Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC guidelines: Reptiles

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PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer-review organization responsible for setting, maintaining, and overseeing the implementation of standards for ethical animal care and use in science throughout Canada. CCAC standards are based on professional expertise and current interpretation of scientific evidence.

The *CCAC guidelines: Reptiles* is part of a series of types of animal guidelines documents that build on the general guidelines documents by providing additional guidance for the ethical care and use of particular species or groups of animals in science. Types of animal guidelines documents provide detailed information for protocol authors, animal care committees, facility managers, veterinarians, technicians, and animal care personnel to help facilitate improvement in both the care given to animals and the manner in which scientific activities are carried out.

This guidelines document details the standards that are expected to be met by holders of the CCAC Certificate of GAP – Good Animal Practice®. For scientific activities conducted within Canada or outside of Canada, protocol authors based at CCAC-certified institutions are subject to these standards. Protocol authors are also subject to any relevant legislation and regulations in the jurisdiction where the scientific activity is conducted.

SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

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.

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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, 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, and the needs of individual animals vary. In laboratory settings, their welfare is influenced by their housing environment and any scientific activities they are used in. Species-specific expertise is often required, and extrapolation from the literature to species other than those involved in a study needs to be done with caution.

The *CCAC guidelines: Reptiles* focuses on reptiles housed in laboratory facilities. For scientific activities involving reptiles in the wild, including short-term holding in the field, see the *CCAC guidelines: Wildlife* (CCAC, 2023). Most reptiles lay eggs, although some bear live young. The developing eggs of oviparous reptiles are not covered by this guidelines document, as the CCAC does not require an animal use protocol for these life stages (CCAC, 2020).

These guidelines are intended to be applied to all reptile species held in Canadian facilities, 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. Because of the diversity within this group of vertebrates, these guidelines provide general standards for their care and use in science, and species-specific expertise is often required for correct interpretation. Those working with a particular 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 who have experience with that species. Where knowledge of optimal conditions for a species is lacking, these guidelines and the species' natural habitat provide an appropriate starting point, which needs to be followed up with careful monitoring and adjustment.

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:

- housing and care requirements vary with species and life stages
- limited knowledge of reptile husbandry and welfare for many species

- limitations in the recognition, evaluation, and alleviation of nociception and negative welfare states
- challenges associated with distinct anatomy for scientific activities involving surgical procedures (e.g., suturing of skin)
- 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
- procurement in general, and a lack of captive-bred, pathogen-free sources in particular

As with all 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. Consideration of the quality, reproducibility, and translatability of scientific activities is critical throughout the design, conduct, and reporting of scientific activities to ensure that anytime an animal is used in science, their life is not taken in vain and the data obtained contributes to the research record.

Replacement and reduction are important considerations in planning animal-based scientific activities. Reduction involves determining the fewest number of animals consonant with the provision of valid information and adequate statistical power, while minimizing the welfare impact for each animal. Sample size calculations should 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 animal-based protocols 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 cognitive abilities of reptiles (Section 1.3, "Cognitive Ability"), the sensory abilities of reptiles (Section 1.4, "Senses"), and potential inter-animal variations (Section 1.5, "Sources of Variation"). This information has an impact on welfare considerations and forms the basis of this document. It is important to incorporate knowledge of the characteristics of the species (and strain where applicable), sex, life stage (age, breeding status, season), prandial status (i.e., when the animal was last fed), and the specific characteristics of the individual animal when considering the impact of a procedure or condition on the welfare of reptiles and on the results of the scientific activity.

1.1 BEHAVIOURAL BIOLOGY

Understanding the behavioural biology of animals used in science is crucial to improving both animal welfare and the quality of scientific activities (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.

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). Some species exhibit parental care or live in social groups (Doody, et al., 2021; While et al., 2009) and require suitable opportunities for engaging in social behaviours (Riley et al., 2017); also see Section 1.1.2, “Social Interactions”).

Reptiles are tetrapod vertebrates (i.e., animals with either four limbs or descended from four-limbed ancestors) and use various forms of locomotion. Tortoises and most lizards are quadruped terrestrial reptiles; however, some lizards 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 semi-aquatic.

Much of reptile behaviour is motivated by hunting, feeding, predation, and post-prandial status. Light also 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 influence behaviours associated with feeding, predation, and ecdysis (shedding of old skin).

1.1.1 Thermoregulatory Behaviour

Reptiles are largely ectothermic and behaviourally thermoregulate under natural conditions by selecting microenvironments in which they can gain or lose heat to maintain their body temperature. Reptiles experience microclimates, especially humidity and airflows, that are very different from those perceived by large animals such as humans. The optimal thermal environment for reptiles differs among species and may include opportunities for basking and cooling.

Brumation is 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 become more sedentary, with or without burrowing. This is both a survival mechanism and a required component of reproductive success for some species.

1.1.2 Social Interactions

Reptiles are frequently regarded as solitary animals that display some level of social interaction in the form of parental care, mating, territoriality, and dominance. However, large, stable social groups and social aggregations occur in some species; these are often kin-based and can be seasonal or remain year-round (Clark et al., 2012; Doody et al., 2013; Gardner et al., 2016). Social interactions can also lead to detrimental effects (e.g., aggressive interactions leading to injury or mortality). Thus, species and individual characteristics (see Section 1.5, “Sources of Variation”) are important considerations when housing individuals together.

Social cognition has been demonstrated in some species of reptiles. For example, bearded dragons have been found to display gaze following and the use of gaze cues of another individual, which is considered cognitively advanced and a requirement for perspective-taking (Siviter et al., 2018). Social learning (i.e., the ability to learn from the behaviour of another individual) has been demonstrated in phylogenetically disparate groups, such as some tortoises (Wilkinson and Huber, 2012) and some lizards (Siviter et al., 2018), and bearded dragons have shown evidence of imitation (Kis et al., 2015).

1.2 ANATOMY AND PHYSIOLOGY

Since reptiles are ectotherms, they do not, in general, produce sufficient metabolic heat to raise their body temperature above ambient temperature. Their 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 such needs as diet, fluid intake, resting time, and habitat for daily 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), which can be a welfare indicator (see 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 (e.g., snake organs are organized sequentially and longitudinally).

1.3 COGNITIVE ABILITY

Some reptiles exhibit feats of learning that are comparable to those in mammals and birds (see Szabo et al., 2021, for a review). For example, some tortoises have been observed to rapidly learn to associate neutral stimuli with positive outcomes (Mueller-Paul et al., 2014; Soldati et al., 2017) and readily discriminate between stimuli that result in different relative outcomes such as a favourite food versus a less-favoured food (Soldati et al., 2017). Some reptiles can learn about contingencies (e.g., tortoises (Bridgeman and Tattersall, 2019)) and learn to use a touchscreen (Mueller-Paul et al., 2014). Therefore, signals can be used to allow these reptiles to predict events such as feeding, but inconsistent signals could lead to frustration if the expected outcome is not provided.

1.4 SENSES

Reptiles have nociceptors, and they respond to nociceptive stimulation; hence, reptiles can experience pain sensation. However, some species may not display a thermal response and 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. For example, a study of bearded dragons shows that they are able to process visual information in a flexible manner, indicating that they interpret sensory information captured by photoreceptors rather than simply detect it (Santacà et al., 2019). In addition, another study suggests that bearded dragons exhibit lateralized processing (i.e., parallel processing of the two brain hemispheres at the same time) (Frohnwieser et al., 2017), indicating sophisticated perceptual and cognitive processing that is akin to mammals and birds.

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, 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. It is important to consider the presence of sensory stimuli that may not be perceptible to humans.

1.5 SOURCES OF VARIATION

While variation between reptile species is easily recognizable, morphological, physiological, and behavioural variation can also exist among individuals within a species. This variation can influence housing and husbandry requirements, the effects of procedures on animal welfare, and the interpretation of scientific activity results. Sources of intra-species variation include strain, natural geographic range, developmental stage, sex, and previous experience of the animal.

1.5.1 Strain

There has been limited artificial genetic modification of reptiles for animal model development (Rasys et al., 2019). Specialized breeding programs, both for scientific activities and hobby interests, have produced strains with distinct phenotypes such as scaleless animals and unique colouration (e.g., albinism, hyper- or hypo-melanism, leucism (reduced pigmentation), and anerythrism (lack of red pigmentation)). Different strains may have significantly different requirements for water, humidity, and light levels than the wild-type. For example, "silkback" and "leatherback" bearded dragons differ in their thermal preferences and evaporative water loss (Sakich and Tattersall, 2021).

1.5.2 Developmental Stage

Reptiles are generally long-lived with a prolonged growth and development period. They are highly developmentally plastic, and their development can be influenced by nutrition, age, social environment (Riley et al., 2017), and temperature (While et al., 2018). Even the temperature the mother is exposed to during gestation impacts the development of her young (Liu et al., 2020).

Nutrition and housing needs, including social or solitary housing, vary with developmental stage. An optimally fed reptile will generally reach developmental maturity and size faster than one sub-optimally fed.

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.

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

Individual differences between reptiles of the same species include sex and health status. While some species are sexually dimorphic, it can be difficult to determine the sex of other species based on visual cues. Sex can 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 scientific activities 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").

Genetically diverse populations and different rearing conditions also contribute to individual behavioural preferences, including general temperament (bold or shy), food preferences, and habitat preferences. It is important to consider reptile welfare at the individual level; for example, 'bold' individuals may seek out opportunities for exploration, while 'shy' individuals may prefer hiding places and a more consistent environment.

