monkey was given food in a clear 28cm square box with a protruding wheel to turn, aligning a hole with a chute that released food into a cup. Each test was measured as the number of seconds it took from presentation of the apparatus to completion of 20 attempts.

(1) Visalberghi E., Valente M. & Fragaszy D. (1998) Social context and consumption of unfamiliar foods by capuchin monkeys (*Cebus apella*) over repeated encounters. *American Journal of Primatology,* 45, 367-380.

(2) Dindo M., Whiten A. & De Waal F. (2009) Social facilitation of exploratory foraging behaviour in capuchin monkeys (*Cebus apella*). *American Journal of Primatology,* 71, 419-426.

## 1.29. Feed individuals in subgroups

- No evidence was captured for the effects of feeding individuals in subgroups.

**Background**

Wild primates have to forage over an area that can meet their energetic and nutritional needs. Therefore, an increase in group size will increase the area that must be covered to find adequate food for the group. Individuals in a group that forage in an area that meets the needs of all group members may have to travel further and spend more time and energy foraging than would be the case if they forage in smaller groups or alone (Chapman 1990). Therefore the aim of this intervention is to provide captive primates with the opportunity to forage in subgroups.

Chapman C, (1990) Ecological constraints on group size in three species of Neotropical primates. *Folia Primatologica*, 55, 1-9

# 2. Species management: *Ex situ* conservation - breeding amphibians

## Author: Fey Young

**Background**
Amphibians have undergone massive declines globally, with the latest assessment indicating that 42% of known amphibian species are threatened with extinction, a higher proportion than that of mammals (26%) or birds (13%) (IUCN 2016). To combat this loss of species, the Amphibian Conservation Action Plan proposed actions to address the amphibian crisis. One of those was *ex situ* conservation breeding programmes, to act as an 'ark'. The aim was to ensure species were saved in captivity, with the goal of future reintroduction, once threats had been eliminated or reduced in the wild. This role of captive breeding remains a controversial topic in conservation (Snyder *et al.* 1996; Griffiths & Pavejeau 2008).

In order to breed amphibians successfully, an understanding of their species specific needs is important. Changes in a captive animal's environment and care regarding, for example, diet, heat or light requirements, humidity or enclosure design, can be either beneficial or detrimental to this process. Different techniques may be used in each facility with different levels of success.

Advances in artificial reproduction have been driven by both the cost of maintaining increasing numbers of genetic lines of amphibians and the difficulties faced with captive breeding of threatened species. With the exception of 2.37, the evidence for interventions in the section Artificial Reproduction has been previously summarized in the Amphibian Conservation synopsis (Smith & Sutherland 2014). Links to key messages and summaries for these interventions are included at the end of this chapter.

For a more cohesive conservation effort, it is vital that information on the effectiveness of breeding interventions are available to practitioners in a manner which is clear, concise and backed up by scientific literature.

Griffiths R.A. & Pavajeau L. (2008) Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, 22, 853-861.

IUCN (2016). The IUCN Red List of Threatened Species. Version 2016-1. <http://www.iucnredlist.org>. Downloaded on 30 June 2016.

Smith, R.K. & Sutherland, W.J. (2014) *Amphibian conservation: Global evidence for the effects of interventions.* Exeter, Pelagic Publishing.

Snyder N.F.R., Derrickson D.R., Beissinger S.R., Wiley J.W., Smith T.B., Toone W.D. & Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conservation Biology*, 10, 338–348.

## Key messages – refining techniques using less threatened species

***Identify and breed a similar species to refine husbandry techniques prior to working with target species***

Two small, replicated interlinked studies in Brazil found that working with a less threatened surrogate species of frog first to establish husbandry interventions promoted successful breeding of a critically endangered species of frog.

## Key messages – changing environmental conditions/ microclimate

***Vary enclosure humidity to simulate seasonal changes in the wild using humidifiers, foggers/ misters or artificial rain***

No evidence was captured for the effects of varying enclosure humidity to simulate seasonal changes in the wild using humidifiers, foggers/ misters or artificial rain.

***Vary enclosure temperature to simulate seasonal changes in the wild***

One small, replicated study in Italy found that one of six females bred following a drop in temperature, and filling of an egg laying pond. One replicated, before-and-after study in 2006-2012 in Australia found that providing a pre-breeding cooling period, alongside allowing females to gain weight before the breeding period, along with separating sexes during the non-breeding period, providing mate choice for females and playing recorded mating calls, increased breeding success.

***Vary quality or quantity (UV% or gradients) of enclosure lighting to simulate seasonal changes in the wild***

One replicated study in the UK found that there was no difference in clutch size between frogs given an ultraviolet (UV) boost compared with those who only received background levels. However, frogs given the UV boost had a significantly greater fungal load than frogs that were not UV-boosted.

***Vary duration of enclosure lighting to simulate seasonal changes in the wild***

No evidence was captured for the effects of varying the duration of enclosure lighting to simulate seasonal changes in the wild.

***Simulate rainfall using sound recordings of rain and/or thunderstorms***

No evidence was captured for the effects of simulating rainfall using sound recordings of rain and/or thunderstorms.

***Allow temperate amphibians to hibernate***

No evidence was captured for the effects of allowing temperate amphibians to hibernate.

***Allow amphibians from highly seasonal environments to have a period of dormancy during a simulated drought period***

No evidence was captured for the effects of allowing amphibians from highly seasonal environments to have a period of dormancy during a simulated drought period.

***Vary water flow/speed of artificial streams in enclosures for torrent breeding species***

No evidence was captured for the effects of allowing varying water flow/speed of artificial streams in enclosures for torrent breeding species.

***Provide artificial aquifers for species which breed in upwelling springs***
One small study in the USA found that salamanders bred in an aquarium fitted with an artificial aquifer.
***Vary artificial rainfall to simulate seasonal changes in the wild***
Two replicated, before-and-after studies in Germany and Austria found that simulating a wet and dry season, as well as being moved to an enclosure with more egg laying sites and flowing water in Austria, stimulated breeding and egg deposition. In Germany, no toadlets survived past 142 days old.

# Key messages – changing enclosure design for spawning or egg laying sites

***Provide multiple egg laying sites within an enclosure***
One replicated study in Australia found that frogs only bred once moved into an indoor enclosure which had various types of organic substrate, allowed temporary flooding, and enabled sex ratios to be manipulated along with playing recorded mating calls. One small, replicated, before-and-after study in Fiji found that adding rotting logs and hollow bamboo pipes to an enclosure, as well as a variety of substrates, promoted egg laying in frogs.
***Provide natural substrate for species which do not breed in water (e.g. burrowing/tunnel breeders)***
Two replicated studies in Australia and Fiji found that adding a variety of substrates to an enclosure, as well as rotting logs and hollow bamboo pipes in one case, promoted egg laying of frogs. The Australian study also temporarily flooded enclosures, manipulated sex ratios and played recorded mating calls.
***Provide particular plants as breeding areas or egg laying sites***
One small, controlled study in the USA found that salamanders bred in an aquarium heavily planted with java moss and swamp-weed.
***Provide particular enclosure furniture for calling sites, breeding areas or egg laying sites***
One replicated study in Fiji found that adding rotting logs and hollow bamboo pipes, as well as a variety of substrates to an enclosure, promoted egg laying for frogs. One before-and-after study in Austria found that captive frogs started breeding when housed in enclosures with more calling, perching and laying sites, and with simulated wet and dry seasons.

# Key messages – manipulate social conditions

***Provide visual barriers for territorial species***
No evidence was captured for the effects of providing visual barriers for territorial species.
***Manipulate adult density within the enclosure***
No evidence was captured for the effects of manipulating animal density within the enclosure.
***Manipulate sex ratio within the enclosure***
One replicated study in Australia found that frogs only bred once sex ratios were manipulated, along with playing recorded mating calls and moving frogs into an

indoor enclosure which allowed temporary flooding, and had various types of organic substrate.

***Separate sexes in non-breeding periods***

One replicated, before-and-after study in Australia found that clutch size of frogs increased when sexes were separated in the non-breeding periods, alongside providing female mate choice, playing recorded mating calls and allowing females to increase in weight before breeding.

***Play recordings of breeding calls to simulate breeding season in the wild***

One replicated study in Australia found that frogs only bred when playing recorded mating calls used, along with manipulating the sex ratio after frogs were moved into an indoor enclosure which allowed temporary flooding, used under-substrate filtration, and had various types of organic substrate. One replicated, before-and-after study in Australia found that clutch size of frogs increased when playing recorded mating calls, along with sexes being separated in the non-breeding periods, providing female mate choice, and allowing females to increase in weight before breeding.

***Allow female mate choice***

One replicated, before-and-after study in Australia found that frogs only bred when females carrying eggs were introduced to males,  recorded mating calls were played, as well as sex ratio manipulation and after frogs were moved into an indoor enclosure which allowed temporary flooding and had various types of organic substrate. One replicated, before-and-after study in Australia found that clutch size of frogs increased when female mate choice was provided, alongside playing recorded mating calls, sexes being separated in the non-breeding periods, and allowing females to increase in weight before breeding.

# Key messages – changing the diet of adults

***Vary food provision to reflect seasonal availability in the wild***

No evidence was captured for the effects of varying food provision to reflect seasonal availability in the wild.

***Formulate adult diet to reflect nutritional composition of wild foods***

No evidence was captured for the effects of formulating diet to reflect nutritional composition of wild foods.

***Supplement diets with vitamins/ calcium fed to prey (e.g. prey gut loading)***

No evidence was captured for the effects of supplementing diets with vitamins/ calcium fed to prey (e.g. prey gut loading).

***Supplement diets with vitamins/ calcium applied to food (e.g. dusting prey)***

No evidence was captured for the effects of supplementing diets with vitamins/ calcium applied to food (e.g. dusting prey)

***Supplement diets with carotenoids (including for colouration)***

One study in the USA found that adding carotenoids to fruit flies fed to frogs reduced the number of clutches, but increased the number of tadpoles and successful metamorphs.

***Increase caloric intake of females in preparation for breeding***

One replicated, before-and-after study in Australia found that clutch size of frogs increased when females were allowed to increase in weight before breeding, alongside having mate choice, recorded mating calls, and sexes separated in the non-breeding periods.

# Key messages – manipulate rearing conditions for young

***Formulate larval diet to reflect nutritional composition of wild foods***
One randomized, replicated, controlled study in the USA found that tadpoles had higher body mass and reached a more advanced developmental stage when fed a control diet (rabbit chow and fish food) or freshwater algae, compared to those fed pine or oak pollen. One randomized, replicated study in Portugal found that a diet high in protein had higher growth rates, survival and body weights at metamorphosis compared to diets containing less protein.

***Leave infertile eggs at spawn site as food for egg-eating larvae***
No evidence was captured for the effects of leaving infertile eggs at spawn site as food for egg-eating larvae.

***Manipulate humidity to improve development or survival to adulthood***
No evidence was captured for the effects of manipulate humidity to improve survival to adulthood.

***Manipulate quality and quantity of enclosure lighting to improve development or survival to adulthood***
No evidence was captured for the effects of manipulate quality and quantity of enclosure lighting to improve survival to adulthood.

***Manipulate temperature of enclosure improve development or survival to adulthood***
Two replicated studies found that tadpoles took longer to reach metamorphosis and higher survival rates when reared at lower temperatures. One replicated study found that the growth rate and development stage reached of tadpoles was higher when reared at steady rather than varied temperatures. One replicated, controlled study found that developing eggs reared within a temperature range had higher survival rates, higher growth rates and lower abnormalities.

***Manipulate larval density within the enclosure***
One randomized study in the USA found that decreasing larval density of salamanders increased larvae survival and body mass.

***Allow adults to attend their eggs***
No evidence was captured for the effects of allowing adults to attend their eggs.

# Key messages – artificial reproduction

***Use artificial cloning from frozen or fresh tissue***
No evidence was captured for the effects of using artificial cloning from frozen or fresh tissue.

# Refining techniques using less threatened species

## 2.1. Identify and breed a similar species to refine husbandry techniques prior to working with target species

- Two small, replicated interlinked studies in Brazil[1,2] found that working with a less-threatened surrogate species of frog first to establish husbandry interventions promoted successful breeding of a critically endangered species of frog.

**Background**

Captive breeding techniques and correct husbandry have only been developed for a relatively small number of amphibian species. It is sometimes necessary to trial husbandry interventions on similar species, which are less threatened in the wild, before trying to breed more challenging and higher risk species.

A small, replicated study in 2009–2010 of *Scinax perpusillus* at São Paulo Zoo, Brazil (1), carried out to develop methods for breeding *Scinax alcatrazin* (2), found that eggs were produced in captivity. Five batches of 4–77 eggs were laid in 2010 by one female. Two of three adult males died during the year. The three males, one female and six larvae were wild caught in 2009. Following five months quarantine, adults were housed at 12–27°C in a glass tank (70 x 30 x 45 cm) with a water dish and plants. Tanks were misted once or twice a day and before breeding an ultra-sonic fogger was turned on for 10 hours overnight three times a week. Management and husbandry protocols were established using this species in preparation for attempted captive breeding of *Scinax alcatrazin*.

A small, replicated study in 2011–2012 of captive *Scinax alcatrazin* at São Paulo Zoo, Brazil (2), having developed methods using *Scinax perpusillus* (1), found that eggs were produced and juveniles maintained in captivity. The first breeding event occurred after 33 days in captivity. One female deposited around 140 eggs, of which 132 hatched. By July 2012, 93 froglets were still alive. Two males and a female died on the first day in captivity. Eleven animals (five males, three females, three tadpoles) were collected from the wild in October 2011 and housed in a biosecure room. Adults were kept in two glass enclosures, with plants and water. An ultra-sonic fogger was used to increase night-time humidity to stimulate breeding. Tadpoles were housed in a plastic enclosure and froglets in plastic cups. Management and husbandry protocols had been established over two years using captive *Scinax perpusillus* (see 1).


(1) Lisboa C.S. & Vaz R.I. (2012) Captive breeding and husbandry of *Scinax perpusillus* at São Paulo Zoo: preliminary action for *ex situ* conservation of *Scinax alcatraz* (Anura: Hylidae). *Herpetological Review*, 43, 435-437.

(2) Lisboa C.S. (2012) Captive breeding and *in situ* monitoring of a critically endangered tree frog species. *Amphibian Ark Newsletter*, 20, 6-8.

## Changing environmental conditions/ microclimate

### 2.2. Vary enclosure humidity to simulate seasonal changes in the wild using humidifiers, foggers/misters or artificial rain

- No evidence was captured for the effects of varying enclosure humidity to simulate seasonal changes in the wild using humidifiers, foggers/misters or artificial rain.

**Background**

For some species, breeding is seasonal and can be triggered by longer periods of sunlight, changing temperatures or increased/decreased rainfall which can change the humidity of their environment. It may be possible to trigger breeding by simulating these 'cues' within an enclosure. Studies investigating the effect of varying artificial rainfall to simulate seasonal changes in the wild are discussed in 2.11.

### 2.3. Vary enclosure temperature to simulate seasonal changes in the wild

- One small, replicated study in Italy[1] found that one of six females bred following a drop in temperature from 20-24 to 17°C, and filling of an egg laying pond.
- One replicated, before-and-after study in Australia[2] that provided a pre-breeding cooling period, alongside allowing females to gain weight before the breeding period, separating sexes during the non-breeding period, providing mate choice for females and playing recorded mating calls, increased breeding success.

**Background**

For some species, breeding is seasonal and can be triggered by longer periods of sunlight, changing temperatures or increased/decreased rainfall. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

A small, before-and-after study in 1993 of parsley frogs *Pelodytes punctatus* at Genoa University, Italy (2) found that one of six females bred following filling an egg laying pond with water and a drop in temperature from 22 to 17°C. Immediately after the drop in temperature and filling of the pond, mating and egg laying occurred when none had occurred previously, no statistical tests were carried out. One clutch (500 eggs) was produced and hatched in a separate tank. Thirty-one tadpoles were obtained from the wild in 1993. Tadpoles were housed in a 400 L tank (20 cm water) and metamorphs in a 50 x 50 x 25 cm tank. From eight months animals were housed in a 120 x 60 x 50 cm glass breeding tank with pebbles, moss and a small pond which could be filled with water for breeding. In March 1995, the terrarium was temporarily moved to a room with a steady temperature (22°C), then to a thermostatically controlled chamber (17°C) in April, at the same time the pond was filled with water.

A replicated, before-and-after study in 2006-2012 in Victoria and New South Wales, Australia (2) reported that providing a pre-breeding cooling period, along with allowing females to gain significant weight before the breeding period, separating sexes during the non-breeding period, providing mate choice for females and playing recorded mating calls, increased clutch size and decreased egg mortality in captive southern corroboree frogs *Pseudophryne corroboree*, although no statistical tests were carried out. In 2006 Melbourne Zoo had no cooling period (average clutch size: 21; egg mortality: 95%). In 2007-2011, cooling periods of 6-9$^0$C for 31-64 days were used (average clutch size: 21; egg mortality: 85%). In 2012 a cooling period of 98-112 days at 5-12$^0$C was used (average clutch size: 46; egg mortality: 27%). At Taronga Zoo the cooling period was 5$^0$C for 56 days for 2010 (average clutch size: 20; egg mortality: 72%), 2011 (average clutch size: 12.2: egg mortality 26%) and 2012 (average clutch size: 17; egg mortality 28%).


(1) Emanueli L., Jesu R., Schimment G., Arillo A., Mamone A. & Lamagni L. (1997) Captive breeding programme of the parsley frog (*Pelodytes punctatus* Daudin, 1803) at Genoa aquarium (Italy). *Herpetologica Bonnensis*, 1997, 115-118.

(2) McFadden M., Hobbs R., Marantelli G., Harlow P., Banks C. & Hunter D. (2013) Captive management and breeding of the Critically Endangered southern corroboree frog (*Pseudophryne corroboree*) at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation,* 5, 70–87.


## 2.4. Vary quality or quantity (UV% or gradients) of enclosure lighting to simulate seasonal changes in the wild

- One replicated study in the UK[1] found that there were no significant differences in frogs given an ultraviolet (UV) boost, compared with those who only received background levels. However, frogs given the UV boost had a significantly greater fungal load in comparison to frogs that were not UV-boosted.

**Background**

For some species, breeding is seasonal and can be triggered by longer periods of sunlight. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

In captivity, metabolic bone disease can be an issue in amphibians if the correct lighting is not used. It develops as a result of an imbalance of vitamin D3, phosphorous or calcium. Amphibians cannot process calcium without vitamin D, which is activated by ultraviolet light. If the correct lighting is not provided, animals can suffer from brittle or deformed bones. Diseased animals are not likely to breed.

