CCAC guidelines: Reptiles

Draft for Public Review

TABLE OF CONTENTS

PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer-review organization responsible for setting, maintaining, and overseeing the implementation of high standards of ethical care and use of animals in science throughout Canada.

The *CCAC guidelines: Reptiles* provides information for investigators, study directors, instructors, animal care committees, facility managers, veterinarians, and animal care personnel to help facilitate improvement in both the care given to reptiles and how experimental procedures are carried out. These guidelines address conditions normally present in laboratories housing reptiles; where experimental conditions required by studies differ from the guidelines, they must be justified to, and approved by, the animal care committee.

The individual guideline statements in the document have been developed based on expert peer advice and current interpretation of scientific evidence.

CCAC guidelines are intended to provide a framework for implementing Russell and Burch's Three Rs: Replacement, Reduction, and Refinement (Russell and Burch, 1959), primarily the principle of Refinement. These practices are constantly evolving, and refinements should result in continual improvement in animal welfare.

For studies outside of Canada, investigators based at CCAC-certified institutions are subject to these guidelines and the relevant legislation and regulations pertaining to ethical animal care and use in the country where the study is conducted.

LIST OF GUIDELINES

The following list of guideline statements serves as an executive summary covering the most important aspects of the care and use of reptiles. These guideline statements are included throughout this document alongside details and references that provide support and context for their implementation. Throughout this document, the term 'should' is used to indicate an obligation, for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

2. Facilities

Guideline 1

Enclosures must provide sufficient space and complexity to enable reptiles to perform behaviours important to their welfare.

Section 2.2 Enclosures

Guideline 2

Regardless of what type of terrestrial cage is chosen, it must be possible to control the environmental conditions within it to meet the needs of the species to be housed.

Section 2.2.3 Terrestrial Holding Systems

3. Facility Management and Personnel

Guideline 3

Laboratory management practices must aim to ensure the macroenvironment (room) and microenvironment (primary enclosure) maintain the health and welfare of both the animals and personnel and provide consistency for research outcomes.

Section 3.1 Managing the Environment

Guideline 4

Water quality must be monitored.

Section 3.1.3.2 Water Quality

Guideline 5

Reptiles must be observed regularly by trained personnel, with minimal disruption to the animals.

Section 3.2 Personnel

4. Procurement

Guideline 6

Facilities and investigators acquiring or transporting reptiles, or conducting research on reptiles, must be familiar with and comply with relevant international, federal, and provincial or territorial legislation and policies.

Section 4.1 Source

Guideline 7

Information relating to the transport, welfare, and care of the reptiles should be communicated between the supplier and receiver before shipment of the reptiles occurs.

Section 4.3 Pre-Shipment Procedures

Guideline 8

The health status of reptiles being received should be reviewed before animals are shipped.

Section 4.4 Transportation

Guideline 9

Animals should not be shipped if weather forecasts predict extreme (very hot or very cold) temperatures.

Section 4.4 Transportation

Guideline 10

The health and welfare of reptiles must be checked upon their arrival by competent animal care personnel.

Section 4.5 Receiving Animals

Guideline 11

Reptiles should undergo quarantine and acclimation after transport and before use in a scientific activity.

Section 4.6 Quarantine and Acclimation

5. Breeding

Guideline 12

Species-specific health assessment benchmarks should be established for breeding animals, and these should be met before initiating breeding.

Section 5.3 Physiological Considerations

6. Husbandry

Guideline 13

Environmental enrichment relevant to the species and life stage should be provided.

Section 6.6 Environmental Enrichment

7. Handling and Restraint

Guideline 14

Reptiles should only be handled when necessary, according to the purpose, and the handling time should be minimized.

Section 7.1 Physical Handling and Restraint

Guideline 15

Reptiles must be continually monitored during anesthesia, with particular attention paid to respiration, heart rate, and depth of anesthesia.

Section 7.2 Chemical Restraint

8. Health and Disease Control

Guideline 16

All reptiles should be included in an animal health program, irrespective of where they are housed.

Section 8 Health and Disease Control

Guideline 17

Strategic measures for disease prevention should include a plan for disease control and a system of regular monitoring and reporting for health assessment purposes.

Section 8.1 Disease Prevention

Guideline 18

SOPs should be developed for assessing animal health, providing health care, and treating common health problems for the animals; these should be reassessed at least every three years to ensure relevance.

Section 8.2 Health Monitoring and Disease Detection

Guideline 19

A response plan must be in place to deal with potential disease outbreaks.

Section 8.3 Disease Management in the Event of an Infectious Outbreak

9. Welfare Assessment

Guideline 20

All reptiles maintained in an animal facility must be subject to routine welfare assessments.

Section 9 Welfare Assessment

10. Experimental Procedures

Guideline 21:

The least invasive method suited to the goals of the study must be used, with consideration of the potential impacts of the procedures on the reptiles and measures taken to reduce those impacts.

Section 10 Experimental Procedures

Guideline 22:

Endpoints must be developed and must be approved by the animal care committee before the commencement of the study to minimize the negative impacts of procedures on the animal.

Section 10 Experimental Procedures

11. Euthanasia

Guideline 23:

Euthanasia of reptiles must only be carried out by competent personnel using an approved method best suited to the particular species and life stage and to the study objectives.

Section 11 Euthanasia

1. INTRODUCTION

Throughout this document, the term 'should' is used to indicate an obligation, for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

Reptiles are a diverse group, with approximately 10,850 known species and new species being described regularly. Reptiles include Squamata – lizards, snakes, and amphisbaenians or "worm-lizards" (approximately 10,500 species); Chelonia – turtles and tortoises (approximately 350 species); Crocodilia – crocodiles, gharials, caimans, and alligators (24 species); and Sphenodontia tuataras from New Zealand (1 species). From a practical perspective, non-avian reptiles (hereafter "reptiles") are best considered as a distinct group of ectothermic (cold-blooded), air-breathing vertebrates that employ internal fertilization and amniotic development. They have keratinized scales covering part or all of their body. Reptiles display a wide range of physiological and behavioural adaptations to specific environmental conditions. Therefore, it is critical to be aware of the animal's individual needs and how their housing environment and any studies they are involved in influence their welfare. These guidelines apply to a diverse classification of species, and therefore species-specific expertise is often required for correct interpretation.

The *CCAC guidelines: Reptiles* focuses on reptiles housed in laboratory facilities. For studies involving reptiles in the wild, including short-term holding in the field, see the *CCAC guidelines on: the care and use of wildlife* (2003). Most reptiles lay eggs, although some bear their young live. Currently, the developing eggs of oviparous reptiles (who produce young by means of eggs that hatch after having been laid by the parent) are not covered by this guidelines document, as the CCAC does not require an animal use protocol for these life stages.

Canadian facilities house a wide range of reptile species, the most reported of which are bearded dragons, leopard geckos, crested geckos, anoles, terrapins, box turtles, garter snakes, gopher snakes, ball pythons, corn snakes and boa constrictors. Reptiles (both captive-bred and wild-caught) contribute to a wide range of studies in regeneration, comparative anatomy, comparative physiology, nutrition, diagnostic imaging, ecology, aggression, stress physiology, reproductive cycles, and the effects of neurotoxins (Crawford et al., 2001).

Some of the challenges associated with reptile-based studies include:

- species-specific housing and care requirements at different life stages;
- limited knowledge of reptile husbandry and welfare for many species;
- limitations in the recognition, evaluation, and alleviation of nociception, discomfort, and distress;
- challenges associated with distinct anatomy (e.g., suturing of skin) for studies involving surgical procedures;
- an inherent difficulty in maintaining asepsis for surgery and recovery in aquatic species;
- potential adverse effects on animal welfare when used as disease models;
- lack of veterinary support and knowledge;
- lack of a consistent genetic background; and
- procurement in general, and a lack of captive-bred, pathogen-free sources in particular.

Those working with a species must have a comprehensive understanding of the animals' housing and husbandry requirements, which can be acquired through literature searches and consultation with investigators, veterinarians, and others having experience with that species. Where knowledge of a species' optimal conditions is lacking, these guidelines and the species' natural habitat provide an appropriate starting point, which should be followed up with careful monitoring and adjustment. Facility-specific standard operating procedures (SOPs) must be developed for the husbandry of each species.

As with any animal-based science, the scientific validity of any protocol involving reptiles must be established carefully, and the Three Rs (Replacement, Reduction, and Refinement) (Russell and Burch, 1959) must guide decisions concerning experimental design and the care of the animals.

Replacement is an important consideration in planning any animal-based study. Consideration must also be given to Reduction, to determine the fewest number of animals appropriate to provide reproducible valid information and statistical power while minimizing the welfare impact for each animal. Sample size calculations must be carried out, and a biostatistician should be consulted when necessary.

The present guidelines focus primarily on Refinement, both in terms of the care of reptiles in a facility and procedures carried out on reptiles as part of an animal-based protocol approved by an animal care committee. Animals living in an environment where facilities and practices are oriented toward promoting good animal welfare are less likely to be stressed and more likely to exhibit normal behaviours and physiology (Poole, 1997).

The following sections provide a brief overview of the behavioural biology important to the welfare of reptiles (Section 1.1, "Behavioural Biology"), the anatomical and physiological characteristics of reptiles (Section 1.2, "Anatomy and Physiology"), the sensory abilities of reptiles (Section 1.3, "Senses"), and potential inter-animal variations (Section 1.4, "Sources of Variation"). This information forms the basis of this document and has an impact on welfare considerations. It is important to consider the characteristics of the species (and strain where applicable), sex, life stage (age, breeding status, season), and prandial status (i.e., when the animal was last fed) as well as the specific characteristics of the individuals when considering the impact of a procedure or condition on the welfare of reptiles and on the research results.

See Appendix 1, "List of Useful Resources", for potential resources to consult for additional background information on particular species.

1.1 Behavioural Biology

Understanding the behavioural biology of experimental animals is crucial to improving both animal welfare and the quality of scientific research (Olsson et al., 2003). While there are many studies of the ecology, biology, and natural history of various species of reptiles, literature on reptile welfare is relatively limited. Addressing the welfare of reptiles in the laboratory environment requires considering their natural behaviours, which vary with species, and providing the opportunity for those behaviours to be expressed where appropriate.

Unlike amphibians, reptiles do not have an aquatic larval stage. Most reptiles are oviparous, producing young by means of eggs that hatch after being laid by the parent. However, some species of squamates are viviparous (i.e., the fetus develops within the mother, rather than externally).

Reptiles are tetrapod vertebrates, animals with either four limbs or, like snakes, they are descended from four-limbed ancestors. Reptiles use various forms of locomotion. Tortoises are quadruped terrestrial reptiles. Although most lizards walk on all fours, some use only their hind limbs when running. Snakes and legless lizards move by applying sequential friction between their body scales and the surface. Snakes and turtles are semi- or fully aquatic, and some lizards are also semi-aquatic.

1.1.1 Thermoregulatory Behaviour

Reptiles are largely ectothermic (i.e., they regulate body temperature by exchanging heat with their environment) and behaviourally thermoregulate under natural conditions, selecting microenvironments in which they can gain or lose heat as required to maintain their body temperature.

The thermoregulatory needs of reptiles differ among species. The optimal thermal environment needs to be considered when designing housing systems for reptiles, including opportunities for basking and cooling. Another behavioural feature associated with thermoregulation is the process of brumation, a form of environmentally induced dormancy experienced by ectothermic reptiles in response to low environmental temperatures and shortened day length in the winter months. Brumating reptiles typically cease eating and drinking and will become more sedentary with or without burrowing; this is both a survival mechanism and a required component of reproductive success for some species.

Reptiles are exposed to microclimates, especially humidity and airflows, that are very different from those perceived by large animals such as humans.

Much of reptile behaviour is motivated by hunting, feeding, predation, and post-prandial status. In addition, light plays a significant role in reptile behaviour. Reptiles live in close association with their structural microenvironments, and subtle cues such as scent, texture, and contact are important aspects of behaviours associated with feeding, predation, and ecdysis (shedding of old skin).

1.1.2 Social Interactions

Reptiles are generally regarded as solitary animals that display some level of social interactions in the form of parental care, mating, territoriality, and dominance. However, there are differing levels of sociality across reptile species, and research suggests that large stable social groups, as well as social aggregations, occur in some species which are often kin-based and can be seasonal or remain year-round (Clark et al., 2012; Doody et al., 2013; Gardner et al., 2016). However, social interactions can also lead to detrimental effects (e.g., aggressive interactions leading to injury or mortality). Thus, species and individual-specific considerations (e.g., age, sex, season, enclosure size, resource availability) must be given before housing individuals together.

1.2 Anatomy and Physiology

As outlined in Section 1.1, "Behavioural Biology", reptiles are ectotherms, meaning they do not, in general, produce sufficient metabolic heat to raise their body temperature above ambient temperature. Reptiles' slow resting metabolic rates result in very different physiological parameters than mammals or birds, including relatively slow heart and respiratory rates and diminished feeding frequency. The slower metabolism and associated physiological features impact appropriate husbandry, including diet, fluid intake, resting time, and habitat for daily life activities.

The heavily keratinized skin of scaled reptiles provides protection from water loss and mechanical abrasion and contributes to vitamin D synthesis and thermoregulation. The shell of chelonians is composed of dermal bone arising from ossified ribs that form a protective carapace and plastron. As reptile skin is relatively inelastic, most species undergo shedding cycles, particularly during growth phases. Some reptiles can change colour rapidly due to neurological control over pigment distribution in the chromatophore (skin pigment cell); this feature can be used in health and welfare assessments (see Section 8, “Health and Disease Control”, and Section 9, “Welfare Assessment”).

Reptiles lack a true diaphragm, and all organs are contained within a coelomic cavity. Post-pulmonary or post-hepatic membranes may separate the coelomic cavity into compartments. Reptiles have evolved to maximize water conservation through the excretion of urate from the cloacal chamber, where water can be reabsorbed. Most reptiles do not have a bladder to store water. Reptile lungs are not highly developed; there is no alveolar system. Due to a three-chambered heart comprising two atria and one ventricle, reptiles have a slightly deoxygenated system that is generally capable of prolonged breath-holding. Internal organs in reptiles are usually arranged to fit the overall morphology of the animal; for example, snake organs are organized sequentially and longitudinally.

1.3 Senses

Nociceptors are present in reptiles and respond to nociceptive stimulation; hence, reptiles can experience pain sensation. Reptiles lack the withdrawal reflex and therefore are susceptible to injury from direct heat sources such as heat lamps.

Most reptiles can see colours (Davies et al., 2009), and some can detect ultraviolet light. Some snakes can ‘see’ heat through heat-sensing pits or scales, which connect via the nervous system to the optical processing areas of the brain. Chelonians, the tuatara, and many lizards have a parietal eye at the top of their head, connected to the pineal gland, which responds to the wavelength and intensity of light. The parietal eye appears to have a role in circadian behaviour, seasonal reproductive cycles, and thermoregulation. Terrestrial reptiles can sense vibrations, and many can hear sound and vocalize.

Among reptiles, chemoreception includes olfaction (smell), vomeronasal detection (for pheromones), and gustation (taste). Olfaction is used to detect volatile (typically airborne) chemicals during inspiration, while vomeronasal detection is used to perceive non-volatile chemicals taken into the oral cavity via stimulation of the vomeronasal (Jacobson’s) organ through the tongue. All senses have multiple roles in foraging, hunting, avoiding predators, social communication, and finding mates. Reptile husbandry must consider the presence of sensory stimuli that may not be perceptible to humans.

1.4 Sources of Variation

While the variation between species of reptiles is easily recognizable, consideration must also be given to the potential morphological, physiological, and behavioural variation that can exist among individuals within a species. This variation can influence housing and husbandry requirements and the effects of certain procedures on animal welfare and the interpretation of study results. Sources of the intra-species variation include the species strain, natural geographic range, previous experience of the animal, sex, and developmental stage.

1.4.1 Strain

There has been limited artificial genetic modification of reptiles for animal model development (Rasys et al., 2019). Specialized breeding programs, both for research and hobby interests, have produced strains with distinct phenotypes such as scale-less animals and unique colour morphologies. Strain differences may have significantly different requirements for water, humidity, and light levels than the wild-type. Unique traits of particular strains must be accommodated to ensure the animals' welfare is maintained.

Some strain differences provide interesting research models. For example, the spider morph ball python has a defect of cerebellar function leading to tremors and problems catching prey. Strain differences include changes in pattern and colouration (albinism, hyper- or hypomelanism, leucism, and anerythrism) as well as changes in the presence of scales themselves.

1.4.2 Developmental Stage

The developmental stages of an animal must be considered for appropriate husbandry and experimental design. Reptiles are generally long-lived with a prolonged growth and development period; development can be influenced by nutrition level and age. An optimally fed reptile will generally reach developmental maturity and size faster than one sub-optimally fed. Nutrition and housing needs, including social or solitary housing, vary with developmental stage. Due to the complex interaction of nutrition and age in development, wild-caught animals may introduce more variation than expected, even if they are developmentally similar.

1.4.3 Individual Differences – Sex, Health Status, Social Status, and Behavioural Preferences

Individual differences between animals – which arise from individual preferences, sex, and health status – must be taken into consideration. These variations result in highly individualistic animals which require consideration for all facets of husbandry, procedures, and experimental design and activities. Due to genetically diverse populations and different rearing conditions, reptiles are likely to demonstrate individual behavioural preferences, including general temperament (bold or shy), food preferences, and habitat preferences.

Sexual differences can be among the most important variables in research. While some reptile species are sexually dimorphic, it can be difficult to determine the sex of certain species based on visual clues. Sex can also affect social behaviour (e.g., males may be more active or aggressive) and health status (e.g., females can suffer from dystocia, or egg binding). Reptile development, particularly before and after sexual maturity, can impact husbandry and handling requirements and behavioural traits.

The health status of reptiles has significant implications for their use in research and how they are housed within a facility. Quarantine and sentinel programs, and other means of monitoring the colony for pathogens, are important in maintaining animals of a particular health status (see Section 8, "Health and Disease Control").

1.4.4 Effects of the Environment and Previous Experience

Differences in housing and husbandry conditions can result in variation between individuals of the same species. Even within the same housing enclosure, changes to an animal's environment, such as an increase or decrease in temperature or an alteration in light intensity, can be a source of stress that affects the animal's behaviour, physiology, or both. Inadequate or inappropriate husbandry is a factor for many reptile diseases. Nervous or aggressive species or individuals may require additional hiding spaces or restricted sightlines to prevent stress from perceived inter- or intra-species interactions from beyond the enclosure. Insufficient nutrition can cause growth retardation, while excessive feeding can cause obesity and physiological abnormalities.

Due to species diversity and high individualism, there can be significant differences in response to the same situation. Reptile handling, procedures, and husbandry should be developed in the context of the species' use and the individuals being handled.

The source of the reptiles can make a difference to their behaviour in the laboratory. Wild-caught versus captive-bred animals could potentially respond differently to the same stimuli. In addition, wild-caught animals may have different health statuses than captive-bred animals requiring different welfare and veterinary considerations.

2. FACILITIES

For general guidance on facilities, see the CCAC *guidelines on: laboratory animal facilities – characteristics, design, and development* (CCAC, 2003). Additional guidance and information of particular concern for reptiles are presented in this section. When planning new facilities to house reptiles, reputable experts in reptile facility design should be consulted for evidence-based approved practices (e.g., provincial or national herpetological societies, the Canadian Association of Zoos and Aquariums, the (American) Association of Zoos and Aquariums).

2.1 Animal Rooms and Procedure Rooms

For reptile facilities, the physical environment must include the general elements expected in a laboratory animal environment. Where possible, procedures should be performed in a separate room from where the animals are housed. Procedures may occur in the same room if barriers can be placed to block relevant stimuli. Racks and tanks for large reptile housing can be particularly heavy; therefore, it is necessary to ensure that the floors can support the weight. It is particularly important that the floors are non-slip, especially for aquatic enclosures.

While the primary containment enclosures should be escape-proof, floors, walls, and ceilings should be constructed in a manner designed to prevent the escape of the animals into the internal infrastructure of the wall or environmental chamber. Seams and small holes must be well sealed, and there must be strong weather stripping under doors. Construction materials need to be able to tolerate high humidity. Drains are required for aquatic and highly humid environments but must be covered with mesh to prevent the escape of snakes in particular. Floor-level refuges may be placed to enable retrieval in case of accidental release of animals. All electrical outlets must have ground fault interrupters. In addition to the general requirements for electricity, lighting, and storage, there must be appropriate areas to maintain live feed, if live feed is required.

The number of reptiles present in an animal facility is frequently insufficient to warrant a separate room for each species or even groups of species with similar environmental requirements. Animals with different environmental requirements may be held in a common room if the macroenvironment is suitable for all species held there, or the microenvironments are independently regulated to accommodate individual species' requirements.

If there are multiple enclosures in the same room, the potential for stress due to the proximity of potential predators or prey should be minimized, irrespective of whether there are different species or conspecifics (Stapley, 2003; Webb et al., 2009). Enclosures located near each other may allow the sight or scent of other animals to cause stress or behaviour that may cause injury. Situations where sightlines can cause these issues can be avoided through enclosure selection, orientation, or using opaque barriers. Situations where scent can cause these issues can be avoided through the separation or spacing of enclosures or through increased ventilation.

Housing recently wild-caught reptiles in the same room as reptiles that have already undergone quarantine can cause problems because of ectoparasite and disease transfer. Adequate space should be provided for quarantine and isolation. All wild-caught animals should be kept under quarantine or isolation conditions for a duration longer than the incubation period of any expected parasites or diseases, and appropriate veterinary screening should be provided (e.g., fecal sample tests).

2.2 Enclosures

<u>**Guideline 1**</u>

Enclosures must provide sufficient space and complexity to enable reptiles to perform behaviours important to their welfare.

The biological needs of each species and the nature of individual projects vary widely; therefore, this section contains only the most general recommendations on housing reptiles. When dealing with unfamiliar species, evaluation of several types of housing may be necessary to find the housing system most appropriate for the animal's needs and the purposes of the study. Husbandry information from zoos or hobbyist publications based on current reputable sources may be helpful in this regard.

Investigators can often infer the requirements for a particular species to thrive from their knowledge of the biology of their animals; such information should be incorporated whenever possible. Restraint and ease of maintenance by animal care personnel should not be the prime determinant of housing conditions. SOPs for housing and husbandry should be developed once appropriate conditions are established.

Similar to the Queensland government's Department of Environment and Science's *Code of Practice for wildlife management* (1992), all housing for captive reptiles must be:

- escape-proof;
- free from sharp edges or coarse wire;
- safe for the research team and animal care personnel by enabling access to the animal without being exposed to danger;
- located and designed to avoid the occurrence of dangerously high temperatures;
- of sufficient size to promote normal behaviour and activity by the inhabitants and accommodate environmental enrichment;
- easy and practical to clean;
- designed to allow safe access by handlers;
- illuminated sufficiently to enable effective and safe husbandry while meeting species-appropriate lighting requirements;
- equipped with ultraviolet lighting – for species requiring it – that must be able to penetrate the enclosure (e.g., through mesh that is not too fine); and
- well-drained (for large reptiles requiring water).

Indoor enclosure walls, floors, and fittings must be constructed from impervious materials that can be easily cleaned (NSW, 2013). Suitable reptile housing options include glass or acrylic aquaria, stackable caging systems, fibreglass tanks, and other types of impervious primary enclosures (O'Rourke et al., 2018).

Sufficient space must be available in the enclosure to create a heat gradient, offering both a warm and cool end of the species-specific thermoregulatory spectrum. As detailed in Section 3.1.2, "Temperature and Relative Humidity", species-appropriate heating must be provided, and enclosures must have the appropriate design and space for thermoregulatory behaviour. However, because the body temperatures maintained by many species of lizards during activity are only a few degrees below their lethal temperatures, overheating is a substantial risk if temperature gradients are poorly designed. The cage must be large enough that one end always remains cool, and there should be adequate ventilation to prevent overheating. Thermal gradients should typically be provided horizontally but may be oriented vertically in the case of climbing species. Shelters should be placed along the gradient length so that animals are not forced to choose between thermoregulation and security (see Section 3.1.2, "Temperature and Relative Humidity").

In most cases, a variable temperature regime is necessary. Any wires, cables, or electrical cords in the enclosure should be securely fastened in a manner that prevents an animal from becoming entangled in or having direct contact with them. A system for monitoring temperatures at both ends of the spectrum and emergency power to ensure environmental consistency must be in place.

2.2.1 Spatial Requirements

Spatial requirements for reptiles vary greatly, depending on species and life stage, that quantifying them is difficult. It is important that the space be large enough to permit free movement and exhibition of reasonable natural daily behaviours of the animals (Kaplan, 2014). The aim should be to promote natural behaviours, although it may be logistically impossible to replicate the animal's natural habitat. The space must also be sufficiently large for a proper temperature gradient to be set up and maintained. The space taken up by items such as feed dishes, water dishes, and environmental enrichment should be discounted from the total space, and these items should not impact the movement of the animal as intended by the space requirement. The space should be appropriately sized to accommodate the above requirements but not so large as to impair the animals' ability to perform observations or hinder successful feeding, especially of live insects.

When calculating a lizard's size for the purposes of determining enclosure size, the tail must be included in the total size as it is just as important in thermoregulation and the manufacturing of the precursors to vitamin D_3 as the rest of the body (Kaplan, 2014).

2.2.2 Enclosure Design

2.2.2.1 Cage Materials

Wood is an acceptable material for terrarium construction, but it must be properly sealed so that it is easy to clean and will withstand water washing; polyurethane or marine epoxy paint or varnish are suitable for sealing, but the safety of all products must be verified before use. Products that are safe for exposure to aquatic life are generally safe for use with reptiles.

Many species will rub their noses raw against wire screen enclosures and glass walls, and animals can injure themselves while attempting to squeeze into cage crevices, such as where the sides and top meet, and where the glass fits into metal frames on aquaria (Greene, 1995). Measures must be taken to mitigate injury (e.g., by covering the glass or moving animals to another type of enclosure).

Terrarium doors and lids should be constructed to facilitate access and cleaning, except for those housing venomous snakes, when the safety of the animal care personnel must be a priority over access and ease of cleaning. While cages should be easy to sanitize, some lizards, such as geckos, need climbing substrate on the enclosure walls.

An opaque top and three opaque side walls are generally preferred for terrariums, although this is not always possible if providing lighting or heating from above. If the top and sides of an enclosure are transparent, most reptiles must be provided with a covered area to shield themselves from light and outside disturbances. When using ultraviolet lighting, ultraviolet rays must be able to penetrate the enclosure (e.g., mesh that is too fine can prevent appropriate penetrance). For most species, one side of the enclosure should be fully or mostly glass or plexiglass to allow easy viewing of the inside of the terrarium. If desired, the clear wall can be provided with a partially or entirely removable covering to reduce negative stimuli, especially for highly irritable or easily frightened reptiles. Reflective surfaces should be avoided in terrariums.

2.2.3 Terrestrial Holding Systems

<u>Guideline 2</u>

Regardless of what type of terrestrial cage is chosen, it must be possible to control the environmental conditions within it to meet the needs of the species to be housed.

Correct arrangement of the cage environment is necessary for the occupants to thrive. Reptiles need a cage where they can perform a range of natural functions; as a result, aesthetic considerations must take second place to functionality. While species-specific requirements will vary, provision of secure shelter sites, perching sites, open areas for foraging, and access to water are generally necessary. At least 30-40% of the floor space should be left open for the reptile to easily move about, feed, water, and defecate (Kaplan, 2014).

Different species require unique, optimized environments, and general recommendations must be adapted for species-specific needs. Most lizards and snakes, as well as the more terrestrial species of chelonians, can be kept in terrariums.

A terrarium may be specially constructed or simply be a modified aquarium or another secure type of enclosure of appropriate size. Enclosures should be designed to balance appropriate ventilation with heat and humidity requirements.

Some reptile species, such as garter snakes and corn snakes, are quite active (Kischinovsky et al., 2018) and require larger enclosures relative to their body size to engage in species-specific behaviours. Enclosures should be of a sufficient height for species that climb or perch, such as iguanas, anoles, rat snakes, and corn snakes (O'Rourke et al., 2018).

Terrestrial reptiles should be provided with a water bowl located sufficiently low that small individuals can easily gain access and leave without drowning (Australian Code of Practice, 1992) or flipping themselves over on their backs (i.e., tortoises). Typical cage designs are depicted by Ewert et al. (2004). Some species like to soak; these species should be housed in an enclosure with room for a sufficiently large bowl for soaking.

A screened area, located at one end of the top portion of the cage, is a desirable location for a basking lamp mounted on the outside of the enclosure, should one be needed for thermoregulation. Basking lamps or "hot spots" are frequently essential for species-appropriate environmental enrichment and can also be important for gravid females and snakes with health problems. As an alternative to basking lamps, many forms of heaters are available, such as heat cables and pads, ceramic bulbs, and plate or radiant heat panels. Basking lamps or heaters should be placed in one location to enable the creation of species-appropriate thermal gradients from cool to warm. Regardless of heater type, it should be regulated, preferably with a thermostat, and animals must not be able to maintain direct contact with the heat source due to their lack of a withdrawal reflex, which can lead to burn injuries (see Section 3.1.2.1, "Temperature").

2.2.3.1 Lizards

Small lizards may be kept in aquaria or terraria to maintain adequate humidity, with a few exceptions. Chameleons and *Abronia* species should be kept in mesh enclosures (flexarium or cages) to allow for appropriate ventilation that decreases the risk of fungal infection. Large lizards (e.g., adult iguanas) may be kept in mews or large cages in rooms with controlled temperature and humidity. Regardless of type, enclosures should balance appropriate ventilation with heat and humidity retention. Lids for lizard cages must be provided, and all access points must be tightly fitted and secured to prevent escape (O'Rourke et al., 2018). Wheler and Fa (1995) provide useful recommendations for enclosure design for geckos.

Most species will drink from water bowls of varying sizes (see Section 6.4.2, "Drinking Water"); however, other species, such as chameleons, generally do not drink from bowls, and a drip-water or misting system should be provided. Some species and individuals can develop harmful behaviours if inappropriate (excessive or unnecessary) stimuli are visible outside the cage. This can include running into the cage wall during fear or territorial aggression. This behaviour can be managed by providing sufficient hides and placing external wall covers to reduce the line of sight to external stimuli and neighbouring animals. Visual barriers (e.g., external wall covers) may also be used to provide background colours as some species, especially colour polymorphs or cryptic species, prefer background colours that match their preferred environment.

2.2.3.2 Snakes

Snakes occupy a wide range of natural habitats, including aquatic, terrestrial, and arboreal. Species' specific characteristics must be considered when designing enclosures to promote natural behaviours while optimizing the health and welfare of the animals. Snakes can be deceptive about their requirements: some small, active snakes such as racers need more room, relative to body length, than do large, and largely sluggish, pythons and boas (Kaplan, 2014; Divers, 2020 – cited by Warwick et al., 2019; Kischinovsky et al., 2018). Regardless of habitat design, snakes should be able to stretch out (i.e., two-thirds of the length of the animal, horizontally or vertically depending on whether they are terrestrial or arboreal) and have adequate space to perform normal behaviours. Snakes will often use provided structures during movement and stretching. Semi-aquatic snakes require larger enclosures to provide a water area large enough for them to comfortably swim in, while the land area should be large enough for sleeping and basking (Kaplan, 2014). There should be sufficient room for the species' required thermal gradient, an adequately sized water bowl, a retreat box, and a place to feed (Kaplan, 2014). Snakes with more space exhibit better growth and muscle tone.

Access doors should be flush with inside surfaces and feature appropriate latching mechanisms for security (AZA, 2009).

Some species that are colour polymorphs or cryptic prefer background colours that are darker or that more closely match their colour morphs (e.g., Juvenile green tree pythons (Garrett and Smith, 1994) and the European adder (Capula and Luiselli, 1995)).

2.2.3.2.1 Housing Venomous Snakes

All the requirements and considerations previously discussed for snakes are equally applicable to venomous snakes. Potentially venomous reptiles with a low risk of causing a medically significant envenomation (e.g., Eastern hognose snake) may be housed and handled as non-venomous reptiles, but bite protocols must be in place. Venomous reptiles with a moderate or greater risk of causing a medically significant envenomation (e.g., Massasauga rattlesnake) must be housed and handled with all procedural and administrative protocols and facility infrastructure to minimize the risk of envenomation and enable prompt response to injury.

Venomous species of snakes should be kept in non-breakable cages that are completely secure. All cages containing venomous animals must have functional double locks; the locks must be secured when there is an animal inside. In addition, the following precautionary criteria should be met.

Ventilation Ports – All openings except the lid should be obstructed in such a way to prevent successful strikes. This is commonly accomplished by utilizing a double layer of screening to ensure that there is no possibility of the snake coming into contact with personnel. Ventilation ports must be clearly marked as possible danger points of exposure to the snake's fangs.

Viewing Walls – Removable opaque covers should be fitted to the outside of the viewing wall to reduce aggression-inducing stimuli. All components of the enclosure, including the viewing wall, should be shatterproof.

Access – The terrarium should be deep enough to at least slow down any attempt by the snake to climb to the top. If floor-level doors are used, it must be possible to see the snake while opening the door. Newer cage designs for venomous species often include shift panels; these are particularly useful as they allow the animal to be segregated from the area being serviced and decrease personnel risk by minimizing animal handling (O'Rourke and Lertpiriyapong, 2015). Common practice is to use "lock boxes" or hide boxes with doors that can be closed (with a snake hook or other tool) before doing any cage maintenance. The housing room and primary enclosure must be secured to prevent unauthorized access.

2.2.3.3 Terrestrial Chelonians (Box Turtles) and Tortoises

Although tortoises are often considered to be slow-moving animals, a healthy individual housed in a proper environment is both active and quick. Terrestrial turtles are also relatively fast-moving. Many terrestrial chelonians range widely throughout their habitat, with several sleeping and basking areas used every day, and species-specific habitat preferences should be considered. Most are burrowers and efficient diggers, easily digging under outdoor pen walls and fences. Chelonians, especially tortoises, are also good climbers. Escapes can be prevented when chelonians are housed indoors by building the enclosure walls higher than they can stretch when they climb on the back of another inhabitant or on top of a rock, log, or hide box (Kaplan, 2014).