Individual differences in behaviour often focus on animal temperament or personality, or behavioural traits (e.g., boldness), and these are likely to play an important role in understanding an animal's ability to cope in captivity. For example, studies on the impact of incubation temperature on bearded dragon behaviour found that altering incubation temperature within the normal range impacted the development of behaviour (Siviter et al., 2017), foraging success, running speed, growth (Siviter et al., 2019), and the cognition of adult lizards (Siviter et al., 2018).

1.5.4 Effects of the Environment and Previous Experience

While there are clear species differences in behaviour (e.g., in response to novelty (Moszuti et al., 2017)), there is also evidence of differences between individuals of the same species, and early life experiences can impact individual reptiles for the rest of their lives. 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. Insufficient nutrition can cause growth retardation, while excessive feeding can cause obesity and physiological abnormalities. Nervous or aggressive species or individuals may require additional hiding spaces or restricted sightlines to prevent stress from perceived inter- or intra-species interactions beyond the enclosure.

Some reptiles show spatial learning and have substantial long-term spatial memory (Mueller-Paul et al., 2012a; Mueller-Paul et al., 2012b; Mueller-Paul et al., 2014), suggesting the long-term impacts of their experiences. For example, a species of tortoise is able to remember spatial tasks for at least three months (Mueller-Paul et al., 2014) and can remember stimuli associated with preferred food for at least 18 months (Soldati et al., 2017). Therefore, even brief experiences of inappropriate housing or poor husbandry can have long-term impacts on their welfare.

The source of the reptiles can affect 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.

Due to species diversity and high individualism, there can be significant differences in response to the same situation. Reptile handling, procedures, and husbandry must be developed in the context of the species and the individual animal.

2 FACILITIES

For general guidance on facilities, see the *CCAC guidelines: Laboratory animal facilities* (CCAC, 2024). 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, and 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 required in a laboratory animal environment. Procedures should be performed in a separate room from where the animals are housed; however, if barriers are in place to block relevant stimuli from other animals, procedures may occur in the same room.

Racks and tanks to house large reptiles are heavy, and the floors must be able to support the weight. Floors should be non-slip, especially for rooms housing aquatic enclosures.

Floors, walls, and ceilings must be designed and constructed to prevent the escape of animals into the internal infrastructure of the wall or environmental chamber. Seams and small holes must be well sealed, and there must be strong weatherstripping under doors. For aquatic and highly humid environments, construction materials that tolerate high humidity must be used and floor drains must be in place with mesh covers to prevent escape (especially for snakes). Floor-level refuges may be useful to enable the retrieval of animals that manage to escape their enclosure.

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.

Room controls, a space heater or other localized heating source, and radiant heat panels installed at the top of enclosures can be used to maintain suitable temperatures when the lights are off. All heat sources should be controlled with a thermostat or routine static monitoring to prevent overheating. Heat sources and regulators should be CSA- or ULC-certified and installed by a qualified electrician if wiring is required. Bulbs should be positioned so that animals can approach and warm themselves easily, but not so close that they can be burned (Pees and Hellebuck, 2019). For aquatic enclosures, heating sources can include titanium or glass heaters in an unbreakable cage, either in line with the life support system or submersed in the tank or filtration system. If the heat source or electrical cable is submerged in the tank, measures must be taken to prevent animals from interacting with the elements. Aquatic heat sources must be connected to an electrical circuit with a ground fault circuit interrupter.

Failure of electrical and heating, ventilation, and air conditioning (HVAC) systems that lead to cooling or overheating can be lethal for reptiles, and backup emergency power should be available (O'Rourke et al., 2018).

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

If multiple enclosures are in the same room, the potential for stress or behaviour that may cause injury due to the sight or scent of potential predators or prey must be minimized, irrespective of whether there are different species or conspecifics (Stapley, 2003; Webb et al., 2009). Sightlines can be controlled through enclosure selection and orientation or the use of opaque barriers. Problems associated with scent can be minimized through the separation or spacing of enclosures or through increased ventilation.

Housing recently wild-caught reptiles in the same room as reptiles that have undergone quarantine can result in ectoparasite and disease transfer. Adequate space should be provided for quarantine and isolation (see Section 4.6, "Quarantine and Acclimation").

2.2 ENCLOSURES

Guideline 1

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

The needs of each species and the requirements of scientific activities vary widely; therefore, this section contains only general guidance on housing reptiles.

Investigators should search the recent literature and communicate with other facilities housing the species to infer the requirements for a particular species to thrive. Knowledge of the biology of the species should be used in determining the requirements of the animal; however, for many species of reptiles, this information is lacking or has been influenced by the act of observing the animals, which can be a source of stress to wild animals (Warwick et al., 2013). When housing unfamiliar species for which such information is not available, information from zoos or reputable hobbyists may be helpful, and it may be necessary to evaluate several types of housing to determine the most suitable system.

Enclosures must be designed to meet the reptiles' needs for good welfare, but do not have to replicate the natural environment. As an illustration, Burghardt et al. (1996) found that items such as hoops and balls, which turtles would not naturally encounter, reduced self-mutilation behaviour for a captive softshell turtle. Reptile enclosures must enable the animals to perform a range of behaviours important to their welfare; as a result, aesthetics must not take priority over functionality for the animals. Additionally, the provision of an aesthetically pleasing natural environment may lead to the assumption that the animal's needs are being met and result in insufficient evaluation of their welfare (Fabregas et al., 2012; Veasey et al., 1996).

Restraint and ease of maintenance by animal care personnel should not be the prime determinant of housing conditions. Standard operating procedures (SOPs) for housing and husbandry should be developed once appropriate conditions are established.

All housing for captive reptiles must be:

- escape-proof
- free from sharp edges or coarse wire
- designed to enable handlers (research teams and animal care personnel) to have safe 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
- illuminated sufficiently to enable effective and safe husbandry while meeting species-appropriate lighting requirements
- equipped with ultraviolet lighting that is able to penetrate the enclosure (e.g., through mesh that is not too fine) when required by the species
- designed with suitable drains if large reptiles requiring water will be housed (Queensland Government, 2010)

Interior enclosure walls, floors, and fittings must be constructed from impervious materials that can be easily cleaned (NSW, 2013). Suitable reptile housing options depend on the species and life stage and may include glass or acrylic aquaria, stackable cages, or fibreglass tanks; other types of impervious primary enclosures may also be suitable (O'Rourke et al., 2018).

Guideline 2

Enclosures must be designed to enable control of the internal environmental conditions to meet the needs of the species to be housed.

Enclosures must have sufficient space and adequate ventilation to create a heat gradient that includes the warm and cool ends of the species' 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. The body temperatures maintained by many species of lizards during activity are only a few degrees below their lethal temperatures, and overheating is a serious risk if temperature gradients are poorly designed. Thermal gradients should typically be horizontal but may be vertical for climbing species. Shelters should be placed along the gradient so that animals are not forced to choose between thermoregulation and security.

A system for monitoring temperatures at both ends of the spectrum and emergency power to ensure environmental consistency must be in place. Any wires, cables, or electrical cords in the enclosure must be securely fastened to prevent animal entanglement or direct contact with an animal.

2.2.1 Spatial Requirements

Spatial requirements for reptiles vary greatly, depending on species and life stage; however, the space must be large enough to permit free movement and the exhibition of daily behaviours important to the welfare of the animals (Kaplan, 2014; Hoehfurtner et al., 2021; Wheler and Fa, 1995; Hollandt et al., 2021). As noted in Section 2.2, "Enclosures", the space must also be sufficiently large to maintain a suitable temperature gradient. The space taken up by items such as feed dishes, water dishes, and environmental enrichment should be discounted from the total space required, and these items should not impact the movement of the animal. However, the space should not be so large as to impair the animals' ability to observe their surroundings, hinder successful feeding (especially of live insects), or prevent observation of the animals by animal care personnel.

When calculating enclosure size for lizards, the tail must be included in the total size of the animal.

2.2.2 Enclosure Materials and Design

Enclosures must be designed to promote species-specific behaviours that optimize the welfare of the animals. Different species require unique, optimized environments, and general guidance must be adapted for each species.

Wood is an acceptable material for terrarium construction, but it must be properly sealed so that it is easy to clean and will withstand 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 enclosure crevices, such as where the sides and top meet or where the glass fits into metal frames (Greene, 1995). Measures must be taken to mitigate injury (e.g., by covering the glass or moving animals to another type of enclosure).

All enclosures for reptiles should have lids, and where enclosure sides are of insufficient height to prevent escape (e.g., some turtles are effective climbers), lids must be in place. Enclosure doors and lids should be constructed to facilitate access and cleaning, except for those housing venomous snakes, for which the safety of the animal care personnel must be a priority. Positioning of doors and lids (i.e., side-opening versus top-opening) should be from the animal's perspective, as approaching the animal at their height rather than from above can be less stressful for some species. However, the risk of the animal escaping must also be addressed. Ventilation ports must be screen-covered to prevent escape.

An opaque top and three opaque side walls are generally preferred for terrariums, although this is not always possible if providing light or heat 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 penetration). For most species, one side of the enclosure should be fully or mostly transparent to allow easy viewing of the inside. A partially or entirely removable covering on the clear wall can be used to reduce negative stimuli, especially for highly irritable or easily frightened reptiles. Reflective surfaces should be avoided.

While cages should be easy to sanitize, some lizards, such as geckos, need climbing substrate on the enclosure walls.

2.2.3 Furnishings

Furnishings include resting spots, platforms, and areas to retreat. Habitat design and enrichment devices may facilitate natural environmental interactions and natural social interactions, which may also allow for reproductive behaviours (Rose et al., 2014).