A replicated, controlled study in 2014 in Manchester, UK (1) found that there was no significant effect on clutch size for red-eyed tree frogs *Agaluchnis callidryas* when given ultraviolet (UV) boost treatment, in conjunction with a diet supplemented with calcium. Two clutches were laid from two females in the background UV group (number eggs laid: 118), and four clutches from seven females in the UV boost group (184). There was no statistically significant effect of UV treatment on clutch size (background UV: 59; UV boost: 74). Frogs given the UV boost had significantly more fungal colony forming units (CFU's) in comparison to frogs that were not UV-boosted (background UV: 2-3 CFU's; pre-UV boost: 2 CFU's; post-UV boost: 13 CFU's). Forty frogs were used in four treatment groups, which started three months after their metamorphosis: background UV, 5% calcium diet; background UV, 10% calcium diet; background UV with UV boost, 5% calcium diet; background UV with UV boost, 10% calcium diet. Frogs receiving the "UV boost" treatment were put under a 300-watt Osram™ Ultra Vitalux mercury vapour sun lamp suspended 40cm above the base of the tank for 20 minutes. Breeding trials were conducted immediately after the UV boost trials.

(1) Antwis R.E., Preziosi R.F. & Fidgett A.L. (2014) Effects of different UV and calcium provisioning on health and fitness traits of red-eyed tree frogs (*Agalychnis callidryas*). *Journal of Zoo and Aquarium Research*, 2, 69-76.

### 2.5. Vary duration of enclosure lighting to simulate seasonal changes in the wild

- No evidence was captured for the effects of varying duration of enclosure lighting to simulate seasonal changes in the wild.

**Background**

For some species, breeding is seasonal and may be triggered by longer periods of sunlight. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

### 2.6. Simulate rainfall using sound recordings of rain and/or thunderstorms

- No evidence was captured for the effects of simulating rainfall using sound recordings of rain and/or thunderstorms.

**Background**

For some species, breeding is seasonal and may be triggered by increased or decreased rainfall. It may be possible to trigger breeding by simulating such 'cues' within an enclosure.

### 2.7. Allow temperate amphibians to go through hibernation period

- No evidence was captured for the effects of allowing temperate amphibians to go through hibernation period.

**Background**

Hibernation is a natural process for some amphibians from colder regions, in which they burrow underground or at the bottom of ponds. They dramatically slow down their metabolic processes to conserve their energy over winter to mate in spring. Breeding is also seasonal and can be triggered by longer periods of sunlight, changing temperatures or increased/decreased rainfall. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

## 2.8. Allow amphibians from highly seasonal environments to have a period of dormancy during a simulated drought period

- No evidence was captured for the effects of allowing amphibians from highly seasonal environments to have a period of dormancy during a simulated drought period.

**Background**

Dormancy (or aestivation) is a natural process for some amphibians from warmer regions, where they move underground and dramatically slow down their metabolic processes in order to save energy in hot, dry weather. Breeding is also seasonal and can be triggered by longer periods of sunlight, changing temperatures or increased/decreased rainfall. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

## 2.9. Vary water flow/speed of artificial streams in enclosures for torrent breeding species

- No evidence was captured for the effects of allowing varying water flow/speed of artificial streams in enclosures for torrent breeding species.

**Background**

Some amphibian species live in habitats where flowing water is a constant or seasonal feature. Varying the flow rate of artificial streams or waterfalls in captivity may simulate seasonal changes in the wild which may signal the onset of breeding.

## 2.10. Provide artificial aquifers for species which breed in upwelling springs

- One small study in the USA[1] found that salamanders bred in an aquarium fitted with an artificial aquifer.

**Background**

Some amphibian species appear to breed underground in places where water flows up through rock fragments to form springs (aquifers). In captivity, artificial systems can be designed to replicate these upflows and may improve breeding success for cave-dwelling species.

A small, before-and-after study in 1990–1994 in Dallas, USA (1) found that Texas salamanders *Eurycea neotenes* bred more successfully in an aquarium with an artificial aquifer compared to without, although no statistical tests were carried out. With access to the aquifer one female deposited eggs in April 1993 (number not reported), a second female laid 40 eggs in May, and 50 in March 1994. Without access to the aquifer one female laid 19 eggs in February 1991 but no other breeding activity was observed. Prior to 1993, salamander pairs had been kept within one of three aquariums, one with gravel substrate, one heavily planted with aquatic plants, and one with partially buried rocks and rock shards. The only eggs laid were in the planted aquarium, fixed singly to live plants. In 1993, two pairs of salamanders were placed in an aquarium with a 1.2 m long acrylic tube filled with limestone shards attached to the bottom. Water was pumped up through this aquifer to replicate an upwelling spring.

(1) Roberts D.T., Schleser D.M. & Jordan T.L. (1995) Notes on the captive husbandry and reproduction of the Texas salamander *Eurycea neotenes* at the Dallas Aquarium. *Herpetological Review*, 26, 23-25.

## 2.11. Vary artificial rainfall to simulate seasonal changes in the wild

- Two replicated, before-and-after studies in Germany[1] and Austria[2] found that simulating a wet and dry season, as well as being moved to an enclosure with more egg laying sites and flowing water in Austria, stimulated breeding and egg deposition. In Germany, no toadlets survived past 142 days old.

**Background**

In some amphibian habitats, rainfall is highly seasonal with the onset of rain following a dry period signaling the start of the breeding season. It may be possible to trigger breeding by simulating these 'cues' within an enclosure.

A replicated, before-and-after study in 2008–2011 in Cologne, Germany (1) found that egg deposition of harlequin toads *Atelopus flavescens* was stimulated by intensive rainfall following a dry period, whereas none had occurred previously. However, no offspring survived past 142 days. The first three breeding trials with no wet or dry seasons resulted in no egg production. Once a wet and dry season was simulated, two clutches of eggs were produced with 400–500 eggs (5–10% unfertilized). On day 43 after egg deposition, only two larvae survived. One tadpole survived to day 112, the other died as a toadlet at day 142. Males were housed in three groups of 12–15 in tanks (100 x 60 x 60 cm) with artificial streams. Four females were transferred to the tanks for breeding. Tanks were misted several times each day. A dry season with reduced water and misting was then simulated for three months followed by a wet season with increased rainfall using an artificial rain system.

A before-and-after study in 2010-2012 in Vienna, Austria (2) found that captive *Staurois parvus* and *Staurois guttatus* started breeding when wet and dry seasons were simulated, and frogs were moved to a larger enclosure with more calling, perching and laying sites, although no statistical tests were carried out. Neither species had bred successfully in captivity before 2011. Once the animals were moved to two breeding enclosures with simulated wet and dry seasons and with more calling perching and laying sites in August 2011, *S. parvis* tadpoles were observed from October 2011 and *S. guttatus* tadpoles from March 2012. By 2012, a total of 285 *S. parvis* froglets, 600 tadpoles and 180 juveniles had been raised and 76 *S. guttatus* tadpoles had been raised. In August 2011, five pairs of each species were moved from a medium-sized (50 × 60 × 70 cm) terraria with tree branches, plants and stones and no simulated wet and dry seasons, to two larger (150 × 120 × 100 cm) enclosures with controllable waterfalls, plants, small burrows, ledges for calling sites and perching sites. The waterfall for *S. guttatus* also had several tree branches. Each enclosure had a simulated 14 day dry period, followed by a 14 day simulated rainy season (4 hours daily rainfall) with the temperature in both enclosures at 22-27°C.


(1) Gawor A., Rauhaus A., Karbe D., Van Der Straeten K, Lötters S. & Ziegler T. (2012) Is there a chance for conservation breeding? *Ex situ* management, reproduction, and early life stages of the harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). *Amphibian & Reptile Conservation*, 5, 29-44.

(2) Preininger D., Weissenbacher A., Wampula T. & Hödl W. (2012) The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. *Amphibian & Reptile Conservation*, 5, 45-56.


# Changing enclosure design for spawning or egg laying sites

## 2.12. Provide multiple egg laying sites within an enclosure

- One replicated study in Australia[1] found that frogs only bred once moved into an indoor enclosure which had various types of organic substrate, allowed temporary flooding, and enabled sex ratios to be manipulated along with playing recorded mating calls.
- One small, replicated, before-and-after study in Fiji[2,3] found that adding rotting logs and hollow bamboo pipes to an enclosure, as well as a variety of substrates, promoted egg laying in frogs.

**Background**

A choice of egg laying sites may be offered which vary in humidity, temperature, water level or protection from predation. This can often be done by using inexpensive, readily accessible natural items. Mimicking an amphibian's natural environment in captivity, to meet species specific needs, could help improve breeding success.

A replicated, before-and-after study in 1994–1996 of roseate frogs *Geocrinia rosea* at Melbourne Zoo, Australia (1) found that frogs only bred after being moved to an indoor enclosure which had a mix of organic substrates with moss, mud, bark and palm peat, temporary flooding of enclosures, manipulated sex ratios, introduced females carrying eggs to males, and played recorded mating calls. The only fertile spawning occurred in spring 1996, in a well-established burrow hidden beneath dry leaf litter and eucalyptus bark which contained 25 eggs, but they were later destroyed by fungus. From 1994-1995, two male and three sub-adult frogs were housed in two outdoor tanks (120 x 60 x 60 cm) with a sub-surface water depth 50-100mm. Males called when they were in outdoor enclosures, but fertile eggs were not produced until animals were moved to indoor tanks. From 1996, 6–7 frogs were housed in each of four indoor tanks (47 x 55 x 36 cm and 180 x 46 x 46 cm).

A small, replicated, before-and-after study in 2004-2007 in Fiji (2,3) found that the provision of egg-laying sites including rotting logs and hollow bamboo stems *Piper aduncum* and various organic substrate in an enclosure resulted in successful breeding for two pairs of Fijian ground frogs *Platymantis vitianus*. A total of 39 froglets were raised after one year. Two egg clutches were found, one in a section of bamboo stem filled with damp soil substrate, and another under a moist rotting log on a mix of soil and leaf litter. A captive breeding program had been running for this species since 2004, but only one froglet was reared after three years of trying. From 2006-2007, five male and five female frogs were placed in a purpose built outdoor enclosure.


(1) Birkett J., Vincent M. & Banks C. (1999) Captive management and rearing of the roseate frog, *Geocrinia rosea,* at Melbourne Zoo. *Herpetofauna*, 29, 49-56.

(2) Narayan E., Christi K. & Morley C. (2007) Provision of egg-laying sites for captive breeding of the endangered Fijian ground frog *Platymantis vitianus,* University of the South Pacific, Suva, Fiji. *Conservation Evidence,* 4, 61-65.

(3) Narayan E., Christi K. & Morley C. (2009) Captive propagation of the endangered native Fijian frog *Platymantis vitiana*: Implications for ex-situ conservation and management. *Pacific Conservation Biology,* 15, 47-55.


## 2.13. Provide natural substrate for species which do not breed in water (e.g. burrowing/tunnel breeders)

- Two replicated studies in Australia[1] and Fiji[2,3] found that adding a variety of substrates to an enclosure, as well as rotting logs and hollow bamboo pipes in one case[2], promoted egg laying of frogs. The Australian study also temporarily flooded enclosures, manipulated sex ratios and played recorded mating calls[1].

**Background**

Breeding may be stimulated by promoting natural breeding behaviours, such as digging or 'nest' building. By providing natural substrate, which may be present in the amphibian's wild environment, natural digging and building behaviour may be triggered, leading to breeding.

A replicated, before-and-after study in 1994–1996 of roseate frogs *Geocrinia rosea* at Melbourne Zoo, Australia (1) found that frogs only bred after being moved to an indoor enclosure which had a mix of organic substrates, temporary flooding of enclosures, manipulated sex ratios, introducing females carrying eggs to males, and recorded mating calls being played. The only fertile spawning occurred in spring 1996, in a well-established burrow hidden beneath dry leaf litter and eucalyptus bark which contained 25 eggs, but they were later destroyed by fungus. From 1994-1995 , two male and three sub-adult frogs were housed in two outdoor tanks (120 x 60 x 60 cm) with a sub-surface water depth 50-100mm. Males called when they were in outdoor enclosures, but fertile eggs were not produced until animals were moved to indoor tanks. From 1996, 6–7 frogs were housed in each of four indoor tanks (47 x 55 x 36 cm and 180 x 46 x 46 cm) with moss, mud, bark and palm peat as substrates.

A small, replicated, before-and-after study in 2004-2007 in Fiji (2,3) found that the provision of egg-laying sites including various organic substrates, rotting logs and hollow bamboo stems *Piper aduncum* in an enclosure resulted in successful breeding for two pairs of Fijian ground frogs *Platymantis vitianus*. A total of 39 froglets were raised after one year. Two egg clutches were found, one in a section of bamboo stem filled with damp soil substrate, and another under a moist rotting log on a mix of soil and leaf litter. A captive breeding program had been running for this species since 2004, but only one froglet was reared after three years of trying. From 2006-2007, five male and five female frogs were placed in a purpose built outdoor enclosure.

(1) Birkett J, Vincent M. & Banks C. (1999) Captive management and rearing of the roseate frog, *Geocrinia rosea*, at Melbourne Zoo. *Herpetofauna*, 29, 49-56.
(2) Narayan E, Christi K. & Morley C. (2007) Provision of egg-laying sites for captive breeding of the endangered Fijian ground frog *Platymantis vitianus*, University of the South Pacific, Suva, Fiji. *Conservation Evidence*, 4, 61-65.
(3) Narayan E, Christi K. & Morley C. (2009) Captive propagation of the endangered native Fijian frog *Platymantis vitiana*: Implications for ex-situ conservation and management. *Pacific Conservation Biology*, 15, 47–55.

## 2.14. Provide particular plants as breeding areas or egg laying sites

- One small, controlled study in the USA[1] found that salamanders bred in an aquarium heavily planted with java moss and swamp-weed.

**Background**

Some amphibian species require particular types of plant to complete their natural breeding cycle. This may be for courtship, eggs laying or larval development. Mimicking an amphibian's natural environment by providing these or similiar plants in captivity may improve breeding success.

A small, controlled study in 1990–1994 of Texas salamanders *Eurycea neotenes* in Dallas, USA (1) found that captive breeding was successful in a heavily planted aquarium but not in aquariums without plants. Between 1990 and 1993, a pair of salamanders housed in a planted aquarium produced 19 eggs (in February 1991) but none were produced by salamander pairs housed in an aquarium containing a gravel substrate or one with partially buried rocks and rock shards over the same period. The eggs were fixed singly to live plants. Four of the eggs hatched and three larvae were reared to maturity. The planted aquarium contained java moss *Vesicularia dubyana* and swamp-weed *Hygrophila* sp.. Eggs were removed to a separate aquarium to avoid predation by the adult salamanders or snails.

(1) Roberts D.T, Schleser D.M. & Jordan T.L. (1995) Notes on the captive husbandry and reproduction of the Texas salamander *Eurycea neotenes* at the Dallas Aquarium. *Herpetological Review,* 26, 23-25.

## 2.15. Provide particular enclosure furniture for calling sites, breeding areas or egg laying sites

- One replicated study in Fiji[1,2] found that adding rotting logs and hollow bamboo pipes, as well as a variety of substrates to an enclosure, promoted egg laying in frogs.
- One before-and-after study in Austria3 found that captive frogs started breeding when animals were housed in enclosures with more calling, perching and laying sites, as well as simulated wet and dry seasons.

**Background**

Specific habitat features may be critical to amphibian breeding by offering sites for calling, courtship or egg laying. Mimicking an amphibian's natural environment in captivity by providing these habitat features or artificial alternatives could improve breeding success.

A small, replicated, before-and-after study in 2004-2007 in Fiji (1,2) found that the provision of egg-laying sites including rotting logs and hollow bamboo stems *Piper aduncum* and various organic substrate in an enclosure resulted in successful breeding for two pairs of Fijian ground frogs *Platymantis vitianus*. Two egg clutches were found, one in a section of bamboo stem filled with damp soil substrate, and another under a moist rotting log on a mix of soil and leaf litter. A total of 39 froglets were raised after one year. A captive breeding program had been running for this species since 2004, but only one froglet was reared after three years of trying. From 2006-2007, five male and five female frogs were placed in a purpose built outdoor enclosure.

A before-and-after study in 2010-2012 in Vienna, Austria (3) found that captive *Staurois parvus* and *Staurois guttatus* started breeding when animals were housed in terraria with more calling, perching and laying sites, and with simulated wet and dry seasons, although no statistical tests were carried out. Neither species bred successfully in captivity before 2011. Once animals were moved to breeding arenas with a more complex habitat in August 2011, *S. parvis* tadpoles were observed from October 2011 and *S. guttatus* tadpoles from March 2012. By 2012, a total of 285 *S. parvis* froglets, 600 tadpoles and 180 juveniles had been raised and 76 *S. guttatus* tadpoles had been raised. In August 2011, five pairs of each species were moved from a medium-sized (50 × 60 × 70 cm) terraria with tree branches, plants and stones and no simulated wet and dry seasons, to larger (150 × 120 × 100 cm) enclosures with controllable waterfalls, plants, small burrows, ledges for calling sites and perching sites, and simulated wet and dry seasons. The waterfall for *S. guttatus* also had several tree branches.


(1) Narayan E, Christi K. & Morley C. (2007) Provision of egg-laying sites for captive breeding of the endangered Fijian ground frog *Platymantis vitianus,* University of the South Pacific, Suva, Fiji. *Conservation Evidence,* 4, 61-68.

(2) Narayan E, Christi K. & Morley C. (2009) Captive propagation of the endangered nativeFijian frog *Platymantis vitiana*: Implications for ex-situ conservation and management. *Pacific Conservation Biology,* 15, 47–55.

(3) Preininger D., Weissenbacher A., Wampula T. & Hödl W. (2012) The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. *Amphibian & Reptile Conservation,* 5, 45-56.


## Manipulate social conditions

### 2.16. Provide visual barriers for territorial species

- No evidence was captured for the effects of visual barriers for territorial species.

**Background**

A captive breeding setup may house multiple pairs of amphibians. In territorial species this could potentially cause aggression between individuals which negatively impacts breeding success. By providing barriers which allow individuals or pairs to avoid visual contact with each other, this aggression may be reduced or eliminated.

### 2.17. Manipulate adult density within the enclosure

- No evidence was captured for the effects of manipulating animal density within the enclosure.

**Background**

By manipulating animal densities, particularly around the breeding season, aggressive behaviour could be minimised. Also, eggs are less likely to be damaged if fewer adults are kept in the same enclosure. Alternatively, some species may require higher densities to trigger breeding.

### 2.18. Manipulate sex ratio within the enclosure

- One replicated study in Australia[1] found that frogs only bred once sex ratios were manipulated, along with playing recorded mating calls and moving frogs into an indoor enclosure which allowed temporary flooding, and had various types of organic substrate.

**Background**

By manipulating sex ratios, increased competition between males for a mate could stimulate more breeding calls or mating. However, males from more aggressive species may need to be separated during this time to minimise injury to tank mates, eggs or larvae.