2.2.4 Aquatic Holding Systems

Aquatic holding systems are needed for semi-aquatic and aquatic turtles, freshwater or seawater snakes and crocodilians. Of these species, freshwater turtles are the only aquatic reptiles commonly held in laboratories in Canada.

Tanks must be of an appropriate size to permit free movement and reasonable daily behaviours of the occupants. Aquatic and semi-aquatic turtles need both a land and water area. The water area must be large enough for them to swim freely through the water. The tank must be deep enough to accommodate silt or sand for burrowing, as appropriate, and in the case of turtles, sufficient water for the animal to submerge and right itself if it becomes turned over. For some species of aquatic turtles, a haul-out area big enough for all the tank inhabitants may be all that is needed in an otherwise completely aquatic enclosure. However, most semi-aquatic species will require a substantial land area as well as a water area. The haul-out and land areas are used for basking, sleeping, and laying eggs.

A good, strong aquarium or prefabricated tub is required for aquatic reptile species. Aquatic tanks are very heavy, and there is tremendous pressure placed on the walls of the enclosure. Aquatic turtle housing may comprise a large stock tank (e.g., for watering cattle), either directly on the floor or on a riser. The surround can be built up to provide an area of land and to enclose the animals. Aquatic turtles should have the opportunity for continuous or uninterrupted swimming, as is found in circular tanks. Those species whose natural habits include burrowing into the silt or sand at the bottom of their native lakes or rivers may have similar substrate layers in the bottom of their enclosure, provided the substrates can be periodically changed or cleaned to ensure appropriate water quality. Natural behaviour may require different water depths and habitat enrichment for different species at different life stages; this should be considered when establishing pool design and water depth.

A platform just clear of the water surface should be provided as a resting board on which the turtles can haul out and bask. It may be necessary to offer a visual barrier to potentially negative external stimuli (e.g., personnel movement) to encourage the use of the basking platform. As resting platforms are continuously water-soaked, the use of wood for these structures is not appropriate; flat rocks or custom-made, water-impervious platforms can be used. As discussed in Section 2.2.2.1, “Cage Materials”, the safety of all products must be verified before use. The stability of resting platforms should be ensured to prevent toppling and potentially trapping animals. A sloping approach is required so that turtles can easily leave the water. Turtles must be able to get a firm hold with their claws to pull themselves out, as it is possible for turtles to drown if they cannot easily leave the water (Queensland, 1992). There should be a basking lamp above this resting area.

Flow-through water systems are suitable for freshwater turtles (CCAC, 2005). Recirculating systems should have robust filtration (e.g., a filter rated for a 400 L aquarium should be used for a 200 L turtle tank). Dechlorinated water must be used.

For the safety of the animals and personnel, lids are recommended on enclosures for all reptile species. Enclosure lids are required if the enclosure sides are of insufficient height to prevent escape as some turtles are effective climbers.

Sea turtles, crocodilians, and aquatic snakes are not commonly held in Canadian laboratories; therefore, if they are to be held, species- and facility-specific SOPs should be developed. These SOPs should ensure the development of appropriately sized enclosures that are safe for occupants and animal users and furnished to permit free movement and appropriate daily behaviours of the animals (Brien et al., 2016). Investigators should consult literature and individuals with relevant expertise if planning to work with these species or others not described in these guidelines.

3. FACILITY MANAGEMENT AND PERSONNEL

3.1 Managing the Environment

Guideline 3

Laboratory management practices must aim to ensure the macroenvironment (room) and microenvironment (primary enclosure) maintain the health and welfare of both the animals and personnel and provide consistency for research outcomes.

The most practical and effective way of providing suitable holding conditions for reptiles within an animal facility is to first establish a set of general environmental conditions for the rooms as a whole, for parameters such as day length (photoperiod), humidity, and temperature range. Secondly, each terrarium or tank should be established as an individual environmental chamber in which temperature, light level, and humidity can be adjusted to suit the requirements of each species.

The microenvironment of the enclosure should meet the physiological needs of the species and the life stage of the animals. Physiological needs can vary widely between species and between different life stages within a species, and facilities must be able to accommodate all physiological needs before housing new species or life stages. Divers (2020) lists husbandry requirements which can be used as a rough guide for preferred optimum lighting, temperature, and humidity for selected reptiles. Special equipment needed for housing reptiles includes humidifiers, room-controlled heating and cooling – most brumation requires low to average temperatures (i.e., 5-15°C), and additional ground fault interrupted outlets (for heat lamps). Specialized plumbing for terrarium drip or misting systems may also be needed. All the equipment needed for aquatic enclosures is listed in the *CCAC guidelines on: the care and use of fish in research, teaching, and testing* (CCAC, 2005).

3.1.1 Lighting

A regular day:night light cycle (e.g., 12 h:12 h) should be maintained, or lighting should follow the seasonality of day length, as many reptiles obtain physiological cues from light:dark cycles. Many reptile species have a circadian rhythm (measured as melatonin level), and photoperiod differences affect the phase, amplitude, and duration of this rhythm. Both constant light and constant dark environments have been shown to induce stress (Bradley Bays and de Souza Dantas, 2019). For example, light during the night can suppress activity, as shown in adult prairie rattlesnakes (Clarke, 1996). If animals are being bred in-house, appropriate lighting is also critical for egg incubation: light exposure accelerates embryonic development but may have negative survival outcomes, depending on the species (Zhang et al., 2016). Wild-caught animals may stop eating as the season changes and need a reduction in photoperiod for a period of time in order for eating to be stimulated once the day length is increased. For captive specimens to thrive, and especially to reproduce in captivity, some exposure to seasonal variation in day length (photoperiod) or temperature may be necessary.

Visible light (400–700 nm) has several effects on reptile behaviour. First, light intensity is used as an indication of temperature; higher intensities are associated with higher temperatures. This has been shown for basking species such as anoles and turtles. Light also impacts thermoregulatory behaviour in the nocturnal Tokay gecko (Sievert and Hutchison, 1988). It is important to understand the needs of the particular species with regard to the light spectrum. Given a choice, iguanas prefer incandescent light over ultraviolet light, likely due to the former's warmth. Dickinson and Fa (1997) recommend using both ultraviolet and incandescent light in the captive environment. Turtles, especially young turtles, must have the option to bask under sunlight or ultraviolet radiation at least three times each week.

3.1.1.1 Ultraviolet Light

While humans can only see visible light, some reptiles can also see within the ultraviolet range (290-400 nm). Before procuring reptiles, it should be determined whether ultraviolet or full-spectrum lighting is required (Ferguson et al., 2010; Baines et al., 2016). Providing ultraviolet light via suitable lamps seems beneficial for most species (Oonincx and van Leeuwen, 2017). Exposure to ultraviolet light must be direct, as normal glass and fine mesh block ultraviolet radiation. Animals should not be permitted to be near sources of ultraviolet light, as high levels of ultraviolet light can be detrimental to some animals (e.g., chameleons, bearded dragons, nocturnal species, and certain morphs (albinos) may experience eye and skin damage). Placement of the ultraviolet B light source is a balance of proximity for effective ultraviolet B exposure and distance for appropriate light intensity.

Varying bulb intensities are available, and it is important that the species-specific bulbs be procured, and that either scheduled ultraviolet tests be performed or scheduled bulb changes documented. Mercury vapour bulbs are often the best option for providing ultraviolet B light, provided they can be used safely. Otherwise, ultraviolet light can be provided by fluorescent bulbs; however, it should be noted that these bulbs have a short lifespan. Compact fluorescent bulbs may have a poor distribution of ultraviolet B light.

In some species, social interactions increase when a source of ultraviolet light is provided (Oonincx and van Leeuwen, 2017; Vergneau-Grosset and Peron, 2020). This suggests that these species have visual sensitivity within the ultraviolet A range. For example, anoles use ultraviolet light for intraspecific communication via dewlap recognition.

Many lizards and chelonians require an ultraviolet B light source for normal calcium metabolism and vitamin D synthesis (Baines et al., 2016; Rossi, 2019). Oral supplementation of vitamin D_3 should not be used as a replacement for providing appropriate ultraviolet B light: oral supplements alone are not as effective and can increase the risk of hypervitaminosis.

3.1.2 Temperature and Relative Humidity

3.1.2.1 Temperature

Thermal considerations are important for the health and well-being of reptiles due to their ectothermic nature (Ferguson et al., 2010; Christian et al., 2016). Taxon-specific ranges of preferred temperature can be obtained from the primary literature (reviewed in Baines et al., 2016; see also Varga, 2019; Rossi, 2019).

Many nocturnal lizards and snakes do not routinely thermoregulate behaviourally and therefore require air temperatures that equate with their natural environment (Ferguson et al., 2010). All diurnal species thermoregulate behaviourally and require cage designs that provide thermal gradients and ample opportunity for animals to thermoregulate behaviourally by choosing from diverse microenvironments (Ferguson et al., 2010; Rossi, 2019; Varga, 2019). Before placing any reptile in a cage, it is necessary to understand the temperature gradients within the cage by monitoring conditions at various locations with a thermometer, recognizing that seasonal change may affect these conditions.

Every effort should be made to ensure that the caging environment provides thermal conditions that are appropriate for the species and that enhance behavioural and physiological function (NSW, 2013). Baines et al. (2016) provide general guidelines for estimating preferred temperature ranges based on characteristics of the animals' natural habitat, and Rossi (2019) and Baines et al. (2016) include information on the preferred optimal temperature zone for many species. Most sources recommend that captive reptiles should experience thermal cycles around this "preferred" temperature. Where possible, such thermal cycles should be based on natural thermal variation during the normal active season of the organism, provided that natural variation does not exceed the critical thermal limits of the animal (ASIH, 2004). Recently fed and gravid animals may seek higher temperatures, while inactive animals may sometimes seek to remain cool, even immersing themselves in water (Australian Code of Practice, 1992). If the animal spends most or all of its time in either the hottest or coldest part of the cage, it may be an indication that the temperature settings need adjustment.

Reptiles are very sensitive to sudden temperature changes. If captive reptiles are forced to experience temperatures substantially outside their preferred ranges, illness or death may result. For example, if inappropriate cooling occurs, reptiles may experience decreased metabolic function including extended gut transit time and lethargy, immune suppression, and potentially, death. Similarly, care must be taken that laboratory enclosures do not become too hot. There must be areas that are sufficiently cool to enable the reptiles to lose and gain heat. The microclimate temperature gradient should stay constant throughout the day. The cool end of the microclimate should be sufficient for nocturnal use, and basking heat should always be available.

It should always be possible to control the temperature independently of light. An incandescent light or sun lamp is a useful source of supplementary warmth for basking reptiles but should never be the sole source of either heat or light. Room controls, a space heater, or other localized heating sources may be used to maintain suitable temperatures when the lights are off. Radiant heat panels installed at the top of an enclosure can also be used. All heat sources should be controlled with a thermostat or routine static monitoring to prevent overheating. The heat sources and regulators should be CSA or ULC certified and properly installed by a qualified electrician if wiring is required. Bulbs should be positioned so that the animal can approach and warm itself easily, but not so close that it can burn itself (Pees and Hellebuck, 2019). For aquatic enclosures, heating sources can include titanium or glass heaters contained in an unbreakable cage, either in line with the life support system or submersed in the tank or filtration system itself. If submersed in the tank, measures should be taken to prevent interaction by the animals with the heat source or electrical cable. Aquatic heat sources should be connected to an electrical circuit with a ground fault circuit interrupter.

The temperature must be monitored in the enclosure, rather than relying solely on heat source controls. Temperature probes should be placed in multiple sites and should be alarmed. In addition, remote thermometers (e.g., infrared heat gun) should be used to verify the probes and additional enclosure temperatures (i.e., basking zone and cool zone) on a regular basis. Failure of electrical and heating, ventilation, and air conditioning (HVAC) systems leading to cooling or overheating can be lethal for reptiles. Therefore, as for other species, animal facilities housing reptiles should have backup emergency power (O'Rourke et al., 2018).

3.1.2.2 Humidity

Low humidity can be hazardous for small individuals and species adapted to humid, tropical conditions, such as chameleons. Some means of controlling air exchange within the enclosure and the room as a whole should be provided. Neither a completely open unit, such as a wire-mesh mammal cage, nor a tightly closed one is desirable. Ventilation ports must always be screen-covered to prevent escape. Ventilation ports may be used to adjust airflow and assist with humidity regulation, but the airflow should not be reduced to a level where it promotes mould or pathogen growth. Consistent airflow should be maintained, with adjustments in humidity provisions as required. Species of snakes that normally live under humid tropical conditions require a relative humidity between 60% and 90% saturation in their enclosure; failure to maintain a high humidity may result in the snake's inability to shed its skin completely (see Section 8, "Health and Disease Control"). Elevated humidity can be maintained by evaporating water from a container placed near the heater or light, by adding a container filled with damp peat moss to the enclosure, or by hanging an absorbent paper wick with one end in a dish of water. Ultrasonic foggers and misting systems can be used to increase humidity if needed, but should be set appropriately so that the substrate does not become saturated. Ultrasonic foggers should not be used in the enclosure itself. While a damp substrate is suitable for some species (e.g., arboreal geckos) or a short-term increase in humidity, in general, the humidity level must not be maintained by allowing the substrate to become soaking wet, as this promotes bacterial growth and may lead to scale rot and other skin problems (Australian Code of Practice, 1992), or respiratory disease.

Humidity requirements should be considered on a case-by-case basis. It is reasonable for the animal care committee to request references that recommend specific humidity guidelines for particular taxonomic groups (ASIH, 2004). The humidity of enclosures should be monitored regularly.

3.1.3 Air and Water Quality

3.1.3.1 Air Quality

Airflow in the room must be sufficient to allow surfaces to dry properly. The optimal turnover of air depends on the requirements of the species, the temperature and humidity of the source air, and the ability to create a microenvironment. A high turnover of dry air can lower humidity enough to dehydrate reptiles and cause dysecdysis or abnormal shedding pattern, while low turnover can result in increased humidity to a level that promotes condensation, microbial growth and contamination, and corrosion of metal. High humidity can also lead to shedding problems. Rooms housing terrestrial species may not require the air change rate to be as high as those housing mammalian species, keeping airflow directions in mind (e.g., AZA (2013)). Pathogens can travel in aerosols over a distance of a few metres, so any sick animals must be moved at least a few metres away from other animals.

For rooms housing aquatic species, a minimum of 12-15 air changes per hour, as recommended by aquatic equipment manufacturers (Cardona, 2017), should be used as a starting point, and monitored.

3.1.3.2 Water Quality

<u>Guideline 4</u>

Water quality must be monitored.

Water quality is very important to the health of aquatic and semi-aquatic animals and must be monitored in line with the capacity of the life support system. The monitoring frequency should be based on the level of assurance that husbandry practices are adequate for maintaining good water quality for the particular animals present in the enclosure. The rate of water changes in an enclosure should be based on maintaining water quality in relation to the animal's needs, which includes the development and maintenance of the skin microbiome. Water should be observed for cleanliness, turbidity, and high-level sedimentation.

The main water variables to be measured daily are temperature, pH, and conductivity or salinity. Measuring conductivity or salinity is essential for aquatic species; they will not survive in water treated with reverse osmosis if it has not been properly reconstituted. The water must also be free of chlorine and chloramine, which can be present in municipally treated water supplies. Chlorine and metals such as copper, which can leach from pipes, are toxic to some animals and life stages. Aquatic and semi-aquatic reptiles obtain oxygen via their lungs and are therefore more resilient to the effects of ammonia, nitrate, and nitrite than fish and amphibians that obtain oxygen via gills or skin. If reptiles are housed in an environmentally controlled room with set temperature and humidity, the water temperature does not need to be monitored as regularly as when in rooms without such environmental control.

3.1.4 Sound and Vibration

Lizards and turtles have similar acoustic physiology to humans and are capable of hearing, albeit at the lower end of the human range (20-20,000 Hz). Turtles such as the red-eared slider, loggerhead, and green sea turtle hear sounds in the 50-900 Hz range (Piniak et al., 2016; Wang et al., 2019; Bartol et al., 1999). Lizards such as the Tokay gecko and green anole hear sounds in the 1-3,000 Hz (Tokay gecko) and 1-7,000 (anole) Hz ranges (Brittan-Powell et al., 2010). Snakes do not have functional outer and middle ears and thus cannot "hear" as many other species do, but have an acute vibration sensitivity that allows them to detect and respond to low range soundwaves in the 80-160 Hz range (Young, 2003). Studies on the impact of mining noise on lizards have shown that high frequency, high amplitude noise elicits fear responses (Mancera et al., 2017), indicating that noise should be taken into consideration when designing and managing reptile facilities.

Some reptiles use vibration as a means of communication (e.g., Barnett et al., 1999; Hill, 2009). The level of vibration in the facility should be assessed, particularly when renovations are taking place, and considered a potential negative welfare concern. Both noise and vibration levels should be minimized; potential solutions include placing rubber tires under tanks or standing racks in buckets of sand. Environmental chambers are particularly noisy, requiring ear protection for people working in them; as a result, they may not be appropriate for studies with reptiles.

3.1.5 Brumation Requirements

It is important that species-specific temperature requirements be understood before initiating brumation as inappropriate brumation can be lethal. Most brumation needs low to normal temperatures (i.e., 5-15°C). Facilities must have the ability to gradually increase animal temperatures back to normal at the end of a period of brumation. In addition, there must be a means of changing the lighting so that a regular photoperiod is resumed at the end of brumation.

3.2 Personnel

Guideline 5

Reptiles must be observed regularly by trained personnel, with minimal disruption to the animals.

Sufficient animal care personnel are needed to ensure: 1) enclosures are cleaned, food and water are provided, and other husbandry requirements are addressed, as appropriate; and 2) animals are observed regularly. Under most circumstances, reptiles must be observed daily. In some circumstances, direct daily observations of the animal may be deleterious to health and welfare, and alternate observation procedures may be approved by the animal care committee. Daily observation of containment and environmental systems supporting the animals must be performed by trained personnel who can recognize welfare concerns and health problems in that species and respond appropriately. Responses may include resolution by following institutional SOPs, proper record keeping, and reporting concerns and procedures to the facility manager, veterinarian, and investigators. The frequency of observation should be described in an SOP for each species. While some animal care may be entrusted to well-trained students or other members of research or testing teams with the approval of the animal care committee, the work by these persons must always be overseen by animal health professionals (CCAC, 2008). All containment and environmental systems supporting the animals must be checked daily.

Working with reptiles can pose challenges for animal care personnel used to working with mammalian species, such as rodents. It is particularly important for the animal care personnel to work closely with the research personnel and take time to fully understand the needs of the animals. Where possible, dedicated personnel should care for the reptiles in the facility. If this is not possible, for their safety, personnel should be careful not to carry the scent of rodents into the reptile facility. An institution that maintains reptiles for research, teaching, or testing must make species-appropriate training resources available to all personnel and investigators (CCAC, 2015).

Where welfare concerns are identified, any additional demands on personnel time to implement appropriate mitigation strategies also need to be considered and accommodated.

All personnel should use appropriate practices that respect the welfare of the animals (e.g., not tapping on the tank, moving tanks in a way that minimizes disturbance).

3.3 Pest and Vermin Control

General information on pest and vermin control is provided in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017). See Section 6.9, “Cleaning and Sanitation”, for more specific information on enclosure disinfection. Insect infestations within enclosures should be managed with mechanical methods (e.g., sticky paper placed out of the reach of the animals). Extreme care must be taken to prevent insect exposure to insecticides when the insect may be consumed by the reptile. A veterinarian must be consulted for any animal experiencing a parasitic infestation or any other pathogenicity.

4. PROCUREMENT

The *CCAC guidelines on: procurement of animals used in science* (CCAC, 2007) should be consulted for general guidelines that apply to all species. This section provides additional considerations that are particular to reptiles.

4.1 Source

<u>Guideline 6</u>

Facilities and investigators acquiring or transporting reptiles, or conducting research on reptiles, must be familiar with and comply with relevant international, federal, and provincial or territorial legislation and policies.

Investigators should be prepared to justify the source of reptiles for their studies, based on the type of research being carried out. Investigators should be aware of local bylaws and regulations that may limit or require exemptions for certain species. Purpose-bred reptiles should be used over animals taken from the wild (Council of Europe, 2004). Where possible, common, readily available species should be selected and should be obtained from reputable breeders rather than breeding in-house. If a reliable and high-quality source of reptiles is not available, in-house breeding may be a valid option (see Section 5, "Breeding").

Individuals of endangered or threatened taxa should not be removed from the wild nor imported or exported, except in cases involving conservation efforts that are in full compliance with applicable regulations.

Reptiles often live many years in captivity, yet particular species may be quite difficult to obtain. Consequently, investigators conducting comparative studies may need to maintain individual reptiles for long periods, during which they may be used for a variety of teaching and research purposes (see the CCAC guidelines on the identification of scientific endpoints, humane intervention points, and cumulative endpoints (in prep.) on establishing time limits and endpoints for the long-term holding of animals). Various species (e.g., bearded dragons, geckos, corn snakes, king snakes, ball pythons) are now bred in captivity, while some are readily available from wild populations (such as green anoles). The procurement of many reptiles is not the same as the procurement of laboratory rodents, which can be ordered for a specific short-term use, often within quite a narrow time frame.

4.1.1 Captive Bred

There are several benefits of using captive-bred reptiles (adapted from Reed, 2005):

- Laboratory or captive-bred individuals have a known life history, age, and diet, and the potential for introducing unwanted diseases or parasites to an existing colony can be reduced.
- Captive-bred animals have the experience of artificial rearing, housing, and husbandry conditions similar to those they are likely to experience for the rest of their lives. Events such as handling by humans and tank and water cleaning practices are likely to cause less stress or health and welfare problems in captive-bred than in naive, wild-caught animals.
- Laboratory-reared reptiles inbred over several generations may reduce the effect of individual genetic variation on experimental outcomes.

- Reptiles are an important part of the ecological balance of many habitats. Removing reptiles from these habitats may disrupt the balance of the local ecosystem.

Reptiles should be obtained from reputable commercial suppliers. The selection of suppliers should be influenced by health and genetic parameters and associated import and export requirements of the reptile in question. Where possible, reputable local suppliers should be used to reduce transport-associated stress. Reptile suppliers acquire their specimens through field capture, trade, or captive breeding. Institutions should recognize that the legality and professionalism of reptile suppliers vary tremendously and should endeavour to ensure that suppliers are reputable, noting that this can change from year to year. Possible indications that a supplier is reputable include:

- transparency – they allow site visits and review of their husbandry records;
- referral from a qualified veterinarian; and
- provision of their permit to operate, if required by local legislation.

Patronizing suppliers who traffic in illegal animals or who fail to maintain healthy stock encourages the continuation of those practices. Institutions should be aware that some licensed suppliers obtain their animals from the pet trade or from the wild. Reputable zoological parks can sometimes provide surplus animals to other professional organizations, although their charters often preclude subsequent invasive experiments with those individuals (Greene, 1995). As many reptiles are long-lived, institutions should develop relationships with breeders to permit healthy animals to be returned once they are no longer needed for scientific studies (see Section 12, “End of Study”).

4.1.2 Wild Caught

The use of wild-caught animals should be considered only when the proposed studies relate to the environment, ecology, welfare, or sustainability of the species, or when animals are not suitable or available as captive-bred. Investigators must be able to provide specific justification for the need to use wild-caught individuals (Reed, 2005), which will be study-dependent. It should be noted that, as for other species bred in captivity, animals bred in-house for long periods of time have been found to have different intestinal flora from their counterparts in the wild and also develop different diseases.

Before removing animals from the wild, every effort should be made to understand the local population status – abundant, threatened, rare, etc. – of the taxa to be studied. The numbers of animals removed from the wild must be kept to the minimum the investigator determines necessary to accomplish the goals of the study. Consideration should also be given to other benefits that can derive from animals removed from the wild, such as providing genetic samples or location data to other investigators, in order to obtain the most benefit and reduce the need for additional wild-caught specimens elsewhere.

It is the responsibility of the investigator to ensure that the capture of animals is done according to CCAC guidelines, whether the collection is done by trained institutional personnel, students, or third-party specialists. The *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003) should be consulted for information on capture. Methods for capturing reptiles include hand capture, sometimes assisted by a hand-held lasso or net; pit-fall trapping; and road driving. The capture of reptiles in the wild should be carried out in a manner that minimizes bycatch and habitat damage;

for example, cover objects should be returned to their exact resting places in order to preserve the microhabitats under them). Pit-fall traps, drift fences, and other indirect methods of capture must be designed and used such that captured animals do not perish. Traps must be checked at least every 24 hours (Agreement on International Humane Trapping Standards), but more frequent checks may be required, depending on the metabolic requirements and natural history of target and potential bycatch species, including any potential mammalian bycatch species. Traps can be designed to capture target species while allowing bycatch to escape, and can be supplemented with species-specific food and habitat to ensure animal health between trap checks. Wild-caught reptiles should normally be released back where they were initially caught if they have been held short term and not been manipulated in a manner that would impair their survivability in that environment. The biosecurity of wild-caught animals intended for release must be maintained to eliminate the risk of introducing pathogens to the environment upon their return. Local, provincial or territorial, and federal wildlife agencies must be contacted to determine the regulatory requirements and restrictions regarding the collection, keeping, and release of wild animals that have been kept captive in an animal facility.

4.2 Regulations

The 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) specifically applies to many reptiles, such that additional federal permits are required when obtaining these species in the wild and transporting them across international boundaries.

Imports involving reptile species are listed in Appendices I-III of the CITES convention (CITES; 2021). Appendix I of the CITES convention lists reptile species that require an import permit from Canadian authorities before entering Canada, as well as an export permit from the country of export. The species listed in Appendices II and III require only an export permit from the country of export or re-export. Non-CITES-listed species do not require these permits but may be subject to other permit requirements. Most reptile species are not listed in CITES; however, many commonly traded species or higher taxonomic groups (i.e., all members of the family) are. Those responsible for importing reptiles should also be aware of the source nation's export requirements (e.g., a Form 3-177 Declaration of Importation or Exportation of Fish or Wildlife is required to export animals from the United States, US government (2005)). Some countries do not allow any export of their wildlife.

Anyone procuring reptiles should also be aware that the Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act requires compliance with all relevant wildlife laws of other countries or provinces and provides for penalties in Canada for anyone contravening laws of other jurisdictions.

Under the Canadian Food Inspection Agency Health of Animals Regulations (Government of Canada, 2021), a permit is required to import all chelonians and their eggs. This permit is normally issued only to zoos and research laboratories and is not issued for commercial purposes. Restrictions on the importation of chelonians and their eggs are due to the risk of transmitting diseases, such as Salmonella. Other types of reptiles are not covered under these regulations, so no Canadian Food Inspection Agency import permit or health certificate is required when other reptiles are brought into Canada.

Institutions must also make themselves aware of any provincial or territorial, or municipal restrictions or licensing requirements for the procurement of reptiles.

4.3 Pre-Shipment Procedures

Guideline 7

Information relating to the transport, welfare, and care of the reptiles should be communicated between the supplier and receiver before shipment of the reptiles occurs.

Any signs of pre-existing physiological stress (e.g., dehydration) should preclude the shipment of animals.

Before shipping a reptile, its food should be withheld for 1-7 days, depending on the species. Fasting duration should be sufficient to allow complete digestion before beginning the trip (e.g., a day for most lizards and at least several days for most snakes).

4.4 Transportation

Guideline 8

The health status of reptiles being received should be reviewed before animals are shipped.

The first requisite of proper shipping is a healthy animal. Small harmless lizards and turtles can be sent by airmail, using the fastest means possible. Larger animals and all snakes should be sent via air freight (either via courier or an airline). Venomous species require special packaging and should only be sent via airline courier when shipped. Carriers should be contacted in advance to learn applicable regulations and schedules for shipping. Recipients should monitor a shipment's routing diligently so that it can be retrieved promptly and unpacked. Reptiles typically do not require feeding or other temporary care during transport, although some species that desiccate easily (e.g., chameleons) will require special moist packing.

Shipping companies should follow the International Air Transport Association (IATA) and the Animal Air Transport Association's (AATA's) recommendations for adequate animal transportation (see *CCAC guidelines on: procurement of animals used in science*, 2007), noting that the larger carriers tend to have better-controlled temperatures (Tetzlaff et al., 2016). Investigators should ensure that couriers transport animals in accordance with IATA and AATA regulations.

IATA Live Animal Regulations are a good source of information on container designs and appropriate animal densities within containers. During transportation, reptiles should be placed in containers that are closed, adequately ventilated, constructed of sturdy non-toxic materials, and insulated to protect the animals against temperature variations (IATA, 2020). Containers should be properly labelled with respect to contents and appropriate handling; prominent notices to keep from extreme heat and cold are especially important.

For unaccompanied long-distance transportation, insulated foam shipping containers – a Styrofoam inner box placed in a water-resistant outer box – are recommended to prevent sudden changes in temperature and to provide a buffer against temperature extremes. Shipping containers must allow sufficient ventilation for proper breathing while preventing the risk of animal escape or injury. Freshwater turtles should travel in a damp environment that minimizes their movement so that they cannot be turned upside down. Fabric should not be used in shipping containers for freshwater turtles as their claws may get snagged. Lizards and snakes can travel in dry cloth bags. Lizards that cannot tolerate dry conditions should be shipped in containers with moistened paper towel or sphagnum moss. Venomous snakes travel well in bags but must be further enclosed in solid but ventilated boxes to prevent escape and to prevent handlers from being bitten through the bag. Venomous snakes should be double-boxed and properly identified (i.e., each snake is confined in a securely knotted cloth bag and placed into a container or box with a secure lid, then placed in a larger insulated box for shipping). Most species should be maintained between 16°C and 25°C.

Guideline 9

Animals should not be shipped if weather forecasts predict extreme (very hot or very cold) temperatures.

Airlines may have specific weather-related policies for shipping animals that should be consulted. Heat packs and cold packs may be placed inside the insulated shipping box to compensate for the external environment. Heat and cold pack quantity and placement are species-specific, and individuals experienced in shipping reptiles should be consulted regarding their inclusion and placement. Temperature packs of varying intensities and durations are available. The use of heat and cold packs should also take into account external environmental factors that may be encountered during transportation. Room temperature gel packs can help to buffer against temperature variations en route.

To prevent predator-prey interactions, territorial aggression, and toxic effects between certain species, crowding should be avoided when packing different species, different size classes (i.e., small and medium), or individuals of the same species that are likely to injure each other. Animals must be transported individually in containers within the shipping box, and the containers packed in a manner that prevents visual interactions. Consideration should be given to whether animals need to be transported individually in a shipping box (i.e., a single animal per shipping box) to avoid olfactory or auditory predator-prey stimuli, even within the same species.

For short distances (e.g., transportation between laboratories in a facility), most species may be accommodated in cotton bags, knotted at the neck, and transported in Styrofoam coolers. Bags should not be left unattended outside the coolers. The bags must be carefully inspected for holes. Proper ventilation and protection from temperature extremes are essential. Bags should be kept out of direct sunlight and away from hot surfaces, as the animals can overheat quickly.

4.5 Receiving Animals

Guideline 10

The health and welfare of reptiles must be checked upon their arrival by competent animal care personnel.

In addition to reviewing the health status of the animals in advance of their arrival, it is also important to obtain as much information as possible on the details of the husbandry and other reptile-related practices of the source institution shipping the animals. This will assist in establishing quarantine conditions for the animals upon arrival.

Reception conditions should be described in an SOP and include steps to be followed upon opening containers, such as:

- verifying that the animals received correspond to the order;
- checking the internal temperature of the container;
- decontaminating the exterior surfaces of non-disposable containers;

- opening the container in such a way as to prevent the animal's escape;
- handling the reptiles in such a way as to prevent contamination (e.g., not touching the reptiles with hands that touched the exterior of the container);
- verifying that the animals are alive (they may be hypothermic);
- verifying that all animals have been removed from the transport container; and
- dealing with animals that are sick or dead upon arrival.

A veterinarian or other qualified and well-trained individual must assess the condition of the animals upon receipt, according to the institution's SOP. A visual examination of the animals upon arrival is valuable to assess any need for immediate treatment (e.g., for dehydration, trauma). It is helpful to communicate with the supplier to provide information about the state of the animals on arrival in case adjustments for future shipping arrangements need to be made. Observation of animals received from a shipper is also important to ensure that the new groupings of reptiles are compatible.

All containers must be thoroughly cleaned and disinfected or sterilized if intended for re-use (IATA, 2020), held for inspection, or destroyed in accordance with relevant regulations. For more information on decontamination procedures, refer to Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada (Canadian Herpetofauna Health Working Group, 2017).

4.6 Quarantine and Acclimation

Guideline 11

Reptiles should undergo quarantine and acclimation after transport and before use in a scientific activity.

Animals brought into the facility must undergo quarantine. Ideally, quarantine should be carried out in a separate room or area, with separate sets of equipment used in each. However, a procedural quarantine (i.e., working with new animals last) may be logistically easier than physical quarantine for most institutions. If procedural quarantine must be used, all equipment must be carefully decontaminated after use in quarantined enclosures.

A period of environmental acclimatization can run concurrently with the period of quarantine. It is important to ensure that any stress associated with transportation has been alleviated and the physiology of the animal has returned to a normal state. The length of time required for quarantine will depend on the conditions of the transport, the age of the animals, and the particular animals involved. During this period, the animals should be habituated to the method of food and water delivery and to the new environment. Post quarantine, animals should also be acclimated to study conditions and any procedures that will be conducted while they are conscious (CCAC, 2017). The minimum acclimation period should range from two weeks (for smaller animals like anoles) to a month (for snakes like ball pythons with slower metabolic rates).

5. BREEDING

5.1 Rationale (When and Why Internal Breeding Programs are Indicated or Acceptable)

When possible, animals should be obtained from reputable, commercial captive breeding operations (see Section 4.1, “Source”). When the use of reputable commercial suppliers is not possible, in-house breeding programs may be developed. In-house breeding can ensure:

- implementation of a prophylactic plan against infectious disease in the facility (including testing and treatment against parasitic, bacterial, and viral diseases);
- avoidance of animal transportation from the breeding facility (reduced stress);
- incubation of an appropriate number of eggs in oviparous reptiles, and selection of the sex of the animal in species with a temperature-dependent sex determination (prevents breeder mistakes on the sex of the reptile in some species);
- knowledge of the nutritional status of the reproductive animals, conditioning the nutritional content of the egg and juveniles; and
- control of the level of inbreeding among animals of the same experiment, which is not always possible when receiving a colony from a single breeder.