Housing enclosures must include a refuge that ideally mimics attributes of the animal's natural habitat (Cooper, 2010). Hiding places enable 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. Aquatic turtles will use ledges, rocks, and other areas to hide. Similarly, tortoises will use hides if provided. Nocturnal species use hides as retreats during the day. When corn snakes were provided with a number of different types of hides and shelters, they used the full range (Hoehfurtner et al., 2021).

Furnishings should offer opportunities for physical and tactile exploration 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 can also assist in the shedding process.

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

2.2.4 Terrestrial Holding Systems

Most lizards, snakes, and the more terrestrial species of chelonians can be kept in terrariums. A terrarium may be specially constructed, a modified aquarium, or another secure type of enclosure of appropriate size. Enclosures must be designed to balance appropriate ventilation with heat and humidity requirements.

Secure shelter sites, perching sites, open areas for foraging, and access to water are generally necessary for terrestrial species. At least 30-40% of the floor space should be open space for the reptile to easily move about, feed, access water, and defecate (Kaplan, 2014).

The natural behaviour of the species should be used in determining appropriate enclosure size. 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 to enable animals easy access without the risk of drowning (Queensland Government, 2010) or flipping themselves over on their backs (i.e., tortoises). Examples of cage designs are depicted by Ewert et al. (2004). Species that soak in water should be housed in an enclosure with room for a sufficiently large bowl for soaking.

Basking lamps or "hot spots" are frequently essential for species-appropriate environmental enrichment, such as for some gravid females and snakes with health problems. A basking lamp can be mounted on the outside of the enclosure above a screened area at one end of the top of the enclosure. Many forms of heaters are also available, such as heat cables and pads, ceramic bulbs, and plate or radiant heat panels. Regardless of heater type, the temperature must be regulated, preferably with a thermostat. Animals must not be able to maintain direct contact with the heat source as they may not display a thermal response, which can lead to burn injuries (see Section 3.1.2.1, "Temperature").

Hide boxes offer animals the opportunity to withdraw visually from activities outside their enclosure and provide tactile security, which can increase comfort, particularly for newly acquired animals, shy animals, or those who react negatively to routine environmental stimuli. Hide boxes or hiding spots should be provided at both the cool and warm ends of the enclosure to allow animals to select their preferred temperature without sacrificing security (AZA, 2009).

Hide boxes should be an appropriate size for the animal. They can be purpose-made (generally plastic or ceramic) or homemade from a variety of materials, including repurposed opaque plastic food containers that are easy to clean inside and out. Disposable hide boxes for use in relatively dry environments can be created from cardboard. Any repurposed materials must be inspected closely to remove potentially harmful elements (e.g., tape, staples, and sharp edges). Hides made from absorbent materials (e.g., wood or cardboard) should not be moved between enclosures nor shared between animals unless they can be autoclaved.

2.2.4.1 Lizards

Small lizards may be kept in aquariums or terrariums to maintain adequate humidity, with a few exceptions. Chameleons and *Abronia* species should be kept in mesh enclosures that allow for appropriate ventilation to decrease the risk of fungal infection. Large lizards (e.g., adult iguanas) should be kept in large enclosures in rooms with controlled temperature and humidity. 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 suggestions for enclosure design for geckos.

Most species will drink from water bowls of varying sizes (see Section 6.4.2, "Drinking Water"); however, some species, such as chameleons, generally do not drink from bowls and require a drip or misting system.

Some reptiles can develop harmful behaviours if inappropriate (excessive or unnecessary) stimuli are visible outside the enclosure. This can include territorial aggression or running into the enclosure wall due to fear. This behaviour can be managed by providing sufficient hides and external wall covers to reduce the sightline to external stimuli and neighbouring animals. 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. 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 environment.

2.2.4.2 Snakes

Snakes occupy a wide range of natural habitats, including aquatic, terrestrial, and arboreal habitats. Snakes can be deceptive about their requirements: some small, active snakes, such as racers, need more room, relative to body length, than large and mostly sluggish pythons and boas (Kaplan, 2014; Divers, 2020; 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 behaviours important to their welfare. Snakes will often use provided structures during movement and stretching. Semi-aquatic snakes require larger enclosures that contain a water area large enough for them to comfortably swim in and a land area 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.

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 of the hide box should be twice the snake's widest girth.

The enclosure environment should be kept simple and safe for snakes, as large specimens can move or overturn furnishings, sometimes creating hazards where tails can become caught (AZA, 2009). Access doors should be flush with inside surfaces and have 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)).

2.2.4.2.1 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 SOPs must be in place. For venomous reptiles with a moderate or greater risk of causing a medically significant envenomation (e.g., Massasauga rattlesnake), housing and facility infrastructure must minimize the risk of envenomation and enable prompt response to injury.

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

- Ventilation ports – These must be clearly marked as possible danger points of exposure to the snake's fangs. All openings except the lid should be obstructed to prevent successful strikes; this can be accomplished with a double layer of screening to ensure that there is no possibility of the snake coming into contact with personnel.
- Viewing walls – These should be fitted with removable opaque covers on the outside to reduce aggression-inducing stimuli. All components of the enclosure, including the viewing wall, should be shatterproof.
- Access – This should be carefully considered: if floor-level doors are used, it must be possible to see the snake while opening the door. Shift panels are useful to segregate the animal from the area being serviced and decrease personnel risk (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 maintenance of the enclosure. The housing room and primary enclosure must be secured to prevent unauthorized access. The enclosure should be deep enough to at least slow down any attempt by the snake to climb to the top.

2.2.4.3 Terrestrial Chelonians (Box Turtles) and Tortoises

Species-specific habitat preferences should be incorporated into the environment. Healthy tortoises and terrestrial turtles that are housed in proper environments are active and relatively fast-moving. Many terrestrial chelonians range widely throughout their habitat and use several sleeping and basking areas every day. Most are burrowers and can easily dig under outdoor pen walls and fences. Chelonians, especially tortoises, are also good climbers. To prevent escape, enclosure walls should be higher than the animals can stretch when they climb on the back of another animal or on a structure in the enclosure (Kaplan, 2014).

2.2.5 Arboreal Reptiles

Enclosure space for arboreal reptiles should be vertically oriented, with objects and surfaces for climbing. Branches and perches of varying diameters should be included to provide appropriate traction and enable animals to grip (Astley and Jayne, 2007). The height of the structures must be suited to the heat gradient and heat sources. Structures should be placed to minimize the risk of trapping animals and allow easy removal for cleaning. They should not be placed over food or water bowls to prevent contamination with feces or food.

Structures can be purchased or manufactured. In general, branches of willow, birch, beech, ficus, and fruit trees, and cork bark hollows provide non-toxic climbing surfaces. All materials should be able to be autoclaved to eliminate pathogens.

2.2.6 Aquatic Reptiles

Aquatic holding systems are required 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.

Aquatic and semi-aquatic turtles need a land area for basking, sleeping, and egg laying, and a water area that is large enough for them to swim freely. The enclosure must be deep enough to accommodate silt or sand for burrowing, as appropriate, and in the case of turtles, sufficient water for the animals to submerge and right themselves if they turn over. Some species of aquatic turtles only require a haul-out area large enough to accommodate all turtles in the enclosure; however, most semi-aquatic species require a substantially larger land area, as well as a water area.

Enclosures for aquatic reptile species must be strong, as they are very heavy due to the volume of water and the presence of animals, and tremendous pressure can be exerted against the walls. Suitable enclosures include a strong aquarium or prefabricated tub. In the case of aquatic turtles, a large stock tank (e.g., for watering cattle), either directly on the floor or on a riser, can be used, with the area surrounding the enclosure built up to provide a land area and to contain the animals.

Aquatic turtles should be housed in circular enclosures to provide the opportunity for continuous, uninterrupted swimming.

The water depth and habitat enrichment required to meet the needs of the species and life stages should be incorporated into the design of the enclosure.

A platform just above the water surface should be provided for turtles to haul out and bask. Visual barriers to potentially negative external stimuli (e.g., personnel movement) may be necessary for turtles to use the platform. Flat rocks or custom-made, water-impervious platforms can be used; however, wood is not appropriate for a platform as it will be continuously water-soaked. As discussed in Section 2.2.2, "Enclosure Materials and Design", 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 exit the water. Turtles must be able to get a firm hold with their claws to pull themselves out, as it is possible for them to drown if they cannot easily leave the water (Queensland Government, 2010). There should be a basking lamp above the resting area.

Flow-through water systems are suitable for freshwater turtles. If recirculating systems are used, they should have robust filtration (e.g., a filter rated for a 400 L aquarium should be used for a 200 L turtle enclosure). Dechlorinated water must be used (see Section 3.1.3.2, “Water Quality”).

3 FACILITY MANAGEMENT AND PERSONNEL

3.1 MANAGING THE ENVIRONMENT

Guideline 3

The macroenvironment (room) and microenvironment (primary enclosure) must maintain the health and welfare of both the animals and personnel and provide consistency for the outcomes of scientific activities.

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 (i.e., parameters such as light level, humidity, and temperature range). Each enclosure is then established as an individual environmental chamber in which these parameters are adjusted to suit the requirements of the species.