A replicated, before-and-after study in 1994–1996 of roseate frogs *Geocrinia rosea* at Melbourne Zoo, Australia (1) found that fertile eggs were only laid after sex ratios were manipulated, females carrying eggs were introduced to males, recorded mating calls were played, and frogs had been moved to an indoor enclosure with trickle water filtration, as well as a mix of organic substrate, temporary flooding of enclosures. The only fertile spawning occurred in spring 1996, which contained 25 eggs, but they were destroyed by fungus. From 1994-1995 , two male and three sub-adult frogs were housed in two outdoor tanks (120 x 60 x 60 cm) with a sub-surface water depth 50-100mm. Males called when they were in outdoor enclosures, but fertile eggs were not produced until animals were moved to indoor tanks. From 1996, 6–7 frogs were housed in each of the five indoor enclosures.

(1) Birkett J., Vincent M. & Banks C. (1999) Captive management and rearing of the roseate frog, *Geocrinia rosea*, at Melbourne Zoo. *Herpetofauna*, 29, 49-56.

## 2.19. Separate sexes in non-breeding periods

- One replicated, before-and-after study in Australia[1] found that clutch size of frogs increased when sexes were separated in the non-breeding periods, alongside providing female mate choice, playing recorded mating calls and allowing females to increase in weight before breeding.

**Background**

Many species of amphibian lead largely solitary lives during non-breeding periods in the wild but may be permanently housed in mixed-sex groups in captivity. In some species, burrows or nests are constructed before the breeding season. Separating the sexes outside of the breeding season allows the males or females time to construct these burrows. The timing of introducing the females to the males alongside could promote breeding.

A replicated, before-and-after study in 2006-2012 in Victoria and New South Wales, Australia (1) reported that providing a pre-breeding cooling period, along with allowing females to gain significant weight before the breeding period, separating sexes during the non-breeding period, providing mate choice for females and playing recorded mating calls, increased clutch size and decreased egg mortality in captive southern corroboree frogs *Pseudophryne corroboree*, although no statistical tests were carried out. From 2006-2010 Melbourne Zoo did not separate sexes in the non-breeding period (average clutch size: 18; egg mortality: 89%). In 2011-2012, sexes were separated in the non-breeding period (average clutch size: 43; egg mortality: 89%). At Taronga Zoo sexes were always kept separate in the non-breeding periods in 2010 (average clutch size: 80; egg mortality: 72%), 2011(average clutch size: 70; egg mortality: 26%) and 2012(average clutch size: 54; egg mortality: 28%).

(1) McFadden M., Hobbs R., Marantelli G., Harlow P., Banks C. & Hunter D. (2013) Captive management and breeding of the Critically Endangered southern corroboree frog (*Pseudophryne corroboree*) [Moore 1953] at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation,* 5, 70–87.

## 2.20. Play recordings of breeding calls to simulate breeding season in the wild

- One replicated study in Australia[1] found that frogs only bred when recorded mating calls were played, as well as manipulating the sex ratio after frogs were moved into an indoor enclosure which allowed temporary flooding, and had various types of organic substrate.
- One replicated, before-and-after study in Australia[2] found that clutch size of frogs increased when playing recorded mating calls, along with the sexes being separated in the non-breeding periods, providing female mate choice, and allowing females to increase in weight before breeding.

**Background**

Calling activity by males is usually prominent in the breeding season, and plays an important part in mate selection. Playback of recorded breeding calls may stimulate breeding, by increasing competition between males.

A replicated, before-and-after study in 1994–1996 of roseate frogs *Geocrinia rosea* at Melbourne Zoo, Australia (1) found that fertile eggs were only laid after recorded mating calls were played, sex ratios were manipulated, females carrying eggs were introduced to males, and frogs had been moved to an indoor enclosure which allowed temporary flooding and had a mix of organic substrates. The only fertile spawning occurred in spring 1996, which contained 25 eggs, but they were destroyed by fungus. From 1994-1995 , two male and three sub-adult frogs were housed in two outdoor tanks (120 x 60 x 60 cm) with a sub-surface water depth 50-100mm. Males called when they were in outdoor enclosures, but fertile eggs were not produced until animals were moved to indoor tanks. From 1996, 6–7 frogs were housed in each of the five indoor enclosures.

A before-and-after study in 2006–2012 in Victoria and New South Wales, Australia (2) found that husbandry interventions such as providing playing recorded mating calls, separating sexes during the non-breeding period, along allowing females to gain significant weight before the breeding period, and providing mate choice for females increased clutch size and decreased egg mortality in captive southern corroboree frogs *Pseudophryne corroboree*, although no statistical tests were carried out. At Melbourne Zoo from 2006–2009 no recorded mating calls were played, (average clutch size: 17; egg mortality: 91%). A recorded tape of mating calls was installed and played in 2010 (average clutch size: 20; egg mortality: 78%), 2011 (average clutch size: 40; egg mortality:

70%) and in 2012 (average clutch size: 46; egg mortality: 27%). At Taronga Zoo a recorded tape of mating calls was installed and played in 2010 (average clutch size: 80; egg mortality: 72%). In 2011, recorded mating calls were not used (average clutch size: 70; egg mortality: 25.5%). In 2012, recorded mating calls were played (average clutch size: 54; egg mortality: 28%). The tape was played for 15 minutes of each hour from 1800 to 2200 hours and was audible to the frogs in all tanks.


(1) Birkett J., Vincent M. & Banks C. (1999) Captive management and rearing of the roseate frog, *Geocrinia rosea,* at Melbourne Zoo. *Herpetofauna*, 29, 49-56.
(2) McFadden M., Hobbs R., Marantelli G., Harlow P., Banks C. & Hunter D. (2013) Captive management and breeding of the Critically Endangered southern corroboree frog (*Pseudophryne corroboree*) [Moore 1953] at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation* 5, 70–87.


## 2.21. Allow female mate choice

- One replicated study in Australia[1] found that frogs only bred after females carrying eggs were introduced to males, sex ratios were manipulated, enclosures were temporarily flooded, recorded mating calls were played, and after being moved to an indoor enclosure which used under-substrate filtration and various types of substrate.
- One replicated, before-and-after study in Australia[2] found that clutch size of frogs increased when female mate choice was provided, alongside playing recorded mating calls, sexes being separated in the non-breeding periods, and allowing females to increase in weight before breeding.

**Background**

Female mate choice has been observed in captivity, with possible factors that are selected including call frequency, territory size or type and body size. By providing multiple males or moving females around enclosures, choice and the probability of compatibility is increased.

A replicated, before-and-after study in 1994–1996 of roseate frogs *Geocrinia rosea* at Melbourne Zoo, Australia (1) found that fertile eggs were only laid after females carrying eggs were introduced to males, recorded mating calls were played, sex ratios were manipulated, and frogs had been moved to an indoor enclosure with trickle water filtration, as well as a mix of organic substrate, temporary flooding of enclosures. The only fertile spawning occurred in spring 1996, which contained 25 eggs, but they were destroyed by fungus. From 1994-1995 , two male and three sub-adult frogs were housed in two outdoor tanks (120 x 60 x 60 cm) with a sub-surface water depth 50-100mm. Males called when they were in outdoor enclosures, but fertile eggs were not produced until animals were moved to indoor tanks. From 1996, 6–7 frogs were housed in each of the five indoor enclosures.

A replicated, before-and-after study in 2006–2012 in Victoria and New South Wales, Australia (2) found that husbandry interventions such as introducing females carrying eggs to males, allowing female mate choice, separating sexes during the non-breeding period, allowing females to gain significant weight before the breeding period, and playing recorded mating calls, increased clutch size and decreased egg mortality in captive southern corroboree frogs *Pseudophryne corroboree,* although no statistical tests were carried out. At Melbourne Zoo from 2006–2009 females were kept in the same breeding enclosure for the season and not moved (average clutch size: 17; egg mortality: 91%). In 2010, females were moved between two breeding enclosures (average clutch size: 20; egg mortality: 78%). In 2011, no female moves are reported, (average clutch size: 40; egg mortality: 70%). In 2012, females were moved between breeding tanks (average clutch size: 46; egg mortality: 27%). At Taronga Zoo in 2010, the three largest females were moved into other breeding tanks mid-season (average clutch size: 80; egg mortality: 72%). Movement of females is not reported for 2011 (average clutch size: 70; egg mortality: 25.5%), or 2012 (average clutch size: 54; egg mortality: 28%).

(1) Birkett J., Vincent M. & Banks C. (1999) Captive management and rearing of the roseate frog, *Geocrinia rosea,* at Melbourne Zoo. *Herpetofauna*, 29, 49-56.
(2) McFadden M., Hobbs R., Marantelli G., Harlow P., Banks C. & Hunter D. (2013) Captive management and breeding of the Critically Endangered southern corroboree frog (*Pseudophryne corroboree*) [Moore 1953] at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation*, 5, 70–87.

## Changing the diet of adults

### 2.22. Vary food provision to reflect seasonal availability in the wild

- No evidence was captured for the effects of varying food provision to reflect seasonal availability in the wild.

**Background**

Food availability may vary considerably for wild amphibians across seasons with consequences for body condition and therefore reproduction. By reflecting this in captivity, amphibians could be ‘cued’ to commence breeding.

### 2.23. Formulate adult diet to reflect nutritional composition of wild foods

- No evidence was captured for the effects of formulating diet to reflect nutritional composition of wild foods.

**Background**

A diet similar to that of wild amphibians may be beneficial in captive scenarios; providing vitamins and nutrients while stimulating natural feeding behaviour.

### 2.24. Supplement diets with vitamins/ calcium fed to prey (e.g. prey gut loading)

- No evidence was captured for the effects of supplementing diets with vitamins/ calcium fed to prey (e.g. prey gut loading).

**Background**

In captivity, amphibian diets are often dramatically simplified, and prey items are often those that can be bred cheaply and efficiently in captivity. By feeding prey a diet rich with vitamins or nutrients (gut loading) before they are fed to amphibians, nutritional deficiencies could be avoided.

In captivity, metabolic bone disease can be an issue in amphibians. It develops as a result of an imbalance of vitamin D3, phosphorous and calcium. Amphibians cannot process calcium without vitamin D, which is activated by ultraviolet light. Health problems may affect reproduction in captive animals.

### 2.25. Supplement diets with vitamins/ calcium applied to food (e.g. dusting prey)

- No evidence was captured for the effects of supplementing diets with vitamins/ calcium applied to food (e.g. dusting prey)

**Background**

In captivity, amphibian diets are often dramatically simplified, and prey items are often those that can be bred cheaply and efficiently in captivity. By dusting prey with vitamins or nutrients, nutritional deficiencies could be avoided.

In captivity, metabolic bone disease can be an issue in amphibians. It develops as a result of an imbalance of vitamin D3, phosphorous and calcium. Amphibians cannot process calcium without vitamin D, which is activated by ultraviolet light. Health problems may affect reproduction in captive animals.

### 2.26. Supplement diets with carotenoids (including for colouration)

- One study in the USA[1] found that adding carotenoids to fruit flies fed to frogs reduced the number of clutches, but increased the number of tadpoles and successful metamorphs.

**Background**

In captivity, amphibian diets are often dramatically simplified, and prey items are often those that can be bred cheaply and efficiently in captivity. By providing amphibians with prey that have been in reared on a substrate dusted with carotenoids, which may not naturally be present in the prey, nutritional deficiencies could be avoided. Carotenoids are found in the yolk of eggs, and are thought to improve neonate health.

A replicated, before-and-after study in 2009-2012 in Louisiana, USA (1) found that covering the rearing area (media) of prey fruit fly with carotenoid supplements increased the rate at which tadpoles successfully completed metamorphosis when fed to Strawberry poison frogs *Oophaga pumilio* compared to a diet of flies not supplemented with carotenoids. Pairs produced fewer clutches on the supplemented diet (average per pair: 14) compared to the unsupplemented diet (8), however, they produced more tadpoles (unsupplemented: 3; supplemented: 6) and more metamorphs (unsupplemented: 1; supplemented: 3). From August 2009 to January 2011 fruit flies were reared in conditions similar to commercially reared flies, from February 2011 to November 2012 the rearing media was augmented with carotenoid supplements Red phaffia yeast, powdered marine algae and Spirulina. The fruit flies were then fed to 50 male and 52 female poison frogs.

(1) Dugas M.B., Yeager J. & Richards-Zawaki C.L. (2013) Carotenoid supplementation enhances reproductive success in captive strawberry poison frogs (*Oophaga Pumilio*). *Zoo Biology*, 32, 655-658.

## 2.27. Increase caloric intake of females in preparation for breeding

- One replicated, before-and-after study in Australia[1] found that clutch size of frogs increased when females increased in weight before breeding, as well as having mate choice, recorded mating calls, and sexes being separated in the non-breeding periods.

**Background**

In the wild, females are thought to consume large amounts of prey prior to the breeding season and during seasonal increases of prey availability. This may act as a breeding 'cue', and help the female meet increased energy demands of the breeding season and egg laying.

A before-and-after study in 2009–2012 in New South Wales, Australia (1) reported that allowing female captive southern corroboree frogs *Pseudophryne corroboree* to gain significant weight before the breeding period, along with separating sexes during the non-breeding period, providing mate choice for females and playing recorded mating calls increased clutch size and decreased egg mortality, although no statistical tests were carried out. At Melbourne Zoo from 2009 to 2010 females were fed a normal diet before the breeding season, average female weight was 2.8g (range: 1.8–3.7g) and average clutch size was 17–20/female, with 70–92% egg mortality. In 2011, females were fed more intensively for a further 16 days after the overwintering period, before being introduced to the males. The average female mass was 3.4g (range 2.7–4.0g) and clutch size was 40 with 70% egg mortality. In 2012, females were again separated from the males to be fed more intensively for 14 days. The average female weight was 3.6g (range: 2.9–4.6g) and average clutch size was 46, with 27% egg mortality.

(1) McFadden M., Hobbs R., Marantelli G., Harlow P., Banks C. & Hunter D. (2013) Captive management and breeding of the Critically Endangered southern corroboree frog (*Pseudophryne corroboree*) [Moore 1953] at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation*, 5, 70–87.

# Manipulate rearing conditions for young

## 2.28. Formulate larval diets to improve development or survival to adulthood

- One randomized, replicated, controlled study in the USA[1] found that tadpoles had a higher body mass and reached a more advanced developmental stage when fed a control diet (rabbit chow and fish food) or freshwater algae, compared to those fed pine or oak pollen. Tadpoles fed only pine or oak pollen did not undergo metamorphosis.
- One randomized, replicated study in Portugal[2] found that tadpoles reared on a diet containing 46% protein had higher growth rates, survival and body weights at metamorphosis compared to diets containing less protein.

**Background**

In captivity, amphibian diets are often dramatically simplified. By mimicking a wild diet, nutritional deficiencies could be avoided.

A randomized, replicated, controlled study in 1996 in Tennessee, USA (1) found that tadpoles of upland chorus frogs *Pseudacris triseriata feriarum* had a higher body mass and reached a more advanced developmental stage when fed a control diet (rabbit chow and fish food) or freshwater algae, compared to those fed pine or oak pollen. Tadpoles fed only pine or oak pollen showed similar survival to the end of the experiment to those fed control or algae diets (at least 48 of 50 tadpoles survived per diet). However, tadpoles on the pine and oak pollen diets were unable to undergo metamorphosis. Average body mass was significantly higher at Day 18 and 27 for control diet and freshwater algae compared to pine or oak pollen (data reported as statistical model results). The development stage (Gosner scale) reached at Day 36 also differed: control diet (stage 40), freshwater algae (stage 38), oak pollen (stage 28) and pine pollen (stage 27). The control diet consisted of Purina rabbit chow pellets and flaked fish food (ratio 3:1). Pine and oak pollen are found naturally in the temporary ponds in which upland chorus frogs breed. Ten tadpoles were randomly assigned to each of twenty containers for five replicates of each diet. An additional 12 feeding treatments were carried out to test the effect of presenting the different diets sequentially over four nine-day periods.

A randomized, replicated study in 2009-2013 in Portugal (2) found that rearing natterjack toad *Epidalea calamita* tadpoles on a diet containing 46%

protein had higher survival, increase in body weight over time, and body weights at onset of metamorphosis compared to diets containing less protein. Tadpoles fed the highest protein content diet had significantly greater survival (46% protein: 19 of 25; 38% protein: 10 of 25; 32% protein: 15 of 25), increase in body weight (46% protein: 677%; 38% protein: 564%; 32% protein: 461%), and body weight at metamorphosis (46% protein: 0.10g; 38% protein: 0.09g; 32% protein: 0.09g). Tadpoles on the high protein diet also had significantly longer bodies and shorter tail fins (mean values not reported). Metamorphs on the high protein diet had significantly longer bodies, wider heads and wider hind legs (mean values not reported). However, there was no significant difference in tadpole total length (mean values not reported) or metamorph body weight (46% protein: 0.08g; 38% protein: 0.07g; 32% protein: 0.07g). Individually housed tadpoles were randomly allocated to three groups of 25 each and fed commercial diets with 46%, 38% or 32% protein content. A randomized block design was used with one replicate of each dietary treatment within every block.


(1) Britson C.A. & Kissel R.E (1996) Effects of food type on developmental characteristics of an ephemeral pond-breeding Anuran, *Pseudacris triseriata feriarum. Herpetologica,* 52, 3, 374-382.

(2) Martins F.M.S, Oom M.M, Rebelo R. & Rosa G.M (2013) Differential effects of dietary protein on early life-history and morphological traits in natterjack toad (*Epidalea calamita*) tadpoles reared in captivity. *Zoo Biology,* 32, 457- 462.


## 2.29. Leave infertile eggs at spawn site as food for egg-eating larvae

- No evidence was captured for the effects of leaving infertile eggs at spawn site as food for egg-eating larvae.

**Background**

Some species of amphibian feed on infertile eggs in their first weeks of life at the spawn site. By leaving these eggs in the enclosure, some tadpoles may flourish. By mimicking a diet tadpoles may experience in the wild, nutritional deficiencies could be avoided.

## 2.30. Manipulate humidity to improve development or survival to adulthood

- No evidence was captured for the effects of manipulating humidity to improve development or survival to adulthood.

**Background**

Amphibian larvae may be more sensitive to dry conditions than adults, due to decreased mobility affecting access to other areas. By finding a humidity level which is suitable, tadpole survival may increase.

## 2.31. Manipulate quality and quantity of enclosure lighting to improve development or survival to adulthood

- No evidence was captured for the effects of manipulating quality and quantity of lighting to improve development or survival to adulthood.

**Background**

By providing adequate light levels during the development period of amphibians, skeletal deformities, which result from metabolic bone disease, could be avoided, increasing survival and improving health.