In-house breeding requires specialized facilities and in-depth knowledge of the species’ requirements. This includes specialized facilities for egg incubation and juvenile rearing, animals of appropriate health and maturity, and the ability to induce breeding conditions, as detailed in Sections 5.2-5.7.

Many reptiles are long-lived, and managing appropriate colony numbers can be difficult. The need for captive-breeding programs should be assessed with great care and consideration, recognizing that there may be a need for a homogenous population of animals or a specific pathogen-free colony. It may be possible to partner with an accredited zoological institution with the appropriate facilities and resources to breed from their reptile collection. Investigators should justify in-house breeding based on the needs before establishing a colony.

If in-house breeding is required, SOPs should be developed before initiating the project. These SOPs should include the health criteria for breeding animals, species-specific requirements for mating, habitat changes (e.g., nest boxes), egg incubation (temperature, humidity), and dietary and habitat needs of the offspring.

Before initiating breeding, it is important to decide the intention for any surplus animals with the animal care committee. The number of surplus animals should be kept to a minimum. A plan should be in place to avoid euthanasia – this should be a last resort only.

Inbreeding should be avoided as much as possible, based on the history available on reproductive animals.

Veterinary assistance must be available to humanely intervene for any breeding-associated pathology, including dystocia, egg retention, and malformations associated with incubator malfunction. The animal care personnel must be well trained and experienced with the breeding of reptiles.

The nutrition of the breeding females must be well adapted to their gestation or post-laying status.

Detailed records are important and should include data on age, ancestry, food consumption, defecation, weight, ecdysis, medical problems, and reproductive output (Wright and Raiti, 2019).

5.2 Specialized Facility Needs (Incubation, Nursery, and Specialized Food Maintenance Requirements)

If breeding is to be undertaken in research, teaching, or testing facilities, there must be an assurance that the necessary infrastructure, equipment, and expertise are available (see Section 2, "Facilities" and Section 6, "Husbandry"). Among other requirements, reptile breeding programs need separate habitats with independent environments, appropriate incubators, and personnel with appropriate training or experience with breeding. Enclosures must be escape-proof.

5.3 Physiological Considerations

Guideline 12

Species-specific health assessment benchmarks should be established for breeding animals, and these should be met before initiating breeding.

Reptiles (especially females) must be in good physical condition, healthy, and disease-free, with sufficient energy and calcium stores to support reproduction (Wright and Raiti, 2019).

Consideration should be given to maintaining genetic diversity in the breeding program by using outbreeding or breeding of least-related pairs to prevent genetic bottlenecks and divergence from wild-type phenotype.

Breeding females should have detailed life history records, including potential exposures to males due to the possibility of sperm retention.

5.3.1 Sexual Maturity

Reptiles become sexually mature at a species-specific combination of age and size. Age and size at maturity can be predicted by linear and mass growth rate (Bjorndal et al., 2013). In the wild, lizards from hotter climates reach sexual maturity earlier than lizards from colder climates (Cabezas-Cartes et al., 2018); however, this may not be the case in captivity. Due to optimized nutrition in captive settings, animals are likely to reach sexual maturity faster than in the wild. Power feeding is a technique where high-calorie food items are offered more frequently than normal, primarily to accelerate the time to sexual maturity. This technique should be used with caution, especially with rodent-fed animals, to ensure that animals remain in healthy body condition (see Section 6.4.1, "Food"). Generally, body size at sexual maturity can be estimated based on maximum adult size; reptiles become sexually mature when they reach between 65-75% of maximum adult size (Shine et al., 2000). Females may be capable of reproducing when they first become sexually mature, but breeding at early sexual maturity entails more risk of dystocia. Dystocia occurs when only a portion of eggs are laid during oviposition or fetuses delivered during parturition, and can result in sterility or death. Females may be physiologically capable of mating before they are capable of gestating.

5.3.2 Reptile Reproductive Strategies

Reptiles exhibit two main forms of parity: oviparity and viviparity. Most reptiles are oviparous, producing eggs with shells that incubate externally (Wright and Raiti, 2019), while some reptiles are viviparous, meaning that offspring are born without an egg, autonomous and free-living (Blackburn, 1994). Of these, some groups develop a reliance on the egg yolk until born (lecithotrophic viviparity), while other groups have a chorioallantoic placenta that provides life support until birth (Wright and Raiti, 2019).

Very rarely, reptiles may reproduce by facultative and obligate parthenogenesis. In facultative parthenogenesis, in some lizards and snakes, individuals switch between sexual and clonal reproduction. Facultative parthenogenesis can occur in captivity and in the wild (Booth et al., 2012; Lampert, 2008; Watts et al., 2006). Obligate parthenogenesis is exclusively clonal reproduction and is even rarer than facultative parthenogenesis. Births attributed to parthenogenesis can also result from sperm retention by females, sometimes for periods of many years (Birkhead and Møller, 1993; Booth and Schuett, 2011).

5.3.3 Reproductive Cycles

Most subtropical or temperate zone reptiles have an associated seasonal reproductive cycle in which sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. Gonad regression is stimulated due to changes in temperature rhythms and photoperiod. These reptiles (e.g., leopard gecko, bearded dragon, blue-tongued skink, rat snakes) originate from places with a long active season and a predictable cycle of active and inactive seasons. These subtropical or temperate zone reptiles generally require a period of brumation to become reproductively active (Wright and Raiti, 2019).

Some reptiles have dissociated reproductive cycles, where mating occurs at the start of the active season, before gonadogenesis. The males use sperm produced during the previous active season or during brumation. Females acquire sperm at the beginning of the active season and store it until they undergo gonadogenesis. A dissociated cycle relies on a short active season and a predictable cycle of active and inactive seasons (e.g., garter snakes (Wright and Raiti, 2019; Krohmer, 2004)). This strategy can result in clutches of eggs or broods with multiple paternities (Uller and Olsson, 2008). When breeding for specific genetic outcomes, highly controlled breeding of specific pairs is required.

5.3.4 Physiological and Environmental Stimulus

There are three main types of reproductive patterns exhibited by reptiles: spring or early summer breeding, autumn or winter breeding, and breeding cycle unrelated to seasonal stimuli (Laszlo, 1979).

For successful breeding, environmental conditions should replicate those found in the animal's natural environment. Many species require either a natural seasonal regime or artificial regimes that mimic seasonality (Shine and Brown, 2008). For aquatic species, water depth can also be a factor (Kennett, 1999). Re-warming and activation may induce maturation in many temperate species which undergo brumation (Cooper, 2010), but species' needs vary, and natural life history must be considered. Most reptilian species are either warm temperate or tropical. These species will not usually experience a sharply changing climatic cycle. Among at least some of these species, sexual maturation seems to be governed by intrinsic rhythms that are retained in captivity, even though cyclic variations in day length, rainfall, and other environmental stimuli are completely absent or greatly disrupted in captivity.

The key steps involved in brumation include: stopping feeding (approximately a week in advance for lizards and three weeks in advance for snakes); lowering heat and light levels; and continuing to provide water, monitor weight, and carry out visual health checks. This should continue for a defined period of time that is highly species-specific (e.g., many reptiles originating from the southern hemisphere may only require a four-to-eight-week period of cooling to induce breeding). Following the period of brumation, the enclosure should be re-warmed and the regular photoperiod resumed. Frequent small meals should be offered, followed by large amounts of food, to encourage gonadogenesis.

5.3.5 Pairing and Mating

The natural pair selection for the particular species should be understood before attempting pairing to improve mating success and to ensure the health and safety of animals. Close monitoring of mating sessions is required to prevent injury to the breeding pair. Mating behaviour may appear aggressive in some species; appropriate training of personnel and familiarity with the species is required to ensure the safety of the breeding pair and successful mating. Safety equipment (gloves, etc.) should be on hand in the event that intervention is needed during an unexpectedly aggressive interaction.

Multiple paternities occur naturally in over 50% of reptile clutches (Uller and Olsson, 2008) due to the potential for multiple matings and the ability of many female reptiles to store sperm.

5.4 Breeding and Mating Conditions

Social behaviour often has a modulating effect on the development of the reproductive condition. The presence of a suitable mate can have a profound impact on the reproductive cycle (Wade, 2011). In some reptiles, males rely on cues from females for gonadal development, whereas in others, the females rely on male cues to trigger development (Wright and Raiti, 2019).

5.4.1 Artificial Induction of Breeding

Mating cues can be artificially manipulated to induce mating behaviours. For example, placing shed skin from a rival male snake in the habitat with a breeding pair may induce mating behaviours in male snakes.

Artificial induction of breeding can be done but is not standard care. Electrostimulation, semen storage, and artificial insemination are all highly specialized techniques that require extensive procedural knowledge and equipment (Juri et al., 2018; Molina et al., 2010).

Female reproductive status may be monitored using ultrasound and changes in hormones (Bertocchi et al., 2018).

5.5 Gestation Monitoring

Gravid females may engage in more basking in the enclosure's hotspot. Nesting boxes (also known as brood or lay boxes) are similar to retreats but typically larger to allow the gestating female to move around comfortably; these boxes should be supplied with substrate and other materials appropriate for the species-specific laying behaviour (Xiang and Du, 2001). Several nesting boxes with different thermal and humidity profiles should be provided: if a suitable nesting box is not provided, dystocia may occur (Wright and Raiti, 2019).

Disturbances should be minimized during gestation, but minimally invasive regular monitoring (e.g., weight, body condition scoring, abdominal circumferential monitoring, ultrasound) is important to ensure normal progression of gestation, with more specific diagnostics typically required only if problems are suspected. Monitoring methods requiring general anesthesia or sedation should generally be avoided during gestation unless clinically required. It is also important to monitor for any signs associated with dystocia, the most common condition associated with gestation. Signs of dystocia include lethargy, anorexia, protruding abdomen, protrusion of the cloacal membrane, cloacal discharge, or evidence of eggs postpartum. If signs of dystocia or other conditions are observed, consultation with a veterinarian or other appropriate reptile expert is advised, as a timely intervention may reduce the level of intervention needed.

Some snakes undergo ecdysis unique to gestation 7-14 days before oviposition or parturition (Wright and Raiti, 2019). The pre-oviposition or pre-parturition ecdysis is primarily characterized by longer duration, particularly of the eye caps, which may remain opaque for up to five days.

Reptiles may undergo species-specific behavioural changes immediately before delivery; these may include: becoming secretive and aggressive; lying upside down; digging exploratory nests; scooping out birthing areas in the substrate; or building false nests (Wright and Raiti, 2019). Most snakes lay their eggs or give birth in the evening or very early morning (Wright and Raiti, 2019) and should be left undisturbed if found mid-lay. It is important that the humidity in the lay box be ideal such that the snake and eggs can be left alone overnight without risking egg desiccation or drowning of hatchlings (i.e., not supersaturated substrate), so as not to disturb the snake during egg-laying as that may cause dystocia.

5.6 Incubation of Eggs and Nursery Activities

The decision to remove or leave the eggs in the nest should be justified to the animal care committee based on species-specific considerations; for example, crocodilians and several snake species guard the nest sites. Under laboratory conditions, eggs should normally be removed as soon as the female has left the nest site and is noticeably thinner. Prompt removal of eggs encourages maternal resumption of normal behaviours such as feeding and basking. Artificial incubation of eggs also prevents hatchling cannibalism by adult animals. Some species (e.g., mud snakes, grass snakes) have eggs that adhere to each other almost immediately post-deposition, but these can be carefully moved as a clutch or are easily separated. If eggs cannot be removed due to adhesion to the habitat (e.g., gecko eggs), they can be protected by securing a small plastic container over them. Unlike bird eggs, reptile eggs should not be rotated during incubation. In most oviparous reptiles, the supportive membranes of the developing embryo adhere to the eggshell early in development. Rotating the egg during development and shifting the weight of the embryo within the egg can tear these membranes, resulting in the death of the embryo (Aubret et al., 2015).

Assessing egg viability should be performed carefully due to the delicate nature of the shell (Wise et al., 2009) and the need to prevent rotation of the eggs. Candling with bright light can allow visualization of the early stages of development, particularly in the first stages when vascularization of the supportive membranes develops. Ultrasound may also provide direct visualization of the developing embryo. A marked change in egg colour, egg texture, or mould growth can indicate a non-viable egg. As eggs should not be rotated during incubation, specific eggs can be identified using an incubator map, placing markers beside eggs, or using non-toxic materials such as pencil, chalk, or marker to mark directly on the eggshell.

All species require a carefully controlled incubation temperature as it can affect development, sex, locomotor abilities, and cognitive abilities (e.g., Bókony et al., 2019; Amiel et al., 2014; reviewed in Singh et al., 2020). The optimal temperature for egg incubation varies among oviparous species and is usually lower than the mean activity temperatures maintained by the adults. More stable temperatures have better outcomes (i.e., even with the same mean temperature, the clutch subject to a lesser temperature variation will generally be larger and show stronger anti-predator behaviour (Webb et al., 2001)). Eggs incubated at temperatures outside the optimum have lower levels of hatching success and may be associated with developmental abnormalities. During the last week or two of incubation, the embryos begin to generate their own heat through metabolic processes; this should be taken into consideration when the incubation container is relatively small in comparison to the eggs or minimally ventilated. Species-specific egg incubation temperatures vary widely, and reliable peer-reviewed resources (e.g., Kohler, 2005) should be consulted as part of the breeding SOP.

In many species of reptiles, the sex of an embryo depends not on sex chromosomes but on the temperature during the early stages (usually the first third) of incubation (Cooper, 2010; Singh et al., 2020). There are three known types of temperature-dependent sex determination:

- Females develop at low incubation temperatures; males develop at high incubation temperatures (alligators and many lizard species).
- Females develop at high incubation temperatures; males develop at low incubations (many chelonians).
- Females develop at either low or high incubation temperatures at the limits for successful incubation; males mostly develop at mid-range temperatures, although some females may also develop in mid-range temperatures (snapping turtles, leopard geckos, crocodilians).

While maintaining humidity is important, it appears that incubation is less sensitive to hydric effects than temperature effects (Du and Shine, 2008; Ji and Du, 2001). Humidity can be controlled either by placing eggs directly on a moistened medium such as vermiculite, sphagnum moss, peat moss, or perlite, or by suspending eggs above a moisture source. In general, moister media is required for leathery eggs, but species-specific requirements vary widely and, as for temperature, reliable peer-reviewed sources should be consulted as part of the breeding SOP. Egg incubation typically requires high humidity, and eggs must be monitored for mould if in direct contact with the medium. Suspending eggs above a moisture source, known as a suspended incubation medium, reduces the risk of mould formation and provides more homogenous humidity concentrations. Appropriate humidity must be maintained concurrently with appropriate ventilation as hypoxic conditions are associated with increasing hatch times and reducing the cognitive function of hatchling neonates (Kohler, 2005; Sun et al., 2014).

Many eggs can withstand temporary cooling, but most have poor tolerance for excessive heat, and overheating can quickly lead to embryo death. Due to the critical effect that temperature and humidity have on hatching success, incubators should be on an emergency (backup) power supply.

5.7 Care of Offspring

Incubation temperature can affect thermoregulation in neonatal reptiles, at least in the short term (Goodman and Walguarnery, 2007). The enclosure should mimic the animal's natural environment to maintain thermoregulation. Supplementary heat sources, possibly at different temperatures from the mature animals, may be required. Due to their small size and mass, neonate and juvenile reptiles are susceptible to temperature and humidity shifts, and close monitoring of these parameters is needed. Some species may engage in parental care (e.g., thermoregulation of the newborn (Alexander, 2018)), but with the exception of social lizards, young should generally not be housed with adult animals, to reduce the risk of cannibalism. For herbivorous species, consideration should be given to whether juveniles require inoculation with symbiont fermenting anaerobes (Morafka et al., 2000). These bacteria are important for the efficient digestion of plant matter and are frequently transferred via coprophagy.

Different species have different levels of yolk reserves for post-partitive nutrition (lecithotrophy), which influences the time of first feeding. The species-specific needs for first and early life feeding should be identified in an SOP. Some neonatal animals may not leave the egg immediately on hatching, staying in the shell for several days, absorbing more of the yolk. These animals should not be disturbed, as removing them too fast can rupture the yolk sac that has not been sufficiently internalized. Care must be taken that first feeding is performed at an appropriate time, in concert with hatching and yolk depletion, as premature feeding may be stressful and delayed feeding may negatively impact health. Reptiles may have difficulty with their first feed, and it should therefore be carefully observed. Uneaten food should be promptly removed to prevent rot in the habitat and potential damage to the juveniles from live prey. A variety of foods and techniques may need to be attempted before a successful first feed is achieved. Juveniles should be periodically weighed to ensure that appropriate growth is occurring. It is normal for hatchlings to lose weight during the first few days due to the absorption of the yolk.

In general, juveniles are neither reproductive nor territorial, often allowing multiple animals to be housed together because they should not fight. Though juveniles may be group or singly housed, the risk of cannibalism and the ability to monitor feeding and growth must be assessed. The provision of a large enclosure with plenty of hiding places and visual barriers and a mixture of thermal moisture and light environments are beneficial for juveniles. Aggressive encounters may be a signal that the enclosure is too small or lacks sufficient hiding places or visual barriers. Group-housed juveniles must be fed more frequently to prevent curiosity and hunger, resulting in tail biting (e.g., bearded dragons and blue tongue skinks).

While some conspecific interaction may be beneficial, care should be taken that animals are not overcrowded as this can be a source of stress.

Although much of the care is similar, juveniles require more attention than adults. Juvenile cages should contain the same furnishings as adult cages, but with an additional number of hides available. They should also contain structures for climbing and basking. Juveniles must be fed size- and species-appropriate food, such as finely chopped vegetables (i.e., leafy greens), pinhead crickets, or neonatal mice. Food may be supplemented with calcium or vitamin powders. These food items can be purchased or supplied by the laboratory (Lovern et al., 2004). Care should be taken to select food items whose width is less than the diameter of the lizard pelvis. Indeed, some juvenile lizards, such as bearded dragons, may eat larger prey items if offered, which may cause pelvic nerve compression and hind limb paralysis.

The availability of appropriate ultraviolet light is critical for many reptile species to produce adequate quantities of vitamin D_3, which is required to absorb and use calcium. Juvenile concentrations of vitamin D_3 are affected by maternal reserves, and optimum concentrations of vitamin D_3 are species-specific.

6. HUSBANDRY

The *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017) should be consulted for general guidelines that apply to all species. This section provides additional considerations that are important for reptiles.

6.1 Identification

All animal enclosures should be clearly marked (as described in Section 2, “Identification of Animals”, in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)). Any need for individual identification of animals should be justified, and the least invasive method suited to the study goals should be used. Many individual reptiles can be identified by a combination of their size, scale colour and pattern, and, in certain lizards, the state of the tail. Photographs or even the shed skin may be attached to records to assist in recognizing specific individuals. When individual reptiles cannot be recognized visually, it may be necessary to mark them. To overcome the issue of ecdysis and acute shedding, external marking must either involve morphological changes to the skin (i.e., scale transplant) or be repeatedly applied. Microchipping can be performed for most species, with subcutaneous placement preferred to intraperitoneal placement, to avoid migration of the chip. Temporary marking can be accomplished using materials such as vegetable or food dye or non-toxic paint, but markings will need to be re-applied as they fade. Turtles and tortoises may be temporarily marked with nail polish on their shells. Use of more invasive methods such as ventral scale clipping (snakes) or shell modification (turtles and tortoises) must be appropriately justified to and approved by the animal care committee.

6.2 Animal Observation

As mentioned in Section 3.2, “Personnel”, reptiles must be observed at least daily by trained personnel. The frequency of observation should be described in an SOP for each species, as species-specific considerations are important. The frequency of direct animal observation may be influenced by the expected physiological state, as animals in torpor or brumation may need less frequent observations. For reclusive species, disturbance to the animals caused by digging them up or removing cover to facilitate observation must be balanced with the need to confirm their health. Other species should be disturbed minimally when in post-prandial or other relevant stages. Minimally intrusive observation may be enhanced using video cameras and, depending on the size and material of the enclosure, utilizing all visual angles (e.g., observing from the sides or bottom of the enclosure). Reptiles tend to hide in the same place, which can facilitate quick observation of the animal. Any unusual odours should be investigated. All containment and environmental systems supporting the animals must be checked daily.

6.3 Housing Management

Most reptile species are solitary, and for these species, mature animals are best kept individually or in pairs. Generally, males are territorial and fight when placed together, but females placed together may also show antagonistic behaviour. Social organization occurs in some species (reviewed by Gardner et al., 2016), and these species benefit from housing with conspecifics. Co-housed animals must be monitored for conspecific aggression or adverse behaviours, to ensure the health of all animals in the social group. During feeding time in particular, group-housed snakes must be observed until the food is either finished or removed.

Brief details of preferred housing parameters for species commonly held in Canadian laboratory animal facilities are listed in Appendix 2, “Basic information on reptiles commonly held in Canadian laboratories”. It is the responsibility of the protocol author to ensure that the requirements for housing the study animals have been fully researched and can be met by the animal facility before the arrival of the animals.

6.4 Feeding and Nutrition

6.4.1 Food

Environmental and food item characteristics play a major role in normal feeding behaviour. Environmental parameters, including heat, light, and ultraviolet B, directly affect reptile metabolism and their ability to obtain and digest food. Feeding responses may be triggered by chemoreception, reflexive hunting responses, temperature, and the size of food items. Determining an appropriate diet can be challenging in some species, particularly those with narrow and specific diet specializations or those with different dietary requirements at different life stages. While reptiles may survive being fed an inadequate diet, this can lead to severe health problems and should be avoided by providing a good diet as soon as the reptiles are procured.

It is important to record measures of nutritional status such as amounts and types of food eaten; weight; snout to vent length; presence and quality of feces and urate, recorded before cleaning the enclosure; body condition score; and general feeding behaviour, so that any nutritional problems can be identified early on. During brumation, food intake and output of excretory products will be reduced or stop altogether.

The key criteria for developing feeding schedules should be the maintenance of appropriate weight and general health, rather than the animal’s willingness to accept food. Insufficient nutrition can cause growth retardation, while excessive nutrition can cause obesity and associated physiological abnormalities. Weight should be monitored quantitatively by weighing animals and observationally based on gross body condition. Animals should be weighed on a regular basis and opportunistically when they are removed from the enclosure for husbandry or procedures. Gross body condition can be evaluated based on a simple scoring system where 1 = emaciated, 2 = underfed, 3 = normal, 4 = well fed, and 5 = obese. Lizards should be assessed using the tail base, snakes using epaxial musculature, and turtles based on palpation of fat pads and musculature of the limbs. Growth curves can also be helpful, but it is important to account for differences caused by sex, season, and housing conditions.

Whether an animal receives the nutrients it requires depends on: 1) the composition of the food items provided; 2) which food items are accepted; 3) to what extent the food items are digested; and 4) the nutrient requirements of the animal (Oonincx and van Leeuwen, 2017). Some reptiles may require vitamin supplements (Boyer and Scott, 2019; ASIH, 2004). Other important factors of reptile nutrition include:

- gut transit time, which varies greatly between species and impacts the frequency of feeding (Rendle, 2019);

- temperature effects – of the enclosure and hence the animal – which can have an impact on the digestibility of food and on gut transit time; suboptimal temperatures can lead to poor digestion of food, bloat, or constipation;
- energy requirements, which should be related to the standard metabolic rate, taking into consideration the environmental temperature; and
- chemoreception, which occurs through the vomeronasal system and triggers feeding responses.

Many species grow more quickly in captivity with appropriate nutrition than under natural conditions; this could be due to the ready availability of food, which allows optimal growth. However, high growth rates are also potentially associated with disease conditions, including obesity, renal disease, metabolic bone disease (Ullrey, 2003; Kumar et al., 2018), and shell deformities. Dietary constituents can be divided into plant material, such as leafy plants and fruits, and animal material, such as arthropods, fish, birds, or mammals. Some reptile species are highly specialized toward one of these categories (herbivore, carnivore, insectivore), while others consume both plant and animal material (omnivore). An animal's dietary preference can change as it matures due to nutritional needs and physiological restraints, an effect called ontogenetic change. Large carnivores (e.g., monitor lizards) change from consuming other lizards and insects to consuming mammals as they mature. Smaller lizards that are primarily herbivorous as adults may consume insects or other animal material as juveniles. A similar ontogenetic shift is known in yellow-bellied sliders. In general, omnivorous species prefer animal material if provided with a choice, and juvenile omnivores tend to consume relatively more animal material compared with their adult counterparts, probably due to protein being the first limiting nutrient for growth and reproduction. When raising juvenile reptiles, a strong understanding and classification of the species-specific juvenile and adult dietary requirements are needed (Oonincx and van Leeuwen, 2017).

In some species, a varied diet that allows self-selection of food items relatively rich in a limiting nutrient and low in an overabundant nutrient is preferable. Calcium, carotenoids, vitamin E, and other limiting nutrients may be provided by feeding the supplements to insects (gut loading) for several hours before feeding the insects to insectivorous species (Latney et al., 2017). Calcium can also be provided by sprinkling it on the food for herbivorous species (palatability should be monitored; e.g., Boyer and Scott, 2019) or by offering whole prey or prey portions with bones to carnivorous species. Calcium may also be provided in the form of a bowl of calcium carbonate, although not all species make use of this. Calcium powder without D_3 should be provided to reduce the risk of vitamin D toxicosis. An appropriate balance of calcium (typically supplemented) and phosphorus (typically obtained from dark leafy greens) should be established for herbivores such as iguanas. Recommended calcium to phosphorus ratios are often known for common species and can be easily researched before writing SOPs and obtaining animals.

Food items can be wild-sourced or commercially raised or manufactured, and can consist of either food items (e.g., leafy greens, fruits, vegetables, insects, earthworms, or other prey) or manufactured food (i.e., pellets). If utilizing wild-sourced food items, it is important to ensure they are free of herbicides, pesticides, or other potentially toxic compounds, and are from a reputable source. A wide variety of food items can be commercially raised, including leafy greens, fruits and vegetables, insects, and other prey. Where possible, prey should be frozen and thawed before feeding to reduce the risk of parasites. When feeding food items, it is important to be aware of the nutritional requirements of the species, to ensure an appropriate diet is offered. Manufactured food pellets provide a complete diet with high consistency and cleanliness, but may not be available or suitable for all species. Where commercially prepared foods are available, they should be used as the staple diet, with a variety of other foods offered. Diversity in the diet is often paramount to complete health, especially when reproduction is the goal.

If thiaminase-rich prey is offered, thiamine supplementation should be given to prevent thiamine deficiency (Honeyfield et al., 2008). Crowe (2012) provides a useful list of fish species that do and do not contain thiaminase. Some insects, such as silkworms, also contain thiaminase (Finke, 2013).

When vertebrate prey animals are used as food, they should be humanely killed in accordance with the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) before being offered to the reptile. If vertebrate prey animals are bred on or transferred from another protocol within the institution, then their breeding, husbandry, and use must be in accordance with the institutional animal care and use program. Genetically modified animals may be used as food, provided that neither the prey animals nor the reptiles ever leave the premises. Different presentations of pre-killed prey can be used to motivate feeding behaviour, including warming the food item, offering a variety of prey, simulating prey movement, and scenting the food item. If the animal is reluctant to feed, a veterinarian or species expert should be consulted. Live vertebrate prey should only be used after specific approval from the animal care committee if the predator reptile is in a life-threatening condition and there is no other viable alternative. The animal care committee should consider species, age, and attempts at introducing dead feed in their evaluation of a live vertebrate prey feeding protocol. If live vertebrate prey is used, the feeding must occur under strictly controlled circumstances and be closely monitored to ensure the health of the reptile and minimal distress to the prey. The exposure to the prey must elicit a near-immediate strike and ingestion by the reptile. If the strike and ingestion are not near-immediate, the prey should be removed and humanely euthanized. Any time that live prey is considered to be necessary to train the reptile to eat, the interactions should be carefully monitored.

After feeding, all food items should be removed from the enclosure to prevent fouling, but also to prevent injury to the reptile if live prey such as insects is used. Even prey as small as crickets can injure much larger reptiles. Food items should be consumed or removed if an animal's metabolism is expected to slow during periods of expected inactivity (i.e., diurnal animals) to allow for appropriate digestion.

6.4.1.1 Lizards

Certain lizard species cannot use dietary vitamin D_3 and must receive ultraviolet light to maintain blood levels of 1,25-dihydroxyvitamin D_3. Other species of lizards have been raised successfully without ultraviolet light, in some cases for two or more generations, using dietary vitamin D_3 supplementation. Dietary supplementation of herbivorous and insectivorous species with vitamin D_3 poses a risk of hypervitaminosis D as reptiles' needs in terms of vitamin D intake are not defined (Baines et al., 2016). To date, all reptile species evaluated have been shown to increase their vitamin D circulating concentration when exposed to ultraviolet B sources (Acierno et al., 2006; Acierno et al., 2008), even when exposed for only two hours per day in the case of nocturnal species (Gould, 2018). Thus, exposure to ultraviolet B should be preferred to oral vitamin D administration in non-carnivorous species (see Section 3.1.1, "Lighting"), whenever compatible with study design and reptile morph or strain (Baines et al., 2016).

6.4.1.1.1 Herbivore Versus Omnivore

Some of the larger-sized lizards of the family Iguanidae are herbivorous and will readily feed on pulpy fruits and leafy green vegetables. These genera of New World lizards include the iguanas (*Iguana*), ground iguanas (*Cyclura*), spiny-tailed iguanas (*Ctenosaura*), and chuckwallas (*Sauromalus*).

A higher dietary protein content facilitates higher growth rates in green iguanas. Plant composition can differ between seasons, and consequently, herbivore diets can also differ. For instance, plants preferentially consumed by chuckwallas contain more protein in early spring than in summer. Chuckwallas also prefer herbage over grass, possibly due to the lower fibre content of herbage, making nutrients more readily available. Fruits and flowers, especially when brightly coloured, are regularly preferred over leafy greens and can form a significant portion of the diet in the wild. Leafy greens contain dietary ingredients such as oxalic acid, tannins, or phytate, which can have toxic effects, including inhibiting calcium absorption and the potential for calculi formation. Hence, cabbage, mustard greens, broccoli, etc., should only be fed sparingly. More specialized feeders might be able to detect harmful substances via tongue flicking; however, as a general rule, it is wise to only provide access to plant material that does not contain any toxins (Oonincx and van Leeuwen, 2017).

Omnivores (e.g., bearded dragons) are typically fed a base herbivorous diet, either fresh or commercial pelletized, which is then supplemented with a species- and life-stage-specific moderate proportions of insects or a small proportion of animal protein. Juvenile animals commonly consume proportionately more animal protein than adults. Most omnivorous animals will have a behavioural preference for insects, so care must be taken to ensure that appropriate quantities of food items from both categories are offered and being consumed.

6.4.1.1.2 Carnivore

Most carnivorous lizards can be acclimated to feed on pre-killed prey.

The diet of carnivorous lizards often consists of a variety of pre-killed vertebrate prey such as mice, rats, or one-day-old chicks. Different prey species, as well as different sizes and maturity of the same prey species, tend to differ in nutrient composition. Hence, the choice of prey affects the nutrient intake of carnivorous lizards. The consequences of such differences in dietary intake for lizard health and fertility are currently unclear (Oonincx and van Leeuwen, 2017).

In carnivorous species, offering whole prey is usually sufficient to cover vitamin A and D requirements.

6.4.1.1.3 Insectivore

Most lizards are insectivores that, in the wild, are often adapted to specific prey types. Restricted diet tolerances are typically related to natural behaviour and the native environment. In captivity, small insectivorous lizards can often be enticed to feed on earthworms or nymph and larval stages of insects.

Narrow diet preferences may be encountered among lizards but are often species-specific. It must also be noted that different investigators may have different experiences with members of the same group. Lizards are a very diverse suborder, and their diets are often poorly understood; thus, there may be very little reliable baseline information available.

Most insectivores eat a variety of insect and spider species in the wild. Studies that compared the suitability of different insect species as food for insectivores are scarce (Finke, 2015). A study that provided only mealworms to Western fence lizards or leopard geckos (Gauthier and Lesbarrères, 2010) found that a diet of only mealworms led to more obese animals than providing only crickets or mixed diets. An advantage of active insect species, such as house crickets, over more passive species, such as mealworms, is that the former is often better accepted. Moreover, they are more suitable for environmental enrichment because they increase insectivore activity, thereby reducing the risk of obesity. Wild insects often have a lower fat content and a higher content of carotenoids and omega-3 fatty acids compared to commercially available insects, which are often low in carotenoids due to their diet. This can lead to hypovitaminosis A for lizards such as green anoles or leopard geckos (Wiggans et al., 2018). As discussed in Section 6.4.1, “Food”, nutrient deficiency in reptiles can be mitigated by feeding insects that have been gut-loaded with limiting nutrients. This works better in juvenile insects than adults, owing to the juvenile’s larger relative gut content (Oonincx and van Leeuwen, 2017). Appropriate feeding with gut-loaded insects is important as over-supplementation can result in diseases including hypervitaminosis A. Thiamine (vitamin B_1) deficiency has also been reported in captive anoles, which were successfully treated with injectable thiamine supplementation (Feldman et al., 2011).

6.4.1.2 Snakes

All snakes are predators, with natural foods ranging from soil-dwelling invertebrates to fish, birds, lizards, rodents, and comparatively large-sized mammals. Most snakes do not dismember or otherwise orally reduce prey items; prey is usually swallowed whole (see Jayne et al., 2018 for examples of exceptions). Most snakes commonly held in captivity will accept pre-killed food. All vertebrate prey should be humanely killed before feeding them to snakes. Snakes will be most receptive to feeding when hungry and when food items are offered at a time and in a manner consistent with their natural history. Most snakes need minimal distraction from movement in the room for them to be able to focus on feeding. Live food should only be offered as a last resort and under strictly controlled circumstances for the health of the snake and should ensure minimal distress for the prey. If the snake does not strike and ingest the prey near-immediately upon presentation, the prey must be removed and killed humanely. Force-feeding is not recommended without suitable justification (e.g., anorexic snake) and requires expert involvement.