The microenvironment of the enclosure must meet the physiological needs of the species and life stage of the animals, and facilities must accommodate these needs before housing a new species or life stage. Special equipment needed for housing reptiles includes humidifiers, room-controlled heating and cooling (most brumation requires low to average temperatures of 5-15°C), and additional ground-fault-interrupted outlets for heat lamps. Specialized plumbing for drip or misting systems may also be needed. See Divers (2020) for general information on optimum lighting, temperature, and humidity for select reptiles. The equipment needed for maintaining environmental conditions in aquatic enclosures is described 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 the light cycle should follow the seasonality of day length, as light cycles provide physiological cues for many reptile species. Photoperiod differences affect the phase, amplitude, and duration of circadian rhythm (measured as melatonin level) in many species, and both constant light and constant dark environments can 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 bred in-house, appropriate lighting must be available for egg incubation: light exposure accelerates embryonic development but may lead to negative survival outcomes, depending on the species (Zhang et al., 2016). Wild-caught animals may stop eating as the season changes, and when the day length increases, they may need a reduction in photoperiod for a period to stimulate eating. For captive reptiles to thrive and reproduce, exposure to seasonal variations in photoperiod or temperature may be necessary.

The effects of light intensity and spectrum on a particular species should be understood. Light intensity is used by basking species such as anoles and turtles as an indication of temperature; higher intensities are associated with higher temperatures. Light also impacts thermoregulatory behaviour in the nocturnal Tokay gecko (Sievert and Hutchison, 1988). Given a choice, iguanas prefer incandescent light over ultraviolet light, likely due to the warmth of incandescent light. Dickinson and Fa (1997) suggest using both ultraviolet and incandescent light in captive environments. Turtles, especially young turtles, must have the option to bask under sunlight or ultraviolet radiation at least three times a week.

3.1.1.1 Ultraviolet Light

While humans can only see visible light (400-700 nm), some reptiles can also see within the ultraviolet range (290-400 nm). Before procuring reptiles, requirements for ultraviolet or full-spectrum lighting should be determined (Ferguson et al., 2010; Baines et al., 2016). Ultraviolet light via suitable lamps appears 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. However, animals should not be permitted to be near sources of ultraviolet light, as high levels can be detrimental to some animals (e.g., chameleons, bearded dragons, nocturnal species, and certain morphs (albinos) may experience eye and skin damage). The placement of an 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 bulbs should be suited to the needs of the species being held. Scheduled ultraviolet tests should be performed or bulb changes should be scheduled and documented. Mercury vapour bulbs are a good option for ultraviolet B light, provided they can be used safely. Ultraviolet light can also be provided by fluorescent bulbs; however, these bulbs have a short lifespan. Compact fluorescent bulbs can have a poor distribution of ultraviolet B light.

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

Many lizards and chelonians require ultraviolet B light for normal calcium metabolism and vitamin D synthesis (Baines et al., 2016; Rossi, 2019). Oral supplementation of vitamin D3 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

Appropriate temperatures should be maintained for the welfare of reptiles due to their ectothermic nature (Ferguson et al., 2010; Christian et al., 2016). All diurnal species and many nocturnal species thermoregulate behaviourally during the day and night and require ample opportunity to thermoregulate by choosing from diverse microenvironments (Ferguson et al., 2010; Rossi, 2019; Varga, 2019; Dayananda et al., 2017; Blumberg et al., 2002; Nordberg and Schwarzkopf, 2019; Arenas-Moreno et al., 2018; Tan and Schwanz, 2015). Nocturnal species that do not routinely thermoregulate behaviourally require air temperatures that equate with their natural environment.

Before placing any reptile in an enclosure, the temperature gradients within the enclosure must be determined by monitoring conditions at various locations with a thermometer, recognizing that seasonal change may affect these conditions. The enclosure should provide thermal conditions that are appropriate for the species and enhance behavioural and physiological function (NSW, 2013). Thermal ranges for reptiles should be determined by consulting the literature and natural history of the species, and by monitoring the animals. Baines et al. (2016) provide general guidelines for estimating preferred temperature ranges based on characteristics of the animal's natural habitat, and Rossi (2019) and Baines et al. (2016) include information on the preferred optimal temperature zone for many species. Most sources advise that captive reptiles experience thermal cycles around their preferred temperature. Where possible, thermal cycles should be based on natural thermal variation during the normal active season of the animal, 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 and even immerse themselves in water (Queensland Government, 2010). If an animal spends most or all of their time in either the hottest or coldest part of the enclosure, it may be an indication that the temperature settings need adjustment.

Reptiles are very sensitive to sudden temperature changes, and temperatures substantially outside their preferred ranges may result in illness or death. For example, if inappropriate cooling occurs, reptiles may experience decreased metabolic function, including extended gut transit time and lethargy, immune suppression, and potentially death. Care must also be taken to ensure enclosures do not become too hot. There must be areas that are sufficiently cool and warm 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.

Temperature and light should always be controlled independently of each other. An incandescent light or sun lamp is a useful source of supplementary warmth for basking reptiles but should never be the sole source of heat or light.

The temperature must be monitored in the enclosure, rather than relying 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 areas of different temperatures (i.e., basking zone and cool zone) on a regular basis.

3.1.2.2 Humidity

Humidity requirements should be assessed on a case-by-case basis, based on evidence for the species (ASIH, 2004). The humidity of enclosures should be monitored regularly. Low humidity can be hazardous for small individuals and for species adapted to humid, tropical conditions, such as chameleons. 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 this high humidity may result in snakes being unable to shed their skin completely (see Section 8, "Health and Disease Control").

Means of controlling air exchange within the enclosure and within the room should be available. A completely open unit, such as a wire-mesh mammal cage, or a tightly closed one is undesirable.

Ventilation ports may be used to adjust airflow and assist with humidity regulation, but airflow should not be reduced to a level that promotes mould or pathogen growth. Consistent airflow should be maintained, with adjustments in humidity provisions as required.

Elevated humidity can be maintained by evaporating water from a container placed near a 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 not be used in the enclosure and should be set to protect the substrate from becoming saturated. While a damp substrate is suitable for some species (e.g., arboreal geckos) or for a short-term increase in humidity, in general, the substrate should not become soaking wet, as this promotes bacterial growth and may lead to scale rot and other skin problems (Queensland Government, 2010) or respiratory disease.

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 patterns, while low turnover can result in increased humidity to a level that promotes condensation, microbial growth, 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; however, the direction of airflow should limit the spread of aerially transmitted pathogens (AZA, 2013). Pathogens can travel in aerosols over a distance of a few metres, and 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 advised by aquatic equipment manufacturers, should be used as a starting point and monitored.

3.1.3.2 Water Quality

Guideline 4

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. The conductivity or salinity of the water must be measured for aquatic species; they will not survive in water treated with reverse osmosis that has not been properly reconstituted. The water must also be free of chlorine and chloramine, which can be present in treated municipal 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, water temperature monitoring may be conducted less frequently than in a room without environmental controls.

3.1.4 Sound and Vibration

Measures should be taken to minimize noise and vibration in facilities housing reptiles. Noise and vibration in the facility should be assessed, particularly when renovations are taking place, as it is a potential welfare concern. Vibration can be reduced by placing rubber under enclosures or placing enclosure stands 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 scientific activities using reptiles.

Lizards and turtles have similar acoustic physiology to humans and are capable of hearing 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), and lizards such as the Tokay gecko and green anole hear sounds in the 1-3,000 Hz (Tokay gecko) and 1-7,000 Hz (anole) ranges (Brittan-Powell et al., 2010). High-frequency, high-amplitude noise can elicit fear responses in lizards (Mancera et al., 2017).

Snakes do not have functional outer and middle ears and cannot "hear", but they have acute vibration sensitivity that allows them to detect and respond to low range soundwaves in the 80-160 Hz range (Young, 2003). Some reptiles use vibration as a means of communication (e.g., Barnett et al., 1999; Hill, 2009).

3.1.5 Brumation Requirements

Species-specific temperature requirements must be determined before initiating brumation, as inappropriate brumation can be lethal. Most brumation requires low to normal temperatures (i.e., 5-15°C). Facilities must have the ability to gradually increase the temperature to the normal range for the animals at the end of the brumation period. Lighting must also change to the normal photoperiod 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 must be available 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. The frequency of observation should be described in an SOP for each species. Under most circumstances, reptiles must be observed daily by competent 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. For some animals, direct daily observations may negatively impact their welfare, and alternate observation procedures should be approved by the animal care committee. In all cases, daily observation of the animal's containment and the environmental systems supporting the animals must be performed.

Working with reptiles can pose challenges for animal care personnel who regularly work with mammalian species such as rodents, and they should work closely with research personnel to fully understand the needs of the animals. 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 personnel should use appropriate practices that respect the welfare of the animals (e.g., not tapping on the enclosure and moving enclosures in a way that minimizes disturbance). Where possible, dedicated personnel should care for the reptiles in the facility. If this is not possible, personnel should be careful not to carry the scent of rodents into the reptile area.

An institution that maintains reptiles for scientific activities 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 must be accommodated.

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 6.8, "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 insects that may be consumed by a reptile from exposure to insecticides. A veterinarian must be consulted for any animal experiencing a parasitic infestation or other pathogenicity (see Section 8, "Health and Disease Control").

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

The procurement of reptiles, including the source of the animals, must be justified, based on the requirements of the scientific activity. Whenever possible, purpose-bred reptiles should be used over animals taken from the wild (Council of Europe, 2004), and common, readily available species from reputable breeders should be used, rather than breeding in-house.

Investigators must be aware of federal, provincial or territorial, and local laws and regulations that limit or require exemptions for the use of certain species. Animals 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 (see Section 4.2, "Regulations").

When procuring reptiles, there must be assurance that appropriate housing and husbandry will be provided for the length of time they will be held. Reptiles can live many years in captivity, and those used in comparative studies may be housed and cared for over long periods, during which they may be used for other scientific activities (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022) for establishing endpoints for the long-term holding of animals).

Planning scientific activities using reptiles must consider the length of time the procurement process may take. The procurement of many reptile species differs from that of laboratory rodents, which can be ordered for a specific short-term use, often within a narrow time frame.