## 2.32. Manipulate temperature of enclosure to improve development or survival to adulthood

- One replicated study in Spain[1] found that salamander larvae had higher survival rates when reared at lower temperatures.
- One replicated study in Germany[2] found that the growth rate and development stage reached by harlequin toad tadpoles was faster at a higher constant temperature rather than a lower and varied water temperature. One replicated study in Australia[3] found that frog tadpoles took longer to reach metamorphosis when reared at lower temperatures.
- One replicated, controlled study in Iran[4] found that developing eggs reared within a temperature range of 12-25°C had higher survival rates, higher growth rates and lower abnormalities than those raised outside of that range.

**Background**

Amphibian larvae may be more sensitive to hot or cold conditions than adults, due to decreased mobility affecting access to other areas. By finding a temperature range which is suitable, tadpole development may improve or survival may increase.

A replicated study in 1994-2004 in Spain (1) found that larvae of fire salamanders *Salamandra salamandra* had higher survival rates with a low temperature and food three times per week compared to a higher temperature and food three times or once per week. No larvae on a high temperature-low food frequency treatment survived to metamorphosis. Larvae on high temperature-high food frequency diets had a significantly lower survival (72%) than those on low temperature-high food frequency treatment (98%) and low temperature-low food frequency treatment (98%). Recently born larvae were kept at one of two temperature treatments, 20–25°C (high temperature) or 15–18°C (low temperature) under a 12h:12h light-dark regime. Larvae were fed to near satiation either once a week (low food frequency) or three times a week (high food frequency) with frozen chironomid worms. This schedule defined four treatments: high temperature-high food, high temperature-low food, low temperature- high food, and low temperature-low food.

A replicated study in 2008–2011 in Cologne, Germany (2) found that the growth rate and development stage reached by harlequin toad *Atelopus flavescens* larvae was faster at a higher constant temperature rather than a lower and varied water temperature, although no statistical tests were carried out. Tadpoles kept at a steady temperature of 24°C reached development stage (Gosner scale) 41 (which includes increases in length, mouthpart development and disappearance of the tail) in 100 days. Those kept at varying temperatures of 22-24°C took longer to reach the same stage (106-130 days). Delayed metamorphosis may be beneficial or detrimental to survival depending on the relative risk associated with different habitats. The tadpoles were from two reproduction events in December 2010 and January 2011.

A replicated study in 1998 of captive great barred frogs *Mixophyes fasciolatus* at Melbourne Zoo, Australia (3) found that tadpoles took longer to reach metamorphosis when kept at lower temperatures. Lower temperatures resulted in later metamorphosis (16-20°C: 120–132 days; 18-22°C- 80 days; 19–22°C: 99–108), although no statistical tests were carried out. Delayed metamorphosis may be beneficial or detrimental to survival depending on the relative risk associated with different habitats. Five groups of 75 tadpoles were housed in five separate tanks, two groups kept at kept at 19-22°C, two groups kept at 16-20°C, and one group kept at 18-22°C.

A replicated study in 2014 in Shiraz, Iran (4) found that developing eggs of green frog *Bufotes viridis* had higher survival rates, higher growth rates and lower abnormalities when reared in temperatures from 12 to 25°C, than those reared outside this range. Survival at different temperatures was as follows: 0°C = 50%, 2°C = 60%, 8°C = 75%, 12-25°C = 95-100%, 30°C = 75%, 33°C = 65%, 37°C = 50%, 40°C = 0%. Embryos developing at different temperatures (8, 15, 18, 25, and 30°C) showed a significant difference in the time required to reach development stage 25. At 8°C, eggs had developed to stage 25 after 280 hours, but after only 52 hours at 30°C. In a separate experiment, eggs reared at 0°C and 40°C exhibited no development and no embryos survived. A thermostat-controlled aquarium housed 18 chambers, starting with 150 eggs each, across a temperature gradient with a heater at one end and a chilling unit at the other.


(1) Alcobendas M., Buckley D. & Tejedo M. (2004) Variability in survival, growth and metamorphosis in the larval fire salamander (*Salamandra salamandra*): Effects of larval birth size, sibship and environment. *Herpetologica*, 60, 232-245.

(2) Gawor A., Rauhaus A., Karbe D., Van Der Straeten K., Lötters S. & Ziegler T. (2012) Is there a chance for conservation breeding? Ex situ management, reproduction, and early life stages of the harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). *Amphibian & Reptile Conservation*, 5, 29-44.

(3) Banks C., Birkett J., Young S., Vincent M. & Hawkes T. (2003) Breeding and management of the great barred frog, *Mixophyes fasciolatus*, at Melbourne Zoo. *Herpetofauna*, 33, 2-12.

(4) Derakhshan Z. & Nokhbatolfoghahai M. (2015) Thermal tolerance limits and effects of temperature on the growth and development of the green toad, *Bufotes viridis. Salamandra,* 51, 129-136.


## 2.33. Manipulate larval density within the enclosure

- A replicated study in the USA[1] found that reducing larval density of spotted salamanders increased larval survival and body mass.

**Background**

Some species of amphibian larvae are known to cannibalize other individuals in the enclosure if animal density is too high. It is also thought that body mass could be decreased when larvae are too densely housed.

A replicated study in 2010 of spotted salamanders *Ambystoma maculatum* in the USA (1) found that housing larvae at low densities resulted in bigger salamanders, higher survival and lower stress levels, similar to larvae in the wild. At different larval densities there were significant differences in body mass (6/tank: 1.8 g; 12/tank: 1.6 g; 30/tank: 0.9 g), survival (94%; 67%; 33% respectively) and stress levels (white blood cell ratios: 0.4; 1.5; 2.2 respectively). At medium larval densities, increased food or habitat complexity had no significant effect on body mass (food: 1.4 g; environment: 1.7 g), survival (89%; 50% respectively), or stress levels (1.3; 0.7 respectively). Egg masses were collected from the wild. Larvae were reared in three replicates of five treatments: starting densities of six, 12 or 30 larvae/1,000 l tank, increased food (12 larvae/tank with triple the zooplankton) or increased habitat complexity (tank filled with sticks and refugia). All tanks had leaf litter on the bottom. Metamorphs were weighed and blood sampled for stress hormone levels.

(1) Davis A.K. (2012) Investigating the optimal rearing strategy for *Ambystoma* salamanders using hematological stress index. *Herpetological Conservation and Biology,* 7, 1, 95-100.

### 2.34. Allow adults to attend their eggs

- No evidence was captured for the effects of allowing adults to attend their eggs.

**Background**

Some amphibian parents defend their clutches, carry them on their backs or directly nurse their young (caecilians). By providing the right conditions for these species to carry out natural parenting behaviour it may be possible to increase tadpole survival.

## Artificial reproduction

### 2.35. Use hormone treatment to induce sperm and egg release

For summarised evidence see Smith, R.K. and Sutherland, W.J. (2014) *Amphibian conservation: Global evidence for the effects of interventions*. Exeter, Pelagic Publishing.

Key messages and summaries are available here:

http://www.conservationevidence.com/actions/883

### 2.36. Use artificial fertilization in captive breeding

For summarised evidence see Smith, R.K. and Sutherland, W.J. (2014) *Amphibian conservation: Global evidence for the effects of interventions*. Exeter, Pelagic Publishing.

Key messages and summaries are available here:

http://www.conservationevidence.com/actions/834

### 2.37. Use artificial cloning from frozen or fresh tissue

- No evidence was captured for the effects of using artificial cloning from frozen or fresh tissue.

**Background**

Conservation breeding programmes are being used more frequently for threatened amphibian species. However, captive breeding often results in loss of genetic variation. This can mean that animals that were bred for release back in to the wild have reduced fitness. Freezing, or 'cryopreservation', of sperm and eggs or tissue, allows them to be stored until they are needed. Gene banks can therefore be created for amphibians ensuring that species' genetic variation is preserved. It also means that the number of a particular species needed in captivity can be reduced and genes can be swapped between captive facilities. Cloning is the process whereby organisms are artificially produced with identical genetic material to the single ancestor from which a tissue sample has been preserved. This technique could be used to produce clones of individuals from threatened or even extinct amphibian species for use in captive breeding or reintroduction projects.

### 2.38. Freeze sperm or eggs for future use

For summarised evidence see Smith, R.K. and Sutherland, W.J. (2014) *Amphibian conservation: Global evidence for the effects of interventions*. Exeter, Pelagic Publishing.

Key messages and summaries are available here:

http://www.conservationevidence.com/actions/876

# 3. Species management: Ex-Situ conservation – Interventions in reference to feeding captive carnivores to improve health and welfare.

## Author: Lydia Timbrell

**Background**

Carnivores in the wild hunt or forage for their survival. In captivity, the ability to express such behaviours may be lost over time, and sometimes abnormal behaviours become prevalent, altering their natural behavioural repertoire and affecting their health and welfare (Bashaw *et al.* 2003). Adequate captive environments along with an appropriate husbandry routine can encourage foraging or hunting behaviours as well as reduce abnormal behaviours such as stereotypical pacing. Not only this, the loss of innate behaviours could reduce conservation efforts made by zoos, where the possibility to eventually release captive species back into their natural environment needs to be maintained. For example, wild cheetahs (*Acyninox jubatus*) chase small antelope and other prey at high speeds and consume skin, bones and cartilage. There is increased recognition that such parts of the carcass have an important role for strict carnivores (Depauw *et al.* 2012). In captivity, cheetahs are regularly fed a commercial or meat only diet, which is presented in bowls or thrown into the enclosure, limiting both the behavioural repertoire as well as the fulfilment of dietary requirements. Separately or combined, these limitations could have a detrimental effect on the health and welfare of the animal. Therefore, efforts should be made by zoos to encourage the use of feeding interventions that promote positive behavioural and physiological functions in captivity as shown by animals in the wild.

Bashaw, M.J, Bloomsmith, M.A., Marr, M.J., Maple, T.L. 2003. To Hunt or not to hunt? A feeding enrichment experiment with captive large felids. *Zoo Biology*, 22, 189-198.

Depauw S., Bosch G., Hesta M., Whitehouse-Tedd K., Hendriks W.H., Kaandorp J., Janssens G.P.J. 2012. Fermentation of animal components in strict carnivores: A comparative study with cheetah fecal inoculum. *Journal of Animal Science*, 90, 2540-2548.

## Key message – Diet and food type

***Feed commercially prepared diets***

One replicated, before-and-after study in the USA found that providing a commercial diet to maned wolves led to similar dry matter intake and digestibility despite having lower protein content.

One replicated, randomized study of African wildcats in the USA found that feeding a commercial diet decreased crude protein digestibility and increased food intake and faecal output compared to raw meat. One controlled study of African wildcats in the USA found lower organic matter digestibility compared to a ground-chicken diet.

One replicated, controlled study in South Africa found that cheetahs fed a commercial diet had a similar likelihood of developing gastritis as those fed horse meat, lower levels of blood protein urea but higher levels of creatine. One study in USA found that cheetahs fed a commercial meat diet or whole chicken carcasses had plasma a-tocopherol, retinol and taurine concentrations within the ranges recommended for domestic cats.

***Feed whole carcasses (with or without organs/gastrointestinal tract)***

Two replicated, before-and-after studies in the USA found that feeding whole carcasses reduced pacing levels in lions, leopards, snow leopards and cougars. However, it increased pacing in tigers.

One replicated, randomized, controlled study in Denmark found that when fed whole rabbit, cheetahs had lower blood protein urea, zinc and vitamin A levels compared to supplemented beef. One replicated before-and-after study in Denmark found that feeding whole rabbit showed lower levels of inflammatory bowel indicators in cheetahs.

One replicated, randomized study and one controlled study in the USA found that when fed whole 1 to 3 day old chickens, ocelots had lower digestible energy and fat compared to a commercial diet and African wildcats had had lower organic matter digestibility compared to a ground-chicken diet.

***Provide bones, hides or partial carcasses***

One replicated, before-and-after study in the USA and one replicated, controlled study in Finland found that the provision of bones decreased the frequency of stereotypic behaviours in lions, tigers and Arctic foxes.

Two replicated, before-and-after studies of felids and red foxes in the USA and Norway found that the provision of bones increased activity and manipulation time.

***Feed plant-derived protein***

One replicated, randomized, controlled study and one replicated, controlled study in the USA found that a plant-derived protein diet increased digestible energy and dry matter digestibility but decreased mineral retention and plasma taurine levels in maned wolves compared to a (supplemented) animal-based protein diet.

***Supplement meat-based diets with prebiotic plant material to facilitate digestion***

One replicated, before-and-after study in India found that providing Jerusalem artichoke as a supplement increased two types of gut microbiota, faecal scores and moisture content in leopards.

***Supplement meat-based diet with vitamins or minerals***

No evidence was captured for the effect of supplementing meat-based diets with vitamins or minerals on captive carnivores.

***Supplement meat-based diet with amino acids***

One replicated, before-and-after, study in the USA found that supplementing an animal-protein diet with taurine, increased plasma taurine levels in maned wolves.

***Supplement meat-based diet with fatty acids***

No evidence was captured for the effect of supplementing diets with fatty acids for the benefit of captive carnivores.

***Increase variety of food items***

No evidence was captured for the effect of increasing the variety of food items for the benefit of captive carnivores.

## Key Messages – Food presentation and enrichment

***Hide food around enclosure***

Four replicated, before-and-after studies in the USA, UK and Germany and one before-and-after study of black bears, leopard cats, bush dogs, maned wolves and Malayan sun bears found that hiding food increased exploring and foraging behaviours.

One replicated, before-and-after study and one before-and-after study in the USA found a decrease in stereotypical pacing in leopard cats and black bear.

One before-and-after study in the USA found that hiding food reduced the time Canadian lynx spent sleeping during the day.

***Change location of food around enclosure***

One replicated, before-and-after study in Ireland found that altering the location of food decreased pacing behaviours in cheetahs.

One before-and-after study in the USA found that altering the location of food alongside altering the time of feeding increased exploring/foraging behaviour in black bears.

***Scatter food around enclosure***

One replicated, before-and-after study in Brazil found that scattered feeding increased locomotion in maned wolves.

One replicated study in Brazil found that maned wolves spent more time in the section of their enclosure with scattered food than in a section with food on a tray.

***Present food in/on water***

No evidence was captured for the effects of presenting food in/on water on captive carnivores.

***Present food frozen in ice***

Two replicated, before-and-after studies in the USA found that when presented with food in frozen ice, abnormal or stereotypic behaviours decreased and activity levels increased in bears and felids.

One replicated, before-and-after study in the USA found that manipulation behaviours increased in lions, whereas a replicated study in the USA found that manipulation behaviours decreased in grizzly bears.

***Provide food inside objects (e.g. boomer balls)***

Two before-and-after studies in Germany and India found that exploratory and foraging behaviours increased and stereotypic behaviours decreased in sloth bears and spectacled bears when presented with food inside objects.

One before-and-after study in the USA found that exploring/foraging behaviours decreased in a sloth bear when presented with food inside objects.

One replicated study in the USA found that grizzly bears spent a similar time manipulating food in a box and freely available food.

***Provide live vertebrate prey***

One small before-and-after study in the USA found that hunting behaviour increased and sleeping decreased when a fishing cat was provided with live fish.

One replicated, before-and-after study in the USA found that there was no change in the occurrence of stereotypical behaviours in tigers when provided with live fish.

***Provide live invertebrate prey***

One replicated study in the USA found that provision of live prey increased explorative behaviours in fennec foxes compared to other types of enrichment.

***Provide devices to simulate live prey, including sounds, lures, pulleys and bungees***

Two before-and-after studies in the USA and the UK found that activity levels and behavioural diversity increased in felids when presented with a lure or pulley system.

One replicated, before-and-after study in the USA found that pacing behaviour decreased and walking increased in cougars, but pacing initially increased in tigers, when provided with a carcass on a bungee.

***Use food as a reward in animal training***

No evidence was captured for the effects of using food as a reward in animal training for captive carnivores.

## Key Messages – Feeding Schedule

***Provide food on a random temporal schedule***

Three replicated, before-and-after studies and one replicated, controlled study found that an unpredictable feeding schedule reduced the frequency of stereotypic pacing behaviours in tigers and cheetahs.

One replicated, before-and-after controlled study in the USA found that an unpredictable feeding schedule increased territorial behaviour in coyotes but did not affect travelling or foraging.

Two before-and-after studies in Switzerland and the USA found that an unpredictable feeding schedule increased behavioural diversity in red foxes and alertness in a black-footed cat.

***Allocate fast days***

One replicated, before-and-after study in the UK found that large felids fed once every three days paced more frequently on non-feeding days.

***Alter food abundance or type seasonally***

No evidence was captured for the effects of providing food abundance or type seasonally on captive carnivores.

***Provide food during natural active periods***

No evidence was captured for the effects of providing food during natural active periods on captive carnivores.

***Use automated feeders***

No evidence was captured for the effects of using automated feeders on captive carnivores.

***Alter feeding schedule according to visitor activity***

No evidence was captured for the effects of altering feeding schedule to visitor activity on captive carnivores.

***Provide food during visitor experiences***

No evidence was captured for the effects of feeding during visitor experiences on captive carnivores.

## Key Messages – Social feeding

***Feed individuals separately***

No evidence was captured for the effects of feeding individuals separately on captive carnivores.

***Feed individuals within social group***

No evidence was captured for the effects of feeding individuals within a social group on captive carnivores.

***Hand-feed***

No evidence was captured for the effects of hand-feeding on captive carnivores.

## Species Management: Diet and food type

### 3.1 Feed commercially prepared diets

- One replicated, before-and-after study in the USA[2] found that providing a commercial diet to maned wolves led to similar dry matter intake and digestibility despite having a lower protein content.
- One replicated, randomized study of African wildcats in the USA[3] found that feeding a commercial diet decreased crude protein digestibility and increased food intake and faecal output compared to raw meat. One controlled study of African wildcats in the USA[5] found lower organic matter digestibility compared to a ground-chicken diet.
- One replicated, controlled study in South Africa[4] found that cheetahs fed a commercial diet had a similar likelihood of developing gastritis as those fed horse meat, lower levels of blood protein urea but higher levels of creatine. One study in USA[1] found that cheetahs fed a commercial meat diet or whole chicken carcasses had plasma a-tocopherol, retinol and taurine concentrations within the ranges recommended for domestic cats.

**Background**

Nutritional requirements of many exotic species are often unknown and therefore are commonly based on the nearest domestic relative. However, felids and canids typically eat many different parts of carcasses in the wild. Therefore, feeding a commercial diet may reduce the health risks to keepers and animals compared to handling raw meat but may not meet the nutritional needs of the animal. Identifying the digestibility and faecal or blood nutrient content may indicate the appropriateness of the diet in reference to the digestive health of the animal.