Some snakes brought into the laboratory from the wild find captive conditions so disruptive that they will either not feed at all or will feed only under conditions of total isolation in which feeding cannot be observed. Different presentations of pre-killed prey can be used to motivate feeding behaviour, including warming the food item, offering a variety of prey, simulating prey movement, and scenting the food item.

Care must be taken to avoid ingestion of particulate substrate during feeding by ensuring the substrate will not affect the gastrointestinal tract if ingested or by using a clean dish, a solid surface, or a designated feeding habitat (i.e., feed box).

Utilizing designated feeding habitats can reduce aggression in the home environment, enable monitored feeding of co-housed animals, and reduce ingestion of particulate bedding substrates. The use of feed boxes also maintains cleaner home environments. However, feed boxes may not be suitable for all species, particularly nervous animals or those otherwise reluctant to feed.

For most snakes, pre-killed prey items should be offered at normal body temperature for the prey. Snakes from the families *Boidae* and *Viperidae* hunt warm-blooded prey in the wild using sensory organs in their upper or lower labial scales, known as "heat pits" that detect infrared radiation as a mechanism of locating endothermic prey in the dark. These animals often require pre-killed prey to be warmed to their natural body temperature to encourage the snake to strike and subsequently ingest the prey item. Although colubrid snakes do not have heat pits, they often require whole-carcass, mammalian prey items to be warmed to ensure proper ingestion. Frozen rodents should be thawed dry at room temperature or in cold water to prevent disintegration. The carcass should then be warmed in hot water for five seconds or by touching the head of the rodent to a heat source before providing it as feed. Incompletely warmed food items may result in refusal and sometimes regurgitation.

For many snakes, mimicking the natural movement of the prey with a set of tongs may also be required to stimulate predatory and striking behaviour. Captive snakes, however, are often irritable and may respond adversely to any stimulus, including food presented using tongs, by assuming a defensive posture or rattling. It is also possible to get a snake to eat by eliciting a defensive strike, then remaining still while the snake realizes it has food in its mouth. In some instances, food is more readily taken when the room is dark rather than when brightly lit. Of the rattlesnakes found in Canada, the massasauga (*Sistrurus catenatus catenatus*) is among the least irritable of all rattlers and usually feeds readily in captivity.

Individuals may eventually be acclimated to take pre-killed prey with minimal preparation in stages, first to accept a warm carcass being moved to mimic live prey movements, then a warm but not moving carcass, and then, if possible, a non-moving carcass at room temperature. Acceptance can also be increased by scenting the food item to mimic native prey and may be required for species requiring specific dietary items. An extended period of time might be needed to transition to pre-killed food. Transitioning from live to pre-killed prey must be done under supervision.

For species that feed on ectothermic animals, offering the pre-killed prey items at room temperature is usually acceptable to the animal, such as with coral snakes (*Micrurus* sp.), which have been successfully raised on an alternative fish-based diet (Chacón et al., 2012). A fish-only diet can lead to deficiencies in some species, so it is important to consult species-specific guidelines.

6.4.1.3 Aquatic Chelonians

In the wild, carnivorous species, including snapping turtles (*Chelydra* spp.), softshell turtles (*Trionyx* spp.), and pond turtles (*Pseudemys* and other genera), feed in the water, consuming aquatic invertebrates, fish, and frogs. Large snapping turtles may occasionally capture and consume larger prey. In captivity, all will feed readily on pre-killed food, with whole fish being preferable, but pieces of fish fillet, liver, and meat are also generally acceptable. Most species will also readily accept manufactured pellets or food gels. Aquatic turtles must be fed in the water, and uneaten food must be removed promptly to prevent fouling. In captivity, turtles, especially juveniles, are prone to calcium deficiencies, generally due to diets that provide improper calcium to phosphorous ratios (Klaphake, 2010)), and due to vitamin A deficiencies (Boyer and Scott, 2019). A common clinical sign of vitamin A deficiency is an infection in the Hadrian gland, causing the eyelids to swell and be unable to open. The effects of the deficiencies are difficult to correct once they become established, and the best method is to avoid the problem by primarily providing appropriately balanced commercial diets. Alternatively, whole prey items contain much of the needed phosphorus and calcium but must be supplemented with vegetables and fruit. A turtle's diet requires a calcium to phosphorous ratio of approximately 1:1. Many vegetables and fruits provide adequate calcium to phosphorus ratios; however, meats without bone are inadequate sources of calcium (Kumar et al., 2018). In addition, vitamin A deficiency can be prevented by feeding vertebrate prey whole – as opposed to dehydrated *Gammarus* shrimp and other invertebrates – or by including vitamin A-supplemented pelleted food in the diet.

The aquatic chelonian diet can be supplemented occasionally with earthworms, fruit, vegetables, or alternative protein sources to enhance variety. If providing earthworms, care should be taken to ensure the appropriate source and species are provided as they can be a source of disease or can be toxic (e.g., they may contain pesticides if obtained from farm fields or lawns).

6.4.1.4 Land Chelonians

Terrestrial turtles (*Terrapene,* some *Rhinoclemmys, Geoemyda,* some *Heosemys,* some *Cuora, Gopherus*, *Geochelone*, *Testudo* and other terrestrial chelonians) are omnivorous or herbivorous and will feed on a mixture of leafy green vegetables and limited soft fruits, and manufactured food pellets. Mealworms (*Tenebrio* spp.) and other adult and larval insects are acceptable dietary components for *Terrapene* and likely to other land turtle species. However, their chitinous exoskeletons do not provide calcium and, as with other insects, provide an inadequate calcium to phosphorus ratio; this can be mitigated with gut loading (see Section 6.4.1, "Food"). Land tortoises are prone to overeating, and their diet must be monitored carefully to ensure appropriate growth rates and body condition.

Herbivores often have a requirement for fibre that they digest via endosymbionts. For example, red-footed tortoises prefer diets high in fibre (e.g., pineapple, dandelion greens). In contrast, desert tortoises seem to select foods with higher protein and magnesium contents and avoid food high in fibre. As with other species, it is important to be aware of the nutritional needs of the species and to provide appropriate food items and quantities to maintain health. Reptiles that have been treated with antibiotics or de-worming agents may need to recolonize their gut flora and fauna by ingesting probiotics or healthy conspecifics' feces.

6.4.1.5 Crocodilia

In Canada, crocodilian species are rarely held in laboratory animal facilities. Similar to Section 6.4.1.2, "Snakes", crocodilians should be fed whole, pre-killed prey to provide sufficient dietary calcium. Caimans (*Caiman* sp.) and the American alligator (*Alligator mississippiensis*) will both readily feed on chunks of meat and fish presented using a long pair of forceps, or on pre-killed food, placed in the water in their tanks. Even when the food is seized out of the water, crocodilians will not eat it until the food is submerged (Honeyfield et al., 2008).

6.4.2 Drinking Water

Drinking water for reptiles should be obtained from a consistent source that is clean and free of contaminants, or checked regularly if there is the potential for variability in dissolved components. Domestic water supplies may be used; if heavily chlorinated, the water may be dechlorinated by allowing it to sit overnight to de-gas. The use of chemicals to dechlorinate the water is not advisable. Where municipal water is has been treated with chloramine, a carbon filtration system should be used for dechlorination.

Water bowls should be checked and cleaned daily when the reptile is active, as the water may also be used by the reptile for soaking and defecating – weekly water bowl checks during brumation are generally sufficient. Water should be lukewarm to prevent accidental changes to the reptile's body temperature.

Water bowls should be heavy – ceramic, glass, heavy plastic – to prevent the animals from overturning them. Many snakes will not drink "stale" water but are attracted to fresh water when changed (AZA, 2009). Water bowls should be large enough to allow the animals to enter the water dish at least partially, with some species needing to be able to immerse themselves completely.

Tortoise and box turtle enclosures need accessible pans of shallow water that they can easily enter and leave without the danger of flipping over. North American box turtles (*Terrapene* spp.) should have water deep enough to submerge their head if desired. The Chinese and Indonesian box turtles (*Cuora* spp.) are more aquatic in their habits and must have deeper water for soaking and feeding (Kaplan, 2014).

Some reptiles that are accustomed to licking condensation from leaves or obtaining water from their food may not be willing to drink from dishes of static water. In these cases, spraying vegetation or using a drip or recirculating waterfall or fountain system may be required.

6.5 Substrates and Furnishings

6.5.1 Terrestrial Environments

Substrates for enclosures include paper, wood chips, corncob, coconut coir, indoor or outdoor carpet, tile, paper towel, or more natural substances such as dirt (topsoil), sphagnum moss, and sand. If using wood chips, highly aromatic species such as cedar and pine should not be used as they can cause respiratory irritation (Rossi, 2019). The selection of the most appropriate material depends on the needs of a given species. Burrowing animals, such as sand boas (*Erycinae*), must be provided with material that is deep enough to be able to dig and hide. Many lizard species such as the horned lizard (*Phrynosoma* sp.) and snakes such as the hognose snake (*Heterodon*) also burrow in loose sand. Coarse, flat wood shavings, such as planings from softwood, make a useful substrate for many species. Fine sawdust should be avoided as there is a risk (with this or any other small particle substrate) that reptiles, snakes in particular, may ingest substrate particles with their food. This can cause serious mouth or internal injuries from wood slivers and, occasionally, a bowel obstruction when large amounts of substrate are ingested. Absorbent paper (such as cage-pan lining paper) or a piece of synthetic fibre indoor or outdoor carpet can be used as a solid substrate. Where a solid substrate has to be used for burrowing reptiles, a shelter box that simulates the darkness of a burrow should be provided.

Shavings permit burrowing behaviours in many species. For water snakes and related species that spend much time in the water bowl, precut indoor or outdoor carpet keeps animals dry, allows traction for movement, and is easily sanitized and replaced. Natural substrates, while aesthetically pleasing, can harbour pathogens and must be disinfected before use; they are also more difficult and labour-intensive to maintain (O'Rourke et al., 2018). Many natural products are heat-treated, which makes them safe for use; if sterility is of particular importance, materials should be autoclaved.

Sphagnum moss can be an excellent substrate in the water and on land for moisture-loving species, but can also be an excellent addition to hide boxes. The moss helps inhibit fungal and bacterial growth, provides a mechanical cleaning of the body, can help with ecdysis, holds moisture, and is easy to burrow in.

Hide boxes act as retreats and offer animals the opportunity to withdraw visually from activities happening outside their cages. Hide boxes also provide tactile security that can be important for comfort, particularly for newly acquired specimens. Hide boxes of a suitable size to provide tactile and visual security are particularly important for animals that are normally shy and retiring or who react negatively to routine environmental stimuli. Hide boxes or hiding spots should be provided at both the cool and warm ends of the cage; this will allow the inhabitants to select their preferred temperature without having to sacrifice security (AZA, 2009). Boxes can be purpose-made (generally plastic or ceramic) or homemade from a variety of materials, including repurposed opaque plastic food containers, but should be easy to clean inside and out, if they are to be kept for longer periods. Disposable hide boxes, which are simply replaced instead of cleaned, can also be created from cardboard for use in relatively dry environments. Any repurposed materials should be inspected closely to remove potentially harmful materials (e.g., tape, staples, sharp edges). Hides made from absorbent materials such as wood or cardboard should not be moved between enclosures nor shared between animals unless they can be autoclaved.

Hide boxes should be an appropriate size for the species and animal. Snake hide boxes should be large enough for the snake to maintain a normal resting coil, but most snakes like to reside in close-fitting hide boxes. Many snakes will wedge themselves tightly into spaces, giving them tactile security and less exposure to predators during resting periods. The opening into the hide box should be twice that of their widest girth. Hide boxes for lizards and chelonians must be large enough for the animal's entire body (including the tail) to enter and for the animal to freely turn around within the hide.

6.5.2 Arboreal Environments

The cage space for arboreal reptiles should be vertically oriented. It should include objects and surfaces for climbing, but these structures should not be placed over food or water bowls to prevent contamination with feces or food. The height of objects must be taken into consideration for the heat gradient and heat sources. In general, willow, birch, beech, ficus, and fruit trees provide non-toxic branches, and cork bark hollows are appreciated by many climbing species. All of these materials should be able to be autoclaved to eliminate pathogens.

Care should be taken to provide branches and perches of varying diameters, as perch size is very important for snakes that use friction to grip onto branches and for lizards to ensure appropriate foot grip (Astley and Jayne, 2007). Structures can be purchased or manufactured as long as appropriate traction and diameters are considered. Structures should be placed to minimize the risk of trapped animals and be able to be easily removed for cleaning.

6.5.3 Aquatic Environments

The *CCAC guidelines on the care and use of fish in research, teaching and testing* (CCAC, 2005) provides information about water source, water quality, and testing parameters that are also suitable for reptiles.

Aquatic turtles should be able to access a basking area easily, as described in Section 2.2.4, "Aquatic Holding Systems". Flat stones, floating docks, or rafts may all be used, but wood is not appropriate as it is susceptible to deterioration and tannin leaching. Floating rafts should be secured so that animals can haul out safely. Basking areas may be constructed of a variety of materials, including stones, plastics, epoxy resin, and metal. If stones are used, heavy stones should be avoided as there may be safety issues related to their removal and cleaning. It is important to use caution with smaller stones, to avoid hazards such as the potential for stacked stones to collapse and trap animals or the ingestion of smaller stones by turtles and juvenile crocodilians. In addition to basking areas, species-specific natural environmental features such as marsh, vegetation, and shallow water depth zones should be considered when selecting substrates and furnishings.

6.6 Environmental Enrichment

Guideline 13

Environmental enrichment relevant to the species and life stage should be provided.

Environmental enrichment covers a multitude of innovative techniques that are aimed at providing adequate social interaction or positively engaging the animal in its environment (Shepherdson, 2001). The aim of enrichment is to increase the overall welfare of animals in captivity, maintaining their health and activity levels, and to promote species-specific behaviours that are similar to wild conspecifics (Bostock, 2001).

The optimal environment provides reptiles with the opportunity to: 1) live in conditions similar to their wild microhabitat (Varga, 2019; Cooper, 2010); and 2) perform enrichment activities that are natural to them (Kuppert, 2013). Environmental enrichment allows the species to have a measure of control over their environment.

Creating environments that allow for the expression of natural behaviours that are part of proper biological function is not necessarily providing enrichment; rather, it is simply good husbandry (Warwick et al., 2013). Providing a range of opportunities for animals to exhibit those behaviours would be considered environmental enrichment. Due to the diversity of species and lower frequency of reptile use in research and teaching, there is still much unknown regarding positive enrichment for reptiles. It is the responsibility of the institution to continuously evaluate and, as appropriate, incorporate new evidence-based methods to enrich the lives of captive reptiles (Rosier and Langkilde, 2011).

Eagan (2019) points out that different forms of enrichment stimulate different aspects of the senses; therefore, providing as many forms of enrichment as possible is most desirable. Reptiles, in particular lizards and turtles, have been shown to be capable of learning, particularly in the context of their sensory abilities and behavioural repertoires (Burghardt, 2013).

Many species of reptiles clearly benefit from environmental enrichment. However, it is important to evaluate environmental enrichment strategies, to ensure that they are beneficial for the animals. The appropriate enrichment items must be carefully selected for each species and for specific individuals. An enriched enclosure could be inherently more dangerous than a sterile enclosure if proper care is not taken. Incorrect enrichment application can cause injuries or even cause death in some cases (Hare et al., 2008); for example, snakes can become trapped and injured in hiding retreats with inappropriately sized entrances. Enrichment studies should take behavioural aspects together with neural, endocrine, reproductive, metabolic, psychological, phylogenetic, and ecological factors because no single measure corresponds directly with an animal's welfare state (Burghardt, 2013).

Enclosures should offer environmental complexity with multiple elements for the animal to choose from to encourage mental stimulation and physical movement. Many reptiles in the wild spend a large portion of their time moving through novel environments, often in search of prey. While an enclosure can rarely provide the same range and novelty as in the wild, it can encourage movement and mental stimulation. A barren environment will be quickly explored, but items and elements for investigation can be added to prolong the exploration, as long as the added items are not disruptive. The benefit or stress associated with a complex static environment or a complex environment with frequent novelty is highly species-specific: different species will be affected differently by a changing environment. Novelty can be accomplished by either placing new objects in the environment or rearranging items in the enclosure to create new settings. Some species (e.g., leopard geckos) benefit greatly from the placement of novel items in the environment (Bashaw et al., 2016). Small European lacertid lizards spend much more time moving if a couple of wooden blocks are added to the environment (Cooper, 2010). In contrast, other species (e.g., rattlesnakes), while able to cope with minor changes in their environment (Holding, 2011), find significant changes to their environment stressful (Heiken et al., 2016).

It is important to remember that reptiles are olfactory animals, and environmental complexity does not need to be limited to tactile items. Providing olfactory experiences can increase exploration, as reptiles use olfaction or additional senses (Clark and King, 2008). For example, prey-scented items stimulate the exploratory behaviour of rattlesnakes for an extended period (Kuppert, 2013). Providing rat snakes with enrichment appeared to result in animals that were less fearful of exploring new environments from which they could extract information more efficiently (Almli and Burghardt, 2006). Providing novel objects or olfactory opportunities for leopard geckos also led to an increase in exploration and behavioural diversity (increased movement, manipulation of the object, and tongue-touching) (Bashaw et al., 2016).

6.6.1 Predatory Behaviour

For some species, engaging with their predatory behaviours can be an excellent mechanism of enrichment. For personnel safety, any predatory behaviour should be stimulated at a distance, using appropriate tools. Many species respond best to simulated prey movements such as jiggling pre-killed rodents via tongs for snakes or jiggling pre-killed fish for turtles. Kuppert (2013) provides examples of enrichment strategies that engage with the olfactory systems of reptiles (e.g., strikes on Tupperware containing mouse bedding).

Staggering and randomizing food availability is a common way of providing behavioural enrichment as it is usually thought to increase the amount of natural behaviour due to the unpredictability of the food source. Scattering feed in multiple locations promotes activity and increased hunting difficulty, partly due to the enclosure design, where a complex physical environment contributes to the difficulty of catching the prey (Januszczak et al., 2016). When providing live insects as food, it is important to provide them in moderate quantities so that animals are not overwhelmed. Insects should be provided sufficiently early in the day to ensure that basking and digestion can occur.

For insectivores, such as lizards, feeding devices containing crickets are a preferred enrichment option (Bashaw et al., 2016). Food enrichment allows reptiles, which are natural hunters and browsers, to participate in active food searches (Kuppert, 2013).

6.6.2 Furnishings – Resting Spots, Platforms, Behavioural Thermoregulation, Areas to Retreat

Structural and habitat design and manufactured and natural enrichment devices may be used to facilitate natural environmental interactions, thereby allowing natural social interactions, which may also allow for reproductive behaviours (Rose et al., 2014). Structural enrichment is the most studied form of enrichment for reptiles (de Azevedo et al., 2007; Eagan, 2019).

Housing enclosures must include a refuge that ideally mimics attributes of the animal's natural habitat (Cooper, 2010). Besides absolute enclosure size, hiding places enable the animals to avoid fighting, seek shelter, and obtain a sense of security. Materials to hide in or under can increase the effective size of an enclosure. Such retreats are forms of environmental enrichment, particularly for animals that are singly housed. Aquatic turtles will use ledges, rocks, and other areas to hide. Similarly, tortoises will take advantage of hides if provided. Nocturnal species in particular use hides as retreats during the day.

Furnishings and props should offer physical and tactile objects to explore while creating areas of security. Small logs, branches, and nonabrasive rocks may be strategically placed in the environment to accommodate the animal's movement and provide a secure resting area when out of the hide box. These objects will also assist in the shedding process. The cage environment should be kept simple and safe for snakes, as large specimens can move or overturn cage furnishings, sometimes creating hazards where tails can become caught under or between cage props (AZA, 2009).

A selection of basking locations at varying levels and varying degrees of exposure to heat is beneficial for many species (Bashaw et al., 2016).

Arboreal species benefit from a selection of branches and vegetation of varying size and complexity or ledges on which to perch (O'Rourke et al., 2018). Branches and perches should be of appropriate diameter and placed at appropriate angles to facilitate movement (Astley and Jayne, 2007). These structures should be easy to sanitize or replace.

6.6.3 Social Interactions

The natural history of the species must be considered when deciding whether or not to provide conspecific social interactions. Males of almost all reptilian species are aggressive to other males, especially during breeding; as a result, it is rarely recommended to co-house male reptiles, and any co-housed animals must be sexed appropriately. Most snakes should not be co-housed, but certain aquatic turtles and lizards, such as the blue-tongued skink, can benefit from social housing (Benn et al., 2019). Social learning by turtles may indicate the importance of social interactions for these species (Davis and Burghardt, 2011). Any cohort of group-housed animals requires more careful monitoring to ensure appropriate distribution of resources for each animal and to monitor for potential aggression. For species that benefit from social interactions, single housing should be appropriately justified. A consideration when deciding on single or group housing should be the duration the animal will be maintained. When multiple species are maintained in the same enclosure, they should be of similar morphology and needs and not susceptible to cross-predation.

Increased vigilance by animal care personnel is required when housing different species together, especially during and immediately after feeding.

While reptiles have historically been considered non-social, a review by Doody et al. (2013) indicates that reptiles engage in some social interactions, particularly in relation to parental care. The application of natural behaviour should be judiciously applied in the captive environment due to the restricted size of the environment and the optimization of light and heating in captive environments. Some rattlesnakes and garter snakes have been shown to form aggregations in the wild that may help with thermoregulation (Clark et al., 2012), which is not necessary for an appropriately controlled captive setting. As more knowledge is gained on the species' natural history and husbandry, it should be incorporated into husbandry practices.

6.6.4 Digging

Some reptiles live the majority of their lives underground, while others dig at the surface or only during certain stages of their life cycle (e.g., when laying eggs). Due to the difficulty of observing animals that are in burrows, substrate burrows are frequently replaced with shallow hide structures. A variety of substrates (see Section 6.5, "Substrates and Furnishings") can be used to encourage digging behaviours and may be used as either the enclosure substrate or in "dig boxes" that can be added to enclosures. Some species, such as tortoises, are powerful diggers and require appropriately strong or reinforced enclosure bases.

6.7 Human Contact and Handling

This section includes information relevant to handling reptiles for husbandry procedures. Section 7, "Handling and Restraint", focuses on additional information that may be required for the safe handling of reptiles used in scientific activities.

Handling should be kept to a minimum, unless that is the purpose of maintaining the reptiles in captivity (e.g., to teach handling techniques), and precautions should be taken to avoid stress or injury to the animals. If animals are to be used for handling, they should be carefully chosen based on species, individual temperament, health, and age or sex where pertinent. Based on the current body of knowledge, most reptiles do not benefit from human social interactions unless they are being trained to tolerate biomedical procedures or if human interaction is used for the purpose of enrichment. Some reptiles can tolerate human contact but do not seek it or require it. Competent handling and restraint are equally important for reptiles as for other species, and personnel should be well trained before working with these animals (Cooper, 2010). Particular attention is needed when caring for venomous reptiles, and personnel should be thoroughly trained, knowledgeable, and comfortable with handling procedures. Venomous animals should only be handled by trained designated personnel (see Section 13.1, "Working with Venomous Reptiles"). Facilities should have SOPs for handling specific species that are being maintained at the institution. An SOP for an emergency response to envenomation must be in place before receiving animals, as should SOPs for the appropriate handling, transfer, and restraint of venomous animals.

Dedicated handling equipment should be available for the room or enclosure, depending on the disease status of the animals, to reduce pathogen transmission through fomites. Most amenable animals can be transferred between enclosures using free handling techniques described in Section 7.1.1, "Free Handling". When necessary, to reduce the risk of escape or bite injury during free handling, snakes and lizards can be grasped firmly behind the head while supporting the body.

Support is particularly important for snakes and legless lizards, which can be injured or stressed if they are allowed to dangle (Cooper, 2010). Alternatively, appropriate handling devices as described in Section 7.1.2, "Handling and Restraint Devices", can be utilized to transfer animals when escape or bite injury is a risk.

Some lizard species will spontaneously drop their tail (autotomy) when they feel threatened. For these species, care must be taken to ensure that the tail is not grasped or pinned. Other species of lizards, such as geckos, must be handled with extreme care as their skin is only loosely attached to the underlying tissue and can tear easily (Cooper, 2010). Day geckos' skin and tails are so delicate and easily torn that transfer should be done by catching the gecko in a small container or fishnet.

Large lizards – iguanas and monitors – have a powerful bite and can also cause injury using their claws and tail. Leather gloves should be worn when handling these animals (Cooper, 2010).

Turtles can generally be handled by their shell. In general, terrestrial turtles do not bite defensively; however, some species of aquatic turtles will bite and vigorously struggle if handled. These animals should always be picked up by the sides of the shell, far enough from the head to prevent biting, with the head pointed away from the handler (Tonge, 2010). Turtles can also be handled with one hand under the plastron and the other hand grasping the rear margin of the carapace or the base of the tail – this is the preferred and safest method for large snapping turtles. Turtles should never be lifted by the tail or inverted unnecessarily.

Training or behaviour modification allows for complex cognitive function to be exercised (Kis et al., 2014; Manrod et al., 2008) and may facilitate husbandry procedures (Augustine and Baumer, 2012; Augustine et al., 2013) while reducing stress for reptiles (Hellmuth et al., 2012). For snakes, feeding can be used as a way of removing the snake for cage maintenance or cleaning.

6.8 Animal Monitoring

Reptiles must be observed daily to ensure the health and welfare of the animals, ongoing suitability of the environment, and appropriate functioning of life support systems (see Section 6.2, "Animal Observation" and Section 10.1, "Health and Behavioural Monitoring" of the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)). Ideally, this will involve direct observation of the animals and their behaviour as well as food and water consumption, soiling of the environment, the status of environmental systems, etc. In rare circumstances (i.e., with burrowing species), disturbing the animal may be deleterious to their health and welfare, and monitoring may be limited to observing the environment and systems. Accommodation for these situations must include a reasonable frequency of direct animal observation and be reviewed and approved by the animal care committee.

6.9 Cleaning and Sanitation

Reptile enclosures should be cleaned by designated animal care personnel. Cleaning is the removal of dirt and organic material, while disinfection is the killing of microbes and spores. Disinfection should always be preceded by thorough cleaning, as all disinfectants have reduced functionality in the presence of organic material and proteins. The choice of disinfectant is important, as some can be toxic, especially to small reptiles (Cooper, 2010). No disinfectant should come into contact with animals. Disinfectant selection should be based on identified and anticipated organisms in the facility and enclosures. Activated peroxides are broad-spectrum antimicrobials and are commonly used in reptile husbandry. Alternative disinfectants may be required depending on the pathogen in question. Knowledgeable individuals such as a biosafety officer or veterinarian should be consulted on disinfectant selection (Rzadkowska et al., 2016; Hemby et al., 2019).

Schedules of cage cleaning should represent a balance between cleanliness and disturbance of the animal. Some species, including rattlesnakes (Heiken et al., 2016), utilize odour and pheromones associated with feces to identify their home environment, and completely cleaning or sanitizing the home enclosure may be stressful to these animals as it removes home territory markings. In some herbivorous cases such as green iguanas (*Iguana iguana*) and some tortoises, juveniles require ingestion of adult feces to achieve appropriate gut floras (Morafka et al., 2000). In these cases, disease and experimental conditions permitting, feces should be removed, and the cage floor spot cleaned, while enrichment items should be cleaned of feces and debris but not sterilized during standard enclosure cleaning. In contrast, other species completely avoid contact with their own feces, and full cage and enrichment item sterilization have no adverse effects. Waste removal and quantity of residual waste left behind are species-specific and should be specified in SOPs. Particular care should be taken regarding the removal of the waste substrate from venomous enclosures, as shed fangs passed through fecal matter still pose a significant health and safety risk.

Bioactive substrates are now available that include isopods and other organisms that reduce the need for bedding changes. While these substrates are becoming more common among hobbyists, they are not recommended for laboratory reptiles as the bedding is not sterile.

6.10 Record Keeping

It is important that all records identified in Section 12, "Record Keeping", of the *CCAC guidelines: Husbandry of animals in science* (2017) are maintained. Individual records are necessary for animals that undergo treatment, procedures, or breeding. Requirements for breeding records are detailed in Section 5.1, "Rationale (When and Why Internal Breeding Programs are Indicated or Acceptable)", of this document.

7. HANDLING AND RESTRAINT

7.1 Physical Handling and Restraint

Guideline 14

Reptiles should only be handled when necessary, according to the purpose, and the handling time should be minimized.

Animal handling skills are techniques used to approach, manipulate, and calm the animal safely. Animal restraint skills are a subset of animal handling that involve the restriction of the animal's movement for a period of time. Reptiles should only be handled when necessary; the method of any handling or restraint and the duration requires justification. Anyone handling reptiles should have appropriate training or qualified supervision, and particular care must be taken when handling venomous species. Appropriate tools must be available and used when handling venomous species.

Techniques used for handling may also be used for restraint. Handling and restraint can be utilized separately or in combination; for example, by using a snake hook to move the anterior portion of the snake while supporting its posterior weight with the other hand. Simple handling and physical restraint should not cause excessive distress, but if this is likely to be the case, then chemical restraint should be considered. Chemical restraint should also be considered for animals that are too small, too quick, or too fragile to manipulate safely. Due to the inherent risks and side effects of chemical restraint, it should not automatically take the place of appropriate physical restraint performed by well-trained personnel.

When appropriate, operant conditioning can be used to facilitate the handling of reptiles and the conduct of some procedures. While not every species or individual is responsive to this technique, animals that have been carefully and selectively trained will be easier to manage. Target training can be utilized to facilitate blood collection, weighing, and animal movements. While animals may seem to solicit tactile interactions, this does not automatically mean they will do so for experimental procedures. Using operant conditioning for desensitization is a critical component to establishing reliable tactile interactions. Animals should be made aware that they are about to be touched, either through a verbal signal or another sensory cue, so that they are not taken by surprise. This strategy can reduce potential reflexive or defensive responses from a surprised animal and help prevent damaging the trust between the trainer and animal (Hellmuth et al., 2012).

Appropriate handling and restraint techniques are critical to prevent injury to the animal. The appropriate time and method of handling will depend on the species, as different species have different vulnerabilities that must be accounted for. The handling and restraint of snakes and lizards should be minimized during peri-ecdysis to reduce the risk of dysecdysis, and avoided for all reptiles during brumation. If possible, snakes should not be handled during the initial digestion of food items (normally 48 hours post-feeding). Scaleless reptiles have fragile skin that is easily damaged, and chameleons are prone to rib fractures from inappropriate handling. Incorrect weight distribution without support can cause injury to the cervical vertebrae in many reptiles. Gripping a lizard by the tail or inducing a flight response should be avoided to prevent autotomy (traumatic dropping of the tail). Autotomy is not universal among lizards and can be prevented by avoiding pressure on the tail and using equipment such as transfer boxes as necessary. While a tail can be regenerated after autotomy, loss of the tail will influence future growth and reproduction by depriving the animal of fat stores (Price, 2017) and may affect the animal's behaviour (Cromie and Chappie, 2012, reviewed by Bateman and Fleming, 2009; Cooper et al., 2009; Michelangeli et al., 2020). The regenerated tail is composed wholly of cartilage (rather than bone) and will likely differ in morphology and function compared to the original appendage.

7.1.1 Free Handling

Free handling refers to the use of hands rather than any tools to handle and manipulate the animal. It can easily be combined with the use of tools when needed. Free handling of animals should only be performed on non-venomous, tractable animals of appropriate size, with consideration that stress or illness may have a deleterious impact on their behaviour. The handler must always maintain awareness and control of the animal's movements and prevent the animal from moving in ways that can cause harm to itself or to the handler (e.g., it is important to maintain the animal's distance from the handler's head and neck, and to monitor tail movements in large lizards, which can deliver an injurious blow). Protective gloves may be used, but should be selected on the basis of the physiological features of the animal and the procedure being performed. Anatomical features that may harm the animal handler (e.g., fangs) or may be harmed by the handler (e.g., epithelium of the animal) should both be evaluated. Handlers must still be able to perform appropriate restraint while wearing protective gloves.

Animals should be handled differently based on their species, size, and whether they are venomous.

- Large lizards can be free handled if they are adequately trained. Tractable animals should be laid along the forearm and can be restrained by holding them close to the handler's body, with lateral pressure from the handler's free arm. Tails can be gently restrained between the handler's body and upper arm. Intractable large lizards should be handled using free handling and a restraint device, and thick leather gloves should be worn by the handler. Small lizards can be picked up with one hand, securing the forelimbs with the thumb and index finger, supporting the weight of the abdomen with the middle and ring finger, and restraining a hind limb with the pinky finger. When restraining small lizards, an open flat hand should be used to apply even pressure over the animal's entire body. Pressure on the tail should be avoided as autotomy can occur, which is traumatic to the animal.
- Snakes should always be handled using at least two points of contact. One point of contact should be approximately one-third of the body length from the head, but the head should also be controlled if there is a bite risk or if hyperactivity is a concern. The other point of contact should be at the posterior third, with the weight distributed as evenly as possible. For large snakes, appropriate support may require multiple handlers (e.g., one point of contact for every three feet of snake) and appropriately sized snake hooks.
- Turtles should be grasped by the sides of the shell, ensuring that the handler's hands are placed far enough away from the head to prevent bites. Alternatively, species with a long cervical reach can be held with one hand supporting the plastron and the other hand grasping the posterior carapace or tail if the turtle's weight is not supported by the tail.

7.1.2 Handling and Restraint Devices

All restraint devices should allow visualization of the animal. It is important to have enough designated instruments and ensure adequate disinfection between uses to prevent cross-contamination.

7.1.2.1 Transfer Boxes

Transfer boxes are typically small, ventilated boxes resistant to animal strikes and forces (e.g., digging) that can be used to transport an animal relatively short distances over a relatively short period of time. Transfer boxes should be designed to be comfortable to the reptile, such as by being warm and dark. Animals can be acclimated to transfer boxes by occasionally placing them in the enclosure and associating it with positive experiences such as feeding, or attaching them to the habitat. The transfer box can be used as a feeding box to reduce potential aggressive behaviours in the home enclosure associated with offering food items.