4.1.1 Captive Bred

The benefits of using captive-bred reptiles include (adapted from Reed, 2005):

- Captive-bred animals have a known life history, age, and diet.
- The potential for introducing unwanted diseases or parasites to an existing colony is reduced.
- Captive-bred animals experience artificial rearing, housing, and husbandry conditions throughout their lives, and events such as handling and enclosure cleaning are likely to cause less stress and negative welfare impacts compared to naive, wild-caught animals.
- The use of reptiles that are inbred over several generations may reduce the effect of individual genetic variation on the outcomes of scientific activities.

In addition, removing reptiles from their natural habitat for use in scientific activities may disrupt the balance of the local ecosystem.

Reptiles should be obtained from reputable commercial suppliers that meet specific health and genetic parameters for the animals and associated import and export requirements. Minimizing travel distance for the animals should also be a consideration to reduce transport-associated stress.

Reptile suppliers acquire animals through wild capture, trade (including the pet trade), or captive breeding, and the legality and professionalism of reptile suppliers must be assured on a continual basis, as it may change from year to year. Reputable zoological parks may also release surplus animals to other professional organizations, although their charters often preclude subsequent invasive scientific activities with those animals (Greene, 1995).

Indications that a supplier is reputable include:

- They are transparent, allowing site visits and review of their husbandry records.
- They have been referred by a qualified veterinarian.
- They show 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.

Many reptiles are long-lived, and the outcomes for these animals following their use in scientific activities must be determined (see Section 12, "End of Study"). One possibility is for institutions to develop relationships with breeders to permit healthy animals to be returned once they are no longer needed for scientific activities.

4.1.2 Wild Caught

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. Wild-caught animals should be procured only when specifically required by a scientific activity (e.g., activities related to the environment, ecology, or sustainability of the species) or when captive-bred animals are not suitable or available. As for other species, animals in the wild have been found to have different intestinal flora and develop different diseases than those bred in captivity over a long period. Investigators must be able to provide specific justification for the use of wild-caught reptiles, based on the objectives of their scientific activity.

Before removing animals from the wild, the local population status (abundant, threatened, rare, etc.) should be understood. The number of animals removed from the wild must be the minimum necessary to accomplish the goals of the scientific activity, and the information derived from removing animals from the wild should be maximized, for example, by providing genetic samples or location data to other investigators, with consideration of cumulative endpoints (CCAC, 2022), to reduce the overall need for wild-caught animals.

Investigators must ensure that the capture of animals is done according to the *CCAC guidelines: Wildlife* (CCAC, 2023), regardless of who is doing the capture. Capture should minimize bycatch and habitat damage (e.g., cover objects should be returned to their exact location to preserve the underlying microhabitats).

Methods of capturing reptiles include hand capture, possibly assisted by a hand-held lasso or net, and indirect methods, such as a pit-fall trap or drift fence. Indirect methods of capture must be designed and monitored to protect the welfare of captured animals. Guidance on trap monitoring is provided in the *CCAC guidelines: Wildlife* (CCAC, 2023). Traps should be designed to capture target species while allowing bycatch to escape, and they should be supplemented with species-specific food and habitat to ensure good animal welfare between trap checks.

If wild-caught reptiles are to be released (see Section 12.3, “Release to the Wild”), they should be released in the location where they were caught if they have been held for a short time and not manipulated in a manner that would impair their survivability in that environment, providing the site is safe and appropriate for the animal. To assist with this, a GPS location should be obtained where the reptile is collected. This is particularly useful for species with a very limited home range, such as Eastern box turtles. 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.

4.2 REGULATIONS

Guideline 6

Facilities and protocol authors acquiring or transporting reptiles, or conducting scientific activities using reptiles, must comply with relevant international, federal, and provincial or territorial legislation and policies.

The 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) specifically applies to many reptiles, and 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 current CITES convention (CITES, 2021). Appendix I of the aforementioned 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 listed. Those responsible for importing reptiles should also be aware of the source nation’s export requirements (e.g., 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 be aware that the *Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act* (WAPPRIITA) 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 (CFIA) *Health of Animals Regulations* (Government of Canada, 2021), a permit is required to import all chelonians and their eggs due to the risk of transmitting diseases, such as salmonella. This permit is normally issued to zoos and research laboratories and is not for commercial purposes.

Institutions must also be aware of any provincial or territorial restrictions, 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.

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

Before shipping a reptile, food should be withheld for 1-7 days, depending on the species. The duration of fasting should allow complete digestion before initiating transportation (e.g., a day for most lizards and at least several days for most snakes).

4.4 TRANSPORTATION

Guideline 8

The health status of the animals should be assessed before they are transported.

The first requisite of proper transportation is a healthy animal.

For air transport, there should be assurance that the shipping company follows the International Air Transport Association (IATA) and the Animal Air Transport Association (AATA) regulations for animal transportation. Large carriers tend to have better temperature control than smaller ones (Tetzlaff et al., 2016), which is an important consideration for some species. Carriers should be contacted in advance regarding applicable regulations and schedules. Small lizards and turtles that do not pose a risk to humans can be transported by airmail, using the fastest means possible. Larger animals and all snakes should be transported via air freight (either via courier or an airline). Venomous species require special packaging and should only be transported via airline courier. Institutional personnel receiving the reptiles should monitor the shipment's routing diligently so that it can be promptly retrieved and unpacked. Reptiles typically do not require feeding or other temporary care during transport; however, species that require high humidity (e.g., chameleons) require special damp packing.

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. This should include identification (such as species), location of origin, final destination, and contact information for responsible persons.

For unaccompanied long-distance transportation, insulated foam shipping containers (i.e., a Styrofoam inner box placed in a water-resistant outer box) should be used 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 turn 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, ventilated boxes that prevent escape and prevent handlers from being bitten through the bag. Venomous snakes should be double-boxed and properly identified (i.e., each snake should be confined in a securely knotted cloth bag and placed in a container with a secure lid, which is then placed in a larger insulated box). Most species should be maintained between 16°C and 25°C.

Guideline 9

Reptiles should not be transported if weather forecasts predict extreme temperatures.

Airlines may have specific weather-related policies for transporting animals that should be consulted.

Heat packs, cold packs, and room-temperature gel packs can be placed inside insulated boxes to compensate for the external environment and buffer against temperature variations en route. Temperature packs of varying intensities and durations are available. Their use should be based on the external environmental factors that may be encountered during transportation and the species being transported. Individuals experienced in transporting the reptile species should be consulted regarding the inclusion and placement of these packs.

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. Animals should be transported individually in a shipping box when appropriate (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 that provide proper ventilation and protection against temperature extremes. The bags must be carefully inspected for holes and should not be left unattended outside the coolers. 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 arrival by competent animal care personnel.

In addition to reviewing the health status of the animals in advance of their arrival, as much information as possible should be obtained on the details of the husbandry and other reptile-related practices of the institution shipping the animals, to assist in establishing quarantine conditions for them 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
- dealing with animals that are sick or dead upon arrival

A veterinarian or other competent individual must assess the condition of all animals upon receipt, according to the institutional SOP. A visual examination of the animals should be conducted to assess any need for immediate treatment (e.g., for dehydration or trauma). The animals should also be observed to ensure that new groups of reptiles are compatible. Communication with the supplier to provide information about the state of the animals on arrival can help inform improvements to future shipping arrangements.

All containers must be thoroughly cleaned and disinfected or sterilized if intended for reuse (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.

Reptiles brought into a facility must undergo quarantine. Quarantine should be carried out in a separate room or area, with separate sets of equipment used in each. Where this is not possible, measures that prevent splashing and aerosol transfer between enclosures must be taken, husbandry procedures for animals in quarantine should be undertaken after the other animals in the facility, and equipment (including personal protective equipment) must be dedicated to the quarantine area or carefully decontaminated after use in the quarantine area. SOPs for cleaning, disinfection, and personal protective equipment should be developed based on the potential diseases the species may carry (Divers, 2019; Rzadkowska et al., 2016).

New reptiles must be kept separate from other animals until they can be appropriately screened and monitored for health concerns (Divers, 2019). The length of time required for quarantine should be based on a risk assessment of the animal, with consideration given to the source, conditions of transport, and the age of the animal. During this period, the animals should be habituated to the method of food and water delivery and to the new environment.

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).

A period of environmental acclimatization can run concurrently with the period of quarantine. Any stress associated with transportation should be alleviated and the physiology of the animal should return to a normal state. The animals should also be acclimated to the conditions of the scientific activity and any procedures that will be conducted while they are conscious (CCAC, 2017). Animals with a low metabolic rate will take longer to acclimate than those with a high metabolic rate, and the minimum acclimation period should range from two weeks (for smaller animals such as anoles) to a month (for snakes such as ball pythons with a slower metabolic rate).

5 BREEDING

5.1 GENERAL CONSIDERATIONS

Animals should be obtained from reputable, commercial captive-breeding operations (see Section 4.1, “Source”). When this is not possible for a scientific activity, in-house breeding programs may be appropriate. 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
- elimination of animal transportation from the breeding facility (reduces stress)
- incubation of an appropriate number of eggs in oviparous reptiles and selection of the sex of the animal in species with temperature-dependent sex determination (prevents breeder mistakes on the sex of the reptile in some species)
- knowledge of the nutritional status of reproductive animals, conditioning the nutritional content of the egg and juveniles
- control of inbreeding among animals used in the same scientific activity, which is not always possible when receiving a colony from a single breeder

Many reptiles are long-lived and managing 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 need before establishing a colony.