A study in 1988 of cheetahs *Acinonyx jubatus* in 13 institutions in the USA [1] found that when fed commercial food (supplemented minced meat products) or whole chicken carcasses, plasma a-tocopherol, retinol and taurine concentrations were within ranges recommended for domestic cats. Seven of 13 facilities maintained one fasting day per week, one had two fasting days per week and the others fed cheetahs daily. Thirteen facilities within the USA were surveyed for diet quantity, feeding schedule, dietary changes during lactation and body mass/condition. This was in conjunction with chemical analysis of 19 diet samples fed as well as blood analysis of 88 cheetahs across the facilities.

A replicated, before-and-after study in 1990–1991 of maned wolves *Chrysocyon brachyurus* in a research facility in the USA [2] found that when fed a diet containing commercial pellets, dry matter feed intake and digestibility were similar compared to a high meat and fruit-based diet despite a lower protein content. Dry matter intake was similar on the commercial diet compared to the high fruit and meat diet (584 vs 389 g per 30 kg of body mass per day) as was digestibility (73 vs 77%) whereas the metabolisable energy derived from protein was lower (28.6 vs 36.4%). Excess dietary protein is associated with the renal disorder cystinuria in maned wolves. Feed intake was monitored for three days in two individually housed wolves and one breeding pair housed together but fed separately, for the high meat and fruit diet and for two breeding pairs, where feed intake was combined, for the commercial diet. The high meat and fruit diet consisted of whole rats (575 g per 30 kg of body mass per day) fed in the morning and a mixed feed of frozen meat mix, bread, rice, oatmeal and fruit fed in the afternoon. For the commercial diet, rats were reduced (50 g) and the afternoon mixed feed consisted of frozen meat mix, rice and dry commercial pellets for dogs. Three other diets were also studied but without digestibility being measured. Faecal samples were collected daily and dried for analysis.

A replicated, randomized study in 2013 of African wildcats *Felis silvestris lybica* in a zoo in the USA [3] found that when fed a high protein commercial extruded diet, crude protein digestibility was lower and food intake and faecal output were higher compared to feeding a raw meat diet. Crude protein digestibility was lower when consuming a commercial diet (84%) compared to a raw meat diet (92%). Food intake and faecal output were higher on a dry matter basis when fed the commercial extruded diet (61.8 and 12.9 g/day respectively) compared to a raw meat diet (43.7 and 6.7 g/day respectively). There were no differences in faecal scores, ammonia or fatty acid concentrations between diets and no differences in the apparent digestibility of other nutrients, most blood metabolite levels or nitrogen retention. If commercial diets are nutritionally similar to raw meat diets then they may be preferred to reduce the risk of pathogens. Five adult wildcats were fed a raw meat or a high-protein dry commercial extruded diet and then switched to the other diet. Food offered and refused was weighed daily. Urine and faeces were collected in metabolism cages. Blood samples were collected from anaesthetised wildcats and serum metabolite was determined.

A replicated, controlled study in 2012 of cheetahs *Acinonyx jubatus* in a wildlife centre in South Africa [4], found that cheetahs fed commercial dry feline food had a similar likelihood of developing gastritis compared to cheetahs fed horsemeat and bone with supplement. Cheetahs on the commercial diet had a daily hazard of developing gastritis 2.2 times higher than cheetahs on the meat-based diet (gastritis grade 3 or above), although this difference was not significant. Serum urea levels were lower (14.76 vs 19.15 mmol/litre) and creatine levels higher (256.9 vs 249.1 umol/litre) on the commercial compared to the meat-based diet.

Serum urea and creatine are expected to increase with renal disease. Forty-eight cheetahs which had a gastritis grade of less than 3 and whose blood protein creatine levels were below 300 umol/litre were studied. They were fed either 4 kg of horsemeat and bone, 5 g of vitamin/mineral supplement and 20 ml of fish-oil supplement (n=26) or 500 g of commercial adult feline food (n=22) daily, both diets included one whole eviscerated chicken twice a week. A concurrent study comparing a meat-based diet with a commercial food formulated for renal disease in cheetahs diagnosed with gastritis and/or renal disease was inconclusive. Gastritis was graded 0-9 based on biopsies, with 9 being most inflamed.

A small, randomized study in 2013 of African wildcats *Felis silvestris lybica* in a zoo in the USA [5] found that canned, dry extruded and whole one to three day old chicken diets had lower organic matter digestibility compared to a ground-chicken diet. Organic matter digestibility was lower when fed canned (87%), extruded (86%) or whole chicks (85%) compared to a ground chicken diet (94%). Canned and extruded diets met macronutrient and mineral recommendations for domestic cat foods and tested negative for all microbes whereas whole one to three day old chicken and ground chicken diets met macronutrient requirements for domestic cats but were below recommendations for some minerals and tested positive for potentially pathogenic microorganisms. If commercial diets are nutritionally similar to raw meat diets then they may be preferred to reduce the risk of pathogens. Four wildcats were each fed four chicken-based diets: whole one to three day old chickens, ground chicken, canned commercial diet and extruded commercial diet. Wildcats were fed daily on each diet for 16 days. Each diet was analysed and apparent total tract macronutrient digestibility was measured daily using food intake and faecal output.

[1] Dierenfeld, E.S.1993. Nutrition of captive cheetahs: food composition and blood parameters. *Zoo Biology*, 12, 143-150.

[2] Barboza, P.S., Allen, M.E., Rodden, M., Projeta, K. 1994. Feed intake and digestion in the maned wolf (*Chrysocyon brachyurus*): Consequences for dietary management. *Zoo Biology*, 13, 375-381.

[3] Vester, B.M., Burke, S.L., Liu, K.J., Dikeman, C.L., Simmons, L.G., Swanson, K.S. 2010. Influence of feeding raw or extruded feline diets on nutrient digestibility and nitrogen metabolism of African wildcats *(Felis lybica). Zoo Biology*, 29, 679-686.

[4] Lane, E.P, Miller, S., Lobetti, R., Caldwell, P., Bertschinger, H.J., Burroughs, R., Kotze, A., van Dyk, A. 2012. Effect of diet on the incidence of mortality owing to gastritis and renal disease in captive Cheetahs (*Acinonyx jubatus*) in South Africa. *Zoo Biology*, 31, 669-682.

[5] Kerr, K.R., Morris, C.L., Burke, S.L., Swanson, K.S.2013. Apparent total tract macronutrient and energy digestibility of 1- to 3-day old whole chicks, adult ground chicken, and extruded and canned chicken-based diets in African wildcats (*Felis silvestis lybica*). *Zoo Biology*, 32, 510-517.

## 3.2 Feed whole carcasses (with or without organs/gastrointestinal tract)

- Two replicated, before-and-after studies in the USA[1,6] found that feeding whole carcasses reduced pacing levels in lions, leopards, snow leopards[1] and cougars[6]. However, it increased pacing in tigers[6].
- One replicated, randomized, controlled study in Denmark[3] found that when fed whole rabbit, cheetahs had lower blood protein urea, zinc and vitamin A levels compared to supplemented beef. One replicated before-and-after study in Denmark[5] found that feeding whole rabbit showed lower levels of inflammatory bowel indicators in cheetahs[5].
- One replicated, randomized[2] study and one controlled study[4] in the USA found that when fed whole 1 to 3 day old chickens, ocelots[2] had lower digestible energy and fat compared to a commercial diet and African wildcats[4] had had lower organic matter digestibility compared to a ground-chicken diet.

**Background**

For most carnivorous species, whole carcasses, including bones, skin and cartilage are a large part of their diet in the wild. These parts of the carcass help facilitate digestion of many species, including cheetahs (Depauw *et al*. 2012); they also include essential vitamins, minerals and amino acids which are not always present in commercial or meat only diets. Furthermore, carcasses require more manipulation before and whilst consuming, potentially allowing more natural feeding behaviours.

Depauw S., Bosch G., Hesta M., Whitehouse-Tedd K., Hendriks W.H., Kaandorp J., Janssens G.P.J. 2012. Fermentation of animal components in strict carnivores: A comparative study with cheetah fecal inoculum. *Journal of Animal Science*, 90, 2540-2548.

A replicated, before-and-after study in 2002 of snow leopards *Panthera uncia*, leopards *Panthera pardus* and lions *Panthera leo* in three zoos in the USA [1] found that when fed large carcasses, the frequency of off-exhibit feeding and natural behaviours increased and stereotypic behaviours decreased compared to when fed a commercial meat-based diet (mean values not reported). There were no differences in these behaviours on exhibit although hiding behaviour increased on-exhibit after carcass feeding. Nine felids were fed intact, eviscerated calf carcasses. Off-exhibit behaviour was recorded using a video camera starting immediately prior to provision of food. Twenty-four instantaneous scan samples were recorded over two hours per individual. Each animal received a carcass once every two weeks between 16.30 h and 17.00 h on scheduled days. On-exhibit behaviour was recorded by direct observation four times a day using instantaneous scan sampling every minute for ten minutes at each exhibit in 1.15 h rounds. Exhibit order was randomly selected.

A replicated, randomized, study in 1994 of ocelots *Leopardus pardalis* in three zoos in the USA [2], found that a diet of whole week-old chickens was lower in digestible energy and fat compared to commercial feline diet, whole mice, rat, rabbit, or quail and lower in dry matter digestibility compared to whole rabbit and quail diets. There were no other differences in energy, fat or protein digestibility between diets (mean values not reported). The diets did not differ in macronutrient content. Whole prey items may be more suitable for enrichment or supplementary feeding if their lower digestibility decreases the risk of overfeeding. Six ocelots in three zoos were fed once daily using six experimental diets: a commercial feline diet, adult mice, immature rabbits, immature rats, week-old domestic chickens and adult quail. Each ocelot received each diet for 1–3 trials in a random order.

A replicated, randomized, controlled study in 2012 of cheetahs *Acinonyx jubatus* in a safari park in Denmark [3], found that when fed whole rabbit, cheetahs had lower daily mean urea and zinc and higher vitamin A blood protein levels compared to being fed a supplemented beef diet. Cheetahs had a lower daily mean urea (12.8 mmol/litre) and higher vitamin A (96 ug/dl) blood protein levels when fed whole rabbit compared to being fed a supplemented beef diet (urea: 16.1 mmol/litre. vitamin A: 70 ug/dl). High levels of urea are potential indicator of chronic renal disease in captive cheetahs, but excessive vitamin A can result in skeletal deformities. Fourteen cheetahs, four housed individually and the rest group housed, were randomly assigned either a supplemented beef diet (1.2–1.6 kg/day/animal of chunk beef with 10 g/kg of multivitamin and mineral premix) or an un-supplemented whole rabbit diet (1.5–3 kg/day/animal). The cheetahs were acclimated to their diets for three weeks before blood samples were taken. Blood samples were collected from ten of the cheetahs. Three feed samples from both diets were collected for diet analysis.

A small, randomized study in 2013 of African wildcats *Felis silvestris lybica* in a zoo in the USA [4] found that canned, dry extruded and whole one to three day old chicken diets had lower organic matter digestibility compared to a ground-chicken diet. Organic matter digestibility was lower when fed canned (87%), extruded (86%) or whole chicks (85%) compared to a ground chicken diet (94%). Canned and extruded diets met macronutrient and mineral recommendations for domestic cat foods and tested negative for all microbes whereas whole one to three day old chicken and ground chicken diets met macronutrient requirements for domestic cats but were below recommendations for some minerals and tested positive for potentially pathogenic microorganisms. If commercial diets are nutritionally similar to raw meat diets then they may be preferred to reduce the risk of pathogens. Four wildcats were each fed four chicken-based diets: whole one to three day old chickens, ground chicken, canned commercial diet and extruded commercial diet. Wildcats were fed daily on each diet for 16 days. Each diet was analysed and apparent total tract macronutrient digestibility was measured daily using food intake and faecal output.

A replicated, before-and-after study in 2014 of cheetahs *Acinonyx jubatus* in a safari park in Denmark [5] found that when fed whole rabbit, the cheetahs exhibited lower faecal S100A12 (indicator of inflamed bowels) concentrations compared to when fed a supplemented beef diet. When fed whole rabbit, cheetahs had lower faecal S100A12 (301 ng/g) compared to a supplemented beef diet (1671 ng/g). There were no significant differences between diet groups for calprotectin or blood S100A12. Twelve cheetahs were part of a cross-over study which investigated the microbial fermentation of two diets. All cheetahs were fed a supplemented beef diet prior to the investigation. The two diets investigated were un-supplemented whole rabbit (2.5–3 kg diet/animal/day) or beef (1.2–1.6 kg diet/animal/day) supplemented with vitamin and mineral premix (10 g/kg meat). Ten of the cheetahs were anaesthetised as part of a routine veterinary procedure during the investigation and four blood samples from cheetahs fed whole rabbit and six from cheetahs fed supplemented beef were taken.

A replicated, before-and-after study in 2006 of tigers *Panthera tigris* and cougars *Felis concolor* in a zoo in the USA [6] found that when fed carcasses on a bungee, pacing behaviour decreased and walking behaviour increased in cougars, while the frequency of pacing and other behaviours increased in tigers. In cougars, pacing behaviours decreased (6%) and walking behaviours increased (6%) when fed a carcass on a bungee compared to pre-enrichment (pacing: 32%, walking 2.5%). Tigers showed an increase in 'other' (aggressive, social, interactive, stalking and jumping) (15%) and pacing (8.5%) behaviours compared to pre-enrichment (other: 11.5%; pacing: 4%). Faecal glucocorticoid metabolites did not vary throughout the testing period. Four felids, housed in pairs were fed a skinned deer rump individually on a 120 cm bungee cord. Behaviour was recorded every two minutes using instantaneous scan sampling over three hours, during pre- and post-treatment. Prior to enrichment felids were feed a routine commercial, supplemented diet. Faecal samples were collected once a week for four months prior to treatment and twice a week from first treatment until one week after the second treatment.


[1] McPhee, M, E. 2002. Intact Carcasses as Enrichment for Large Felids: Effects on On-and Off-Exhibit Behaviours. *Zoo Biology*, 21**,** 37-47.

[2] Bennett, C.L., Booth-Binczik, S.D., Steele, S.R.E. 2010. Nutritional composition and digestibility by Ocelots (*Leopardus pardalis*) of whole animals and commercial diet. *Zoo Biology*, 29, 753-759.

[3] Depauw, S., Hesta, M., Whitehouse-Tedd, K., Stagegaerd, J., Buyse, J., Janssens, G.P.J. 2012. Blood values of adult captive cheetahs *(Acinonyx jubatus*) fed either supplemented beef or whole rabbit carcasses. *Zoo Biology*, 31, 629-641.

[4] Kerr, K.R., Morris, C.L., Burke, S.L., Swanson, K.S. 2013. Apparent total tract macronutrient and energy digestibility of 1- to 3-day old whole chicks, adult ground chicken, and extruded and canned chicken-based diets in African wildcats (*Felis silvestis lybica*). *Zoo Biology*, 32, 510-517.

[5] Depauw, S., Heilmann, R.M, Whitehouse-Tedd, K., Hesta, M., Steiner, J.M., Suchodolski, J.S., Janssens, G.P.J. 2014. Effect of diet type on serum and faecal concentration of S100/calgranulins in the captive cheetah. *Journal of Zoo and Aquarium Research*, 2, 33-38.

[6] Ruskell, A.D., Melers, S.T., Jenkins, S.E., and Santymire, R.M. 2015. Effect of Bungee-Carcass Enrichment on Behavior and Fecal Glucocorticoid Metabolites in Two Species of Zoo-Housed Felids. *Zoo Biology*, 34, 170-177.


### 3.3 Provide bones, hides or partial carcasses

- One replicated, before-and-after study in the USA[1] and one replicated, controlled study in Finland[3] found that the provision of bones decreased the frequency of stereotypic behaviours in lions, tigers[1] and Arctic foxes[3].
- Two replicated, before-and-after studies of felids[2] and red foxes[4] in the USA[2] and Norway[4] found that the provision of bones increased activity[2] and manipulation time[4].

**Background**

In captivity, mental and physical stimulation can be unfulfilled due to the limiting environment. Many animals in captivity are fed unnatural food items that may inhibit the expression of appetitive behaviours, this can cause a deterioration in oral health and the expression of abnormal, repetitive behaviours as a coping mechanism. Providing items such as bones aims to promote natural foraging or gnawing behaviours.

A replicated, before-and-after study in 2003 of lions *Panthera leo* and tigers *Panthera tigris* in a zoo in the USA [1] found that when presented with bones twice a week as enrichment in addition to a commercial diet, stereotypic behaviours decreased compared to a commercial diet without bones. The presentation of bones twice a week on fed days, reduced the frequency of stereotypic behaviours (0.17 proportion of scans) compared to their routine commercial diet with one bone presented on fast days (0.28 proportion of scans). When presented in the morning, the frequency of resting (65%), standing (14%) and consumptive (3%) behaviours increased compared to when fed the commercial diet (resting: 35%; standing: 5%; consumptive: 0%). Before the experiment, the five cats were fed ground beef-based commercial diet six days a week and were not fed on the seventh day, instead they received a horse leg bone. There were four conditions in the experiment, each condition lasted four weeks 1) baseline data 2) bones were supplied twice per week 3) Live fish were supplied twice per week which were placed inside water pools. 4) A post-manipulation baseline was measured (for tigers only). Each cat was observed for one-hour sessions using instantaneous scan sampling at one-minute intervals for a minimum of six hours per cat.

A replicated, before-and-after study in 2007 of tigers *Panthera tigris,* ocelots *Leopardus pardalis,* cougars *Puma concolor,* cheetahs *Acinonyx jubatus* and lions *Panthera leo* in a zoo in the USA [2] found that when provisioned with bones active behaviours increased compared to no added enrichment. Active behaviours, excluding pacing, increased when provisioned with bones (31%) compared to no added enrichment (16%). This study was conducted on fourteen felids. Baseline data was collected before the investigation and two weeks of no enrichment occurred between each treatment. Horse knuckle or shank bone were provided daily for seven consecutive days (four days for tigers). Each cat was observed for one 30-minute session/day for ten days over a period of four weeks prior to enrichment and for two 30-minute session/day over three days during treatments using instantaneous scan sampling.

A replicated, controlled study in 2009 of Arctic foxes *Vulpes lagopus* in a research facility in Finland [3] found that when a cattle femur bone was provided as enrichment, foxes performed less oral stereotypies and engaged in more solitary play (mean values not reported). Foxes in the enrichment group interacted with bones for 50 minutes a day (3–4% of the observations). Sixteen fox families were used in this experiment. A frozen and defrosted cattle femur was placed into every second cage of a row. Foxes that were not provided with a bone could still see and smell the bones. All foxes were fed twice daily. Instantaneous focal sampling from video recordings were undertaken at five minute intervals for three 24-hour periods over four months.