7.1.2.2 Snake Bag

A snake bag is a temporary enclosure consisting of a tightly closed, appropriately permeable fabric bag (e.g., pillowcase of appropriate breathable material). The animal cannot be seen directly, but the body position can be visualized when handling the pillowcase and animal. It may be necessary to place the pillowcase in a secondary, hard-sided container to limit animal movement or prevent accidental damage to the snake or bag. While the use of a snake bag does not protect the handler from being bitten, animals are generally reluctant to strike blindly, minimizing the opportunity for bites.

7.1.2.3 Snake Hooks

A snake hook is a C-shaped blunt hook at the end of a handle. The size of the hook used should be appropriate for the size of the animal, and the length of the handle should be matched to the procedure being performed. The snake must be supported at two contact points: first, placing a snake hook at the anterior end to control the head, then using a second hook or a hand at the posterior third of the body to distribute the weight appropriately. Large snakes require specialized, thicker snake hooks, to prevent injury to the snake's ribs and spine from the weight being supported. Large snakes may also require additional points of contact, at least one for every three feet of body length. Snake hooks are useful when free handling as they communicate to the animal that it is about to be manipulated rather than fed. The combination of a snake hook followed by free handling is useful for acclimating the snake to being moved.

7.1.2.4 Pinning Tool

A pinning tool can be used for snakes or lizards and consists of a padded or elasticized fork to immobilize the animal at the base of the head. The fork should be the appropriate size for the cervical area of the animal, and animals should always be pinned against a soft surface to minimize the risk of injury. The body of the animal should be restrained as needed to control thrashing.

7.1.2.5 Tongs or Forceps

Tongs or forceps should be used with care as the animal's weight and the pinching mechanisms of the tool can work together to cause injury. The tips of the tongs or forceps should be padded or covered in rubber to reduce injury risk to the animal. Rubberized tongs or forceps can be used as snake hooks for appropriately sized snakes as the rubberized tips increase friction and can slow the snake's movements. Specialized tongs are available and can be useful when handling venomous snakes. Neither tongs nor forceps should ever be used to pick up an animal by the tail.

7.1.2.6 Snake Tubes

Snake tubes must be made of clear plastic or acrylic, open on one end, and either completely or partially open on the other to allow for unimpaired breathing – it can also be used for anesthetic induction. The tube should be sufficiently long to enclose the first third of the snake, and the diameter should be wide enough to accommodate the thickest part of the snake without allowing the snake to turn its head around in the tube. Snake tubes should be checked for structural integrity and rounded edges before every use; the edges should be smooth to prevent injury to the snake's scales. A snake hook can be used to guide the snake into the completely open end, up to 50-75% of the way through the tube. The tube and snake should then be grasped together to prevent the snake from moving either forward or backward. For venomous species, venom may be deposited in the tube; it is therefore important to clean the snake tube with species-specific venom neutralization protocols at the end of every use.

7.1.2.7 Body Socks

Mildly elastic cloth tubes such as casting stockinettes can be used to restrain smaller lizards. A section of a casting stockinette can be rolled down from the head to the tail, keeping the legs proximal to the body. Care must be taken that the claws do not snag in the stockinette material and lead to broken toes. Body socks can be removed by cutting them open with bandage scissors.

7.1.2.8 Boards

Larger crocodilians can be secured to wooden boards or placed inside tubular structures (culverts) for transportation. Jaws should be secured shut, typically with tape, while ensuring that the nares are unobstructed. The material used to secure the jaws should be such that it cannot slip down over the nostril "button" over time, as this can quickly result in the death of the animal. Even with secured jaws, crocodilians' protruding teeth can cause scratches; it is therefore important to control crocodilian heads to prevent this.

7.1.3 Safety

The safety of both the handler and the animal must be considered during physical handling and restraint procedures. Appropriate use of handling and restraint devices will minimize both animal and human injury. Handlers can potentially be injured by the claws of lizards and turtles; the spines of some reptiles; bites from snakes, lizards, or turtles; and tail whipping by lizards. When handling larger animals and those with a greater safety risk (i.e., venomous reptiles, larger constrictor snakes, and aggressive or defensive reptiles), two or more people may be required. Animals can potentially be injured by inappropriate handling techniques, as discussed in Section 7.1, "Physical Handling and Restraint", and by inappropriate contact with elements in their environment. Physical handling of venomous animals requires extensive training in addition to specific procedural and infrastructure requirements discussed in Section 7.1.3.2, "Venomous Animals".

7.1.3.1 Personal Protective Equipment

When selecting personal protective equipment, the temperament of the species and the individual animal must always be taken into consideration, as well as safety considerations for the procedure being performed. Puncture-resistant gloves may be required when handling intractable animals. The handler's external layer of clothing should be tightly woven, with minimal tags, flaps, hooks, etc., to reduce the risk of claw snags that can lead to broken toes in the animal. Any individual with a compromised immune system or other health concerns should consult with the institutional health and safety officer or equivalent individual to identify appropriate personal protective equipment.

7.1.3.2 Venomous Animals

Venomous animals should only be handled by designated personnel, and additional venomous-species-specific training is required for all animal handlers. There should always be at least two qualified personnel present when handling venomous reptiles in case assistance is needed. Having a second person close by is crucial should envenomation occur. Procedures should be chosen to minimize handling time and reduce or eliminate contact between the handler and the animal. The use of additional personal protective equipment, such as an eye shield and puncture-resistant gloves, and use of appropriate restraint and transfer devices should be implemented unless sufficient justification is provided otherwise. It can also be helpful for handlers to clearly display a written description of the species in use (e.g., on an ID badge) should the handler become non-verbal in an emergency.

All appropriate tools for handling should be prepared before starting any procedure involving a venomous reptile. Cages should remain locked until they are ready to be opened. Announcing that the cage is about to be opened is important so that the attention of personnel is immediately focused on the procedure (Lock, 2008). Venomous snakes can be safely restrained in transfer boxes or clear plastic tubes. When it is necessary to restrain a venomous snake for hands-on procedures such as blood collection, sexing, or force-feeding, it is recommended that the snake be directed into a snake tube. This can be done on a work table, on the floor, in a bucket, or directly from a snake bag. Even if a snake appears dead, tongs or a snake hook should be used to test for movement. The mouth of a dead specimen should be carefully taped closed to avoid unintentional contact with the fangs (Lock, 2008). Venom may be deposited on tools and in enclosures used to house and manipulate venomous animals; they should be considered contaminated with venom until appropriately cleaned.

7.2 Chemical Restraint

Chemical restraint may be used when animals are too small, too quick, too fragile, or too dangerous to safely restrain for transfer or short, relatively painless procedures. Chemical restraint protocols for reptiles are challenging due to the wide variety of species involved; different species and even different individuals may respond differently to sedatives, hypnotics, tranquilizers, and anesthetics. Chemical restraint protocols for mammals are difficult to extrapolate to reptiles, and it is preferable to consult published studies of sedative protocols for reptiles. Currently, all drugs used for the chemical restraint of reptiles are used off-label. A veterinarian experienced with the use of sedatives in reptiles should be consulted when planning for their use in a new project or on a new species.

Chemical restraint may be delivered via injection or inhalation. Some chemicals produce an initial excitement phase, and the use of tranquilizers in conjunction with the sedating agent may be indicated. Preference should be given to sedative drugs or combinations that are partially or completely reversible to avoid prolonged or unpredictable recoveries associated with reptile-specific metabolism. Sedative drugs or combinations should be selected based on the desired level of sedation (mild for brief restraint to deep for prolonged periods of restraint), the general condition of the animal, and the procedure to be performed (see Sladky and Mans, 2012 for suggested sedation protocols). Injectable drugs can have prolonged effects on reptiles, and careful drug selection is required. There is currently no evidence to support cold narcosis due to hypothermia as a safe and humane method for restraint or anesthesia.

Alpha-2 adrenergic agonists such as dexmedetomidine and xylazine are commonly used sedatives for reptiles and provide a moderate duration of sedation. They are completely reversible using atipamezole or yohimbine.

Isofluorane or sevoflurane are commonly used inhalation anesthetics in veterinary medicine. While these gases induce general anesthesia, they are commonly used for chamber inhalation deep sedation of reptiles when other methods of handling and restraint for injection are not practical. The benefits of using inhaled volatile gases are that they are very safe, immediately reversible, easily titratable, and can be used in a contactless chamber. However, these gases require specialized equipment, including oxygen, gas vaporizer, delivery circuits, gas scavenging, and appropriate ventilation for human safety. Inhalation anesthetics should not be used without all of this equipment in place.

Local anesthesia may be used as an adjunct during anesthesia and restraint or as a standalone for minor procedures for reptiles that are easily restrained. The toxic dosage of local anesthetics does not appear to have been investigated in reptiles, and care should be taken to use the minimum dose required. It is recommended that small volume syringes and small gauge needles be used.

<u>Guideline 15</u>

Reptiles must be continually monitored during anesthesia, with particular attention paid to respiration, heart rate, and depth of anesthesia.

Manipulation of body and ambient temperature is part of the reptile anesthetic regimen. Higher temperatures generally increase the metabolic rate and are frequently used during induction and recovery, while lower temperatures generally decrease the metabolic rate and are frequently used during maintenance. The time required for recovery from anesthesia depends on the life stage of the animal, anesthetic, temperature, species, and depth of anesthesia. When an animal is anesthetized, relevant information such as the type of anesthetic, method of administration, and any complications or welfare concerns must be documented in the animal records.

A method of artificial respiration, including manual bagging, and the ability to quickly adjust the body temperature, should be available to improve patient survival in the advent of an adverse reaction to anesthesia.

8. HEALTH AND DISEASE CONTROL

<u>**Guideline 16**</u>

All reptiles should be included in an animal health program, irrespective of where they are housed.

Veterinary professionals must be engaged in the development of the health program, which should be approved by the animal care committee and overseen by people competent in evaluating the health of reptiles. The animal health program should include:

- good record keeping in accordance with the *CCAC guidelines: Husbandry of animals in science* (2017);
- adherence to the Canadian Association of Laboratory Animal Medicine (CALAM/ACMAL) *Standards of Veterinary Care* (2020), as applied to reptiles;
- good biosecurity to limit disease introduction and transmission, and minimize contamination of the environment;
- health monitoring and detection of latent disease by systematic evaluation of individual animals and the health status of each colony; and
- a response plan for when a potential infectious disease outbreak is identified – execution of this plan should limit disease propagation and spread until the outbreak is confirmed, the pathogen is identified, and a veterinarian can recommend pathogen-specific measures (see Section 8.3, “Disease Management in the Event of an Infectious Outbreak”).

8.1 Disease Prevention

<u>**Guideline 17**</u>

Strategic measures for disease prevention should include a plan for disease control and a system of regular monitoring and reporting for health assessment purposes.

Animals should be free of unwanted pathogens and clinical diseases. A veterinarian should be integral in developing a disease prevention and control plan, with supporting SOPs to limit the risk of introducing a disease into the facility, and should be available for consultation on all matters relating to the health of the animals.

The disease prevention and control plan should address the following:

- procurement – reptiles coming from a supplier should have as complete a history as possible, with particular attention to wild-caught versus captive-bred status (see Section 4.5, “Receiving Animals”);
- quarantine – newly arrived animals should be kept separate from other animals in the facility pending physical exam and routine screening measures (see Section 4.5, “Receiving Animals”);
- facilities and their management – facilities, equipment, and management practices should be in place to prevent airborne, waterborne, direct contact or fomite transmission of microorganisms, water contamination, pest infestations, and contaminants from external sources (see Section 2, “Facilities”);

- husbandry – reptiles should be fed a high-quality diet, and practices should be in place for effective sanitation and prevention of overcrowding (see Section 6, "Husbandry");
- biosecurity for the animals – SOPs should limit access to the animal facilities (see Section C.2, "Location" of the *CCAC guidelines: laboratory animal facilities – characteristics, design and development* (CCAC, 2003); and
- isolation procedures – plans should be in place for holding contaminated animals separate from other animals in the facility in the event of a disease outbreak; the plans should include a disease prevention strategy.

It is important that all of these components are included in the disease prevention and control program. As noted by Suedmeyer (1995), incorrect husbandry accounts for the majority of diseases in captive reptiles.

8.2 Health Monitoring and Disease Detection

<u>Guideline 18</u>

SOPs should be developed for assessing animal health, providing health care, and treating common health problems for the animals; these should be reassessed at least every three years to ensure relevance.

SOPs should be developed for routine health checks and welfare assessment for individual animals and each colony, based on the species, sex, life stage, age, and health status of the animals; the housing system; the type of research; and the potential effects on other animals in the facility and on the research itself. Animal monitoring requirements for health and disease control will also depend on the length of time the animals are housed. Health monitoring programs may include the use of environmental monitoring of both the room (e.g., temperature and humidity) and the enclosure. Evaluation procedures, including test intervals, selection of agents, and verification, need to be determined. The selection of agents to test requires consultation with a qualified veterinarian and should be based on the history of the facility and animals and the probability of the organism being present. It is important that testing methods and samples are appropriate to the conditions of particular interest. Where possible, the methods should adhere to the Three Rs principles of Reduction and Refinement to minimize the impact on the animals. In most scenarios, sentinel animals are not appropriate or required.

Disease prevention and control programs and SOPs should be updated regularly and particularly in response to the prevalence of diseases for particular species and new information on reptile health. The literature should be reviewed for information on diseases affecting the species and procedures for their detection. When possible, the use of validated molecular assays to test directly for pathogens is strongly encouraged. Diagnostic laboratories capable of assessing reptile samples can be rare, and long wait times for results may occur. Prior to disease outbreaks, the veterinarian or facility manager should develop a relationship with a diagnostic laboratory capable of analyzing reptile samples for relevant conditions. Knowing what samples need to be collected and where they can be analyzed expedites analysis during potential disease outbreaks. Institutions may be able to utilize in-house testing capabilities, but the assay(s) should be developed and verified prior to diagnostic need. It is important to implement quarantine or isolation procedures when an infectious condition is suspected, to prevent pathogen spread while diagnostic testing occurs.

There should be procedures in place to ensure any animal health concerns or other potential animal welfare issues are documented and promptly communicated to the veterinarian.

Reptiles should be fed appropriately and maintained in a low-stress environment to minimize health and disease problems (Tonge, 2010). Signs of illness in reptiles are rarely specific to a particular disease. General signs of illness are outlined in Mader (2019), and more detailed information about chelonian diseases can be found in McArthur et al. (2004). Diagnostic testing for diseases in reptiles is discussed by Hernandez-Divers et al. (2004), Cooper (1999), Campbell and Ellis (2007), Diethelm and Stein (2006), Garner and Jacobson (2020), and Jacobson and Garner (2020).

It is important to understand that several reptile species will commonly harbour opportunistically pathogenic organisms (e.g., *Oxyuris* spp. in bearded dragons). Complete eradication of these commensals can be challenging, invasive, and unnecessary. A veterinarian should be consulted for any treatment plan.

8.2.1 Common Diseases and Conditions

Maintaining reptiles in captivity imposes physical, behavioural, and physiological constraints on reptiles that may result in suppression of their immune systems, increasing the possibility of infection (Cooper, 2010). The immune responses of reptiles are temperature-dependent and therefore function more effectively at the higher range of the preferred optimum temperature zone, underling the importance of providing an appropriate temperature and temperature gradient in enclosures. Some species will respond to bacterial infection by actively seeking higher temperatures to raise their body temperature (Evans et al., 2015).

Due to the unique life patterns of reptiles (i.e., brumation, sporadic eating, and ecdysis), any potential signs of ill-health must be interpreted in the context of the animal's environment and life cycle. A veterinarian or reptile specialist experienced with the reptile in question should be consulted before an intervention. Signs of a potentially sick reptile include:

- anorexia
- lethargy
- closed eyelids
- poor body condition
- seizures
- lameness
- ataxia
- open mouth gasping
- regurgitation
- laboured breathing
- discharge upon breathing
- swollen or misshaped mouth

Common categories of reptile diseases or conditions include:

- fungal dermatitis

- necrotic dermatitis (scale or shell rot), generally related to inadequate husbandry
- pneumonia (from viral or bacterial etiology)
- stomatitis (from viral or bacterial etiology)
- acariasis (species-specific mites)
- abscesses or fibriscesses
- aural or tympanic abscessation
- bite wounds and prey-induced trauma
- cloacal prolapse
- diarrhea
- dystocia
- gout
- hypovitaminosis B and D
- hypervitaminosis A
- nutritional secondary hyperparathyroidism
- salmonellosis
- metabolic bone disease

Common lizard-specific diseases or conditions include:

- dysecdysis
- hepatic lipidosis
- periodontal disease
- thermal burns

Common turtle-specific diseases or conditions include:

- shell abnormalities (nutritional, fungal, and bacterial)
- hypovitaminosis (vitamin A deficiency)
- respiratory disease
- turtle herpes
- mycoplasma

Common snake-specific diseases or conditions include:

- blister disease (related to excessive humidity, with subsequent infection by bacteria)
- dysecdysis
- cloacal scent gland adenitis
- snake cryptosporidiosis
- ophidiomycosis (snake fungal disease)
- thermal burns

For information on these and other common diseases or conditions affecting reptiles, see Divers and Stahl (2019) or Girling and Raiti (2019).

8.3 Disease Management in the Event of an Infectious Outbreak

Guideline 19

A response plan must be in place to deal with potential disease outbreaks.

A response plan must be developed to deal with potentially serious disease outbreaks within the facility and from outside sources, and to prevent pathogen transmission within the colony and infection recurrence. These plans should be developed in anticipation of an outbreak, with the ability to modify based on the nature of the disease or outbreak. Plans should include a communication strategy involving veterinarians, veterinary and animal care personnel, investigators, the facility manager, and the animal care committee. Restrictions on the experimental use of animals and animal movement and contact during a disease outbreak should be described in the response plan. Access to quarantine facilities or a means of isolating the animals must be available (described in Section 4.6, “Quarantine and Acclimation”).

For infectious disease outbreaks, the veterinarian must be consulted to ensure that the techniques employed will eradicate the pathogens. Typical procedures may include quarantining the room where the disease is discovered and tracking and testing any animals that were recently moved from that “source” room. Follow-up actions, such as treatment, depopulation, etc., will depend on the nature and extent of the outbreak, the health status of the animals, and the type of research. If infected animals are to be euthanized, proper containment measures must be in place for handling and disposal of the animals and the contents of the enclosure, and for the decontamination of the enclosure and room to prevent the spread of disease.

9. WELFARE ASSESSMENT

Guideline 20

All reptiles maintained in an animal facility must be subject to routine welfare assessments.

General guiding principles for welfare assessment of all animals used for scientific purposes are described in the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021). Information in this section builds on the general guidelines by focusing on indicators for assessing the welfare of reptiles, keeping in mind that any indicator must be tailored to the species and life stage of the animal of interest.

An animal welfare assessment plan should include the use of observations and other tools that collectively provide information on the health, behaviour, and physiology of the animal. As noted in the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021), information should be obtained through a mixture of animal-based measures, resource-based measures, and management-based measures.

Animal-based measures include observation of the animal within the enclosure (see Section 6.2, “Animal Observation”), health assessment upon receipt of the animal at the institution (see Section 4.5, “Receiving Animals”) and as part of the animal health program (see Section 8, “Health and Disease Control”), and any additional information on the health, behaviour, or physiology of the animal obtained during experimental procedures. While it is important that reptiles are observed regularly, some animal-based measures can cause disturbance (e.g., for species that hide, digging the animals up, or removing cover to facilitate observation). Thus, the frequency of such disturbance must be carefully considered when designing the welfare assessment plan. Combining animal observation with resource-based measures and management-based measures can help minimize disturbance of the animal while obtaining the necessary information to assess their welfare. Generally, animal-based (output) measures are the best for identifying the actual welfare status of the animals but are less useful in identifying specific causes of poor welfare than input evaluation.

Resource-based measures evaluate the suitability of the enclosure for the particular animal being housed. Warwick et al. (2018) describe key elements for a range of species, including reptiles, that should be assessed in the captive environment. Criteria for the development of resource-based (input) measures are described in Section 2.2, “Enclosures” (e.g., enclosure size and design), Section 3, “Facility Management and Personnel” (e.g., water quality, lighting, and temperature), and Section 6, “Husbandry” (e.g., housing management, nutrition, and environmental enrichment). Input measures are most useful in identifying potential causes of poor welfare.

Management-based measures focus on assessing records (husbandry records, medical records, mortality and morbidity records, experimental records, etc.) to identify potential sources of welfare impacts to animals. Like resource-based measures, management-based factors are useful for identifying potential causes of poor welfare; they are particularly useful in tracking these potential causes over time.

9.1 Welfare Indicators

Reptiles include a wide range of species, which collectively occupy very diverse habitats in nature (i.e., from tropical regions to temperate climates with cold winters). Determination of suitable welfare indicators must take both of those factors into consideration.

It is important for investigators to have a good understanding of the biology and behaviour of the species that they are working with in order to make appropriate assessments of their welfare. It is also important to recognize how different factors could affect behaviour and physiological parameters in different species. While some species may appear healthy despite living in poor conditions, their welfare may be significantly compromised. Thus, health does not necessarily equate with good welfare.

Changes in behaviour or unexpected behaviours warrant further investigation into environmental conditions to assess their relevance to the animal's welfare. Behavioural welfare indicators often fall within a spectrum and cannot be evaluated by a simple checklist. Additionally, behavioural welfare indicators must be considered within the context of the animal's environment, as a particular change in behaviour may be indicative of varying stress levels, depending on the situation. For example, a diminished avoidance response could indicate lethargy or mean that the reptile is habituated to personnel. Activity levels can also fluctuate in response to factors that do not necessarily relate to the welfare of an individual (e.g., seasonal changes). All assessments should consider the individual animal to be the baseline and apply general behavioural biology from the species as a whole. The ability to detect changes in behaviours relies heavily on regular monitoring and observation by individuals working closely with the animals to capture behavioural nuances.

Warwick et al. (2013) provide a list of behavioural signs that may indicate captivity-related stress in reptiles. In general, behavioural signs of stress in reptiles may include anorexia; freezing; death-feigning; prolonged retraction of head, limbs, or tail; and pigmentation changes. Indicators of positive welfare are more difficult to ascertain and are currently focused on providing enrichment (Benn et al., 2019; Eagan, 2019); however, behaviours such as exploration and normal levels of feeding activity indicate a non-stressed reptile. Physiological indicators of welfare assume that a sick animal is in a state of diminished welfare.

In general, the following behaviours and physiological parameters could be used to make welfare assessments on individuals or group-housed reptiles:

- Possible behavioural indicators of welfare:
  - general activity level (including gait and escape behaviours);
  - location of the animal within the enclosure (whether the animal is using the enclosure space as expected);
  - utilization of enrichment such as furniture and hides (including use of the water dish);
  - changes to feeding behaviour (e.g., time to feed, amount of food consumed in relation to pre- or post-procedure or during acclimation or feed transitions (James et al., 2017));
  - unexpected behavioural response
    - knowing what behaviours are 'expected' under typical captive settings (e.g., healthy individuals should exhibit a startle response when their environment is suddenly

    disturbed, whereas they should be less reactive (no startle response should be seen) to daily routine husbandry practices like feeding); and
  - social interactions
    - ensuring that a colony is not exhibiting aggression.
- Possible physiological indicators of welfare:
  - growth and reproduction (Vitousek et al., 2010);
  - weight, physical appearance, and subjective body assessment;
  - colour and appearance of the skin, depending on the species (Lewis et al., 2017; Greenberg, 2002);
  - appearance of fecal pellets; and
  - corticosterone levels
    - some studies have looked at corticosterone in relation to bacterial infection susceptibility and its modulation by immune challenge (Meylan et al., 2010); plasma corticosterone levels can also be correlated with diminished growth and reproductive success (Vitousek et al., 2010).

Investigators should consult the literature and use the least invasive indicators appropriate for the particular animals and research being conducted.

10. EXPERIMENTAL PROCEDURES

Guideline 21

The least invasive method suited to the goals of the study must be used, with consideration of the potential impacts of the procedures on the reptiles and measures taken to reduce those impacts.

Measures should be taken to reduce the potential impacts of experimental procedures on other reptiles in the room (see Section 2.1, “Animal Rooms and Procedures Rooms”), as they may be affected by the production of alarm pheromones (e.g., Mason and Parker, 2010).

The institutional animal care committee must review all experimental procedures contained within animal use protocols. For routine procedures, SOPs approved by the animal care committee should be available to all personnel involved with the animals to ensure consistency of procedures and animal care. Where new procedures are proposed, SOPs should be developed in consultation with an expert in the subject matter, and input should be sought from stakeholders (e.g., investigators, safety officers, animal care personnel) before they are approved and implemented. SOPs should be reviewed regularly and updated as new information becomes available (CCAC, 2006). All procedures should be documented, and records should be kept in electronic files or close to the housing or procedure areas and be accessible to the veterinary team, animal care personnel, animal care committee, and the research team.

Institutions should have a policy or SOP for repeated procedures on animals, including reptiles. The frequency, duration of intervals between procedures, and the total number of procedures that may be performed on the same animal during its lifetime must be considered. The SOPs must take into account the invasiveness, pain, and distress associated with those procedures and their impact on the welfare of the reptile, both in the short and long term (see the CCAC guidelines on the identification of scientific endpoints, humane intervention points, and cumulative endpoints (in prep.)).

Procedures that adversely affect animals should be avoided where alternative methods effectively achieve the study outcomes.

All procedures have the potential to cause pain and distress. It can be difficult to assess pain and suffering in reptiles, so it should be assumed that a procedure that would cause discomfort in a mammal is likely to cause the same discomfort to a reptile. Many seemingly routine procedures are complicated when conducted on reptiles because of the difficulty in handling them, particularly venomous species. Procedures must be performed by competent people that have been properly trained by personnel with appropriate expertise. It is preferable to use the expertise of the veterinarian and experienced animal care personnel to carry out these procedures.

As techniques advance, refinements will continue to evolve in many areas, and investigators, veterinarians, and animal care committees should evaluate new evidence on refinements and consider their implementation.

Equipment should be made of non-porous materials and thoroughly cleaned and disinfected between uses to minimize the possibility of cross-contamination, especially when sharing equipment.

<u>Guideline 22</u>

Endpoints must be developed and must be approved by the animal care committee before the commencement of the study to minimize the negative impacts of procedures on the animal.

The CCAC guidelines on the identification of scientific endpoints, humane intervention points, and cumulative endpoints (in prep.) indicate that protocol authors must establish appropriate and study-specific endpoints (e.g., initiation of treatment, termination of a procedure, and euthanasia) and plans for monitoring in consultation with the veterinarian. Key references relevant to the particular study should be consulted in determining the earliest practical endpoints.

Defining humane intervention points (i.e., clinical signs that indicate an intervention is required to humanely treat an animal by relieving pain and distress) can be challenging, as reptiles generally do not display the range of clinical signs found in other laboratory animals, and it may be difficult to interpret the severity of a particular condition when signs are presented (Benn et al., 2019). Welfare indicators, such as inappetence, changes in the skin, changes in body weight, body condition scores, loss of ambulatory function, and levels of stress hormones (see Section 9, "Welfare Assessment"), can provide a basis for defining endpoints. However, since many reptiles do not eat regularly and may not be very mobile normally, indicators linked to feeding behaviours and movement are often difficult to assess (Warwick et al., 2013). When assessing the behaviour of an animal, it is important that personnel be familiar with normal or expected behaviour so that they can recognize deviations.

When an animal model is in development or new to a researcher, pilot studies should be performed to establish scientific endpoints and humane intervention points.

Appropriate monitoring frequency must be established based on the level of invasiveness of the protocol and expected clinical (or other) signs, as well as the progression of the animal's condition, the animal model, and the individual animal (e.g., previous experience). Monitoring should be documented.

Monitoring for endpoints should be a cooperative effort involving investigators, veterinarians, and veterinary and animal care personnel. Where appropriate, and in accordance with the level of invasiveness of the protocol, monitoring score sheets incorporating several parameters of assessment can be helpful in monitoring for endpoints.

Animals should be removed from the study or euthanized when endpoints are met or after consultation with appropriately trained veterinarians. Animals experiencing pain or distress that cannot be relieved and that are not approved as part of the animal use protocol must be euthanized promptly.

10.1 Animal Models

Reptiles are used as animal models for a wide range of studies in fundamental research such as genetics, immunology and toxicology (Poletta et al., 2012), regeneration (McLean and Vickaryous, 2011; Fisher et al., 2012; Sun et al., 2018), and evolutionary development (Nomura et al., 2013; Woolley et al., 2004). Reptiles may also be used in clinical veterinary research (Balko and Chinnadura, 2017; Carsia et al., 2018; Skovgaard et al., 2018).

Investigators or study directors should decide whether reptiles are required for the study and, if so, which species, strains, and life stage provide the best model of the biological processes involved in their work, taking into account the special needs of the species, strain, life stage; the ethical or welfare considerations of working with those animals for a given experiment or study; and their availability. Animals must not be obtained until measures are in place to care for them appropriately. Particular studies may need to be redesigned if those requirements could pose difficulty with maintaining the health and welfare of the animals or be intensified as a result of the experimental interventions. The measures required in these situations may include special or additional technical expertise and highly trained personnel.

10.2 Administration of Substances

General information concerning the administration of substances can be found in the CCAC guidelines on experimental procedures – administration of substances and biological sampling (in prep.). This section provides additional species-specific information for reptiles. The route of administration depends on the chemical characteristics of the substance to be administered, including absorption rate, site of action, the potential for tissue irritation, and the practicalities of administration (e.g., Knotek, 2019; Divers and Stahl, 2019). For systemic distribution of a substance, parenteral injections are often preferred over an oral route. Substances are commonly delivered intramuscularly, intracoelomically, subcutaneously, intravenously, or orally. Injections in the larger muscle areas of the anterior half (towards the head) of the snake's body are preferred to avoid potential interference from the renal portal system. For all injection routes, administration sites caudal to the kidney (e.g., hind limb) may reduce the bioavailability of certain substances due to first-pass metabolism, but the level of reduction varies with different compounds (Giorgi et al., 2015; Holz et al., 1997; Kummrow et al., 2008; Scheelings, 2013; Fink et al., 2018). Nephrotoxic substances with tubular excretion should be administered in the anterior third of the animal, thus avoiding the kidney.

When determining appropriate doses of substances for administration, it is important to make sure that the dose is appropriately scaled for the individual animal, considering its metabolic rate at the particular holding temperature (see Mayer, 2019).

Adequate hydration and body temperature of the animal are required before the intramuscular administration of any compounds.

Routes of drug administration for reptiles have been reviewed by Coutant et al. (2018). Intramuscular and subcutaneous injections are frequently selected because of injection site access and rapid compound uptake and distribution. Repeated intramuscular injections should generally not be carried out at the same site, especially into small muscles, as they may cause muscle damage. Subcutaneous injections may be technically difficult to administer to small reptiles. In addition, mammalian and reptilian subcutaneous space functions quite differently, and extrapolation of subcutaneous therapeutics between these animals should be done with great care (Mathews, 2011; Turner and Cassano, 2004). Many reptiles have relatively inelastic skin, which restricts the volume that can be delivered subcutaneously to approximately 1% of body weight (Perry and Mitchell, 2019); as a result, a slow administration of a small volume using a small gauge needle is recommended. Injection sites should be held off as the needle is withdrawn to prevent backflow of the administered compound.

Several studies use the subcutaneous route (e.g., Hawkins et al., 2019); subcutaneous injections can be spread over multiple sites in order to reduce the volume per site. Intracoelomic injections deliver compounds to the coelomic cavity, a large, highly absorptive space in the abdomen. Due to the absence of a diaphragm, intracoelomic volumes should be limited to avoid lung compression. Compounds that cause tissue irritation should not be administered via this route as they can cause long-term damage to the viscera and cause fluid accumulation in the coelomic cavity. Intravenous administration is only feasible on medium-sized and larger reptiles; some intravenous catheters may require incisions and sutures for placement.

The use of intraosseous catheters should be discussed with a veterinarian. Intraosseous catheters may be beneficial in smaller reptiles and for delivering large volumes of fluid but must be performed on an anesthetized animal using surgical aseptic techniques. Drug diffusion may be slower than intravenous administration but is usually faster than intracoelomic administration (Young et al., 2012).

Some species may discolour their skin after any type of injection; it is important to differentiate this response from an adverse reaction.

Oral administration may involve the manual administration of a compound or treated food items in the buccal cavity, pharynx, esophagus, or stomach (gavage). Manual administration may be accomplished by opening the mouth with rounded implements and gentle pressure, followed by compound administration with a rounded blunt object such as a blunt syringe. Care should be taken to avoid damage to the oral mucosa, as this can lead to stomatitis. Administration of compounds directly to the stomach (gavage) requires veterinary consultation. In all manual administration situations, it is important to avoid the glottis to prevent accidental respiratory administration. Compounds may be incorporated into food items if eating is monitored appropriately.

10.2.1 Lizards

Intramuscular injections should be given in the cranial epaxial muscle or the forelimb muscles (e.g., triceps or biceps; the area of the arm above the elbow and below the shoulder). Injection at the pelvic limb should be avoided for substances that are nephrotoxic or subject to active clearance by the kidney. Injection at the base of the tail should be avoided for those species that demonstrate autotomy or use the tail as a fat storage site.

Intracoelomic injections are administered in the right caudal quadrant at a level even with the cranial aspect of the rear leg, with the lizard in dorsal recumbency (Knotek, 2019). The needle should be positioned at a shallow angle, from caudal to cranial, with a shallow puncture depth to reduce the risk of damage to the underlying tissues and organs. Care must be taken to avoid the ventral midline abdominal vein.

Subcutaneous injections and fluids are commonly administered at the thoracic limbs and caudal aspect of the pelvic limbs for non-nephrotoxic substances (Knotek, 2019).

The use of intravenous injection sites is limited by the size of the lizard, availability of injection sites, and physiological characteristics such as autotomy. Intravenous injections can only be performed on medium-sized and larger lizards. The ventral coccygeal vein is the preferred site for intravenous injections for medium-sized and larger lizards that do not demonstrate autotomy (Knotek, 2019). Alternative intravenous injections sites include the jugular vein and cranial vena cava.

When orally administering compounds, the handler must be aware that some lizards have a reflexive increase in bite pressure when objects are inserted into the mouth.