In-house breeding requires specialized facilities (e.g., to induce breeding conditions, for egg incubation, and for rearing juveniles), in-depth knowledge of the species’ requirements, and animals of appropriate health and maturity (see Sections 5.2, “Physiological Considerations”, and 5.3, “Breeding and Mating Conditions”). Animal care personnel must be well trained and experienced with the breeding of reptiles, and veterinary assistance must be available to humanely intervene for any breeding-associated pathology, including dystocia, egg retention, and malformations associated with incubator malfunction. 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. If breeding is to be undertaken, there must be an assurance that the necessary infrastructure, equipment, and expertise are available to care for the animals (see Section 2, “Facilities”, and Section 6, “Husbandry”).

If in-house breeding is required, SOPs should be developed before initiating breeding. These SOPs should include 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. The outcome for surplus animals should also be determined before initiating breeding. Surplus animals should be kept to a minimum, and a plan should be in place to avoid euthanasia whenever possible.

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

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

Breeding females should have detailed life history records, including potential exposures to males due to the possibility of sperm retention. Records should also include details on age, ancestry, food consumption, defecation, weight, ecdysis, medical problems, and reproductive output (Wright and Raiti, 2019).

5.2.1 Sexual Maturity

Reptiles become sexually mature at a species-specific combination of age and size, which 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 and Feeding"). Generally, body size at sexual maturity can be estimated based on maximum adult size; reptiles become sexually mature when they reach 65-75% of their 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 a higher risk of dystocia. Dystocia occurs when only a portion of the eggs are laid during oviposition or the fetuses are delivered during parturition, and this can result in sterility or death. Females may be physiologically capable of mating before they are capable of gestating.

5.2.2 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 others are viviparous, having autonomous, free-living offspring born without an eggshell (Blackburn, 1994). In addition, some species develop a reliance on the egg yolk until birth (lecithotrophic viviparity), while others have a chorioallantoic placenta that provides life support until birth (Wright and Raiti, 2019).

In very rare cases, reptiles reproduce by facultative and obligate parthenogenesis. In facultative parthenogenesis, individuals switch between sexual and clonal reproduction. Facultative parthenogenesis can occur in some lizards and snakes in captivity and in the wild (Booth et al., 2012; Lampert, 2008; Watts et al., 2006).

Obligate parthenogenesis is exclusively clonal reproduction and is more rare 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).

5.2.3 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 by changes in temperature rhythms and photoperiod. These reptiles (e.g., leopard gecko, bearded dragon, blue-tongued skink, and 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.

5.2.4 Physiological and Environmental Stimulus

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

For successful breeding, environmental conditions should replicate the animal's natural environment. Many species require a natural seasonal regime or an artificial regime that mimics natural 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 that undergo brumation (Cooper, 2010), but species' needs vary, and natural life history must be considered. Most reptilian species are either warm-temperate or tropical and 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) and lowering heat and light levels, while continuing to provide water, monitor weight, and carry out visual health checks. This should continue for a defined period that is highly species-specific (e.g., many reptiles originating from the southern hemisphere may only require a 4-8-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 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, females rely on male cues to trigger development (Wright and Raiti, 2019).

5.3.1 Pairing and Mating

Natural pair selection for the species should be understood before attempting pairing, to improve mating success and to ensure the health and safety of the animals. For example, multiple paternities occur naturally in over 50% of reptile clutches (Uller and Olsson, 2008) due to the potential for multiple mating and the ability of many female reptiles to store sperm.

Close monitoring of mating sessions is required to prevent injury to the breeding pair. Mating behaviour may appear aggressive in some species, and appropriate training of personnel and familiarity with the species is required to ensure the safety of the breeding pair and successful mating. Safety equipment, such as gloves, should be readily available in case intervention is needed during an unexpectedly aggressive interaction.

5.3.2 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 must be performed by personnel trained in conducting the procedure on the species involved. 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 can be monitored using ultrasound and changes in hormones (Bertocchi et al., 2018).

5.4 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' 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, and ultrasound) should be conducted to ensure normal progression of gestation, with more specific diagnostics only if problems are suspected. Monitoring methods requiring general anesthesia or sedation should be avoided during gestation unless clinically required. Monitoring must also be done 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, a veterinarian or other appropriate reptile expert should be consulted, as 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). Pre-oviposition or pre-parturition ecdysis is primarily characterized by a 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, such as 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 not be disturbed if found mid-lay, as disturbance may cause dystocia. The humidity in the lay box should be optimal to allow the snake and eggs to be left alone overnight without risking egg desiccation or drowning of hatchlings (i.e., not supersaturated substrate).

5.5 INCUBATION OF EGGS AND NURSERY ACTIVITIES

The decision to remove or leave the eggs in the nest should be justified 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 and 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 to prevent rotating the egg. Candling with a bright light can enable visualization of the early stages of development, particularly 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. 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 should have a carefully controlled incubation temperature, as it can affect physical development, sex, locomotor abilities, behavioural development, and cognitive abilities (e.g., Bókony et al., 2019; Amiel et al., 2014; reviewed in Singh et al., 2020; Siviter et al., 2017, 2018, 2019). Eggs incubated at temperatures outside the optimum have lower levels of hatching success and may be associated with developmental abnormalities. 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 result in 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)). During the last 1-2 weeks of incubation, 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 and males develop at high incubation temperatures (alligators and many lizard species).
- Females develop at high incubation temperatures and males develop at low incubation temperatures (many chelonians).
- Females develop at either low or high incubation temperatures at the limits for successful incubation and males mostly develop at mid-range temperatures, although some females may also develop in mid-range temperatures (snapping turtles, leopard geckos, crocodilians).

Egg incubation typically requires high humidity; however, incubation appears to be less sensitive to hydric effects than temperature effects (Du and Shine, 2008; Ji and Du, 2001). Humidity can be controlled by placing eggs directly on a moistened medium (e.g., 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 reliable peer-reviewed sources should be consulted as part of the breeding SOP.

Eggs that are in direct contact with a moist medium must be monitored for mould. Suspending eggs above a moisture source (known as a suspended incubation medium) reduces the risk of mould formation and provides more homogenous humidity conditions. 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. 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.

5.6 CARE OF OFFSPRING

Incubation temperature can affect thermoregulation in neonatal reptiles, at least in the short term (Goodman and Walguarnery, 2007). The enclosure should provide an environment that supports thermoregulation through the inclusion of a thermal gradient that is based on the species' natural history, geographical ranges, and monitoring. Supplementary heat sources, possibly at different temperatures than for mature animals, may be required. Due to their small size and mass, neonate and juvenile reptiles are susceptible to temperature and humidity shifts, and these parameters must be closely monitored.

Some species may engage in parental care (e.g., thermoregulation of the newborn (Alexander, 2018)); however, there is limited information about the importance of the social environment during the development of many species. The natural history of the species and experts experienced with housing the species should be consulted regarding the risks of cannibalism and mitigation measures, such as providing sufficient refuges to allow the escape of aggressive advances or not housing young with adult animals.

For herbivorous species, consideration should be given to whether juveniles require inoculation with symbiont-fermenting anaerobes (Morafka et al., 2000) for the efficient digestion of plant matter; these bacteria are frequently transferred via coprophagy.

The level of yolk reserves for post-partitive nutrition (lecithotrophy), which influences the time of first feeding, differs among species. 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 and 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 that is 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 be carefully observed. Uneaten food should be promptly removed to prevent rot and potential damage to juveniles from live prey. It may take several attempts of foods and feeding techniques 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. However, the risk of cannibalism and the ability to monitor feeding and growth must be assessed. A large enclosure with plenty of hiding places and visual barriers and a mixture of thermal, moisture, and light environments is beneficial for juveniles. Aggressive encounters may 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-tongued skinks).

While some conspecific interaction may be beneficial, care should be taken to ensure that animals are not overcrowded, as this can be a source of stress. Measures to prevent overcrowding should be taken, such as consulting the literature and those with experience breeding the species to determine optimal density, and the animals should be monitored.

Although much of their care is similar to that of adults, juveniles require more monitoring. Juvenile enclosures should contain the same furnishings as for adults, plus additional hides. 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. Care should be taken to select food items that are appropriate for the size of the animal; for example, lizards require foods of a size that is less than the diameter of their pelvis. Some juvenile lizards, such as bearded dragons, may eat larger prey items if offered, and these 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. The concentration of vitamin D_3 in juveniles is affected by maternal reserves and the optimum concentration is 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. Facility-specific SOPs must be developed for the husbandry of each species.

6.1 IDENTIFICATION

All animal enclosures should be clearly marked (as described in Section 2, "Identification of Animals", of 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 scientific activity 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 shed skin may be attached to records to assist in recognizing individuals. When individual reptiles cannot be recognized visually, it may be necessary to mark them. To overcome the issue of ecdysis and acute shedding, the external marking must either involve morphological changes to the skin (i.e., scale transplant) or be repeatedly applied. Microchipping can be used 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 these markings fade over time and can be re-applied as necessary. Turtles and tortoises may be temporarily marked with nail polish on their shells. The use of more invasive marking methods, such as ventral scale clipping (snakes) or shell modification (turtles and tortoises), must be justified to and approved by the animal care committee.

6.2 ANIMAL OBSERVATION

As mentioned in Section 3.2, "Personnel", reptiles must be observed regularly, and in most cases at least daily, by trained personnel to ensure the health and welfare of the animals. In all cases, there must be daily observation of the containment of the animals, suitability of the environment, and appropriate functioning of life support systems. The frequency of observation should be described in an SOP for each species. This should involve direct observation of the animals and their behaviour with minimal disturbance, as well as food and water consumption, soiling of the environment, and the status of environmental systems. Any unusual odours should be investigated.