A replicated, before-and-after study in 2013 of red foxes *Vulpes vulpes* in a research facility in Norway [4], found that presenting a cattle bone increased time interaction/manipulation time and reduced latency to contact than presenting foxes with a pulling device, straw, rawhide bone or a plastic cube. Within the first hour of presentation, foxes spent more time (1751 seconds/hour) manipulating the cattle bone and the latency to contact was shorter (mean values not reported) compared to a pulling device (62.4 s/hour), straw (31 s/hour), rawhide bone (313 s/hour) and cubes (24 s/hour). On the second day, time spent in manipulation was higher for the cattle bone (70.7 s/hour) than the other objects (0-21.7 s/hour), except for the rawhide (111.9 s/hour), and was again highest for the cattle bone on the fourth day (152.3 s/hour). Thirty juvenile foxes were individually housed and fed a food paste daily as desired. Enrichment objects included a pulling device (metal wire and plastic tubes), straws, pressed rawhide dog bone, cattle femur bone, and plastic cube. The experiment included an adjustment, habituation, deprivation, and reintroduction period. All objects were made available to the foxes at the same time. Continuous sampling was used for the first hour in the reintroduction period and then for one hour after access on the second and fourth day.

[1] Bashaw, M.J., Bloomsmith, M.A., Marr, M.J., Maple, T.L. 2003. To hunt or not to hunt? A feeding enrichment with captive large felids. *Zoo Biology*, 22, 189-198.
[2] Skibiel, A.L., Trevino, H.S., Naugher, K. 2007. Comparison of several types of enrichment for captive felids. *Zoo Biology*, 26, 371-381.
[3] Koistinen, T., Turunen, A., Kiviniemi, V., Ahola, L., Mononen, J. 2009. Bones as enrichment for farmed blue foxes (*Vulpes lagopus*): interaction with the bones and preferences for a cage with the bones. *Applied Animal Behaviour Science*, 120, 108-116.
[4] Hovland, A.L., Marit, Rod, A, M, S., Koistinen, T., Ahola, L. 2016. Preference for and use of oral enrichment objects in juvenile silver foxes (*Vulpes vulpes*). *Applied Animal Behaviour Science*, 180, 122-129.

## 3.4 Feed a plant-derived protein diet

- One replicated, randomized, controlled study[1] and one replicated, controlled study[2] in the USA found that a plant-derived protein diet increased digestible energy and dry matter digestibility[1] but decreased mineral retention[1] and plasma taurine levels[2] in maned wolves compared to a (supplemented) animal-based protein diet.

**Background**
Many captive, exotic animals are fed diets based on the nutritional requirements of their closest domestic relative. However, these diets for domestic animals are not always suitable for non-domestic species with different wild feeding behaviours. This can cause long-term health problems such as cystinuria, a metabolic defect in maned wolves.

A replicated, randomized, controlled study in 2006 of maned wolves *Chrysocyon brachyurus* and domestic dogs *Canis lupus* in a research centre in the USA [1] found that feeding maned wolves a plant-derived protein diet resulted in higher energy digestibility and dry matter digestibility but lower mineral retention compared to an animal-based protein diet. When maned wolves were fed a plant-derived protein diet, apparent digestible energy (3,510 kcal/kg) and apparent dry matter digestibility (67%) were higher compared to an animal-based protein diet (apparent digestible energy: 3,331 kcal/kg; apparent dry matter digestibility: 65%). Apparent retention was lower on the plant-derived protein diet for copper (7% vs 12%), iron (9% vs 12%), magnesium (26% vs 32%) and sodium (42% vs 53%). The plant-derived protein diet had previously been shown to raise the low urine pH associated with the renal disorder cystinuria in maned wolves. Diet did not affect transit time. Six wolves and six dogs were randomly assigned to be fed a diet containing plant-derived protein (soybean meal) or animal-based protein (meat meal and low ash poultry meat meal) for a period of 16 days. After 16 days, the animals were switched to the alternative diet. Faecal samples were collected on two consecutive days immediately after 12 days of being fed the diets. Dry matter, energy, protein, and minerals contained in the faeces were measured and chromic oxide was used as a marker to detect digestibility.

A replicated, controlled study in 2001 of maned wolves *Chrysocyon brachyurus* in a research centre in the USA [2], found that when fed a plant-derived protein diet, plasma taurine levels were lower compared to a supplemented animal-protein based diet. When fed a plant-derived protein diet or animal-based protein diet (4.03 nmol/ml), average plasma taurine was lower compared with a supplemented animal-based protein diet (66.68 nmol/ml). The plant-derived protein diet was not within target domestic canine reference ranges (60–120 nmol/ml). Deficient concentrations of plasma taurine levels responded within four months of supplementation. In the original experiment, four wolves were maintained on the commercial diet (animal-based protein) and two were maintained on an experimental diet (plant-derived protein). Four weeks prior and at the end of the diet trial, animals were restrained and sedated to collect a 12 ml blood sample. Due to clinical signs including weight loss and decreased appetite, the trial was terminated early and taurine supplementation (0.3% concentration) was deemed necessary. Taurine concentrations were monitored over a four-month period.

[1] Childs-Sanford, S.E., Angel, C.R. 2006. Transit time and digestibility of two experimental diets in the Maned Wolf (*Chrysocyon brachyurus*) and domestic dog (*Canis lupus*). *Zoo Biology*, 25, 369-381.
[2] Childs-Sanford, A.E., Angel, C.R. 2006. Taurine deficiency in maned wolves (*Chrysocyon brachyurus*) maintained on two diets manufactured for prevention of cystine urolithiasis. *Zoo Biology*, 25, 87-100.

## 3.5 Supplement meat-based diets with prebiotic plant material to facilitate digestion

- One replicated, before-and-after study[1] in India found that providing Jerusalem artichoke as a supplement increased two types of gut microbiota, faecal scores and moisture content in leopards.

**Background**

Certain carbohydrates are beneficial for the growth of healthy bacteria in the gut. Strict carnivores consume connective tissues that have similar affects as these carbohydrates. However, these animal products are not always easy to obtain and can be unhygienic if animals refuse them in captive conditions. Therefore, a plant alternative aims to replicate the digestive functions of animal tissues in obligate carnivores.

A replicated, before-and-after study in 2012 of Indian leopards *Panthera pardus fusca* in a zoo in India [1], found that supplementing food with Jerusalem artichoke *Helianthus tuberosus*, increased two types of gut microbiota, faecal scores and moisture content. *Lactobacillus* (8.24 $log_{10}$cfu/g faeces) and *Bifidobacterium* spp. (13.04 $log_{10}$cfu/g faeces) and mean faecal scores (2.39) and faecal moisture content (232.1 g/kg) were higher compared to a diet with no Jerusalem artichoke (*Lactobacillus*: 7.15 $log_{10}$cfu/g faeces; *Bifidobacterium* spp.: 12.13 $log_{10}$cfu/g faeces; faecal score: 1.80; faecal moisture content: 183.7 g/kg). The Jerusalem artichoke appears to improve gut health by promoting beneficial bacteria in the colon. Prior to treatment, eleven leopards housed individually were fed their normal diet of buffalo meat-on-bone with no supplement and during treatment a supplement of Jerusalem artichoke (2% of the diet dry matter basis) was added. Each trial consisted of 18 days of adaptation followed by four days of data collection (blood and faecal samples). Faecal scores were recorded daily (1-5, 1 being the most firm) and blood samples were taken using physical restraint in crush cages on the 22$^{nd}$ day of each trial.

[1] Pradhan, S.K., Das, A., Kullu, S.S., Saini, M., Pattanail, A.K., Dutta, N., Sharma, A.K. 2015. Effect of feeding Jerusalem artichoke (*Helianthus tuberosus*) root as a prebiotic on nutrient utilisation, fecal characteristics and serum metabolite profile of captive Indian leopard (*Panthera pardus fusca*) fed a meat-on-bone diet. *Zoo Biology*, 34, 153-162.

## 3.6 Supplement meat-based diet with vitamins or minerals

- No evidence was captured for the effect of supplementing meat-based diets with vitamins or minerals on captive carnivores.

**Background**
The diet offered to captive carnivores can vary and often not replicate their wild-type diet which includes bones, cartilage, and skin. This can cause negative effects on their gastrointestinal health. Supplementing diets is common practice to promote the overall health of captive animals, however there can still be differences in the fermentation process of these diets. See 2.2 'Feed whole carcasses with or without organs/gastrointestinal tract)'.

## 3.7 Supplement meat-based diet with amino acids

- One replicated, before-and-after, study in the USA[1] found that supplementing an animal-protein diet with taurine, increased plasma taurine levels in maned wolves.

**Background**
Some amino acids cannot be made in the body and are therefore essential within the diet. For felids, taurine is an essential amino acid and a deficiency in taurine can cause diseases if not present in the diet.

A replicated, before-and-after study in 2001 of maned wolves *Chrysocyon brachyurus* in a research centre in the USA [1], found that supplementing a protein-based diet with taurine increased the average plasma taurine levels.

When fed a supplemented animal-based protein diet, average plasma taurine was higher (91.18 nmol/ml), and within target domestic canine reference ranges, compared to no supplement (animal-based protein: 18.79 nmol/ml; plant-based mean: 4.03 nmol/ml). Four wolves were maintained on the commercial diet (animal-based protein) and two were maintained on an experimental diet (plant-based protein). Four weeks prior and at the end of the trial, animals were restrained and sedated to collect a 12 ml blood sample. Due to clinical signs including weight loss and decreased appetite, the diet trial was terminated early and taurine supplementation (0.3% concentration) was deemed necessary. Taurine concentrations were monitored over a four-month period.

[1] Childs-Sanford, A.E., Angel, C.R. 2006. Taurine deficiency in maned wolves (*Chrysocyon brachyurus*) maintained on two diets manufactured for prevention of cystine urolithiasis. *Zoo Biology,* 25, 87-100.

## 3.8 Supplement meat-based diet with fatty acids

- No evidence was captured for the evidence of supplementing diets with fatty acids on captive carnivores.

**Background**
Some animals cannot synthesize certain fatty acids and therefore, they are essential within the diet. Fatty acids have a structural role in cell membranes and are essential for maintaining skin structure (Watson 1998).

Watson, T.D.G. 1998. Diet and skin disease in dogs and cats. *Journal of Nutrition*, 128, 27835—27895.

## 3.9 Increase variety of food items

- No evidence was captured on the effect of increasing the variety of food items on captive carnivores.

**Background**
In the wild, animals have a varied diet due to food abundance and availability. In captive environments, the same food is routinely given, with little variety. Essential amino acids, fatty acids, vitamins and minerals may not be provided in one type of feed. The aim of a varied diet is to supply an animal with all nutrients essential for health, growth and maintenance.

# Species Management: Food Presentation and Enrichment

## 3.10 Hide food around enclosure

- Four replicated, before-and-after studies in the USA[1,2,5], UK[4] and Germany[6] and one before-and-after study[1] of a black bear[1], leopard cats[2], bush dogs[4], maned wolves[5] and Malayan sun bears[6] found that hiding food increased exploring[1,2,4,5] and foraging behaviours[1,6].
- One replicated, before-and-after study and one before-and-after study in the USA[1,2] found a decrease in stereotypical pacing in leopard cats[2] and black bear[1].
- One before-and-after study[3] in the USA found that hiding food reduced the time Canadian lynx spent sleeping during the day.

**Background**

In the wild, many felids, canids and ursids have to forage for food which may be hidden or difficult to access. In captivity, food may be presented to the animal directly without any need for active searching. Enrichment, including hiding food, is aimed at promoting natural foraging behaviours and decreasing abnormal behaviours.

A small before-and-after study in 1988 of a black bear *Ursus americanus* in a zoo in the USA [1] found that when food was hidden around the enclosure (including inside objects) walking and stereotypical pacing behaviours decreased and exploring/foraging behaviours increased compared to when food was not hidden. Stereotypical pacing was lower when food was hidden around the enclosure (median 20 minutes/day) compared to being placed on the floor of the indoor area (median 125 minutes/day) as was walking whereas exploring/foraging was higher (mean values not reported). One black bear was fed once or twice daily before the study and during baseline data collection (8 days). During the first condition (6 days), the bear was still given the morning feed as well as a feeder tree containing snacks. The feeder tree released snacks at scheduled times of day, releasing food at six different locations. The second condition (8 days) included hiding all food apart from the meat around the enclosure, under rocks, in logs and in Boomer balls. Video recordings were taken for 12 hours starting 06:00 each and continuous focal sampling method was used.

A small replicated, before-and-after study in 1991 of leopard cats *Felis bengalensis* in off-exhibit enclosures in the USA [2] found that multiple feeds hidden around the enclosure, increased time spent locomoting/exploring, increased behavioural diversity and decreased stereotypic pacing. Time spent locomoting (15.5 %/h) and behavioural diversity (0.547 Shannon index) increased and stereotypic pacing decreased (9.5%/h) compared to non-hidden food (locomoting: 6%/h; behavioural diversity: 0.458 Shannon index; pacing: 18.5%/h). Four cats were singularly housed and baseline data was collected for eight days before the treatment, when fed their regular diet of 0.25 kg of a commercial meat diet, an egg and one or two dead mice once daily. The hidden food treatment (5 days) included four feeds of either a mouse, chick and egg, or 0.125 kg of feline diet hidden in one of two piles of branches at irregular times of day. Continuous focal sampling from 24-hour video recordings was used to record behaviour and location.

A small before-and-after study in 1995 of Canadian lynx *Lynx canadensis* in the USA [3] found that hiding food around the enclosure reduced the time spent sleeping compared to when food was not hidden. The male lynx and first female lynx showed a reduction in time spent sleeping (1.1%) when food was hidden around the enclosure compared to when food was not hidden (30%). Before the study, the lynxes were fed each morning (processed feline meat, supplemented with dead day-old chicks, trout and mice). During the treatment phase, processed meat was fed each morning and dead prey items were hidden within the enclosure. Food was hidden daily for 17 days and hidden for two to three days a week for 10 days. Video recordings were taken and continuous sampling was used for four 30-minute sessions per day during public opening times. There were two study periods 26-months apart, only the first period showed significant results but this may have been due to the death of the original female lynx.

A replicated, before-and-after study in 1997 of bush dogs *Speothos venaticus* in a zoo in the UK [4] found that when food was hidden around the enclosure, searching behaviour increased compared to when food was thrown into the enclosure. Searching behaviour increased when food was hidden (6.1%) compared to baseline data (2.7%). Searching behaviour decreased as the treatment progressed. Eleven bush dogs in two enclosures were involved in the experiment. Their regular feeding regime consisted of meat chunks being thrown into the enclosure twice daily (chicken on bone, week old chicks, horse meat or unskinned rabbit). Instantaneous sampling with 15 second intervals was used for 30-minutes per day for each dog. During enrichment phase the entire daily food allowance was chopped into small chunks and hidden in the vegetation, rock crevices, under logs and within specially constructed wood-piles. Baseline data was collected for ten days, enrichment data for 20 days and post enrichment for ten days.

A small replicated, before-and-after study in 2007 of maned wolves *Chrysocyon brachyurus* in a research centre in the USA [5] found that when dead mice were hidden around the enclosure, activity rates increased for three out of four wolves and exploratory behaviours increased for all wolves compared to when no mice were hidden. There was an increase in activity (proportion of active time per individual: 0.6, 0.8 and 0.7) and exploratory rates (events/minute for each individual: 0.9, 1.3, 1.7 and 2.3) when presented with hidden mice compared to no enrichment (proportion of active time per individual: 0.3, 0.2 and 0.5; exploratory: 0.13, 0.06, 0.07 and 0.05 events/minute). Four individually housed wolves were observed daily using focal sampling during 30-minute observation sessions for two weeks of no enrichment, two weeks of hiding mice, a further two weeks of no enrichment and two weeks of boomer balls.

A small replicated, before-and-after study in 2003 of Malayan sun bears *Helarctos malayanus* in a zoo in Germany [6] found that hidden food once or twice per day with scent tracks leading to empty and filled food hiding places increased foraging behaviours and behavioural diversity compared to when food was not hidden. Foraging behaviours and behavioural diversity increased when feeds were hidden once daily (foraging: 40.15%/h; behavioural diversity: 2.3

Shannon Index) or twice daily (foraging: 43.73%/h; behavioural diversity: 2.9 Shannon Index) compared to when food was not hidden (foraging: 29%/h; behavioural diversity: 1.05 Shannon Index). For three out of four bears, walking behaviour increased (21.7%/h compared to when food was not hidden (6.8%/h). Four female sun bears were observed twice daily using continuous focal sampling to assess behavioural diversity and instantaneous scan sampling to assess activity budgets. Before treatments, baseline behavioural data were recorded for the standard feeding regime. The two treatments had the same conditions once or twice per day. This included hidden food in eight locations around the enclosure and up to 12 scent tracks of cinnamon dissolved in water.

[1] Carlstead, K., Seidensticker, J., Baldwin, R. 1991. Environmental enrichment for zoo bears. *Zoo Biology*, 10, 3-16.

[2] Shepherdson, D. J., Carlstead, K., Mellen, J.D., Seidensticker, J. 1993. The Influence of food presentation on the behaviour of small cats in confined environments. *Zoo Biology*, 12, 203-216.

[3] Gilkison J.J., White, B.C., Taylor, S. 1997. Feeding enrichment and behavioural changes in Canadian lynx at Louisville Zoo. *International Zoo Yearbook*, 35, 213-216.

[4] Ings, R., Waran, N.K., Young, R.J. 1997. Effect of wood-pile feeders on the behaviour of captive bush dogs (*Spepthos venaticus*). *Animal Welfare*, 6,145-152.

[5] Cummings, D., Brown, J.L., Rodden, M.D., Songsasen, N. 2007. Behavioural and physiologic responses to the environmental enrichment in the maned wolf (*Chrysocyon brachyurus*). *Zoo Biology*, 26, 331-343.

[6] Schneider, M., Nogge, G., Kolter, L. 2014. Implementing unpredictability in feeding enrichment for Malayan sun bears (*Helarctos malayanus*). *Zoo Biology*, 33, 54-62.

## 3.11 Change location of food around enclosure

- One replicated, before-and-after study in Ireland[1] found that altering the location of food decreased pacing behaviours in cheetahs.

**Background**

Predictability in feeding regimes has been linked to potential anticipatory behaviours such as pacing and higher levels of aggression. Varying the location of feeding may make the feeding routine less predictable and therefore reduce pacing and increase foraging behaviours.

A replicated, before-and-after study in 2010 of cheetahs *Acinonyx jubatus* in a wildlife park in Ireland [2] found that when feeding was spatially varied, pacing behaviour decreased compared to using a predictable location. Pacing frequency was reduced when food was spatially varied (0.04 mean proportion of scans) compared to predictable feeding (0.08 mean proportion of scans). Ten cheetahs were housed in five enclosures (one solitary male, two solitary females, a male pair and a mother and four cubs). Instantaneous scan sampling was used at five-minute intervals and a total of 48 scan samples were carried out per enclosure each day. Eight days of data were collected per enrichment technique, including eight baseline days. Spatial feeding involved altering feed between the back and front of the enclosure at the regular feeding time of 16:00 h. Their diet consisted of one whole dead rabbit or chicken six days per week.