10.2.2 Snakes

Intramuscular injections should be given in the epaxial muscles on either side of the snake's vertebral column. Injections in the larger muscle areas of the anterior half (towards the head) of the snake's body are preferred to avoid the renal portal system. The needle should be inserted between the scales at a 45-degree angle into the middle of the muscle. Firm restraint is necessary as snakes will shift when injected. Injections in more caudal locations might be necessary when restraining using a snake tube (James et al., 2018).

Unlike in many other reptiles, the skin on the lateral body wall in most snakes is elastic and provides a convenient location for subcutaneous administration of larger volumes of fluid.

For intracoelomic administration, snakes should be placed on their right side and injected from the left into the caudal quarter of the body in the ventrolateral area (Girling and Raiti, 2019). The needle should be positioned at a shallow angle, from caudal to cranial, with a shallow puncture depth to reduce the risk of damage to the underlying tissues and organs.

Intravenous administration commonly uses the ventral tail vein.

Due to snake anatomy, oral administration of compounds is simpler in snakes than in other reptiles. The mouth can be opened by gentle pressure on the lateral caudal-most aspects of the jaw and the mandible gently drawn down, either with a finger or semi-rigid (soft) speculum. Oral compounds can be administered in the buccal cavity or pharynx as previously described, or a blunt-ended rubber catheter can be guided past the oropharynx and the glottis to the esophagus or stomach (gavage). Treated food items may be considered, but compound delivery can be compromised due to extended transit and digestion period.

10.2.3 Turtles

Intramuscular injections should be given in the thoracic or pelvic limbs of turtles. Injection at the pelvic limb should be avoided for substances that are nephrotoxic or subject to active clearance by the kidney or liver. Adequate hydration and body temperature of the animal are required prior to intramuscular administration of any compounds. Repeated intramuscular injections at the same site, especially into small muscles, should be performed with care as they may cause muscle damage and abscess.

Compounds are commonly delivered subcutaneously at the loose skin between the neck and thoracic limbs. Alternatively, non-nephrotoxic compounds and compounds that will have reduced bioavailability due to the kidneys may also be administered in front of the pelvic limbs.

For intracoelomic administration, the turtle should be placed on its side to encourage the urinary bladder to fall away from the injection site. The needle should be inserted proximally to the hind limb, angled caudal to cranial, and deeper than for lizards and snakes to penetrate the body wall.

Intravenous injection is limited by the size of the turtle and the availability of injection sites. The jugular and brachial veins are common intravenous injection sites for turtles (Mans, 2008). The femoral vein and dorsal tail vein can also be used, but these blood vessels are very close to the lymphatic system, and incorrect administration may occur. The subcarapacial plexus was a common intravenous injection site but is no longer recommended due to common adverse effects, including hind limb paralysis and intrapulmonary injection (Innis et al., 2010).

Manual oral administration of compounds is difficult in turtles due to bite risks, neck retraction, and tortuous curvature of the neck. If oral administration is required, treated food items should be used.

10.2.4 Crocodiles

Intramuscular injections are commonly given in the muscles of the front legs.

Subcutaneous and intracoelomic routes of administration are infrequent in crocodiles.

Intravenous injections can be delivered to the ventral tail vein. Dorsal recumbency is not recommended for crocodilians as it is very stressful for the animal. A recommended method of accessing the ventral tail vein begins by securing the animal to an elevated table with the tail hanging off the edge. A team of personnel can then manually restrain the tail and access the dorsal tail vein. It is not possible to visualize the tail vein as it sits within a groove of the vertebrae. The injection site should be placed approximately 1/3 down the length of the tail on the midline. The needle should be inserted at an angle until the vertebral structures are felt, and then the needle retracted slightly until a flash of blood is obtained.

Manual, oral administration is rare, but treated food items may be used for oral administration of compounds.

10.3 Collection of Body Fluids or Tissue

General information concerning biological sampling can be found in the CCAC guidelines on experimental procedures – administration of substances and biological sampling (in prep.). This section provides additional species-specific information for reptiles. Methods for sampling for research purposes or for health monitoring depend on several factors, including the temperament, size, and anatomy of the reptile (see Divers and Stahl 2019; Girling and Raiti, 2019).

10.3.1 Blood Collection

Anesthetics may need to be used to restrain the animal and limit stress, and methods for proper visualization of blood vessels may be needed. The selection of appropriate-sized needles is essential to minimize tissue trauma.

It should be noted that there can be an impact of puncture site on blood parameters, which should be considered when selecting the appropriate site for blood collection (Bonnet et al., 2016; Mans, 2008). At some sites, the lymphatic system lies close to the circulatory system and may be accessed inadvertently. Inaccurate blood parameters can occur due to inadvertent lymph contamination during diagnostic blood sample collection.

Consideration should also be given to the anticoagulant used during blood collection. Blood from some species of chelonians may develop hemolysis with Ethylenediaminetetraacetic acid (EDTA), and thus lithium heparin should be used as the anticoagulant. However, EDTA generally provides superior blood samples with fewer white blood cells and platelet clumping (Divers, 2019).

10.3.1.1 Survival Blood Collection

When drawing blood from reptiles, it is important to be aware of how much blood can be drawn (Sykes and Klaphaké, 2008). The total blood volume of a reptile varies with species (approximately 5-8% of the body weight). As a rule, up to 0.5% of total body weight may be taken, or 10% of the total blood volume (Redrobe and MacDonald, 1999). It is also important to be aware that a lesser amount of blood should be taken if the reptile is stressed, as these animals can partition their body fluids, reducing their blood volume (Redrobe and MacDonald, 1999). Care must be taken when performing serial blood collections as reptiles generally have prolonged erythrocyte turnover rates, for example, up to 800 days in box turtles (Campbell, 2014). Peak reticulocyte response to blood loss takes up to five weeks to achieve, and it may take four months for red blood cell numbers to return to normal after repeated blood draws (Campbell, 2014; Flanagan, 2015).

The skin of reptiles should have gross contaminants removed, and efforts should be made to disinfect the site before any venipuncture as the inelasticity of the skin creates the possibility for pathogens to enter (Eatwell et al., 2014).

Common sites for blood collection in reptiles include the following.

10.3.1.1.1 Lizards

The ventral coccygeal (tail) vein is the most common and convenient site for venipuncture in lizards without autotomy and has the fewest complications. Venipuncture of the site is considered a blind technique as the vein cannot be visualized. The vein can be accessed anywhere from 20-80% down the tail. Care should be taken to avoid the paired hemipenes of the male at the proximal part of the tail. As it is partially protected by the ventral spinous processes, if vertebrae are hit, the needle should be withdrawn slightly and redirected (Divers, 2019). In geckos, the risk of tail autotomy can be minimized by prior sedation and withdrawing the needle if tail vibration is noted (Cojean et al., 2020). However, other sites such as the cranial vena cava and jugular may be favoured in lizards capable of autotomy.

The ventral abdominal vein is commonly used in smaller lizards such as geckos, in lizards with shorter tails, or when the ventral tail vein is not productive. Lizards have large abdominal veins just under the skin, along the ventral midline. This vein is easily located visually, but it is also easily damaged by venipuncture (Divers, 2019). Care should be taken not to puncture any underlying coelomic organ accidentally.

The jugular vein is another site that may be used for venipuncture in lizards such as iguanas, monitor lizards, or chameleons (Eshar et al., 2018). The jugular veins are located laterally and deep within the neck. They are seldom visible, even when pressure is applied to occlude them. In species with an external ear opening, the tympanic membrane can be used as a landmark (Divers, 2019). Transillumination may also be used in chameleons (Eshar et al., 2018). The jugular vein has also been recommended as a potential site for small lizards, using a method adapted from the venipuncture of small birds (Di Giuseppe et al., 2017).

The cranial vena cava is also a safe venipuncture site in geckos when shallow sticks are used (Mayer et al., 2011; Cojean et al., 2020). A 27 to 29-gauge needle may be inserted at a 45-degree angle from the midline, in the centre of the triangle formed by the cranial part of the sternum (manubrium), the shoulder, and the vertebral column on the midline. Care should be taken to introduce the needle only 1-2 mm in depth to avoid accidental cardiac puncture.

10.3.1.1.2 Snakes

Two common venipuncture sites in snakes are the caudal (ventral) tail vein and the heart. The jugular vein is also used in some species with well-described anatomy (e.g., ball python). Drawing blood from the tail vein is best accomplished in large snakes and snakes with longer tails, as it can be difficult to draw blood in small snakes or short-tailed snakes due to the size of the vessel. Sampling from the caudal vein should be carried out caudal to the cloaca, approximately 25-50% down the tail. Care should be taken to avoid the paired hemipenes of the male and the paired musk glands present in both sexes by maintaining a distance of at least ten ventral scales' width caudal from the cloaca. Cross-contamination with cerebrospinal fluid or lymph is possible using this method (Divers, 2019).

Cardiac puncture can be carried out on alert or anesthetized animals as a survival procedure, according to the competence and preference of the animal user (Isaza et al., 2004; Brown, 2010; McFadden et al., 2011). The snake should be restrained on its back to carry out a cardiac puncture. The heart can then be located in the cranial third of the body. The heart can move both cranially and caudally and should be immobilized between the thumb and index finger before attempting to insert the needle at a 45-degree angle. Access to the heart should be obtained with a single cranial advancement of the needle, preferably guided by ultrasound. Slight negative pressure in the syringe will allow the blood to slowly fill the syringe with each heartbeat (Divers, 2019).

10.3.1.1.3 Chelonians

Sedation may be necessary, particularly for large chelonians, to enable the legs, tail, or head to be exteriorized for access to the vein of choice (Perpiñán, 2017).

In turtles, the left or right external jugular veins are commonly used for blood sampling because there is less chance of contamination with lymphatic or cerebrospinal fluid. The vein can generally be occluded at the base of the neck or by restraining the animal in a 30-degree head-down position. Following venipuncture, the animal should be held head up, with pressure applied to the jugular vein to prevent the formation of a hematoma (Divers, 2019).

Less common but still a site for venipuncture in large turtles is the brachial plexus in the forearm. This site is located near the shoulder joint caudal aspect of the humerus. However, lymph fluid contamination is common at this site (Divers, 2019).

The dorsal coccygeal vein can also be sampled from the dorsal midline of the tail in larger species. This vessel's position varies between species, and there is a significant risk of contamination with lymphatic or cerebrospinal fluid (Divers, 2019). Due to potential hemodilution at the coccygeal site, López-Olvera et al. (2003) recommend sampling from the brachial vein. However, the dorsal midline may be advantageous in large tortoises (such as *Centrochelys sulcata*) as it is accessible in conscious tortoises, providing the tail can be withdrawn from under the carapace. This technique can also be used with chelonians that are prone to biting. The tortoise may be propped onto a chair or table and the needle inserted on the dorsal part of the tail on the midline, with the needle pointing dorsally. The subcarapacial plexus is no longer recommended due to common adverse effects, including hind limb paralysis and accidental intrapulmonary injections (Innis et al., 2010; Coutant et al., 2018). If it must be used, good monitoring of the animal, adequate anti-nociceptive drugs, and prompt investigation of any changes in respiration are required.

10.3.1.1.4 Crocodiles

Blood collection from crocodiles can be performed using a similar technique as described for administering substances through the tail vein (see Section 10.2.4, “Crocodiles”; Divers, 2019). If animals are to be held long term, they can be target trained to accept blood sampling, thus minimizing the need for forcible restraint, which can impact blood parameters (Augustine and Baumer, 2012).

10.3.1.2 Terminal Blood Collection

In all reptile species, cardiac puncture under general anesthesia can be used as a terminal procedure and should be followed by a secondary method (e.g., an overdose of an anesthetic or a physical method) to ensure death without recovery (CCAC, 2010).

10.3.2 Urine and Feces

Cloacal wash, described by Divers (2019), is commonly performed to collect fresh fecal samples due to the slow gut transit time in reptiles. This process involves placing a well-lubricated catheter of appropriate size into the cloaca to infuse a small amount of saline that is aspirated back out. A relatively large catheter should be used to prevent kinking of the tube and prevent any damage to the thin intestinal wall.

10.3.3 Tissue Biopsy

Tissue biopsy should be treated as a surgical procedure using an aseptic technique and wound closure as appropriate. Certain types of research, especially involving regeneration, may require biopsy sites to be left open to monitor healing (Keller et al., 2014; Wu et al., 2014; Peacock et al., 2015; Subramaniam et al., 2018).

10.4 Implants

Implanted telemetry devices are frequently used in field research, and techniques can readily be translated for use in the laboratory setting (Ferrell et al., 2005). Care must be taken to ensure the implant is placed in a location that does not impede locomotion or other normal behaviours (Norton et al., 2018). The implant may need to be surgically attached to the body wall to prevent expulsion via the alimentary tract (Bryant et al., 2010).

10.5 Procedures for Genetically Modified Reptiles

Genetically and phenotypically unique reptiles may be created either through artificial genetic modifications or through specialized breeding programs. These modified reptiles may have special needs.

The introduction of technology that permits gene editing directly in the embryo has led to an interest in genetic modification of a wide variety of taxa, including reptiles (Nomura et al., 2015; Rasys et al., 2019). Selection of methods to generate new genetically modified strains should be made considering the Three Rs principles of Reduction and Refinement. For example, some methods are more efficient than others, thus reducing the number of animals used in creating and maintaining each line, and some methods may have more significant negative welfare impacts. Procedures for the generation of genetically modified animals should be reviewed by the animal care committee during protocol review, keeping with the rapidly evolving nature of genetic modification and advances in research on animal welfare. Submission of protocols for renewal by the animal care committee should include a report from the investigator on the efficiency of the methods used to produce new strains.

Cryopreservation of sperm or eggs can reduce and refine animal use; expertise in this area should be sought when developing a new line.

In addition to genetically modified reptiles, breeders are continuously developing programs for specific phenotypes, including colouration or pattern and scale variation (e.g., scale-less bearded dragons and snakes), which may have a place in the laboratory setting. Unique phenotypes may require special husbandry needs, whether the altered phenotype is acquired through genetic modification or manipulated breeding.

Reptiles to be involved in procedures for genetic modification should be in good health and exhibit normal behaviour.

10.5.1 Collecting Samples for Genotyping

The sampling method should be the least invasive method that can provide the quantity and quality of tissue required for the particular genotyping method. For example, shed skin can frequently supply sufficient genetic material for genotyping snakes and lizards, with no stress to the animal. It should be verified that the shed material is compatible with the method employed as, for example, highly keratinized materials may not be suitable for DNA extraction. If DNA sequencing is not carried out soon after shedding, the shed skin should be stored dry in a freezer. The use of invasive methods that involve the removal of tissue from the tail or digits must be justified in the animal use protocol.

10.5.2 Phenotyping

Some acceptable procedures for animals that have not undergone genetic modification may not be acceptable for genetically modified reptiles with altered phenotypes. Procedures may need to be modified or avoided when the animals' ability to respond to stress is compromised. This includes the choice of procedures for phenotyping.

Once the animals are phenotyped, any additional information related to animal welfare should be given to the animal care committee as soon as possible. Stable germ-line transmission does not necessarily mean a stable phenotype or stable animal welfare since phenotypes can change (e.g., be age-dependent, have background effects, require homozygosity, or require breeding to other mutant lines). Appropriate monitoring is needed for the animal's lifespan or when the genetic background is changed.

Investigators should take reasonable steps to share all available phenotypic and welfare information with the research community, along with strategies for mitigating problems with genetically modified strains.

Genetically modified reptiles may respond differently to drugs and food and some experimental conditions compared to animals of the same species that have not undergone genetic modification. These changes in response may result from differences in the animal's metabolism and are particularly relevant to the use of anesthetics and the use of the animals for testing new drugs or toxicity studies.

10.6 Imaging

Imaging may include conventional radiography, ultrasound, computerized axial tomography (CAT) scan, magnetic resonance imaging (MRI), and fluorescent imaging. Plans for imaging must be developed in consultation with a veterinarian. Although studies involving repeated imaging can reduce the number of animals required for a study, the procedures create numerous occasions for animals to be stressed. Factors to consider include: repeated injections; anesthesia; handling and transportation; experimental conditions (e.g., tumour burden or surgery); and fasting (Cojean et al., 2018; Williams et al., 2019). These factors should be addressed in relation to both the welfare of the animals and the validity of the imaging results. In particular, given the significant impact of repeated anesthesia on the physiology of an animal, consideration should be given to the number of times and frequency of imaging. For serial imagery, it is essential that animals are monitored between imaging sessions.

Chemical restraint may be required, depending on the nature of the image, the activity level of the reptile, and the risk to human safety. However, these needs must be balanced with inherent risks and distress to an animal during chemical restraint.

The imaging schedule should be developed based on the welfare of the animals and anticipated physiological changes.

10.7 Behavioural Studies

General guidelines to principles of behavioural studies can be found in textbooks such as Martin and Bateson (2007). Behavioural studies on reptiles include mating and courtship, social behaviour, feeding behaviour, environmental preference, and predator/prey behaviour. A healthy animal with a good welfare status that is well acclimated to the housing environment is critical to achieving a valid and interpretable outcome of any behavioural testing regime.

Aversive stimulation and deprivation or restriction of resources must only be used when there is no alternative. When possible, a reward strategy (e.g., highly preferred food) should be used to motivate an animal rather than using aversion. Positive operant conditioning strategies have been published for many species (Emer et al., 2015; Hellmuth et al., 2012; Weiss and Wilson, 2003; Fleming and Skurski, 2012) and can be adapted for the needs of the study and species. Motivational studies using shock, aversion stimuli, or food restriction require sound scientific justification to the animal care committee. If these techniques are a focus of the research study, they must be used in the least invasive fashion and for the shortest duration possible.

10.8 Food and Fluid Intake Regulation

Food and fluids may be regulated for metabolic studies or as part of operant conditioning, but this requires careful knowledge of the species and individual animal physiology. Aggressive searching for food may cause distress to animals. Reptiles that have been food-restricted too severely may stop eating. Anorexic reptiles may not recover even when they start eating again due to the metabolic demands of food digestion. In some cases, provision of smaller food items more frequently is preferred to completely fasting animals. Studies involving food or fluid intake regulation require the establishment of humane intervention points (e.g., body condition or skin tenting, in certain species, consistency of saliva) and close monitoring of the animals (see Section 9, “Welfare Assessment”). Knowledge of the individual animals is critical, as there is a large spectrum of requirements for reptiles in terms of hydration, feeding, and digestion. The digestive system of reptiles is considerably different from that of warm-blooded animals, and reptiles do not eat as regularly as warm-blooded animals, in general. Reptiles on food restriction diets should be carefully monitored for any cachexia regarding weight and body condition. Weight can be affected by factors such as the presence or absence of feces in the system, when the animal was last fed, and the brumation state. How quickly an animal will lose weight is also highly variable among species. If a consistent weight loss is observed, the frequency of monitoring should be increased.

10.9 Anesthesia and Analgesia

10.9.1 Anesthesia

General anesthesia typically involves an initial induction phase where the animal is rendered unconscious, maintenance of the anesthetic plane, then recovery. Reptilian physiology and anatomy differ substantially from that of mammals; therefore, it is not appropriate to extrapolate directly from mammalian practices for reptile anesthesia. The ectothermic condition, a typically lower rate of metabolism, and a reduced level of tissue perfusion all contribute to these differences and affect drug action in reptiles during all phases of anesthesia. A veterinarian must be consulted for the appropriate anesthetic regimen for the particular reptile. Within and between species, the rate at which anesthetics take effect can vary greatly, depending on the animal's body temperature; animals should therefore be maintained within their preferred optimal temperature zone, where the effects of the anesthetics are better understood and managed (Mans et al., 2019). Anesthetic induction and recovery frequently are longer processes in reptiles than in mammals; appropriate planning should be in place, particularly for the recovery phase. There is no evidence currently to support cold narcosis (via hypothermia) as a safe and humane method for anesthesia.

10.9.1.1 Inhalation Anesthetics

Volatile anesthetic gases are particularly useful for prolonged procedures or if a rapid recovery is desired. Isofluorane with oxygen as a carrier gas is the most used inhaled anesthetic in reptiles due to more widespread data and safety for the animals and human operators. Inhaled anesthetics can be successfully used with a mask or chamber for most non-aquatic species and with a secured airway for all reptiles. In general, inhaled anesthetic should not be used for species capable of prolonged breath-holding (e.g., aquatic chelonians, bearded dragons). Intubation is easily achieved for most species of reptiles due to their highly pronounced glottis, may be required for prolonged procedures, and is required for mechanical ventilation. Veterinarian guidance should be sought when considering intubation and mechanical ventilation. Due to the anatomical features of the heart in reptiles, cardiac shunting (movement of blood within the heart between pulmonary and systemic flows) influences inhaled anesthetic uptake and elimination, potentially leading to both delayed induction and delayed or an unexpectedly rapid recovery (Greunz et al., 2018).

Inhaled volatile gases are generally very safe, immediately reversible, easily titratable, and can be used in a contactless chamber for most species. However, these gases require specialized equipment, including oxygen, gas vaporizer, delivery circuits, gas scavenging, and appropriate ventilation for human safety. Inhalation anesthetics should not be used without all of this equipment in place and maintained according to the manufacturer's specifications.

10.9.1.2 Injectable Induction and Anesthesia

Induction of anesthesia with injectable anesthesia is commonly performed, and the duration of surgical plane may be sufficient for the length of the procedure. However, recovery of full normal function may extend for hours or days (Mosley, 2005; Mans et al., 2019; Preston et al., 2010). The use of lower concentrations of several drugs with synergistic actions (balanced anesthesia) and the use of readily reversible drugs may be more efficacious and provide more assurance of safety (Mans et al., 2019). Mans et al. (2019) provide a list of suggested anesthetic protocols for many species.

Propofol and alfaxalone are two of the most common injectable anesthetics for reptiles. Neither alfaxalone nor propofol should be used as sole agents for painful procedures (Balko and Chinnadurai, 2017) but can be used in combination with inhaled anesthetics via a secured airway. Propofol is an injectable anesthetic that must be administered intravenously or by intraosseous injection. It provides a rapid and secure induction, minimal accumulation from repeat injections, relatively long-lasting, minimal excitatory side effects, and rapid recovery with little residual effect. Propofol is rapidly metabolized and is noncumulative but does produce a dose-dependent cardiopulmonary depression. Apnea is common after the initial administration of propofol and is dependent on the speed of injection and dose. Alfaxalone, a neurosteroid with no analgesic properties, can be administered intramuscularly or intravenously. While alfaxalone has a short duration of action in mammals, it can have prolonged effects in reptiles. Current commercial preparations of alfaxalone require large volumes to be administered to obtain the desired concentration; this may make intramuscular site selection challenging. Alfaxalone can be used for a brief period of sedation and restraint, for example, for blood sampling (Bertelsen and Sauer, 2011; Hansen and Bertelsen, 2013; Kischinovsky et al., 2013).

10.9.1.3 Anesthesia Monitoring and Recovery

Anesthesia monitoring can be accomplished using pulse (via Doppler, cardiac ultrasound, or pulse oximetry in certain species), stimulus-response, and respiration rate (via a capnometer or direct visualization). Blood oxygen levels can be monitored through blood gas measurement, but the interpretation is complicated by the particular physiology of reptiles, including the fact that many reptiles are hypoxia tolerant. Pulse oximetry reading should not be over-interpreted, as the hemoglobin dissociation curve is different in reptiles. In addition, cardiac shunting has an impact on blood pressure and blood oxygen levels, which should be taken into consideration when monitoring animals undergoing anesthesia (Mans et al., 2019). If required, assisted breathing may be accomplished manually or with a ventilator. Intubation for securing an airway is relatively easy in post-induction animals, and maintenance of an appropriate plane of anesthesia can then be accomplished with an anesthetic machine.

During recovery from anesthesia, reptiles should be kept in a temperature-controlled environment. The species-specific preferred optimum temperature zone should not be exceeded so as not to increase the animal's metabolic rate and oxygen demand. Reptiles recovering from general anesthesia may be apneic and may require assisted breathing. Recovery is complete once the animal is moving normally (Mans et al., 2019). The recovery area should be secured to prevent the inadvertent release of the animal.

The anesthetic process and recovery must be documented in the appropriate medical or experimental record for the animal (CCAC, 2017).

10.9.2 Analgesia and Anti-Nociception

Nociceptors are sensory neurons that respond to physically damaging or potentially damaging stimuli. Many analgesics block the nociceptor pathway, but other analgesics alter the perception of pain (Williams et al., 2019; Perry and Nevarez, 2017). While there is debate in the scientific community on whether “analgesia” or “anti-nociception” is more appropriate for reptiles, the term “analgesia” will be used in this section as a more familiar term for accomplishing a very similar outcome, thus preventing confusion that could lead to unnecessary pain and suffering. Procedures that can be reasonably expected to cause pain in mammals can be reasonably expected to be aversive stimuli for reptiles and must be relieved by analgesia unless contraindicated by the nature of the experiment.

There is limited information on analgesia in reptiles; however, recommendations on using some analgesics in particular species are reviewed by Sladky and Mans (2012) and Chatigny et al. (2017). Long-term use of some analgesics may impact feeding behaviours; feeding routines should be adjusted accordingly.

The route of administration of an analgesic should take into consideration the size and temperament of the individual reptile. In addition, the choice of route may have an impact on the effectiveness of an analgesic drug; administration to the hind limbs or tail of a reptile may cause rapid clearance by the renal portal system or, in the case of opioids, the hepatic first-pass effect (e.g., Kummrow et al., 2008).

10.10 Surgery

Surgery must only be carried out by veterinarians or personnel who have been trained in aseptic surgical techniques and verified as competent by a veterinarian.

Reptiles rely on external regulation for temperature; thus, assisted temperature regulation is critical for surgical procedures. Heating pads and lights can generate considerable heat, but it is important to be aware of the temperature of any fluids used on or administered to the animal and their potential impact on cooling. It is important to understand the unique preferred optimum temperature zone for the species.

Reptiles should be fasted before surgery, with the length of fasting time based on the particular animal. In general, animals should miss one feeding cycle (Divers, 2019).

Reptiles should be kept hydrated during surgery. Subcutaneous, intracoelomic, or intravenous fluids at an appropriate temperature should be administered before, during, and after surgery, as needed.

Appropriately sized sandbags, foam supports, and adhesive tape may be used to maintain the reptile’s position during the surgical procedure.

Conventional aseptic surgical practices and techniques should be employed during reptile surgery. A sterile field at the surgical site should be established with drapes, and the site surface should be aseptically prepared with appropriate disinfectant (e.g., betadine, chlorhexidine, and alcohol). Sterile gloves and instruments should be used by the surgeon, and efforts must be made to maintain the sterility of the field until the procedure is complete. Consideration should be given to making incisions between, not through, scales in the relevant species. Maintenance of a sterile field with aquatic species can pose a challenge due to the sensitive nature of the protective layers of the epidermis that should not be overly disrupted. In these scenarios, being overly aggressive with harsh disinfectants and excessive removal of outer mucous and epidermis can lead to an increased risk of secondary bacterial colonization of the skin. Sutures should be made with a monofilament material using an everting pattern, as reptile skin tends to invert naturally (McFadden et al., 2011).

Records must be kept for anesthesia, surgery, and post-operative care. The type, dosage, site, and route of anesthetic, analgesic, or sedative drugs must be recorded, the animal must be monitored, and records kept before, during, and after surgery for depth of anesthesia, vital signs, and general condition, as deemed appropriate for the study and as approved by the animal care committee (CCAC, 2017).

10.11 Monitoring and Post-Procedural Care

Reptiles should be monitored until anesthetic recovery is complete and the animal is moving normally. The animal should be maintained in an environment supportive of the species' preferred optimum temperature zone at all times. The recovery environment should be appropriately designed to ensure the incision site can be monitored and, if possible, kept dry. Some reptiles may retreat and immerse themselves in water, which can be detrimental to suture material and incision sites. The recovery area should be secured to prevent the inadvertent release of the animal.

Post-procedural analgesic must be provided when pain can be reasonably expected, unless it is contraindicated by the nature of the experiment. Antibiotic therapy may be necessary for some types of surgery but should only be provided when advised by the veterinarian, based on current recommended practices to limit antibiotic resistance. All monitoring and post-procedural care must be documented on the animal's medical or experimental record (CCAC, 2017).

11. EUTHANASIA

The General Guiding Principles outlined in the *CCAC guidelines on: euthanasia of animals used in science* (2010) apply to the euthanasia of all animals in science. This section provides additional information that is specific to the euthanasia of reptiles.

If the brain is needed for study purposes, brain destruction can be avoided if the method of euthanasia has been well studied in the reptile species used in the study, and has been confirmed to be a reliable method at the temperature that the euthanasia would be taking place. If the method of euthanasia selected has not been studied in this particular species, a persistent cardiac arrest should be confirmed for at least two hours with the reptile placed at its preferred optimal temperature zone.

Guideline 23

Euthanasia of reptiles must only be carried out by competent personnel using an approved method best suited to the particular species and life stage and to the study objectives.

For all methods of euthanasia, the following are important requirements:

- SOPs should be developed for euthanasia and disseminated throughout the institution to ensure consistency;
- personnel involved in the procedure must be trained and have their competency assessed with regard to the performance of the procedure on the particular species involved and their ability to confirm the death of the reptile;
- equipment must be appropriately maintained and cleaned before use or reuse;
- death should be confirmed with a secondary method, such as destruction or removal of the brain or exsanguination – for appropriate species and life stages, death may also be confirmed by freezing (see Section 11.6, "Verification of Death");
- animals must not be housed with unfamiliar animals before euthanasia; and
- stress caused by handling should be minimized.

Institutions must have an SOP for emergency euthanasia prepared to address severe, unanticipated health or welfare concerns when immediate veterinary consultation is not available.

11.1 Injection

The *CCAC guidelines on: euthanasia of animals in science* (2010) lists intravenous injection of barbiturates as an acceptable method of euthanasia for all reptiles. Nevarez (2019) recommends pentobarbital injection (60-100 mg/kg) via intravenous injection as being suitable for most reptiles.

While barbiturate injection is the preferred approach to euthanasia of reptiles, it is only suitable when there is adequate venous or intraosseous access, and when the animal can be suitably restrained.

Intracoelomic or intrahepatic injections are also acceptable when other routes are not accessible (Laferriere et al., 2020). While injection of euthanasia solutions into the intracoelomic space is a possible means of euthanasia, attention must be paid to the irritating nature of the compound, and dilution or buffering may be required (Nevarez, 2019).

Intracardiac administration with barbiturates is a possible means of euthanasia, but animals should be deeply sedated or anesthetized before administration.

When venous or intraosseous access is not easily obtained, or when dealing with potentially dangerous or unrestrainable animals, a two-step euthanasia procedure should be performed. The reptiles should be anesthetized or heavily sedated then injected with the euthanasia solution through any suitable route (e.g., intravenous, intracardiac, intracranial, intrahepatic, or intracoelomic).

Injection of potassium chloride into fully anesthetized reptiles is also an acceptable method of euthanasia. The route of administration should take into account the volume of potassium chloride required. Extra attention must be paid to verification of death; potassium chloride stops cardiac function, and animals may demonstrate prolonged, very slow heart rates before death. A secondary physical method of euthanasia should follow, and death should be verified as described in Section 11.6, “Verification of Death”.

Tricaine methanesulfonate (TMS or MS222) can be used as a two-stage procedure to euthanize reptiles (AVMA, 2020). First, dilute, buffered TMS is administered intracoelomically, then, once the reptile is anesthetized, a more concentrated solution of unbuffered 50% TMS is also administered intracoelomically. There appears to be a substantial species difference in sensitivity, and usage must be supported by species-specific evidence (Conroy et al., 2009).

T-61 is a combination of paralytic, narcotic, and local anesthetic that is available in Canada and may be used off-label for the euthanasia of reptiles. This drug, when used alone, can cause involuntary vocalizations and movements and must be injected intravenously at a slow rate (AVMA, 2020). While T-61 may be used in the field for reptiles, alternatives should be considered before using T-61in institutional environments.

11.2 Inhalant Anesthetics

Due to their ability to breath-hold and their general tolerance to hypoxia, an overdose of inhalant anesthetics is not a suitable method of reptile euthanasia. However, the use of inhaled anesthesia is appropriate before conditional methods of euthanasia. A reptile formulary should be consulted for the appropriate dosage.

11.3 Physical Methods

Due to the increased risk of operator error leading to potential animal suffering, the use of physical methods of euthanasia must be scientifically justified. Reptiles should be anesthetized before using a physical euthanasia method. The *CCAC guidelines on: euthanasia of animals used in science* (2010) lists penetrating captive bolt as an acceptable method for larger species of reptiles. Decapitation of anesthetized, small reptiles may also be acceptable (AVMA, 2020) but must be immediately followed by destruction or removal of the brain.

11.4 Freezing

Freezing is a method of euthanasia that refers to a rapid drop of the animal’s internal temperature to an extreme low, typically through immersion in liquid nitrogen. Rapid freezing may be used to euthanize reptiles less than 4 g if specific scientific justification is provided and the procedure is approved by the animal care committee. Before freezing, animals should be rendered unconscious, either through induction of hypothermia (e.g., placed in a refrigerator) or by general anesthesia (AVMA, 2020; Green, 2010). Once the primary method of euthanasia is complete, confirmation of death may be obtained by freezing reptiles of all sizes to a body temperature of less than or equal to -15°C for at least 24 hours. Freezing is not appropriate for species and developmental stages that are resistant to freezing, such as juvenile painted and snapping turtles (Packard et al., 1999; Constanzo et al., 1995).

11.5 Euthanasia of Eggs

There appears to be emerging evidence indicating that oviparous species are conscious at hatching and during the last few days before hatching; this should be considered when developing the protocol (CCAC, 2010). Animals in late incubation (the latter third) and new hatchlings should be euthanized as described in Section 11, "Euthanasia" – this may require opening the shell to access the animal.

Scientific work with eggs do not need be described in protocols or approved by animal care committees unless the institution in question and its animal care committee choose to review such protocols. Freezing of eggs is a common method of euthanasia during early and mid-incubation (i.e., the first two-thirds). Any unwanted or unanticipated eggs should be frozen before disposal.

11.6 Verification of Death

It is important to verify that the animals are dead before their disposal. Due to the unique physiology of many reptiles, it is difficult to consistently verify death based on physical parameters such as corneal reflex, heartbeat, or breathing, especially if the animal was anesthetized before euthanasia. Death should always be confirmed by a physical intervention such as pithing, removal of the brain, or perfusion with a fixative (AVMA, 2020). Alternatively, the death of cold-sensitive species can be confirmed by freezing to a body temperature of -15°C or lower for 24 hours (Nevarez, 2019), or longer for larger species.