The frequency of direct animal observation may be influenced by the expected physiological state; for example, animals in torpor or brumation may require less frequent observation. For reclusive species, disturbance to the animals caused by digging them up or removing cover to facilitate observation must be balanced with confirming their health. Some species should be disturbed minimally when in post-prandial or other relevant stages. Observation may be enhanced using video cameras and all visual angles (e.g., observing from the sides or bottom of the enclosure) when enabled by the type of enclosure. Reptiles tend to hide in the same place, which can facilitate quick observation of the animal.

6.3 HOUSING MANAGEMENT

The natural history of the species must be considered when deciding whether to provide conspecific social interactions. Most reptile species are solitary, and for these species, mature animals are best kept individually or in pairs. Social organization occurs in some species (reviewed by Gardner et al., 2016), and these species benefit from housing with conspecifics. 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).

Males of almost all reptilian species are aggressive to other males, especially during breeding; as a result, co-housing male reptiles is rarely encouraged, and any co-housed animals must be sexed appropriately. However, females placed together may also show antagonistic behaviour.

Any cohort of group-housed animals must be monitored for potential aggression or adverse behaviours, and to ensure the health of all animals in the group and the appropriate distribution of resources for each animal. During feeding time, group-housed snakes must be observed until the food is either consumed or removed.

When multiple species are maintained in the same enclosure, they should have similar morphology and needs and not be susceptible to cross-predation. Increased vigilance by animal care personnel is required when housing different species together, especially during and immediately after feeding.

For species that benefit from social interactions, single housing should be appropriately justified. The duration for which the animal will be maintained should be considered when deciding on single or group housing. See Section 6.6.1, "Social Enrichment", for information on enriching the environment of singly housed animals.

The protocol author must ensure that the requirements for housing the animals have been fully researched and can be met by the animal facility before the animals' arrival.

6.4 FEEDING AND NUTRITION

Each animal's diet should be based on the nutritional requirements of the species and life stage.

6.4.1 Food and Feeding

The environment and characteristics of the food item affect feeding behaviour. Environmental parameters, including heat, light, and ultraviolet B, directly affect reptile metabolism and the animal's ability to obtain and digest food. Feeding responses may be triggered by chemoreception, reflexive hunting responses (i.e., feeding in response to the movement of prey), 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; thus, reptiles must be provided with a good diet as soon as they are procured. To reduce potential aggressive behaviours in the home enclosure associated with offering food items, a transfer box can be used as a feeding box.

Measures of nutritional status should be recorded, 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 nutritional problems can be identified early. During brumation, food intake and output of excretory products are reduced or completely stopped. The literature and species experts should be consulted to develop individual scoring systems.

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 retard growth, while excessive nutrition can cause obesity (particularly for lizards) 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 activities or scientific procedures. Gross body condition can be evaluated based on a simple scoring system (e.g., 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 differences due to sex, season, and housing conditions should be accounted for.

Whether an animal receives the required nutrients depends on: 1) the composition of the food items, 2) acceptance of food items, 3) the extent to which food items are digested, and 4) the nutritional 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 of the enclosure, and hence the animal, which can have an impact on the digestibility of food and 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
- chemoreception, which occurs through the vomeronasal system and triggers feeding responses

Reptiles that have been treated with antibiotics or deworming agents should be provided with probiotics or the feces of a healthy conspecific, as necessary, to recolonize their gut flora and fauna.

Many species grow more quickly in captivity with appropriate nutrition than under natural conditions; this could be due to the 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.

Some reptile species are highly specialized as herbivores (eating plant material, such as leafy greens and fruits), carnivores (consuming animal material, such as fish, birds, mammals, and some arthropods), or insectivores (consuming insects), while others are omnivores (consuming both plant and animal material). An animal's dietary preference can change as they mature due to nutritional needs and physiological restraints. For example, large carnivores (e.g., monitor lizards) change from consuming other lizards and insects to consuming mammals as they mature, and some small lizards and yellow-bellied sliders that are primarily herbivorous as adults consume insects or other animal material as juveniles. 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 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. For insectivores, calcium, carotenoids, vitamin E, and other limiting nutrients can be provided by feeding supplements to insects (gut loading) for several hours before feeding (Latney et al., 2017). For herbivores, calcium can be sprinkled on food items (palatability should be monitored; for example, see Boyer and Scott, 2019), and for carnivores, calcium can be provided by offering whole prey or prey portions with bones. 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 D3 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. Information on calcium-to-phosphorus ratios is available for common species and should be incorporated into SOPs before obtaining animals.

For some species, fasting is part of their normal life cycle. If an animal is not eating, the veterinarian and species experts should be consulted, and consideration should be given to the animal's life cycle, weight, body condition, etc. to determine if it is a concern.

Food items can be wild-sourced or commercially raised (e.g., leafy greens, fruits, vegetables, insects, earthworms, or other prey) or manufactured (i.e., pellets). If using wild-sourced food items, there must be assurance that they are free of herbicides, pesticides, or other potentially toxic compounds, and are from a reputable source. All vegetable material should be rinsed prior to feeding to reduce parasites, toxins, fecal contaminants, etc. Where possible, prey should be frozen and thawed before feeding to reduce the risk of parasites. 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 good health and reproduction.

If thiaminase-rich prey is offered, thiamine (vitamin B_1) 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).

If 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 on a breeding protocol or transferred from another protocol within the institution, 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 institution. Different presentations of dead 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 must only be used if specifically approved by the animal care committee for a predator reptile that is in a life-threatening condition and there is no viable alternative. The animal care committee should consider the species and age of the animal and the attempts that have been made to introduce alternative feed. If live vertebrate prey is used, feeding must occur under strictly controlled circumstances and be closely monitored to ensure the health of the reptile and minimal negative welfare impacts for the prey. Exposure to the prey must elicit a near-immediate strike and ingestion by the reptile. If this does not occur, the prey should be removed and humanely killed.

After feeding, all remaining food items should be removed from the enclosure to prevent fouling and injury to the reptile if live prey is used. Even small prey such as crickets can injure larger reptiles. Food items should be consumed or removed if an animal's metabolism is expected to slow during periods of 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 because their needs for vitamin D intake are not defined (Baines et al., 2016). All reptile species evaluated have been shown to increase vitamin D circulating concentration when exposed to ultraviolet B (Acierno et al., 2006; Acierno et al., 2008), even for only two hours of exposure 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 the scientific activity and the morph or strain of reptile (Baines et al., 2016).

As discussed in Section 6.4.1, "Food and Feeding", nutrient deficiency in lizards can be mitigated by feeding insects that have been gut-loaded with limiting nutrients.

6.4.1.1.1 Herbivores and Omnivores

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

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, and phytate, which can have toxic effects, including inhibiting calcium absorption and the potential for calculi formation. Hence, cabbage, mustard greens, broccoli, etc., should be fed sparingly. Some specialized feeders might be able to detect harmful substances via tongue flicking; however, as a general rule, plant material that does not contain any toxins should be provided (Oonincx and van Leeuwen, 2017).

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

6.4.1.1.2 Carnivores

Most carnivorous lizards can be acclimated to feed on dead prey. The diet of carnivorous lizards often consists of a variety of dead vertebrate prey such as mice, rats, or one-day-old chicks. The nutrient composition of prey differs with species, size, and maturity. The consequences of differences in dietary intake for lizard health and fertility are currently unclear (Oonincx and van Leeuwen, 2017). For carnivorous species, offering whole prey is usually sufficient to cover vitamin A and D requirements.

6.4.1.1.3 Insectivores

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

Lizards are a very diverse suborder, and their diets are often poorly understood; thus, there may be very little reliable baseline information available.

Studies that compared the suitability of different insect species as food for insectivores are scarce (Finke, 2015). One study found that providing only mealworms to Western fence lizards or leopard geckos led to more obese animals than providing only crickets or mixed diets (Gauthier and Lesbarrères, 2010). Active insect species (e.g., house crickets) are often better accepted than passive species (e.g., mealworms), and they can provide environmental enrichment by increasing the insectivore's 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. Many captive-bred insects have a lower level of carotenoids, which 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 and Feeding", nutrient deficiency in reptiles can be mitigated by feeding them 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 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 their natural foods ranging from soil-dwelling invertebrates to fish, birds, lizards, rodents, and comparatively large-sized mammals. For most snakes, prey is usually swallowed whole (see Jayne et al. (2018) for examples of exceptions). Those caring for snakes must consult the literature, experts on the species, and the veterinarian to determine the most appropriate diet, as some diets, such as a fish-only diet, can lead to deficiencies in some species.

Most snakes commonly held in captivity will accept dead prey, which should be offered at the prey's normal body temperature. All vertebrate prey should be humanely killed before being fed to snakes. Snakes are 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 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 negative welfare impacts 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.

Snakes should not be force-fed without justification based on the animal's health (e.g., anorexic snake) and expert involvement.

Some snakes brought into the laboratory from the wild will either not feed at all or will feed only under conditions of total isolation where feeding cannot be observed. Various presentations of dead 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 the snake's ingestion of particulate substrate during feeding by using a substrate that will not affect the gastrointestinal tract if ingested or by using a clean dish, a solid surface, or a designated feeding habitat (e.g., feed box). These designated feeding habitats can also reduce aggression in the home environment and enable monitored feeding of co-housed animals. The use of feed boxes maintains cleaner home environments; however, feed boxes may not be suitable for all species, particularly nervous animals or those otherwise reluctant to feed.