[1] Quirke, T., O'Riordan, R. 2011. The effect of a randomized enrichment treatment schedule on the behaviour of cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, 135, 103-109.

### 3.12 Scatter food around enclosure

- One replicated, before-and-after study in Brazil[1] found that scattered feeding increased locomotion in maned wolves.
- One replicated study in Brazil[2] found that maned wolves spent more time in the section of their enclosure with scattered food than in a section with food on a tray.

**Background**
In the wild, many felids, canids and ursids have to forage for food over large areas whereas in captivity, food is often presented to animals directly without any need for active searching. Potentially, more natural foraging conditions can be simulated by scattering food throughout enclosures and hiding food in materials covering the floor (e.g. straw) for longer periods. Scatter feeds aim to increase foraging and exploratory behaviours potentially providing a more stimulating environment.

A replicated, before-and-after study in 2003 of maned wolves *Chrysocyon brachyurus* in three zoos in Brazil [1] found that scattering food throughout the enclosure increased locomotory behaviour in the observation period immediately following feeding compared to food provided on trays (mean values not reported). There were also significant individual differences between scattered food and food on trays in foraging, aggression, resting and 'out of view'. However, these differences were not consistent for all wolves. There was no difference in faecal glucocorticoid metabolites or pacing between feeding conditions. Eleven maned wolves were housed in pairs except for one individual. The wolves were fed once daily with mixed fruit and mixed meat. In the baseline condition food was placed in trays whereas in the scattered food condition half the regular diet was provided on a tray and the rest hidden in 12 locations. Instantaneous focal sampling every 30 seconds was used for 20-minutes per individual for five periods per day.

A replicated study in 2012 of maned wolves *Chrysocyon brachyurus* in a zoo in Brazil [2] found that when given a choice, the wolves spent more time in the section of their enclosure with scattered food compared to the section with food on a tray (mean values not reported). There was no difference between the number of times the wolves chose to enter the scattered or tray section at the start of each session. There were no differences in intake between scattered and tray sections between pairs or throughout the months. Prior to the study, eight maned wolves housed in mixed sex pairs were fed mixed fruit and mixed meats once a day. Sixteen 30-minute videotaped sessions were conducted over four months. The enclosures comprised of a starting compartment, a choice area and a scattered and tray section. Tray and scattered food conditions were alternated on each side of the enclosure. The section chosen at the beginning, the time spent in each area, the number of shifts between sections and the intake of both animals were all recorded.

[1] Vasconcellos, A.S. Guimarães, M.A.B.V., Oliviera, C.A., Pizzutto, C.S., Ades, C. 2009. Environmental enrichment for maned wolves (*Chrysocyon brachyurus*): group and individual effects. *Animal Welfare*, 18, 289-300.
[2] Vasconcellos, A. S., Adania, C.H., Ades, C. 2012. Contrafreeloading in maned wolves: implications for their management and welfare. *Applied Animal Behaviour Science*, 140, 85-91.

## 3.13 Present food in/on water

- No evidence was captured on the effects of presenting food in/on water on captive carnivores.

**Background:**
Many species of carnivore, particularly bears, hunt or forage in water. Many countries have regulations against feeding live vertebrate animals, including fish, to predatory species in captivity. Therefore, providing food in or floating on water aims to simulate a wild-type situation, encouraging natural foraging and hunting behaviours.

## 3.14 Present food in frozen ice

- Two replicated, before-and-after studies in the USA[1,3] found that when presented with food in frozen ice, abnormal[1] or stereotypic[3] behaviours decreased and activity levels increased[1,3] in bears[1] and felids[3].
- One replicated, before-and-after study in the USA[2] found that manipulation behaviours increased in lions, whereas a replicated study in the USA found that manipulation behaviours decreased in grizzly bears[4].

**Background:**
Presenting an animal with enrichment items that encourage manipulation, such as food frozen in ice, aims to increase engagement of a captive animal, encouraging active behaviours.

A small replicated, before-and-after study in 1989 of a Kodiak brown bear *Ursus arctos middendorffi,* an Asiatic black bear *Selenarctos thibetanus* and two polar bears *Ursus maritimus* in a zoo in the USA [1] found that providing food inside ice blocks increased active behaviours and decreased passive and abnormal behaviours in the first year of observation compared to non-enriched conditions. When provided with fish in frozen iceblocks, bears spent more time engaged in behaviours classed as active (49.6% of observations), less time in passive behaviours (47.6% of observations) and performed fewer abnormal behaviours (2.8% of observations) compared to non-enriched conditions (active: 19.4%, passive: 73.3%, abnormal: 7.3% of observations). These results were only significant in the first year of observations. Four solitary-housed bears were studied using instantaneous focal sampling at one-minute intervals, each exhibit was sampled once every five minutes during two 30 minute sessions a day, three days a week, over five months in 1989 and four months in 1990. For the Kodiak and polar bears the enrichment consisted of plain ice blocks and whole mackerel frozen inside ice blocks. For the Asiatic black bear the ice blocks contained peanuts, apples, raisins, peanut butter and grape jelly. These items were also scattered around the enclosure for the black bear.

A small replicated, before-and-after study in 1994 of African lions *Panthera leo* in a zoo in the USA [2] found that when presented with fish frozen in ice, manipulation and gnaw/licking, stand/locomoting and sniff/flehmen behaviours increased compared to no enrichment. The frequency of paw manipulation (22.3 per 30 minutes), gnaw/lick (36.67 per 30 minutes), stand/locomote (26 per 30 minutes) and sniff/flehmen (6.33 per 30 minutes) all increased when presented with fish in ice-blocks compared to baseline data (paw manipulation: 0; gnaw/lick: 0; stand/locomote: 6.33; sniff/flehmen: 1.13). Two adult lions and two sub-adults were all fed at 17:00 h daily (3.1 kg chopped horse meat) and were fasted one day a week. Instantaneous scan sampling was used at 20-second intervals for two-hour periods for 24 days and included baseline data when no enrichment was provided. One-zero sampling was also used on specific behaviours to measure the occurrence of rare behaviours, including: licking/gnawing, paw manipulation, sniffing/flehmen and face rubbing/back roll. Different enrichments were provided at random due to availability. Fish frozen in balls of ice were presented to the lions, hanging logs and various scents were also placed around the enclosure on different occasions.

A replicated, before-and-after study in 2007 of tigers *Panthera tigris,* ocelots *Leopardus pardalis,* jaguars *Panthera onca,* cougars *Puma concolor,* cheetahs *Acinonyx jubatus* and lions *Panthera leo* in a zoo in the USA [3] found that when provisioned with fish frozen in ice, active behaviours increased and stereotypic behaviours decreased compared to no enrichment. Active behaviours (51%) increased and stereotypic behaviours decreased (4%) when provided with fish frozen in ice compared to the baseline (active: 16%; stereotypic: 27%). Active behaviours also increased with the provision of bones and scattered spices and stereotypic behaviours decreased with the provision of scattered spices. The study was conducted on fourteen cats. Baseline data was collected before the investigation and two weeks of no enrichment occurred between each treatment. The three treatments included: 1) horse knuckle or shank bone daily for seven consecutive days (tiger was four days and jaguar not given); 2) Trout in a frozen soda bottle, daily for eight consecutive days (tiger was five days); 3) 30 ml of cinnamon, chili powder and cumin were sprinkled around the enclosure daily for nine consecutive days (tiger was five days). Each felid was observed for one 30-minute session/day for ten days over a period of four weeks prior to enrichment and for two 30-minute session/day over three days during treatments using instantaneous scan sampling.

A small replicated study in 2010 of grizzly bears *Ursus arctos horribilis* in a research facility in the USA [4], found that when food was presented simultaneously in ice-blocks and unfrozen, the bears spent less time manipulating and investigating frozen food compared to the unfrozen food. When apples and salmon were presented in ice blocks, bears spent less time manipulating (apples: 27 seconds; salmon: 76 seconds) and investigating (apple: 4 seconds; salmon: 2 seconds) compared to the same items that were freely available (manipulate: 1122 seconds (apple), 874 seconds (salmon); investigate:

8 seconds (apples), 15 seconds (salmon)). Four bears were routinely fed at 07:00 h daily and given a snack at 15:00 h on their regular schedule. During the experimental condition, bears were presented with five foraging choices simultaneously: apples, apples in ice, salmon, salmon in ice and plain ice. Behaviour was recorded using continuous focal sampling for six one-hour observation periods per bear.

[1] Forthman, D.L., Elder, S.D., Bakeman, R., Kurkowski, T.W., Noble, C.C., Winslow, S.W. 1992. Effects of feeding enrichment on behaviour of three species of captive bears. *Zoo Biology*, 11, 187-195.

[2] Powell, D.M. 1995. Preliminary evaluation of environmental enrichment techniques for African lions (*Panthera leo*). *Animal Welfare*, 4, 361-370.

[3] Skibiel, A.L., Trevino, H.S., Naugher, K. 2007. Comparison of several types of enrichment for captive felids. *Zoo Biology*, 26, 371-381.

[4] McGowan, R.T.S., Robbins, C.T., Alldredge, R., Newberry, R.C. 2010. Contrafreeloading in Grizzly Bears: Implications for captive foraging enrichment. *Zoo Biology*, 29, 484-502.

## 3.15 Present food inside objects (e.g. Boomer balls)

- Two before-and-after studies in Germany[2] and India[3] found that exploratory[3] and foraging[2] behaviours increased and stereotypic[3] behaviours decreased in sloth bears[3] and spectacled bears[2] when presented with food inside objects.
- One before-and-after study in the USA[1] found that exploring/foraging behaviours decreased in a sloth bear when presented with food inside objects.
- One replicated study in the USA[4] found that grizzly bears spent a similar time manipulating food in a box and freely available food.

**Background:**
Providing food inside objects provides an animal with the opportunity to manipulate their food, increasing engagement and foraging behaviours see 3.16.

A small before-and-after study in 1988 of a sloth bear *Melursus ursinus* in a zoo in the USA [1] found that the first and second presentation of honey-filled logs decreased walking/pacing and explore/foraging behaviours compared to before enrichment. Both walking/pacing (first presentation: 10 min/day); second presentation: 71 min/day) and explore/foraging (first presentation: 13 min/day; second presentation: 35 min/day) behaviours decreased when presented with honey-filled logs (walk/pace: 124 min/; explore/forage: 60 min/day). Explore/forage behaviour was lower on the third presentation compared to before enrichment, however walk/pacing was not significantly lower. One sloth bear was presented with a honey-filled log after six days of baseline observations. The log was presented for five days and was refilled twice. The log was then removed for two days and re-presented for a further four days. Post test data was collected for five days and lastly the log was presented for five continuous days. A camera recorded six hours daily 09:30 h to 15:30 h and continuous focal sampling was performed.

A small before-and-after study in 1994 of spectacled bears *Tremarctos ornatus* in a zoo in Germany [2] found that providing multiple feeding enrichments simultaneously, including logs filled with honey and raisins, increased the time bears spent foraging in the mornings compared to when the bears were fed conventionally, without enrichment. Using food as enrichment increased the time bears spent foraging in the morning (average number of scans: 24) compared to conventional feeding (10.6). There were no significant differences at other times of day. Feeding routine for three bears was switched weekly between an enriched routine, which included food holes, honey inside tree cavities and logs filled with raisins, and a conventional routine consisting of fruit, vegetables, bread and pellets fed twice daily. Behavioural observations were performed four days per week for three hours per day using instantaneous scan sampling, which started after they entered the exhibit in the morning for a total of 114 observational hours. Three 60 minute observation periods were carried out daily: between 08:30 h and 09:45 h, before noon, and early afternoon.

A before-and-after study in 2007 of sloth bears *Melursus ursinus* in a rescue centre in India [3] found that providing honey-filled logs both intermittently or daily reduced the frequency of stereotypic behaviour and increased exploratory behaviour compared to no enrichment. The amount of time devoted to stereotypic behaviours was lower (intermittent: 22%; daily: 22%) and exploratory behaviours were higher (intermittent: 21%; daily: 17.5%) than without honey filled logs (stereotypic: 32.5%: explorative: 14.1%). Fourteen bears were fed three times daily which included porridge and mixed scattered fruit. Behaviour was recorded using instantaneous scan sampling with two minute intervals. Baseline data were collected for ten days before treatment. Enrichment regime included logs which could hold up to 200 g of honey. Two conditions were studied: 1) logs were introduced for five days in a row; 2) logs were introduced on days one, three and five. Behaviour was monitored between 15:00 h and 17:30 h on all five days of a treatment period, behaviour was monitored for another 6-10 days.

A replicated study in 2010 of grizzly bears *Ursus arctos horribilis* in a research facility in the USA [4], found that when presented with apples in boxes, they spent more time manipulating the object compared to an empty cardboard box but a similar time manipulating freely available apples. The bears spent more time manipulating a cardboard box filled with apples (524 seconds) and free apples (1060 seconds) compared to an empty cardboard box (105 seconds). Six bears were fed simultaneously over three days, consisting of three one hour trials. Each bear was presented with free apples, apples in cardboard box and a cardboard box. Behaviour was recorded using continuous focal sampling for one hour observation periods.

[1] Carlstead, K., Seidensticker, J., Baldwin, R. 1991 Environmental enrichment for zoo bears. *Zoo Biology*, 10, 3-16.

[2] Fischbacher, M., Schmid H. 1999. Feeding enrichment and stereotypic behaviour in spectacled bears. *Zoo Biology*, 18, 363-371.

[3] Anderson, C., Arun, A.S., Jensen, P. 2010. Habituation to environmental enrichment in captive sloth bears- effect on stereotypies. *Zoo Biology*, 29, 705-714.

[4] McGowan, R.T.S., Robbins, C.T., Alldredge, R., Newberry, R.C. 2010. Contrafreeloading in Grizzly Bears: Implications for captive foraging enrichment. *Zoo Biology*, 29, 484-502.

## 3.16 Provide live vertebrate prey

- One before-and-after study in the USA[1] found that hunting behaviour increased and sleeping decreased when a fishing cat was provided with live fish.
- One replicated, before-and-after study in the USA[2] found that there was no change in the occurrence of stereotypical behaviours in tigers when provided with live fish.

**Background:**
In the wild, carnivores hunt both terrestrial and aquatic vertebrate prey. In captivity, carnivores are provided with meat or carcasses and are not able to practice their natural hunting behaviours. Providing captive carnivores with the opportunity to hunt is possible although in some countries there are regulations against feeding live vertebrate prey.

A small before-and-after study in 1991 of a fishing cat *Felis viverrina* in an off-exhibit zoo enclosure in the USA [1] found that when provisioned with live fish, sleeping behaviour decreased and hunting behaviour increased compared to behaviour before the provision of live fish. Sleeping behaviour decreased (21%) and hunting behaviour increased (39%) when provisioned with live fish compared to before live fish were provided (sleeping: 67%, hunting: 0%). One fishing cat was observed for six non-consecutive days before the treatment followed by six non-consecutive days where one or two live fish were placed in pools in the enclosure. A second baseline period of six days followed this. The cat was fed its regular diet of processed meat daily between 08:30 h and 09:30 h. Observations began 30 minutes after the fishing cat re-entered the enclosure after the fish were released into the pools. Instantaneous focal sampling at 30 second intervals for 30 minute periods were recorded twice daily over a period of two months.

A small replicated, before-and-after study in 2003 of Sumatran tigers *Panthera tigris sumatrae* in a zoo in the USA [2] found that when presented with live fish, there were no significant differences in the frequency of stereotypical behaviours compared to days without live fish. Two tigers were fed a commercial feline diet six days a week and were not fed on the seventh day, instead they received a horse leg bone. There were four conditions, each lasting four weeks: 1) baseline, 2) bones were supplied twice per week, 3) live fish were placed inside shallow water pools twice per week, and 4) post-manipulation baseline. Each tiger was observed for nine one-hour sessions in each condition using instantaneous scan sampling at one-minute intervals.

[1] Shepherdson, D. J., Carlstead, K., Mellen, J.D., Seidensticker, J. 1993. The Influence of food presentation on the behaviour of small cats in confined environments. *Zoo Biology*, 12, 203-216.
[2] Bashaw, M.J., Bloomsmith, M.A., Marr, M.J., Maple, T.L. 2003. To hunt or not to hunt? A feeding enrichment with captive large felids. *Zoo Biology*, 22, 189-198.

## 3.17 Provide live invertebrate prey

- One replicated study in the USA[1] found that provision of live prey increased explorative behaviours in fennec foxes compared to other types of enrichment.

**Background:**
For some carnivores, providing live invertebrates is a good form of feeding enrichment as it stimulates natural hunting behaviours. For example, providing crickets in a dispenser which is put under a mound, or placing crickets inside a pumpkin are methods which have been tried with some small felid species (Wooster 1997)

Wooster, D.S. 1997. Enrichment techniques for small felids at Woodland Park Zoo, Seattle. *International Zoo Yearbook*, 35, 208-212.

A small replicated study in 1986 of fennec foxes *Fennecus zerda* in a zoo in the USA [1] found that feeding live crickets increased explorative behaviours compared to meat being cut into smaller pieces, extra sand in the enclosure and less noise during cleaning. When fed live crickets, explorative behaviours increased (80 minutes/day) compared to the median of other conditions (40 minutes/day). Four fennec foxes housed in pairs were fed daily at 13:30 h on a commercial diet, fruit, eggs, fish bones and mealworms (only the two males' data statistically analysed). Behaviours were assessed based on 24 hour video recordings during five to eight 10-day periods. The conditions included: 1) added sand, 2) provision of live crickets, 3) meat was cut into smaller pieces, and 4) no excessive noise made during cleaning. Behavioural categories included pacing, digging, exploring, burying food and resting/sleeping.

[1] Carlstead, K. 1991. Husbandry of the Fennec fox (*Fennecus zerda*): environmental conditions influencing stereotypic behaviour. *International Zoo Yearbook*, 30, 202-207.

## 3.18 Provide devices to simulate live prey, including sounds, lures, pulleys and bungees

- Two before-and-after studies in the USA[1] and the UK[2] found that activity levels[1] and behavioural diversity[2] increased in felids when presented with a lure[1] or pulley system[2].
- One replicated, before-and-after study in the USA[3] found that pacing behaviour decreased and walking increased in cougars, but pacing initially increased in tigers, when provided with a carcass on a bungee.

**Background:**
Providing food that simulates wild-type prey aims to encourage natural feeding behaviours and reduce stereotypical behaviours as it mimics wild situations.