12. END OF STUDY

12.1 Transfer of Reptiles Between Facilities or Protocols

For reptiles that are to be transferred to another institution at the end of a study, see Section 4, “Procurement”, particularly regarding regulations, documentation, and transportation. As mentioned, this applies to reptiles that have not been subject to major invasive procedures and are fit to travel.

If reptiles are transferred to an institution that is not CCAC-certified, it is the responsibility of the institution sending the reptiles to verify before placement that the animals will receive appropriate care. For example, transfer to institutions accredited by the Canadian Association of Zoos and Aquariums provides assurance that the needs of the animals will be met once they are released from the research or teaching facility.

12.2 Rehoming

Where permitted by regulatory authorities (e.g., provincial or territorial laws and local bylaws), institutions may release healthy reptiles used for scientific activities that are commonly accepted pet or companion species and strains to individuals who have the knowledge and ability to provide proper care to the animals. No genetically modified reptiles may be moved from research facilities to private premises, as no organism that has had its genome manipulated through artificial genetic manipulation may be released into the environment (Government of Canada, 2005). If reptiles are to be released to the care of an individual as companion animals, the institution should develop an appropriate policy describing the conditions that need to be fulfilled before the release of the animal. Institutions should ensure those adopting the reptiles are aware of the care required.

12.3 Release to the Wild

The release of captive wildlife, including reptiles, is discussed in the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003). Release of any animal must adhere to federal, provincial or territorial, and local laws and regulations. In addition, there must be an evaluation of the benefits and risks to the animal, to other animals at the release site, and to the ecological conditions of the release site. No reptiles that have been subject to artificial genetic manipulation may be released from research facilities. Captive-bred reptiles should not be released into the wild due to their insufficient survival instincts and poor adaptation to the natural environment.

12.4 Disposal of Dead Reptiles

Reptiles must be disposed of according to the relevant federal, provincial or territorial, and municipal regulations for the disposal of biological materials.

13. HUMAN SAFETY

Institutions must have occupational health and safety programs that are specifically tasked with addressing the topic of human safety through risk assessments. The responsibility of the animal care committee extends to ensuring there is an institutional occupational health and safety program in place to properly assess any risks to human health and safety.

Those working with animals must follow institutional policies and SOPs outlining appropriate prevention and protection measures. They should seek professional knowledge on animal-specific physiological risks (e.g., venom), animal allergens, zoonotic diseases, and other risks or hazards that may be associated with a particular study (e.g., exposure to radiation, anesthetic gas, chemical hazards, and human cell lines).

People working with reptiles should take precautions against bites and scratches, as appropriate. Any bite or scratch should be monitored, especially over the first few hours, for allergic reaction and over subsequent days for infection. In addition, caution should be taken when using needles or sharp instruments on reptiles, as their tough skin may increase the risk of personnel poking or cutting themselves.

Several pathogens can be transmitted between reptiles and humans (e.g., *Salmonella* spp., *Escherichia coli*, *Mycobacterium fortuitum*). Handling protocols should include handwashing immediately after handling the animals, and in particular between handling different animals. While animals may be screened for any potential pathogens, it should be assumed that any reptile is a potential carrier, regardless of screening results.

Facilities housing reptiles may have high humidity, high temperatures, and the presence of habitat heating devices that can present physical hazards for personnel. SOPs must be in place to minimize any associated risks.

Personnel who will be moving tanks should be trained in ergonomically correct methods. Tanks with soil or water are heavy and require proper preparation for moving.

Common compounds such as isoflurane and pentobarbital can present risks to human health and safety. Equipment must be calibrated and maintained according to the manufacturer's requirements. SOPs for handling such substances must be followed, utilizing engineering and administrative controls in conjunction with personal protective equipment to reduce personnel risk.

13.1 Working With Venomous Reptiles

The use of venomous species in research, teaching, or testing requires appropriate justification of the inherent risks of their use. Guidance on the safe handling of venomous reptiles can be found in Section 7.1.3.2, "Venomous Animals". All primary personnel and support personnel must be familiar with and use proper equipment for animal capture and handling. Personnel should never work with venomous snakes when in a hurry, distracted, fatigued, or taking medication that may impair alertness (Lock, 2008).

Risks associated with venomous animals vary widely and can be affected by venom characteristics, animal size and life stage, and physiological characteristics (i.e., fang structure and placement). These factors should be assessed by the investigator, facility manager, university veterinarian, and biosafety officer to determine the risk of the animal delivering a medically significant envenomation that would cause a clinically detectable local or systemic physiological change. The risk of medically significant envenomation should inform the risk mitigation strategies implemented.

For reptiles capable of causing medically significant envenomation, additional health and safety measures and venomous species-specific training is required for all animal handlers. Additional personal protective equipment, such as an eye shield and puncture-resistant gloves, and appropriate restraint and transfer devices and techniques should be implemented unless sufficient justification is provided otherwise.

Written envenomation procedures should be posted. An SOP for an emergency response to envenomation must be available, as should SOPs for appropriate handling, transfer, and restraint of venomous animals. In any area where venomous animals are being handled, a landline to contact emergency services must be available. Adequate supplies of antivenin – appropriate for the species and stored appropriately and within the expiry date – must be available. A mechanism must be in place to ensure that the antivenin is always available, either at a local health provider or institutionally – antivenin is a regulated biological product in Canada, an appropriate licence is required to hold it. It is also advisable to notify local medical authorities of the potential risks with venomous species and familiarize them with envenomation protocols if necessary; antivenin should only be administered by a licensed medical professional in a hospital or ambulance.

REFERENCES

More information about documents marked “in prep.” can be found in the Guidelines section of the CCAC website.

Acierno M.J., Mitchell M.A., Roundtree M.K. and Zachariah T.T. (2006) Effects of ultraviolet radiation on 25-hydroxyvitamin D_3 synthesis in red-eared slider turtles (*Trachemys scripta elegans*). *American Journal of Veterinary Research* 67(12):2046–9.

Acierno M.J., Mitchell M.A., Zachariah T.T., Roundtree M.K., Kirchgessner M.S. and Sanchez-Migallon Guzman D. (2008) Effects of ultraviolet radiation on plasma 25-hydroxyvitamin D_3 concentrations in corn snakes (*Elaphe guttata*). *American Journal of Veterinary Research* 69(2): 294-297.

Alexander G.J. (2018) Reproductive biology and maternal care of neonates in southern African python (*Python natalensis*). *Journal of Zoology* 305:141-148.

Almli L.M. and Burghardt G.M. (2006) Environmental enrichment alters the behavioral profile of rat snakes (*Elaphe*). *Journal of Applied Animal Welfare Science* 9(2):85-109.

American Society of Ichthyologists and Herpetologists – ASIH (2004) *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research* (accessed on 2021-12-08).

American Veterinary Medical Association – AVMA (2020) *AVMA Guidelines for the Euthanasia of Animals* (accessed on 2021-12-08).

Amiel J.J., Lindstrom T. and Shine R. (2014) Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. *Animal Cognition* 17:337-347.

Association of Zoos and Aquariums – AZA (2009) *Suggested Guidelines for Reptile Enrichment* (accessed on 2021-12-08).

Association of Zoos and Aquariums – AZA (2013) *Eastern Massasauga Rattlesnake Care Manual* (accessed on 2021-12-08).

Astley H.C. and Jayne B.C. (2007) Effects of perch diameter and incline on the kinematics, performance and modes of arboreal locomotion of corn snakes (*Elaphe guttata*). *Journal of Experimental Biology* 210:3862-3872.

Aubret F., Bonnet X., Shine R. and Maumelat S. (2003) Clutch size manipulation, hatching success and offspring phenotype in the ball python (*Python regius*). *Biological Journal of the Linnean Society* 78:263–272.

Aubret F., Blanvillain G. and Kok P.J.R. (2015) Myth busting? Effects of embryo positioning and egg turning on hatching success in the water snake *Natrix maura*. *Nature Scientific Reports* 5:13385 (accessed on 2021-12-08).

Augustine L. and Baumer, M. (2012) Training a Nile crocodile to allow for collection of blood at the Wildlife Conservation Society’s Bronx Zoo. *Herpetological Review* 43(3):432.

Augustine L., Titus V. and Foster C. D. (2013) Color recognition as a management tool with a female Nile crocodile (*Crocodylus niloticus*) at the Wildlife Conservation Society’s Bronx Zoo. *Herpetological Review* 44(3):445–447.

Baines F.M., Chattell J., Dale J., Gill I., Goetz M., Skelton T. and Swatman M. (2016) How much UVB does my reptile need? The UV-tool, a guide to the selection of UV lighting for reptiles and amphibians in captivity. *Journal of Zoo and Aquarium Research* 4(1):42-63.

Balko J.A. and Chinnadurai S.K. (2017) Advancements in Evidence-Based Anesthesia of Exotic Animals. *Veterinary Clinics of North America: Exotic Animal Practice* 20(3):917-928.

Barnett, K.E., Cocroft R.B. and Fleishman L.J. (1999) Possible Communication by Substrate Vibration in a Chameleon. *Copeia* 1:225-228.

Bartol S.M., Musick J.A. and Lenhardt M.L. (1999) Auditory evoked potentials of the loggerhead sea turtle (*Caretta caretta*). *Copeia* 3:836-840.

Bashaw M.J., Gibson M.D., Schowe D.M. and Kucher A.S. (2016) Does enrichment improve reptile welfare? Leopard geckos (*Eublepharis macularius*) respond to five types of environmental enrichment. *Applied Animal Behaviour Science* 184:150-160.

Bateman P.W. and Fleming P.A. (2009) To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology* 277:1-14.

Benn A.L., McLelland D.J. and Whittaker A.L. (2019) A Review of Welfare Quality Assessment Methods in Reptiles and a Preliminary Application of the Welfare Quality® Protocol to the Pygmy Blue-Tongue Skink, *Tiliqua adelaidensis*, Using Animal-Based Measures. *Animals* 9(1):27 (accessed on 2021-12-08).

Bertelsen M.F. and Sauer C.D. (2011) Alfaxalone anaesthesia in the green iguana (*Iguana iguana*). *Veterinary Anaesthesia and Analgesia* 38:461-466.

Birkhead T.R. and Møller A.P. (1993) Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. *Biological Journal of the Linnean Society* 50:295-311.

Bertocchi M., Pelizzone I., Parmigiani E., Ponzio P., Macchi E., Righi F., Di Girolamo N., Bigliardi E., Denti L., Bresciani C. and Di Ianni F. (2018) Monitoring the reproductive activity in captive bred female ball pythons (*P. regius*) by ultrasound evaluation and noninvasive analysis of faecal reproductive hormone (progesterone and 17β- estradiol) metabolites trends. *PLOS ONE* 13(6):e0199377.

Bjorndal K.A., Parsons J., Mustin W. and Bolten A.B. (2013) Threshold to maturity in a long-lived reptile: interactions of age, size and growth. *Marine Biology* 160:607-616.

Blackburn D.G. (1994) Review: Discrepant Usage of the Term 'Ovoviviparity' in the Herpetological Literature. *Herpetological Journal* 4:65-72.

Bókony V., Milne G., Pipoly I., Székely T. and Liker A. (2019) Sex ratios and bimaturism differ between temperature-dependent and genetic sex-determination systems in reptiles. *BMC Evolutionary Biology* 19:57.

Bonnet X., El Hassani M.S., Lecq S., Michel C.L., El Mouden E.H., Michaud B. and Slimani T. (2016) Blood mixtures: impact of puncture site on blood parameters. *Journal of Comparative Physiology* 186(6):787-800.

Booth W. and Schuett G.W. (2011) Molecular genetic evidence for alternative reproductive strategies in North American pitvipers (Serpentes: Viperidae): long-term sperm storage and facultative parthenogenesis. *Biological Journal of the Linnean Society* 104:934-942.

Booth W., Smith C.F., Eskridge P.H., Hoss S.K., Mendelson J.R.III. and Schuett G.W. (2012) Facultative parthenogenesis discovered in wild vertebrates. *Biology Letters* 8:983-985.

Bostock, S.S.C. (2001) Captivity. In: *Encyclopedia of the World's Zoos, A-F*, vol. I. (Bell C.E., ed.). pp. 215-216. Chicago IL: Fitzroy Dearborn Publishers.

Boyer T.H. and Scott P.W. (2019) Nutrition. In: *Mader's Reptile and Amphibian Medicine and Surgery* (Divers S.J. and Stahl S.J., eds.). Chapter 27. St. Louis MO: Elsevier.

Bradley T. and Naives D. (1999) Leopard Gecko, *Eublepharis macularius* captive care and breeding. *Bulletin of the Association of Reptilian and Amphibian Veterinarians* 9(3):36-40.

Bradley Bays, T. and de Souza Dantas L.M. (2019) Clinical Behavioral Medicine. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 83, pp. 922-931. Elsevier: Amsterdam.

Brittan-Powell E.F., Christensen-Dalsgaard J., Tang Y., Carr C., and Dooling R.J. (2010) The auditory brainstem response in two lizard species. *Journal of the Acoustical Society of America* 128(2):787-794.

Brien M.L., Webb G.J., McGuinness K.A. and Christian K.A. (2016) Effect of housing density on growth, agonistic behaviour, and activity in hatchling saltwater crocodiles (*Crocodylus porosus*). *Applied Animal Behaviour Science* 184:141-149.

Brown C. (2010) Cardiac blood sample collection from snakes. *Lab Animal* 38(7):208-209.

Burghardt G. (2013) Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Applied Animal Behaviour Science* 147:286-298.

Bryant G.L., Eden P., De Tores P. and Warren K. (2010) Improved procedure for implanting radiotransmitters in the coelomic cavity of snakes. *Australian Veterinary Journal* 88(11):443-8.

Cabezas-Cartes F., Boretto J.M. and Ibargüengoytia N.R. (2018) Effects of Climate and Latitude on Age at Maturity and Longevity of Lizards Studied by Skeletochronology. *Integrative and Comparative Biology* 58:1086-1097.

Campbell T.W. and Ellis C.K. (2007) *Avian and Exotic Animal Hematology and Cytology*, 3rd Edition. 286pp. Ames IA: Blackwell Publishing.

Campbell T.W. (2014) Clinical Pathology. In: *Current Therapy in Reptile Medicine and Surgery* (Mader D.R. and Divers S.J, eds.). Chapter 8, pp 70-92. Amsterdam NL: Elsevier.

Canadian Association for Laboratory Animal Medicine – CALAM (2020) *CALAM Standards of Veterinary Care*. Toronto ON: CALAM (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2003a) *CCAC guidelines on: laboratory animal facilities – characteristics, design and development*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2003b) *CCAC guidelines on: the care and use of wildlife*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2005a) *CCAC guidelines on: the care and use of fish in research, teaching and testing*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2005b) *CCAC species specific recommendations on amphibians and reptiles*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2006) *CCAC policy on: Terms of Reference for Animal Care Committees* Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2007) *CCAC guidelines on: procurement of animals used in science*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care − CCAC (2008) *CCAC policy statement for: senior administrators responsible for animal care and use programs*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2010) *CCAC guidelines on: euthanasia of animals used in science*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2017) *CCAC guidelines: Husbandry of animals in science*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Council on Animal Care – CCAC (2021) *CCAC guidelines: Animal welfare assessment*. Ottawa ON: CCAC (accessed on 2021-12-08).

Canadian Herpetofauna Health Working Group (2017) Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada. (accessed on 2021-12-08).

Cannon M.J. (2003) Husbandry and Veterinary Aspects of the Bearded Dragon (*Pogona spp.*) in Australia. *Seminars in Avian and Exotic Pet Medicine* 12(4):205-214.

Capula M. and Luiselli L. (1995) Is there a different preference in the choice of background colour between melanistic and cryptically coloured morphs of the adder, *Vipera berus? Italian Journal of Zoology* 62:253-256.

Carsia R.V., McIlroy P.J. and John-Alder H.B. (2018) Modulation of adrenal steroidogenesis by testosterone in the lizard, *Coleonyx elegans*. *General and Comparative Endocrinology* 259:93-103.

Chacón D., Rodríguez S., Arias J., Solano G., Bonilla F. and Gómez A. (2012) Maintaining Coral Snakes (*Micrurus nigrocinctus, Serpentes: Elapidae*) for venom production on an alternative fish-based diet. *Toxicon* 60:249-253.

Chatigny F., Kamunde C., Creighton C.M. and Stevens E.D. (2017) Uses and doses of local anesthetics in fish, amphibians, and reptiles. *Journal of the American Association for Laboratory Animal Science* 56(3):244-253.

Christian K.A., Tracy C.R. and Tracy C.R. (2016) Body temperature and the thermal environment. In: *Reptile Ecology and Conservation: A Handbook of Techniques* (Dodd C.K, Jr., ed.). pp. 337-351. Oxford University Press.

Clark F. and King, A. J. (2008) A critical review of zoo-based olfactory enrichment. In: *Chemical Signals in Vertebrates 11* (Hurst J.L., Beynon R.J., Roberts S.C. and Wyatt T.D., eds.). pp. 391-398. New York NY: Springer.

Clark R.W., Brown W.S., Stechert R. and Greene H.W. (2012) Cryptic sociality in rattlesnakes (*Crotalus horridus*) detected by kinship analysis. *Biology Letters* 8:523-525.

Clarke J.A., Chopko J.T. and Mackessy S.P. (1996) The Effect of Moonlight on Activity Patterns of Adult and Juvenile Prairie Rattlesnakes (Crotalus viridis viridis). *Journal of Herpetology* 30(2):192-197.

Cojean O., Vergneau-Grosset C. and Masseau, I. (2018). Ultrasonographic anatomy of reproductive female leopard geckos (*Eublepharis macularius*). *Veterinary Radiology & Ultrasound* 59(3):333-344.

Cojean O., Alberton S., Froment R., Maccolini E., Vergneau-Grosset C. (2020) Leopard gecko (*Eublepharis macularius*) packed cell volume and plasma biochemistry reference intervals and reference values. *Journal of Herpetological Medicine and Surgery* 30(3):156-164.

Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES (1973) (accessed on 2021-12-08).

Convention on International Trade in Endangered Species of Wild Fauna and Flora – CITES (2021) Appendices I, II, and III (accessed on 2021-12-08).

Cooper J.E. (1999) Reptilian microbiology. In: *Laboratory Medicine, Avian and Exotic Pets*. (Fudge A.M., ed.). pp. 223-227. Philadelphia PA: Sanders.

Cooper J.E. (2010) Terrestrial reptiles: lizards, snakes and tortoises. In: *The Care and Management of Laboratory and Other Research Animals*, 8th Edition. (Hubrecht R. and Kirkwood J., eds.). Chapter 46, pp. 709-730. Chichester, West Sussex: Wiley-Blackwell.

Cooper W.E. Jr., Wilson D.S. and Smith G.R. (2009) Reproductive Status, and Cost of Tail Autotomy via Decreased Running Speed in Lizards. *Ethology* 115:7-13.

Conroy C.J., Papenfuss T., Parker J. and Hahn N.E. (2009) Use of Tricaine Methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the. American. Association of Laboratory Animal Science* 48:28-32.

Constanzo J.P., Iverson J.B., Wright M.F. and Lee R.E. Jr. (1995) Cold hardiness and overwintering strategies of hatchlings in an assemblage of northern turtles. *Ecology* 76(6):1772-1785.

Council of Europe (2004) *Revision of Appendix A of the Convention ETS123 – Species specific provisions for reptiles: background information for the proposals presented by the group of experts on amphibians and reptiles (Part B)* (draft – 4th meeting of the working party). Strasbourg: Council of Europe.

Coutant T.,Vergneau-Grosset C. and Langlois I. (2018) Overview of drug delivery methods in exotics, including their anatomical and physiological considerations. *Veterinary Clinics of North America: Exotic Animal Practice* 21(2):215-259.

Crawford R.L., Jensen D'A. and Allen T. (2001) Information Resources on Amphibians, Fish & Reptiles used in Biomedical Research. AWIC Resource Series No. 10.

Crews D., Bergeron J.M., Bull J.J., Flores D., Tousignant A., Skipper J.K. and Wibbels T. (1994) Temperature-Dependent Sex Determination in Reptiles: Proximate Mechanisms, Ultimate Outcomes, and Practical Applications. *Developmental Genetics* 15:297-312.

Crowe J. (2012) All About Thiaminase (accessed on 2021-12-08).

Cromie G.L. and Chappie D.G. (2012) Impact of Tail Loss on the Behaviour and Locomotor Performance of Two Sympatric Lampropholis Skink Species. *PLOS ONE* 7(4):e34732.

Davies W.L., Cowing J.A., Bowmaker J.K., Carvalho L.S., Gower D.J. and Hunt D.M. (2009) Shedding light on serpent sight: the visual pigments of henophidian snakes. *Journal of Neuroscience* 29(23):7519-7525.

Davis K.M. and Burghardt G.M. (2011) Turtles (*Pseudemys nelson*) learn about visual cues indicating food from experienced turtles. *Journal of Comparative Psychology* 125:404-410.

de Azevedo C.S., Cipreste C.F. and Young, R.J. (2007) Environmental enrichment: A GAP analysis. *Applied Animal Behaviour Science*, 102(3):329-343.

Dickinson H.C. and Fa F.E. (1997) Ultraviolet Light and Heat Source Selection in Captive Spiny-Tailed Iguanas (*Oplurus cuvieri*). *Zoo Biology* 16:391-401.

Diethelm G. and Stein G. (2006) Hematologic and Blood Chemistry Values. In: *Reptile Medicine and Surgery*, Second Edition. (Divers S.J. and Mader D.R., eds.). Chapter 88, pp.1103-1118. Elsevier.

Di Giuseppe M., Morici M., Martinez-Silvestre A. and Spadola F. (2017) Jugular vein venipuncture technique in small lizard species. *Journal of Small Animal Practice* 58:249 (accessed on 2021-12-08).

Divers S.J. (2019) Diagnostic Techniques and Sample Collection. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (eDivers S.J. and Stahl S.J., eds.). Chapter 43, pp. 405-421. Elsevier: Amsterdam.

Divers S.J. (2020) Management and Husbandry of Reptiles (accessed on 2021-12-08).

Divers S.J. and Stahl S.J. eds. (2019) Reptile Formulary. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 127. Amsterdam NL: Elsevier.

Doneley B. (2018) Taxonomy and introduction to common species. In: *Reptile Medicine and Surgery in Clinical Practice*. (Doneley B., Monks D., Johnson R. and Carmel B., eds.). Chapter 1, pp. 1-14. Oxford: Wiley-Blackwell.

Doody J.S., Burghardt G.M. and Dinets V. (2013) Breaking the social-non-social dichotomy: a role for reptiles in vertebrate social behavior research. *Ethology* 119:95-103.

Du W-G. and Shine R. (2008) The influence of hydric environments during egg incubation on embryonic heart rates and offspring phenotypes in a scincid lizard (*Lampropholis guichenoti*). *Comparative Biochemistry and Physiology Part A* 151:102-107.

Eagan T. (2019) Evaluation of enrichment for reptiles in zoos. *Journal of Applied Animal Welfare* 22(1):69-77.

Eatwell K., Hedley J. and Barron R. (2014) Reptile haematology and biochemistry. *In Practice* 36:34-42.

Emer S.A., Mora C.V., Harvey M.T. and Grace M.S. (2015) Predators in training: operant conditioning of novel behavior in wild Burmese pythons. *Animal Cognition* 18:269-278.

Eshar D., Lapid R. and Head V. (2018) Transilluminated jugular blood sampling in the common chameleon. *Journal of Herpetological Medicine and Surgery* 28(1-2):19-22.

Evans S.S., Repasky E.A. and Fisher D.T. (2015) Fever and the thermal regulation of immunity: the immune system feels the heat. *Nature Reviews Immunology* 15(6):335-349.

Ewert J-G., Cooper J.E., Langton T., Matz G., Reilly K. and Schwantje H. (2004) Background information on the species-specific proposals for reptiles, Presented by the Expert Group on Amphibians and Reptiles.

Ezaz T., Quinn A.E., Miura I., Sarre S.D., Georges A. and Marshall Graves J.A. (2005) The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. *Chromosome Research* 13:763-776.

Feldman S.H., Formica M. and Brodie E.D. (2011) Opisthotonus, torticollis and mortality in a breeding colony of *Anolis* sp. lizards. *Lab Animal (NY)* 40(4):107.

Ferguson G.W., Brinker A.M., Gehrmann W.H., Bucklin S.E., Baines F.M. and Mackin S.J. (2010) Voluntary Exposure of Some Western-Hemisphere Snake and Lizard Species to Ultraviolet-B Radiation in the Field: How Much Ultraviolet-B Should a Lizard or Snake Receive in Captivity? *Zoo Biology* 29:317–334.

Ferrell S.T., Marlar A.B., Alberts A.C., Young L.A., Bradley K., Hurlbut S.L. and Lung N.P. (2005) Surgical technique for permanent intracoelomic radiotransmitter placement in anegada iguanas (*Cyclura pinguis*). *Journal of Zoo and Wildlife Medicine* 36(4):712-715.

Fink D.M., Doss G.A., Sladky K.K. and Mans C. (2018) Effect of injection site on dexmedetomidine-ketamine induced sedation in leopard geckos (*Eublepharis macularius*). *Journal of the American Veterinary Medical Association* 253(9):1146-1150.

Finke M.D. (2013) Complete nutrient content of four species of commercially available feeder insects. *Zoo Biology* 32:27-36.

Finke M.D. (2015) Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology* 34(6):554-64 (accessed on 2021-12-08).

Fisher R.E., Geiger L.A., Stroik L.K., Hutchins E.D., George R.M., Kusumi K., Rawls J.A. and Wilson-Rawls J. (2012). A histological comparison of the original and regenerated tail in the green anole, *Anolis carolinensis*. *Anatomical Record* 295(10):1609-1619.

Flanagan J.P. (2015) Chelonians (Turtles, Tortoises). In: *Fowler's Zoo and Wild Animal Medicine*, Volume 8. (Miller E.R. and Fowler M.E.). Chapter 4, pp. 27-37. Amsterdam NL: Elsevier.

Fleming G.J. and Skurski M.L. (2012) Behavioral training of reptiles for medical procedures. In: *Fowler's Zoo and Wild Animal Medicine*, Volume 7. (Miller E.R. and Fowler M.E.). Chapter 27, pp. 212-216.Amsterdam NL: Elsevier.

Gardner M.G., Pearson S.K., Johnston G.R. and Schwarz M.P. (2016) Group living in squamate reptiles: a review of evidence for stable aggregations. *Biological Reviews* 91:925-936.

Garner M.M. and Jacobson E.R. (eds.) (2020) *Noninfectious Diseases and Pathology of Reptiles: Color Atlas and Text, Diseases and Pathology of Reptiles*, Volume 2. Boca Raton FL: Taylor & Francis.

Garrett C.M. and Smith B.E. (1994) Perch color preference in juvenile green tree pythons, *Chondropython viridis*. *Zoo Biology* 13:45-50.

Gauthier C. and Lesbarrères D. (2010) Growth rate variation in captive species: the case of leopard geckos, *Eublepharis macularius*. *Herpetological Conservation and Biology* 5(3):449-455.

Giorgi M., Salvadori M., De Vito V., Owen H., Demontis M.P. and Varoni M.V. (2015) Pharmacokinetic/pharmacodynamic assessments of 10 mg/kg tramadol intramuscular injection in yellow-bellied slider turtles (*Trachemys scripta scripta*). *Journal of Veterinary Pharmacology and Therapeutics* 38(5)488-496.

Girling S. and Raiti P. (eds.) (2019) *BSAVA Manual of Reptiles*, 3rd Edition. Gloucester UK: Wiley.

Goodman R.M. and Walguarnery J.W. (2007) Incubation temperature modifies neonatal thermoregulation in the lizard *Anolis carolinensis*. *Journal of Experimental Zoology* 307A:439-448.

Gould A. (2018). Evaluating the physiologic effects of short duration ultraviolet B radiation exposure in leopard geckos (*Eublepharis macularius*). *Journal of Herpetological Medicine and Surgery* 28(1-2):34-39.

Gouvernement du Québec (2018) Loi sur la conservation et la mise en valeur de la faune (chapitre C-61.1) *Animaux en captivité. Décret* 1065-2018.

Government of Canada (2005) New Substance Notification Regulations (Organisms) (accessed on 2021-12-08).

Government of Canada (2021) Health of Animals Regulations (accessed on 2021-12-08).

Greenberg N. (2002) Ethological Aspects of Stress in a Model Lizard, *Anolis carolinensis*. *Integrative & Comparative Biology* 42(3):526-540.

Greene H.W. (1995) Nonavian Reptiles as Laboratory Animals. *ILAR Journal* 37:182-186.

Greunz E.M., Williams C., Ringgaard S., Hansen K., Wang T. and Bertelsen M.F. (2018) Elimination of intracardiac shunting provides stable gas anesthesia in tortoises. *Nature Scientific Reports* 8:17124.

Hansen L.L. and Bertelsen M.F. (2013) Assessment of the effects of intramuscular administration of alfaxalone with and without medetomidine in Horsfield's tortoises (*Agrionemys horsfieldii*). *Veterinary Anaesthesia and Analgesia* 40:e68-e75.

Hare V.J., Rich B. and Worley K.E. (2008) Enrichment Gone Wrong! *The Shape of Enrichment* 35-45.

Hawkins S.J., Cox S., Yaw T.J. and Sladky K. (2019) Pharmacokinetics of subcutaneously administered hydromorphone in bearded dragons (*Pogona vitticeps*) and red-eared slider turtles (*Trachemys scripta elegans*). *Veterinary Anesthesia and Analgesia* 46(3):352-359.

Heiken K.H., Brusch G.A. IV., Gartland S., Esccallón C., Moore I.T. and Taylor E.N. (2016) Effects of long distance translocation on corticosterone and testosterone levels in male rattlesnakes. *General and Comparative Endocrinology* 237:27-33.

Hellmuth H., Augustine L., Watkins B. and Hope K. (2012) Using operant conditioning and desensitization to facilitate veterinary care with captive reptiles. *Veterinary Clinics of North America: Exotic Animal Practice* 15(3):425-443.

Hemby C., Keller K., Guzman D., Paul-Murphy J., Byrne B.A., Raudabaugh D.B., Miller A.N. and Allender M.C. (2019) Effectiveness of common disinfecting agents against isolated for *Nannizziopsis guarroi*, Exoticscon conference, Saint-Louis, MO:567.

Hernandez-Divers S.J., Cooper J.E. and Cooke S.W. (2004) Diagnostic techniques and sample collection in reptiles. *Compendium on Continuing Education of the Practicing Veterinarian* 26:470-483.

Hill P.S.M. (2009) How do animals use substrate-borne vibrations as an information source? *The Science of Nature* 96(12):1355-1371.

Holding M.L. (2011) Short-distance translocation of the northern pacific rattlesnake (*Crotalus o. oreganus*): Effects on volume and neurogenesis in the cortical forebrain, steroid hormone concentrations, and behaviors. MSc Thesis Faculty of California Polytechnic State University, San Luis Obispo.

Holz P., Barker I.K., Burger J.P., Crawshaw G.J. and Conlon P.D. (1997) The effect of the real portal system on pharmacokinetic parameters in the red-eared slider (*Trachemys scripta elegans*). *Journal of Zoo and Wildlife Medicine* 289(4):386-393.

Honeyfield D.C., Ross J.P., Carbonneau D.A., Terrell S.P., Woodward A.R., Schoeb T.R., Perceval H.F. and Hinterkopf J.P. (2008) Pathology, physiologic parameters, tissue contaminants, and tissue thiamine in morbid and healthy central Florida adult American alligators (*Alligator mississippiensis*). *Journal of Wildlife Disease* 44(2):280-294.

Innis C., DeVoe R., Myliczenko N., Young D. and Garner M. (2010) A call for additional study of the safety of subcarapacial venipuncture in chelonians. Proceedings, Association of Reptilian and Amphibian Veterinarians.

International Air Transport Association – IATA (2020) Live Animal Regulations (accessed on 2021-12-08).

Agreement on International Humane Trapping Standards (accessed on 2021-12-08).

Isaza R., Andrews G., Coke R. and Hunter R.P. (2004). Assessment of multiple cardiocentesis in ball pythons (*Python regius*). *Contemporary Topics in Laboratory Animal Sciences* 43(6):35-38.

Jacobson E. and Garner M.M. (eds.) (2020) *Infectious Diseases and Pathology of Reptiles*, 2nd Edition. Boca Raton FL: Taylor & Francis.

James L.E., Williams C.J.A., Bertelsen M.F. and Wang T. (2017) Evaluation of feeding behavior as an indicator of pain in snakes. *Journal of Zoo and Wildlife Medicine* 48(1):196-199 (accessed on 2021-12-08).

James L.E., Williams C.J.A., Bertelsen M.F. and Wang T. (2018) Anesthetic induction with alfaxalone in the ball python (*Python regius*): dose response and effect of injection site. *Veterinary Anesthesia and Analgesia* 45:329-337.

Januszczak I.S., Bryant Z., Tapley B., Gill I., Harding L. and Michaels C.J. (2016) Is behavioural enrichment always a success? Comparing food presentation strategies in an insectivorous lizard (*Plica plica*). *Applied Animal Behaviour Science* 183:95-103.

Jayne B.C., Voris H.K. and Ng P.K.L. (2018) How big is too big? Using crustacean-eating snakes (*Homalopsidae*) to test how anatomy and behaviour affect prey size and feeding performance. *Biological Journal of the Linnean Society* 123(3):636-650.

Ji X. and Du W-G. (2001) The effects of thermal and hydric environments on hatching success, embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata*. *Comparative Biochemistry and Physiology Part A* 129(2-3):461-471.

Juri G.L., Chiaraviglio M. and Cardozo G. (2018) Electrostimulation is an effective and safe method for semen collection in medium sized lizards. *Thierogenology* 118:40-45.

Johnson J.H. (2004) Husbandry and Medicine of Aquatic Reptiles. *Seminars in Avian and Exotic Pet Medicine* 13(4):223-228.

Kaplan M. (2014) Reptile Housing: Size, Dimension and Lifestyle (accessed on 2021-12-08).

Keller K.A., Paul-Murphy J., Weber E.P. 3rd, Kass P.H., Guzman S.M., Park S.A., Raghunathan V.K., Gustavsen K.A. and Murphy C.J. (2014) Assessment of platelet-derived growth factor using A splinted full thickness dermal wound model in bearded dragons (*Pogona vitticeps*). *Journal of Zoo and Wildlife Medicine* 45(4):866-874.

Kennett R. (1999) Reproduction of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet–dry tropics of northern Australia. *Journal of Zoology* 247(4):457-473.

Kis A., Huber L. and Wilkinson A. (2014) Social learning by imitation in a reptile (*Pogona vitticeps*). *Animal Cognition* 18(1):325-331.

Kischinovsky M., Duse A., Wang T. and Bertelsen M.F. (2013) Intramuscular administration of alfaxalone in red-eared sliders (*Trachemys scripta elegans*) – effects of dose and body temperature. *Veterinary Anaesthesia and Analgesia* 40:13-20.

Kischinovsky M., Raftery A. and Sawmy S. (2018) Husbandry and Nutrition. In: *Reptile Medicine and Surgery in Clinical Practice*. (Doneley B., Monks D., Johnson R. and Carmel B., eds.). Chapter 4, pp. 45-60. Wiley-Blackwell.

Klaphake É. (2010) A fresh look at metabolic bone disease. *Veterinary Clinics of North America: Exotic Animal Practice* 13(3):375-392.

Knotek S. (2019) Therapeutics and medication. In: *BSAVA Manual of Reptiles*, 3rd Edition. (Girling S.J. and Raiti P., eds.). Chapter 11, pp. 176-199. Gloucester, UK: Wiley.

Kohler G. (2005) *Incubation of Reptile Eggs* UK: Krieger Publishing Company.

Kramer M.H. (2005) What veterinarians need to know about red-eared sliders. *Exotic DVM* 7(6): 38-43.

Krohmer R.W. (2004) The Male Red-sided Garter Snake (*Thamnophis sirtalis parietalis*): Reproductive Pattern and Behavior. *ILAR Journal* 45(1):65-74.

Kumar R., Tiwari R.K., Kumar Asthana R., Kumar P., Shahi B. and Saha S.K. (2018) Metabolic bone diseases of captive mammal, reptile and birds. *Approaches in Poultry, Dairy and Veterinary Sciences*. 3(3) (accessed on 2021-12-08).

Kummrow M.S., Tseng F., Hesse L. and Court M. (2008) Pharmacokinetics of buprenorphine after single-dose subcutaneous administration in red-eared sliders (*Trachemys scripta elegans*). *Journal of Zoo and Wildlife Medicine* 39:590-595.

Kuppert S. (2013) Providing enrichment in captive amphibians and reptiles: is it important to know their communication? *Smithsonian Herpetological Information Service* 142:1-42.

Laferriere C.A., Leung V.S. and Pang D.S. (2020) Evaluating intrahepatic and intraperitoneal sodium pentobarbital or ethanol for mouse euthanasia *Journal of the American Association for Laboratory Animal Science* 59(3):264-268.

Lampert K.P. (2008) Facultative parthogenesis in vertebrates: reproductive error or chance. *Sexual Development*:290-301.

Latney L.V., Toddes B.D., Wyre N.R., Brown D.C., Michel K.E. and Briscoe J.A. (2017) Effects of various diets on the calcium and phosphorus composition of mealworms (*Tenebriomolitor* larvae) and superworms (*Zophobas morio* larvae). *American Journal of Veterinary Research* 78(2):178-185.

Lee J.C., Clayton D., Eisenstein S. and Perez I. (1989) The reproductive cycle of *Anolis sagrei* in Southern Florida. *Copeia* 4:930-937.

Lewis A.C., Rankin K.J., Pask A.J. and Stuart-Fox D. (2017) Stress-induced changes in color expression mediated by iridophores in a polymorphic lizard. *Ecology and Evolution* 7:8262-8272.

Lock B. (2008) Venomous snake restraint and handling. Topics in Medicine and Surgery *Journal of Exotic Pet Medicine* 17(4):273-284.

López-Olvera J.R., Montané J., Marco I., Martinez-Silvestre A., Soler J. and Lavin S. (2003) *Journal of Wildlife Diseases* 39(4):830-836.

Lovern M.B., Holmes M.M. and Wade J. (2004) The Green Anole (*Anolis carolinensis*): A Reptilian Model for Laboratory Studies of Reproductive Morphology and Behavior. *ILAR Journal* 45(1)54-64.

Mader's Reptile and Amphibian Medicine and Surgery. (2019) (Ed S.J Divers and S.J. Stahl). Elsevier: Amsterdam.

Manrod, J.D., Hartdegen, R., and& Burghardt, G. M. (2008). Rapid solving of a problem apparatus by juvenile black-throated monitor lizards (*Varanus albigularis albigularis*). *Animal Cognition* 11(2):267-273.

Mancera K.F., Murray P.J., Lisle A., Dupont C., Fauceux F. and Phillips C.J.C. (2017) The effects of acute exposure to mining machinery noise on the behaviour of eastern blue-tongued lizards (*Tiliqua scincoides*). *Animal Welfare* 26:11-24.

Mans C. (2008) Venipuncture techniques in chelonian species. *Lab Animal (NY)* 37(7):303-304.

Mans C., Sladky K.K. and Schumacher J. (2019) General Anesthesia. In*: Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 49, pp. 447-464. Amsterdam NL: Elsevier.

Martin P. and Bateson P. (2007) *Measuring Behaviour: An Introductory Guide*. Cambridge, UK: Cambridge University Press.

Mason R.T. and Parker M.R. (2010) Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology A* 196(10):729-749.

Mathews K.A. (2011) Monitoring fluid therapy and complications of fluid therapy. In: *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice*, 4th Edition. (DiBartola S.P., ed.). pp. 386-404. Amsterdam NL: Elsevier.

Mayer J., Knoll J., Wrubel K.M. and Mitchell M.A. (2011) Characterizing the hematologic and plasma chemistry profiles of captive crested geckos (*Rhacodactylus ciliatus*). *Journal of Herpetological Medicine and Surgery* (21)2-3:68-75.

Mayer J. (2019) Allometric scaling. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 126, pp. 1186-1190. Amsterdam NL: Elsevier.

McArthur S., Wilkinson, R., and Meyer J. (2004) *Medicine and Surgery of Tortoises and Turtles*. Oxford: Blackwell Publishing.

McFadden M.S., Bennett R.A., Kinsel M.J. and Mitchell M.A. (2011) Evaluation of the histologic reactions to commonly used suture materials in the skin and musculature of ball pythons (*Python regius*). *American Journal of Veterinary Research* 72(10):1397-1406.

McLean K.E. and Vickaryous M.K. (2011) A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius*. *BMC Developmental Biology* 11:50 (accessed on 2021-12-08).

Meylan S., Haussy C. and Voituron Y. (2010) Physiological actions of corticosterone and its modulation by an immune challenge in reptiles. *General and Comparative Endocrinology* 169(2):158-166.

Michelangeli M., Melki-Wegner B., Laskowski K., Wong B.B.M. and Chapple D.G. (2020) Impacts of caudal autotomy on personality. *Animal Behaviour* 162:67-78.

Mitchell M.A. (2004) Snake care and husbandry. *Veterinary Clinics: Exotic Animal Practice* 7:421-446.

Molina F.C., Bell T., Norbury G., Cree A. and Gleeson D.M. (2010) Assisted breeding of skinks or how to teach a lizard old tricks! *Herpetological Conservation and Biology* 5(2):311-319.

Morafka D.J., Spangenberg E.K. and Lance V.A. (2000) Neonatology of reptiles. *Herpetological Monographs* 14:353-370.

Morrill B.H., Rickords L.F., Sutherland C., Julander J.G. (2011) Effects of captivity on female reproductive cycles and egg incubation in ball pythons (*Python regius*). *Herpetological Review* 42(2):226-231.

Mosley C.A.E. (2005) Anesthesia and Analgesia in Reptiles. *Seminars in Avian and Exotic Pet Medicine* 14(4):243-262.

Nevarez J.G. (2019) Euthanasia. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J.). Chapter 47, pp. 437-440. Amsterdam NL: Elsevier.

Nomura T., Kawaguchi M., Ono K. and Murakami Y. (2013) Reptiles: a new model for evo-devo research. *Journal of Experimental Zoology Part B. Molecular and Development Evolution*: 320(2)57-73.

Nomura T., Yamashita W., Gotoh H. and Ono K. (2015) Genetic manipulation of reptilian embryos: toward an understanding of cortical development and evolution. *Frontiers in Neuroscience* 9:45.

Norton T.M., Andrews K.M. and Smith L.L. (2018) Working with free-ranging amphibians and reptiles. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 175. Amsterdam NL: Elsevier.

Olsson I.A., Nevison C.M., Patterson-Kane E.G., Sherwin C.M., Van de Weerd H.A. and Würbel H. (2003) Understanding behaviour: the relevance of ethological approaches in laboratory animal science. *Applied Animal Behaviour Science* 81(3):245-264.

Oonincx D. and van Leeuwen J. (2017) Evidence-based reptile housing and nutrition. *Veterinary Clinics: Exotic Animal* 20:885-898.

O'Rourke D.P., Cox J.D. and Baumann D.P. (2018) Nontraditional species. In: *Management of Animal Care and use programs in Research, Education and Testing*, 2nd Edition. (Weichbrod R.H., Thompson G.A.H. and Norton J.N., eds.). Chapter 25. Boca Raton FL: Taylor & Francis.

O'Rourke, D.P. and Lertpiriyapong K. (2015) Biology and diseases of reptiles. In: *Laboratory Animal Medicine*, 3rd Edition. (Fox J., Anderson L., Otto G., Pritchett-Corning K. and M. Whary, eds.). pp. 967-1013. London, UK: Elsevier.

Packard G.C., Packard M.J., Lang J.W. and Tucker J.K. (1999) Tolerance for freezing in hatchling turtles. *Journal of Herpetology* 33(4):536-543.

Peacock H.M., Gilbert E.A. and Vickaryous M.K. (2015) Scar-free cutaneous wound healing in the leopard gecko, *Eublepharis macularius*. *Journal of Anatomy* 227(5):596-610.

Pees M. and Hellebuyck T. (2019) Thermal Burns. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers, S.J. and Stahl S.J., eds.). Chapter 170, pp. 1351-1352. Amsterdam NL: Elsevier.

Perpiñán D. (2017) Chelonian haematology 1. Collection and handling of samples. *In Practice* 39:194-202.

Perry S.M. and Mitchell M.A. (2019) Routes of Administration. In: *Mader's Reptile and Amphibian Medicine and Surgery*. (Divers S.J. and Stahl S.J., eds.). Chapter 115, pp. 1130-1138. Amsterdam NL: Elsevier.

Perry S.M. and Nevarez J.G. (2017) Pain and its control in reptiles. Exotic Animal Neurology, An Issue of Veterinary Clinics of North America: Exotic Animal Practice, E-Book. 21(1):1.

Piniak W.E.D., Mann D.A., Harms C.A., Jones T.T. and Eckert S.A. (2016) Hearing in the Juvenile Green Sea Turtle (*Chelonia mydas*): A Comparison of Underwater and Aerial Hearing Using Auditory Evoked Potentials. *PLOS ONE* 11(10):e0159711.

Poletta G.L, Siroski P., Amavet P., Ortega H.H. and Mudry M.D. (2012) Reptiles as Animal Models: Examples of Their Utility in Genetics, Immunology and Toxicology. In: *Reptiles Across Research Fields*. (Siroski, P., ed.). Chapter 21, pp. 2-39.

Poole T. (1997) Happy animals make good science. *Laboratory Animals* 31(2):116-124.

Portas T.J.. (2018) Reproduction. In: *Reptile Medicine and Surgery in Clinical Practice*. (Doneley B., Monks D., Johnson R. and Carmel B., eds.). Chapter 7, pp. 91-104. Oxford UK: Wiley-Blackwell.

Preston D.L., Mosley C.A.E. and Mason R.T. (2010) Sources of Variability in Recovery Time from Methohexital Sodium Anesthesia in Snakes. *Copeia* 3:496-501.

Price E.R. (2017) The physiology of lipid storage and use in reptiles. *Biological reviews of the Cambridge Philosophical Society* 92(3):1406-1426.

Queensland Government: Department of Environment and Science (1992) Code of Practice: Captive Reptile and Amphibian Husbandry (accessed on 2021-12-08).

Raiti P. (2012) Husbandry, diseases, and veterinary care of the Bearded Dragon (*Pogona vitticeps*). *Journal of Herpetological Medicine and Surgery* 22(3-4):117-131.

Rasys A.M., Park S., Ball R.E., Alcala A.J., Lauderdale J.D. and Menke D.B. (2019) CRISPR-Cas9 gene editing in lizards through microinjection of unfertilized oocytes. *Cell Reports* 28(9):2288-2292.

Redrobe S. and MacDonald J. (1999) Sample collection and clinical pathology of reptiles. *Veterinary Clinics of North America: Exotic Animal Practice* 2(3):709-730.

Reed B. (2005) Guidance on the housing and care of the African clawed frog, *Xenopus laevis*. 84pp. RSPCA: Horsham, UK (accessed on 2021-12-08).

Rendle M. (2019) Nutrition. In: *BSAVA Manual of Reptiles*. (Girling S.J. and Raiti P., eds.). Chapter 4, pp. 49-69. Gloucester UK: Wiley.

Rizzo J.M. (2014) Captive care and husbandry of ball pythons (*Python regius*). *Journal of Herpetological Medicine and Surgery* 24(1-2):48-52.

Rose P., Evans C., Coffin R., Miller R. and Nash S. (2014). Using student-centred research to evidence-base exhibition of reptiles and amphibians: Three species-specific case studies. *Journal of Zoo and Aquarium Research* 2(1):25-32.

Rosier R.L. and Langkilde T. (2011) Does environmental enrichment really matter? A case study using the eastern fence lizard, Sceloporus undulatus. *Applied Animal Behavior Science* 131(1):71-76.

Rossi J.V. (2019) General husbandry and management. In: *Mader's Reptile and Amphibian Medicine and Surgery*. Chapter 16, pp. 109-130. (Divers S.J. and Stahl S.J., eds.). Amsterdam NL: Elsevier.

Rowland M. (2009) Veterinary care of bearded dragons. *In Practice* 31: 506-511.

Russell W.M.S. and Burch R.L. (1959) *The Principles of Humane Experimental Technique*, special edition, UFAW, 1992. London UK: Universities Federation for Animal Welfare.

Rzadkowska M., Allender M.C., O'Dell M. and Maddox C. (2016) Evaluation of common disinfectants effective against *Ophidiomyces ophiodiicola*, the causative agent of snake fungal disease. *Journal of Wildlife Diseases* 52(3):759-62 (accessed on: 2021-12-08).

Scheelings T.F. (2013) Use of Intravenous and Intramuscular Alfaxalone in Macquarie River Turtles (*Emydura macquarii*). *Journal of Herpetological Medicine and Surgery* 23(3-4):91-94.

Shepherdson D. (2001) Environmental Enrichment. In: *Encyclopedia of the World's Zoo's, Vol 1. A-F*. (Bell C.E., ed.). pp. 421-424. Chicago IL: Fitzroy Dearborn Publishers.

Shine R. and Brown G.P. (2008) Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet–dry tropics. *Philosophical Transactions B* 363(1490):363-373.

Shine R., Olsson M.M., Moore I.T., LeMaster M.P., Greene M.J. and Mason R.T. (2000) Body size enhances mating success in male garter snakes. *Animal Behavior* 59:F4-F11.

Skovgaard N., Abe A.S., Taylor E.W. and Wang T. (2018) Cardiovascular effects of histamine in three widely diverse species of reptiles. *Journal of Comparative Physiology B* 188:153–162.

Sievert L.M. and Hutchison V.H. (1988) Light versus heat: thermoregulatory behavior in a nocturnal lizard gecko (*Gekko gecko*). *Herpetologica* 44(3):266-273.

Singh S.K., Das D. and Rhein T. (2020) Embryonic temperature programs phenotype in reptiles. *Frontiers in Physiology*. 11:35.

Sladky K.K and Mans C. (2012) Clinical analgesia in reptiles. *Journal of Exotic Pet Medicine Topics in Medicine and Surgery* 21:158-167.

Stapley J. (2003) Differential avoidance of snake odours by a lizard: evidence for prioritized avoidance based on risk. *Ethology* 109:785-796.

State of NSW and Office of Environment and Heritage – NSW (2013) Code of Practice for the Private Keeping of Reptiles (accessed on 2021-12-08).

Subramaniam N., Petrik J.J. and Vicaryous M.K. (2018) VEGF, FGF-2 and TGFβ expression in the normal and regenerating epidermis of geckos: implications for epidermal homeostasis and wound healing in reptiles. *Journal of Anatomy* 232(5):768-782.

Suedmeyer Wm. K. (1995) Noninfectious diseases of reptiles. *Seminars in Avian and Exotic Pet Medicine* 4(1):56-60.

Sun A.X., Londono R., Hudnall M.L., Tuan R.S. and Lozito T.P. (2018) Differences in neural stem cell identity and differentiation capacity drive divergent regenerative outcomes in lizards and salamanders. *Proceedings of the National Academy of Sciences USA* 115(35):E8256-E8265.

Sun B-J., Wang T-T., Pike D.A., Liang L. and Du W-G. (2014) Embryonic oxygen enhances learning ability in hatchling lizards. *Frontiers in Zoology* 11(1):21 (accessed on 2021-12-08).

Sykes J.M. and Klaphake É. (2008) Reptile hematology. *Veterinary Clinics of North America Exotic Animal Practice* 11:481-500.

Tetzlaff S.J.,Tetzlaff K.E. and Connors R.J. (2016) Evaluation of thermal regimes for transported ambassador ectotherms: One size does not fit all. *Zoo Biology* 35(4):339-345.

Thorogood J. and Whimster I.W. (1979) The maintenance and breeding of the Leopard gecko as a laboratory animal. *International Zoo Yearbook* 19(1):74-78.

Tonge S. (2010) Aquatic reptiles. In: *The UFAW Handbook on The Care and Management of Laboratory and Other Research Animals,* 8th Edition. (Hubrecht R. and Kirkwood J., eds.). Chapter 47, pp. 731-740. Chichester UK: Wiley-Blackwell.

Turner T. and Cassano A.M. (2004). Subcutaneous dextrose for rehydration of elderly patients – an evidence-based review *BMC Geriatrics* 4:2.

US government (2005) Fish and Wildlife – [The Lacey Act] (accessed on 2021-12-08).

Uller T. and Olsson M. (2008) Multiple paternity in reptiles: patterns and processes. *Molecular Ecology* 17:2566-2580.

Ullrey, D.E. (2003) Metabolic Bone Disease. *In: Zoo and Wild Animal Medicine.* (Fowler, M., and Miller R.E., eds.). Chapter 80. St. Louis MO: Saunders.

Varga M. (2019) Captive Maintenance. In: *BSAVA Manual of Reptiles,* 3rd Edition. (Girling S.J. and Raiti P., eds.). Chapter 3. Gloucester UK: Wiley.

Vergneau-Grosset C. and Péron F. (2020) Effect of ultraviolet radiation on vertebrate animals: update from ethological and medical perspectives. *Photochemical & Photobiological Sciences* 19:752-762.Vitousek M.N., Mitchell M.A., Romero L.M., Awerman J. and Wikelski M. (2010) To breed or not to breed: physiological correlates of reproductive status in a facultatively biennial iguanid. *Hormones and Behavior* 57(2):140-146.

Wade J. (2011) Relationships among hormones, brain and motivated behaviors in lizards. *Hormones and Behavior* 59:637-644.

Wang T., Li H., Cui J., Zhai X., Shi H. and Wang J. (2019) Auditory brainstem responses in the red-eared slider *Trachemys scripta elegans* (Testudoformes: Emydidae) reveal sexually dimorphic hearing sensitivity. *Journal of Comparative Physiology A* 205:847-854.

Warwick C., Arena P., Lindley S., Jessop M. and Steedman C. (2013) Assessing welfare using behavioural criteria. *In Practice* 35:123-131.

Warwick C., Bates G., Arena P.C. and Steedman C. (2018) Reevaluating the use of hypothermia for anesthetizing and euthanizing amphibians and reptiles. *Journal of the American Veterinary Association* 253:1536-1539.

Warwick C., Arena P. and Steedman C. (2019) Spatial considerations for captive snakes. *Journal of Veterinary Behavior* 30:37-48.

Watts P.C., Buley K.R., Sanderson S., Boardman W., Ciofi C. and Gibson R. (2006) Parthenogenesis in Komodo dragons. *Nature* 444:1021-1022.

Webb J.K., Brown G.P. and Shine R. (2001) Body size, locomotion, speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii colubridae*): the influence of incubation environments and genetic factors. *Functional Ecology* 15(5):561-568.

Webb J.K. Guo Du W., Pike D.A. and Shine R. (2009) Chemical cues from both dangerous and nondangerous snakes elicit antipredator behaviours from a nocturnal lizard. *Animal Behaviour* 77:1471-1478.

Weiss E. and Wilson S. (2003) The use of classical and operant conditioning in training Aldabra tortoises (*Geochelone gigantea*) for venipuncture and other husbandry issues. *Journal of Applied Animal Welfare Science* 6(1):33-38.

Wheler C.L. and Fa J.E. (1995) Enclosure utilization and activity of Round Island geckos. *Zoo Biology* 14:361-369.

Wiggans K.T., Sanchez-Migallon Guzman D., Reilly C.M., Vergneau-Grosset C., Kass P.H., Hollingsworth S.R. (2018) Diagnosis, treatment, and outcome of and risk factors for ophthalmic disease in leopard geckos (Eublepharis macularius) at a veterinary teaching hospital: 52 cases (1985-2013). *Journal of the American Veterinary Medical Association* 252(3):316-323.

Williams C.J.A., Greunz E.M., Ringgaard S., Hansen K., Bertelsen M.F. and Wong T. (2019) Magnetic resonance imaging (MRI) reveals high cardiac ejection fractions in red-footed tortoises (*Chelonoidis carbonarius*). *Journal of Experimental Biology* 222: jeb206714.

Williams C.J., James L.E., Bertelsen M.F. and Wang T. (2019) Analgesia for non-mammalian vertebrates. *Current Opinion in Physiology*11:75-84.

Wise P.A.D., Vickaryous M.K. and Russell A.P. (2009) An embryonic staging table for in ovo development of *Eublepharis macularius*, the leopard gecko. *The Anatomical Record* 292:1198-1212.

Wright K. and Raiti P. (2019) Breeding and neonatal care. In: *BSAVA Manual of Reptiles*, 3rd Edition. (Girling S.J. and Raiti P., eds.). Gloucester UK: Wiley.

Woolley S.C., Sakata J.T. and Crews D. (2004) Tracing the evolution of brain and behavior using two related species of whiptail lizards: *Cnemidophorus uniparens* and *Cnemidophorus inornatus*. *ILAR Journal*, 45(1):46-53.

Wu P., Alibardi L. and Chuong C-M. (2014) Regeneration of reptilian scales after wounding: neogenesis, regional difference, and molecular modules. *Regeneration* 1(1):15-26.

Xiang J. and Du W-G. (2001) The effects of thermal and hydric environments on hatching success, embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata. Comparative Biochemistry and Physiology A* 129:461-471.

Young B.A. (2003) Snake bioacoustics: toward a richer understanding of the behavioural ecology of snakes. *The Quarterly Review of Biology* 78(3):303-325.

Young B.D., Stegeman N., Norby B. and Heatley J.J. (2012) Comparison of intraosseous and peripheral venous fluid dynamics in the desert tortoise (*Gopherus agassizii*). *Journal of Zoo and Wildlife Medicine* 43(1):59-66.

Zhang Y-P., Li S-R., Ping J., Li S-W., Zhou H-B., Sun B-J. and Du W-G. (2016) The effects of light exposure during incubation on embryonic development and hatchling traits in lizards. *Nature: Scientific Reports* 6:38527.

APPENDIX 1
LIST OF USEFUL RESOURCES

References for minimal space in reptile enclosures may be found in the new Quebec legislation - décret 1065-2018 (Gouvernement du Québec, 2018)

Divers (2019) provides recommended minimum space requirements for reptiles

Association of Zoos and Aquariums – AZA (2013) *Eastern Massasauga Rattlesnake Care Manual* (accessed on 2021-12-08).

APPENDIX 2
BASIC INFORMATION ON REPTILES COMMONLY HELD IN CANADIAN LABORATORIES

Leopard Gecko (*Eublepharis macularius*)

PHYSIOLOGY	
Mature size	40-100 g and >20 cm
Estimated lifespan	Up to 20 years in captivity
Food	Mealworms, crickets, and wax moth larvae; gut-loaded crickets ideal Supplement with calcium to phosphorus ratio of 1.25:1
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Terrestrial
POTZ[1]	25-30°C
Optimum humidity	30-40%
Photoperiod	Crepuscular
Brumation	Yes, 22-24°C for 2-8 weeks
Solitary or social	Solitary
Habitat preferences	Hiding areas, dark, dampened microclimate to facilitate shedding, branch or rock for basking
REPRODUCTION	
Age/size at sexual maturity	40-50 g Males: 18 months Females: 9 months

[1] Preferred optimum temperature zone: the range that the thermal gradient should offer.

Associated/dissociated reproductive cycle[2]	Associated
Seasonal influence on reproductive cycle	Brumation and decreasing light length (<12 hrs)
Duration of incubation	150-170 days to hatch, 28-32°C
Clutch size	10-14; two egg clutches per year
Effect of incubation temperature on sex of offspring	27-29°C female 29.5°C equal ratio of male to female 32-33°C male
Juvenile food	Crickets 1.3-1.9 cm and standard-sized mealworms every 1-2 days

References: Bradley and Naives, 1999; Thorogood and Whimster, 1979; Varga, 2019 (Chapter 3 in BSAVA)

Bearded Dragon (*Pagona* spp.)

PHYSIOLOGY	
Mature size	33-61 cm
Estimated lifespan	5-10 years
Food	Insects (crickets, mealworms, superworms), leafy vegetables (dandelion, Swiss chard, escarole, endive, romaine, spring mix, chicory, mustard, beet tops, bok choy, etc.), carrots, squash, and zucchini, 2-3 times/week
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Semi-arboreal
POTZ[3]	25-35°C
Optimum humidity	30-40% (more during ecdysis)
Photoperiod	Diurnal

2 Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

3 Preferred optimum temperature zone: the range that the thermal gradient should offer.

Brumation	Yes, 4-6 weeks at 60-70°C with no lighting during the late fall or early winter
Solitary or social	Adults are kept alone or as sexed pairs. Adult males should not share the same cage
Habitat preferences	Basking and resting is often spent on upright posts or trees
REPRODUCTION	
Age/size at sexual maturity	18-24 months
Associated/dissociated reproductive cycle[4]	Associated, 1 per year
Seasonal influence on reproductive cycle	Breeding stimulated following brumation
Duration of incubation	65-115 days, 28-32°C
Clutch size	20-25 eggs, prefer deeper area of slightly moistened sand in more secluded area
Effect of incubation temperature on sex of offspring	None
Juvenile food	30% vegetables and 70% appropriately sized crickets (< width of dragon's head), every 1-2 days

References: Ezaz et al., 2005; Raiti, 2012; Cannon, 2003; Rowland, 2009; Varga, 2019 (Chapter 3 in BSAVA)

[4] Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

4002 Anolis (*carolinensis* and *sagrei*)

PHYSIOLOGY	
Mature size	5 g, <100 mm snout-to-vent length
Estimated lifespan	In wild, 1-2 breeding seasons after summer of hatch; may live longer in captivity
Food	Crickets, mealworms, calcium and vitamin supplement
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Semi-arboreal
POTZ[5]	24°C day, 15°C night
Optimum humidity	70%
Photoperiod	Diurnal
Brumation	Not required
Solitary or social	Males are territorial; females may co-exist with sufficient space
Habitat preferences	Structures for hiding, climbing, and basking
REPRODUCTION	
Age/size at sexual maturity	Males: 39 mm Females: 34-35 mm
Associated/dissociated reproductive cycle[6]	Associated
Seasonal influence on reproductive cycle	Increase temperature to 28°C day, 19°C night; in the wild, breed from April to July.
Duration of incubation	4-6 weeks
Clutch size	Single egg clutches every 7-14 days; buried in damp substrate
Effect of incubation temperature on sex of offspring	None

5 Preferred optimum temperature zone: the range that the thermal gradient should offer.

6 Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

Juvenile food	Baby or one-week-old crickets, fruit flies, calcium and vitamin supplement

References: Lovern et al., 2004; Lee et al., 1989

Red-Eared Slider (*Trachemys scripta elegans*)

PHYSIOLOGY	
Mature size	Carapace length 5-14 inches
Estimated lifespan	30-50+ years
Food	A diverse and balanced diet composed of mostly vegetation, occasional calcium and phosphorus gut-loaded minnow, insect, or crawfish and supplemented with commercial turtle diets (pellets, gels, etc.)
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Semi-aquatic, freshwater
POTZ[7]	20-30°C water temperature; basking temperature: 5-10°C above water temperature
Optimum humidity	
Photoperiod	12:12 or 14:10 light cycle; diurnal
Brumation	Yes; only required if breeding
Solitary or social	Social, but suitability of conspecifics should be regularly monitored
Habitat preferences	Haul-out area(s) for basking
REPRODUCTION	
Age/size at sexual maturity	3-5 years
Associated/dissociated reproductive cycle[8]	

7 Preferred optimum temperature zone: the range that the thermal gradient should offer.

8 Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

Seasonal influence on reproductive cycle	Post-brumation in wild; may be unregulated in captive environment
Duration of incubation	60-90 days
Clutch size	1-22 eggs
Effect of incubation temperature on sex of offspring	26°C (males) 28.6°C (males and females) 31°C (females)
Juvenile food	More carnivorous than adult

References: Johnson, 2004; Crews et al., 1994; Doneley, 2018 (Chapter 1 Reptile Medicine and Surgery in Clinical Practice); Portas, 2018 (Chapter 7 Reptile Medicine and Surgery in Clinical Practice); Kramer, 2005

Ball Python (*Python regius*)

PHYSIOLOGY	
Mature size	1.1-1.5 m and > 1.5 kg typical
Estimated lifespan	20-30 years
Food	Pre-killed rodents; prey size should be same or smaller than the widest part of the snake’s body (adult mice or small rats)
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Terrestrial
POTZ[9]	25-30°C (day), 22-25°C (night); basking zones of 32-35°C
Optimum humidity	50-80%
Photoperiod	12:12 day:night cycle; nocturnal
Brumation	Yes; 3 months with high end preferred optimum temperature zone; reduced 5-6°C
Solitary or social	May be solitary in captivity; in the wild, may be social unless brooding
Habitat preferences	Hides at various points along the temperature gradient
REPRODUCTION	

9 Preferred optimum temperature zone: the range that the thermal gradient should offer.

Age/size at sexual maturity	Males: 12-18 months Females: 24-36 months
Associated/dissociated reproductive cycle[10]	Associated; captive animals can breed year-round
Seasonal influence on reproductive cycle	Post-brumation
Duration of incubation	60-63 days, 31°C; female will incubate eggs if allowed
Clutch size	5-12 eggs
Effect of incubation temperature on sex of offspring	None
Juvenile food	Fuzzy or hopper mice

References: Aubret et al., 2003; Rizzo, 2014; Mitchell, 2004; Morrill et al., 2011; Varga, 2019 (Chapter 3 in BSAVA)

Corn Snake (*Pantherophis guttatus*)

PHYSIOLOGY	
Mature size	76-122 cm
Estimated lifespan	20-25 years
Food	Mice and small rats
Oviparous or viviparous	Oviparous
HUSBANDRY	
Habitat	Terrestrial
POTZ[11]	25-30°C (day), 20-25°C (night)
Optimum humidity	30-70%

[10] Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

[11] Preferred optimum temperature zone: the range that the thermal gradient should offer.

Photoperiod	12:12 light cycle; diurnal
Brumation	10 weeks at 10°C
Solitary or social	Solitary
Habitat preferences	Hide box and substrate appropriate for shallow burrowing
REPRODUCTION	
Age/size at sexual maturity	Approximately 18-24 months 75 cm or 250 g
Associated/dissociated reproductive cycle[12]	
Seasonal influence on reproductive cycle	Post-brumation
Duration of incubation	55-70 days to hatch; 28-30°C
Clutch size	20-25
Effect of incubation temperature on sex of offspring	None
Juvenile food	Pinky mice

References: Varga, 2019 (Chapter 3 in BSAVA); Wright and Raiti, 2019 (Chapter 5 in BSAVA); Doneley, 2018 (Chapter 1 Reptile Medicine and Surgery in Clinical Practice); Portas, 2018 (Chapter 7 Reptile Medicine and Surgery in Clinical Practice)

[12] Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

Garter Snake (*Thamnophis* spp.)

PHYSIOLOGY	
Mature size	
Estimated lifespan	
Food	Amphibians and fish; if fed only fish, thiamine deficiency may result
Oviparous or viviparous	Viviparous
HUSBANDRY	
Habitat	Terrestrial, semi-aquatic
POTZ[13]	21-28°C
Optimum humidity	50-80%
Photoperiod	Diurnal
Brumation	Yes
Solitary or social	
Habitat preferences	
REPRODUCTION	
Age/size at sexual maturity	
Associated/dissociated reproductive cycle[14]	Dissociated
Seasonal influence on reproductive cycle	Insemination pre-brumation; fertilization post-brumation
Duration of incubation	90-100 days, 24-29.5°C
Clutch size	
Effect of incubation temperature on sex of offspring	None

13 Preferred optimum temperature zone: the range that the thermal gradient should offer.

14 Associated reproductive cycle is where sex hormone secretion and gonadogenesis stimulate copulation, followed by egg or fetal development. In a dissociated reproductive cycle, mating occurs before gonadogenesis using sperm stored by males from the previous season and after mating, that sperm is then stored by the female until gonadogenesis is complete.

Juvenile food	

4016 **References:** Varga, 2019 (Chapter 3 in BSAVA), Kischinovsky et al., 2018 (Chapters 4 and 7
4017 Reptile Medicine and Surgery in Clinical Practice)