A small before-and-after study in 1977 of servals *Felis serval* in a zoo in the USA [1] found that when provided with a lure and rewarded for hunting behaviour, activity levels of one female serval increased compared to no enrichment. When presented with a lure, the female's activity levels increased (59.2 min/h) compared to no enrichment (18.2 min/h). Two females and one male, housed in an outdoor enclosure, were presented with a lure, which triggered the delivery of a food reward when struck. The food would come out at the far end of the run and would often get consumed by a different animal to that which struck the lure. One of the females died before the second study period. Two observation periods were performed one year apart. Frequency of jumping, contact with lure, food consumed, threat displays and general activity were recorded using one minute intervals for one or two twenty minute sessions per day and compared to session with no enrichment.

A small before-and-after study in 1996 on cheetahs *Acinonyx jubatus* in a zoo in the UK [2] found that when a whole rabbit was presented on a pulley, behavioural diversity, time spent visible to the observer, and frequency of sprinting behaviour increased and feeding and affiliation behaviours decreased compared to feeding without the pulley. Behavioural diversity (1.74 Shannon Index), time visible (5%) and frequency of sprinting (data not reported) all increased and feeding (8%) and affiliation (26%) behaviours decreased compared to baseline data (behavioural diversity: 1.49 Shannon Index; time visible: 1%; feeding: 11%; affiliation: 24%). Two cheetahs housed together were fed one whole rabbit each once daily. Instantaneous focal sampling occurred at 30 second intervals and was undertaken one hour before, 20 minutes during cleaning and feeding and one hour after cleaning had finished. During the treatment, a whole rabbit was attached to the wire of the pulley system which moved around the enclosure on release. Baseline data was collected for ten days before the treatment, ten days during and five days post treatment. Cheetahs were trained for two weeks on chase and capture of moving bait before the study.

A small replicated, before-and-after study in 2006 of tigers *Panthera tigris* and cougars *Felis concolor* in a zoo in the USA [3] found that when fed a partial carcass on a bungee, pacing behaviour decreased and walking behaviour increased in cougars, while the frequency of pacing and other behaviours increased in tigers compared to before providing a bungee carcass. In cougars, pacing behaviours decreased (6%) and walking behaviours increased (6%) when fed a carcass on a bungee compared to pre-enrichment (pacing: 32%, walking: 2.5%). Tigers showed an increase in pacing (8.5%) and 'other' behaviours (aggressive, social, interactive, stalking and jumping) (15%) compared to pre-enrichment (pacing: 4%; other: 11.5%). The tigers pacing behaviour increased during the first provision and decreased on the second provision, indicating the initial increase was due to unfamiliarity with the object. Faecal glucocorticoid metabolites did not vary throughout the testing period. Four felids, housed in pairs were fed a skinned deer rump individually on a 120 cm bungee cord. Behaviour was recorded every two minutes using instantaneous scan sampling over three hours, during pre- and post-treatment. Faecal samples were collected once a week for four months prior to treatment and twice a week from first treatment until one week after the second treatment.

[1] Mellen, J.D., Stevens, V J., Markowitz, H. 1981. Environmental enrichment for servals, Indian elephants and Canadian otters at Washington Park Zoo, Portland. *International Zoo Yearbook*, 21,196-201.

[2] Williams, B.G., Waran, N.K., Carruthers, J., Young, R. J. 1996. The effect of a moving bait on the behaviour of captive cheetahs (*Acinonyx jubatus*). *Animal Welfare*, 5, 271-281.

[3] Ruskell, A.D., Melers, S.T., Jenkins, S.E., and Santymire, R.M. 2015. Effect of Bungee-Carcass Enrichment on Behavior and Faecal Glucocorticoid Metabolites in Two Species of Zoo-Housed Felids. *Zoo Biology*, 34, 170-177.

### 3.19 Food as a reward in animal training

- No evidence was captured for the effects of using food as a reward in animal training on captive carnivores.

**Background:**
Positive human-animal relationships are discussed as a form of enrichment for captive animals. Using food as positive reinforcer for animal training is commonly used to help with veterinary and husbandry procedures. This form of training aims to reduce the potential stress experienced by an animal when in close proximity to humans (Claxton 2011).

Claxton, A.M. 2011. The potential of the human-animal relationships as an environmental enrichment for the welfare of zoo-housed animals. *Applied Animal Behaviour Science*, 133,1-10.

## Species Management: Feeding schedule

### 3.20 Provide food on a random temporal schedule

- Three replicated, before-and-after studies in Switzerland[1], Ireland[5] and Canada, UK, Ireland, Namibia and South Africa[5] and one replicated, controlled study in Ireland[6] found that an unpredictable feeding schedule reduced the frequency of stereotypic pacing behaviours in tigers[1] and cheetahs[4, 5, 6].
- One replicated, before-and-after controlled study in the USA[2] found that an unpredictable feeding schedule increased territorial behaviour in coyotes but did not affect travelling or foraging.
- Two before-and-after studies in Switzerland[3] and the USA[7] found that an unpredictable feeding schedule increased behavioural diversity in red foxes and alertness in a black-footed cat.

**Background:**
Environmental enrichment variability and novelty is important for captive animals. Predictable regimes may cause negative impacts due to habituation, such as an increase in pacing. Creating an unpredictable regime aims to keep enrichment items novel and maintain long-term positive impacts on the behaviour and welfare of captive animals.

A small replicated, before-and-after study in 1998 of Amur tigers *Panthera tigris altaica* in an outdoor enclosure in a zoo in Switzerland [1], found that feeding boxes with random opening times reduced the frequency of stereotypic pacing. When housed separately and fed at a random time and place of the enclosure, time spent stereotypic pacing by the female tiger (1%) was reduced compared to a conventional feeding schedule (16%) but not by the male (random placement: 0%; conventional feeding: 3%). When housed as a pair, stereotypic pacing was reduced in both tigers (0%) compared to conventional feeding (female: 7%; male: 10%). Two tigers were studied both when housed alone and as a pair. Several electronically controlled feeding boxes were installed at different places within the outdoor areas of the enclosure. Before 09:00 h, meat was distributed to all boxes and the doors were closed. The tigers were only able to open the boxes during two random 15 minute periods between 09:00 h and 17:30 h when the doors were unlocked. Tigers were fed on three day feeding regimes including a three-day baseline (fed at 14:30 h for two days and one fast day), three days conventional feeding (fed at 14:30 h) and lastly box feeding. Behaviour was recorded for six hours on the third day of each regime using focal sampling. The study was replicated when housed as a pair.

A replicated, before-and-after controlled study in 2009 of coyotes *Canis latrans* in a research centre in the USA [2], found that when fed using automated feeders on an unpredictable schedule, marking and howling behaviour increased compared to a predictable schedule. Frequency of marking behaviour (118 events/observation session) and howling behaviour (81 events/observation session) was higher when fed using an unpredictable regime compared to a predictable regime (marking: 42; howling: 24). There were no differences in time spent foraging, travelling, resting or standing. Twelve coyotes were housed individually in 0.1 ha experimental pens. Using automated feeders, one group of coyotes were fed daily on a predictable schedule at 08.00 h and 08.05 h and one group were fed twice daily on an unpredictable schedule. Observations included both pre- and post-feeding activity and non-feeding times using continual focal sampling for two hours per individual per day for ten days.

A small before-and-after study in 2005 of red foxes *Vulpes vulpes* in a wildlife park in Switzerland [3] found that when foxes were provided with unpredictable automated feeds, behavioural diversity and activity increased compared to scheduled feeding but not in relation to other feeding enrichment methods. Behavioural diversity (Shannon index: 2) and time spent in active behaviours (45%) increased when fed unpredictably, compared to predictable feeds before (Shannon index: 1.5; activity: 14%) and after (Shannon index: 1.8; activity: 26%) enrichment was presented. Activity was defined as all behaviours except resting and sleeping. Four adult foxes, housed together, were fed daily except Saturdays, on 400 g of meat, 200 g of fruits and 200 g of dried dog food, raisins, sunflower seeds and nuts. Behaviour was observed for one-hour sessions four times a day. During each observation hour, each fox was continuously observed for 15 minutes to assess behavioural diversity while instantaneous scan sampling at 2.5 minute intervals was used to assess general activity. Data were collected for five days in six conditions: 1) scheduled feeding times; 2) electronic feeders, each randomly dispensing 1/3 of all meat feed between 10:00 h and 18:00 h; 3) electronic feeders and a self-service food box; 4) electronic feeders plus scattered and hidden food; 5) electronic feeders and an electronic dispenser which dispersed food around the enclosure; and 6) a second period of scheduled feeding times

A replicated, before-and-after study in 2010 of cheetahs *Acinonyx jubatus* in a wildlife park in Ireland [4] found that varying the time of feeding decreased pacing behaviour. Pacing frequency was reduced when food was temporally varied (0.02 mean proportion of scans) compared to predictable feeding (0.17 mean proportion of scans). Ten cheetahs were housed in five enclosures (solitary and mixed housing). Instantaneous scan sampling was used at 5-minute intervals and a total of 48 scan samples were carried out per enclosure each day. Eight days of data were collected per enrichment technique, including eight baseline days. Temporal variation included feeding at an alternative time to 16:00 h.

A replicated, controlled study in 2012 of cheetahs *Acinonyx jubatus* in Canada, UK, Ireland, Namibia and South Africa [5] found that an unpredictable feeding schedule decreased the levels of stereotypic pacing compared to a predictable regime (results of statistical models). Increasing size of enclosure also decreased stereotypic pacing, whilst being solitary and having the ability to view other cheetahs in adjacent enclosures increased it. Factors that did not have a significant effect included visual barriers, presence of raised areas, visitor numbers, sex, vehicle disturbance, presence of enrichment, age and diet diversity. One hundred and twelve cheetahs in 88 enclosures, maintained in nine zoological institutions were studied. Data were collected on stereotypical pacing using instantaneous scan sampling with five-minute intervals. Each day was divided in to two-hour periods consisting of eight 15-minute time periods.

A replicated, before-and-after study in 2010 of cheetahs *Acinonyx jubatus* in a wildlife park in Ireland [6] found that varying the time of feeding decreased levels of locomotion and stereotypical behaviours and increased time devoted to other behaviours. In two out of five comparisons the proportion of scan samples in which locomotion was observed was lower (0.13–0.15) than under a fixed schedule (0.22) and in one out of five comparisons the proportion of scan samples in which stereotypical behaviour was observed was lower under a varied (0.03) than under a fixed schedule (0.09). However, in four out of five comparisons the proportion of scan samples in which 'other' behaviours were observed was higher under a varying (0.15–0.22) than under a fixed feeding schedule (0.05–0.09). Eight cheetahs housed in five enclosures were studied, three housed solitarily, two males housed together and one female with two cubs. Cheetahs were fed whole rabbits or chickens once daily at the same time, for six days a week. Data was collected over 16 days, during which food was temporally varied in eight randomly selected days. Instantaneous scan sampling was used every five minutes in varying degrees of sampling effort (ranging from 24 to nine scan samples) within a four-hour time period. Behavioural categories included exploratory, inactive, locomotion, stereotypical and vigilance. 'Other' behaviour category included aggression, allo-grooming, feeding, playing, standing and vocalisation.

A before-and-after study in 2013 of a black-footed cat *Felis nigripes* in a zoo in the USA [7] found that when feeding schedule was unpredictable, alertness increased compared to the baseline of no enrichment. Alertness increased (24%) compared to a baseline of no enrichment (16%). Additionally, locomotion (48%) and investigatory (4%) behaviours increased and alertness decreased (24%) compared to when diet was changed from dry and wet commercial cat food and mice to a low starch diet (locomotion: 28%; investigating: 1%; alertness: 44%). Instantaneous focal sampling was used every 30-seconds during 20-minute periods to record behavior of an individually housed cat. Data was collected at least once per week over 10 months. Four treatments included: 1) baseline, 2) random feeding times twice daily, 3) increased exhibit complexity, 4) changed diet to completely wet food (lower starch).

[1] Jenny, S., Schmid, H. 2002. Effect of feeding boxes on the behaviour of stereotyping Amur tigers (*Panthera tigris altaica*) in the Zurich zoo, Zurich, Switzerland. *Zoo Biology*, 21, 537-584.

[2] Gilbert-Norton, L.B., Leaver, L.A., and Shivik, J.A. 2009. The effect of randomly altering the time and location of feeding on the behaviour of captive coyotes (*Canis latrans*). *Applied Animal Behaviour Science*, 120, 179-185.

[3] Kistler, C., Hegglin, D., Wurbel, H., Konig, B. 2009. Feeding enrichment in an opportunistic carnivore: The red fox. *Applied Animal Behaviour Science*, 116, 260-265.

[4] Quirke, T., O'Riordan, R. 2011. The effect of a randomised enrichment treatment schedule on the behaviour of cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, 135, 103-109.

[5] Quirke, T., O'Riordan, R.M., Zuur, A. 2012. Factors influencing the prevalence of stereotypical behaviour in captive cheetahs (*Acinonyx jubatus*). *Applied Animal Behaviour Science*, 142, 189-197.

[6] Quirke, T. and O'Riordan R. 2013. Evaluation and Interpretation of the Effects of Environmental Enrichment Utilizing Varying Degrees of Sampling Effort. *Zoo Biology*, 32, 262–268.

[7] Leeds, A., Stone, D., Johnson, B., Less, E., Schoffner, T., Dennis, P., Lukas, K., Wark, J. 2016. Managing repetitive locomotor behaviour and time spent off exhibit in a male black-footed cat (*Felis nigripes*) through exhibit and husbandry modifications. *Journal of Zoo and Aquarium Research*, 4, 109-114.

## 3.21 Allocate fast days

- One replicated, before-and-after study[1] in the UK found that large felids fed once every three days paced more frequently on non-feeding days.

**Background**

In the wild, a carnivore's diet depends on the abundance and availability of prey. Therefore, many species do not feed every day. While it is not fully understood why captive animals perform stereotypic behaviours, it is thought they can be influenced by food restriction, causing appetitive behaviours that are deemed as repetitive and abnormal. In captivity, larger carnivore species are often fasted for one or more days per week the aim of replicating natural feeding behaviour.

A replicated, before-and-after study in 1994 of jaguars *Panthera onca,* leopards *P. pardus* and snow leopards *P. uncia* in a zoo in the UK [1] found that felids fed once every three days paced more on non-feeding days than on feeding days. Percentage of total scans spent pacing was higher in non-feeding days (jaguar: 11–18; leopard: 8–18; snow leopard: 8–11) than in feeding days (jaguar: 3; leopard: 4–7; snow leopard: 1–2). Eight felids (two jaguars, three leopards and three snow leopards) were observed using instantaneous scan sampling every 15 minutes during four 1-hour sessions each day (total 560 scans per enclosure). Felids were fed every third day.

[1] Lyons, J. Young, R.J., Deag, J.M. 1997. The effects of physical characteristics of the environment and feeding regime on the behaviour of captive felids. *Zoo Biology*, 16, 71-83.

### 3.22 Alter food abundance or type seasonally

- No evidence was captured for the effects of altering food abundance or type seasonally on captive carnivores.

**Background**

The diets of many wild carnivores change across different seasons but this may not be reflected in their captive husbandry. There is evidence to suggest stereotypic behaviours in captivity can alter seasonally due to innate urges to mate and forage at different times of the year (Carlstead & Seidensticker 1991). Not only this, weight management can also be an issue due to a lack of understanding of the yearly feeding ecology as well as food availability in each zoo (Lisi *et al.* 2013). Altering food type seasonally aims to maintain the health of the animal as well as encourage natural annual feeding cycles.

[1] Carlstead, K., Seidensticker, J. 1991. Seasonal variation in stereotypic pacing in an American black bear *Ursus americanus*. Behavioural Processes, 25, 155-161.

[2] Lisi, K.J., Barnes, T, L., Edwards, M.S. 2013. Bear weight management: a diet reduction plan for an obese spectacled bear (*Tremarctos ornatus*). Journal of Zoo and Aquarium Research, 1, 81-84.

### 3.23 Provide food during natural active periods

- No evidence was captured for the effects of providing food during natural active periods.

**Background**

In the wild, carnivorous species have a range of different circadian rhythms, hunting or foraging at different times of the day. This could be due to food abundance, temperature or physical adaptations. Providing food during natural active periods aims to replicate wild-type behavioural rhythms.

### 3.24 Use of automated feeders

- No evidence was captured for the effects of using automated feeders on captive carnivores.

**Background**

Automated feeders can remove human contact for animals being prepared for reintroduction and could reduce stress for animals nervous of human interaction. Additionally, automatic feeders allow food quantity and intake to be monitored to maintain animals at their healthy weight. They are also utilized as a method to schedule feeding times, see 2.21. Provide food on a random temporal schedule.

### 3.25 Alter feeding schedule according to visitor activity

- No evidence was captured for the effects of altering feeding schedule to visitor activity on captive carnivores.

**Background**
Animal behaviour can be influenced by the presence of zoo visitors [1]. Responses to visitor activity are often species- and individual-specific and consequently adjusting visiting times according to the needs of specific animals might improve their welfare.

[1] Margulis, S.W., Hoyos, C., Anderson, M. 2003. Effect of felid activity on zoo visitor interest. *Zoo Biology*. 22, 587-599.

### 3. 26 Provide food during visitor experiences

- No evidence was captured for the effects of feeding during visitor experiences on captive carnivores.

**Background**
Visitor experiences are increasingly popular events in zoos and give visitors the opportunity to get closer to animals in ways that would not normally be possible. These events often involve feeding the animals, including large carnivores. This may have a positive or negative affect on the health and welfare of the animal. For example, getting closer to the animals allows better health observations and positively reinforces the animals to remain relaxed around humans. However, excessive feeding can cause health problems and it is not always easy to monitor food intake, especially for animals living in social groups. Some species or individuals of the species may also find the close proximity to unfamiliar humans stressful. Also see background 2.27 Alter feeding schedule according to visitor activity.

## Species Management: Social Feeding

### 3.27 Feed individuals separately

- No evidence was captured for the effects of feeding individuals separately on captive carnivores.

**Background**
Feeding animals separately rather than in a group can reduce aggression as well as enable keepers and veterinarians to monitor food intake per animal, promoting the health and welfare of the individuals.

### 3.28 Feed individuals within a social group

- No evidence was captured for the effects of feeding individuals within a social group.

**Background**
Feeding within a social group is natural for many species. For example, wolves have a strong social dynamic, and feeding behaviours reinforce social ranking. In captivity, animals may be housed individually either routinely (e.g. in some laboratory contexts) or temporarily (e.g. for medical treatment). Also refer to 2.29 Feed individuals separately.

### 3. 29 Hand-feed

- No evidence was captured for the effects of hand-feeding on captive carnivores.

**Background**
In recent years, minimal human contact is encouraged for many animals in zoos. Many species of carnivore are considered dangerous and human contact can be considered stressful. However, hand-feeding aims to allow keepers to monitor the health of an animal, administer medication easily and monitor food intake. Also see 2.27 Provide food during visitor experiences' and 2.19 Food as a reward in animal training.