Offering dead prey items at the normal body temperature of the prey is particularly important for snakes from the families Boidae and Viperidae, which 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 to locate endothermic prey in the dark. Although colubrid snakes do not have heat pits, they often require whole-carcass, mammalian prey items to be warmed to ensure proper ingestion. If frozen rodent carcasses are used, they should be thawed dry at room temperature or in cold water to prevent disintegration then 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 or regurgitation.

For many snakes, mimicking the natural movement of the prey may be required to stimulate predatory and striking behaviour. However, captive snakes may respond adversely to any stimulus, including the movement of a food item, 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 they have food in their mouth. In some instances, food is more readily taken when the room is dark. Of the rattlesnakes found in Canada, the massasauga (*Sistrurus catenatus*) is among the least irritable and usually feeds readily in captivity.

Individuals may be acclimated to eating dead prey in stages: 1) movement of a warm carcass to mimic live prey; 2) presentation of a warm, non-moving carcass; and 3) if possible, presentation of a non-moving carcass at room temperature. Scenting the food item to mimic native prey may increase acceptance and may be required for some species. Transitioning from live to dead prey must be done under supervision.

Species that feed on ectothermic animals (e.g., coral snakes (*Micrurus* sp.) which have been successfully raised on an alternative fish-based diet (Chacón et al., 2012)) should usually be offered dead prey at room temperature.

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 dead prey, with whole fish being preferred, but pieces of fish fillet, liver, and meat, and manufactured pellets or food gels are also generally accepted.

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 these deficiencies are difficult to correct once they become established, and the problem should be avoided by providing appropriately balanced diets, either as commercial diets or whole prey items 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). Vitamin A deficiency can be prevented by feeding vertebrate prey whole (as opposed to dehydrated *Gammarus* shrimp and other invertebrates) or by including pelleted food that has been supplemented with vitamin A.

The diet of aquatic chelonians can be supplemented occasionally with earthworms, fruit, vegetables, or alternative protein sources to enhance variety. However, earthworms must be of an appropriate source and species, as they can be a source of disease or toxic (e.g., they may contain pesticides if obtained from farm fields or lawns).

6.4.1.4 Terrestrial 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, limited soft fruits, and manufactured food pellets. Mealworms (*Tenebrio* spp.) and other adult and larval insects are acceptable dietary components for *Terrapene* and likely for other terrestrial 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 and Feeding").

The nutritional needs of the species should be known, and animals should be provided with appropriate food items and quantities to maintain health. Terrestrial tortoises are prone to overeating, and their diet must be monitored carefully to ensure appropriate growth rate and body condition. Herbivores often have a requirement for fibre that they digest via endosymbionts; however, this is species specific. For example, red-footed tortoises prefer diets high in fibre (e.g., pineapple and dandelion greens), while desert tortoises select foods with higher protein and magnesium content and avoid foods high in fibre.

6.4.1.5 Crocodilia

In Canada, crocodilian species are rarely held in laboratory animal facilities. As for snakes (see Section 6.4.1.2, "Snakes"), crocodilians should be fed whole, killed prey to provide sufficient dietary calcium. Caimans (*Caiman* sp.) and American alligators (*Alligator mississippiensis*) will readily feed on chunks of meat and fish presented using a long pair of forceps or on dead prey placed in the water. Even when the food is seized out of the water, crocodilians will not eat 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; it should be checked regularly if there is the potential for variability in dissolved components. Domestic water supplies may be used; however, water that is heavily chlorinated should sit overnight to degas. The use of chemicals to dechlorinate water is not advisable. Where municipal water has been treated with chloramine, a carbon filtration system should be used for dechlorination.

Water should be lukewarm to prevent changes to the reptile's body temperature. Many snakes are attracted to fresh water and will not drink "stale" water (AZA, 2009). Some reptiles that are accustomed to licking condensation from leaves or obtaining water from their food may not drink from dishes of static water. For these animals, spraying vegetation or using a drip, recirculating waterfall, or fountain system may be required.

Water bowls should be checked and cleaned daily when the reptile is active, as the water may also be used for soaking and defecating. During brumation, weekly water bowl checks are generally sufficient.

Water bowls should be heavy (e.g., ceramic, glass, or heavy plastic) to prevent animals from overturning them. For some animals, water bowls should be large enough to allow them to enter and submerse themselves, with some species needing to be able to immerse themselves completely (e.g., corn snakes, see (Hoehfurtner et al. (2021)). However, this is specific to the life history of the animal; for some species, a large water bowl would not be appropriate, as it would contribute to undesirable changes to their environment, such as increased humidity.

Tortoise and box turtle enclosures must have 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. Chinese and Indonesian box turtles (*Cuora* spp.) are more aquatic in their habits and must have deeper water for soaking and feeding (Kaplan, 2014).

6.5 SUBSTRATE

6.5.1 Terrestrial Environments

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). The selection of the most appropriate substrate material depends on the needs of the 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*), burrow in loose sand.

Possible substrates for enclosures include paper (e.g., cage-pan lining paper), wood chips or coarse shavings, corncob, coconut fibre, carpet, tile, paper towel, or more natural substances such as dirt (topsoil), sphagnum moss, and sand. If using wood, highly aromatic species such as cedar and pine should not be used as they can cause respiratory irritation (Rossi, 2019). Fine sawdust and other small particles should be avoided as there is a risk that reptiles, snakes in particular, may ingest substrate particles with their food, which can cause serious mouth or internal injuries and bowel obstruction. Where a solid substrate needs to be used for burrowing reptiles, a shelter box that simulates the darkness of a burrow should be provided.

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

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 importance, materials should be autoclaved.

For species that spend much time in the water bowl, a carpet surface keeps the animals dry, allows traction for movement, and is easily sanitized and replaced.

Due to the difficulty of observing animals that are in burrows, substrate burrows are frequently replaced with shallow hide structures. Some species, such as tortoises, are powerful diggers and require appropriately strong or reinforced enclosure bases.

See Section 2.2.3, "Furnishings", and Section 2.2.4, "Terrestrial Holding Systems", for guidance on furnishings.

6.5.2 Aquatic Environments

Species whose natural habits include burrowing into silt or sand should have a similar substrate in the bottom of their enclosure, provided it can be periodically changed or cleaned to maintain appropriate water quality.

Aquatic turtles should be able to access a basking area easily, as described in Section 2.2.6, "Aquatic Reptiles". Flat stones, floating docks, or rafts may be used, with floating rafts 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, but wood is not appropriate as it is susceptible to deterioration and tannin leaching. If stones are used, they should be of appropriate size: heavy stones pose safety issues related to their removal and cleaning, and small stones can be a hazard to animals if they are stacked and collapse or are ingested by animals (e.g., turtles and juvenile crocodilians).

When selecting substrates and furnishings, species-specific natural environmental features such as marshes, vegetation, and shallow water zones should be considered in addition to basking areas.

6.6 ENVIRONMENTAL ENRICHMENT

Guideline 13

Environmental enrichment relevant to the species and life stage should be provided, and the reaction of the animal should be monitored to ensure there are welfare benefits.

Environmental enrichment aims to improve the welfare of animals in captivity by maintaining their welfare and activity levels and promoting species-specific behaviours that are similar to wild conspecifics (Bostock, 2001). It includes a multitude of techniques that are aimed at positively engaging the animal in their environment and providing appropriate social interaction (Shepherdson, 2001).

Optimal environments provide reptiles with the opportunity to: 1) live in conditions that meet their needs for good health (Varga, 2019; Cooper, 2010); 2) perform a range of activities that they are motivated to perform and that provide positive welfare benefits (Kuppert, 2013); and 3) have a measure of control over their environment. While the natural history of an animal can provide information to promote positive welfare, some natural conditions would contribute to poor welfare in captivity (e.g., conspecific aggression, large fluctuations in temperature and humidity, and periods without food or water). Additionally, focusing on replicating natural conditions, rather than addressing the specific needs of the animals, can lead to important conditions and behaviours not being met (Fabregas et al., 2012; Alligood and Leighty, 2015).

Creating environments that allow for the expression of a diversity of behaviours that are part of proper biological function may not be providing enrichment; rather, it is simply good husbandry (Warwick et al., 2013). However, providing a range of additional opportunities for animals to exhibit behaviours that result in positive welfare benefits would be considered environmental enrichment. Due to the diversity of species and low use of reptiles in scientific activities, there is limited knowledge of enrichment for reptiles. It is the responsibility of the institution to continually evaluate and, as appropriate, incorporate, new evidence-based methods to enrich the lives of captive reptiles (Rosier and Langkilde, 2011).

Environmental enrichment strategies must be evaluated to ensure that they are not detrimental to the individual animals. Appropriate enrichment items must be carefully selected for each species and for the individuals. Incorrect enrichment application can cause injuries or death (Hare et al., 2008); for example, snakes can become trapped and injured in hiding retreats with inappropriately sized entrances. Enrichment studies should evaluate behavioural, 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. Structural enrichment is the most studied form of enrichment for reptiles (de Azevedo et al., 2007; Eagan, 2019). Section 2.2.3, "Furnishings", provides a starting point for physical additions to enclosures.

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 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), and 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) are able to cope with minor changes in their environment (Holding, 2011) but find significant changes to their environment stressful (Heiken et al., 2016).

Different forms of enrichment stimulate different aspects of the senses (Eagan, 2019); therefore, reptiles should be provided with a variety of forms of enrichment. Some lizards and turtles have been shown to be capable of learning, particularly in the context of their sensory abilities and behavioural repertoires (Burghardt, 2013), and cognitively complex housing should be provided to allow choice and control over the environment. Where scientific activities limit the type of enrichment, pictures (Wilkinson et al., 2011, 2013; Frohnwieser et al., 2017) and models (Frohnwieser et al., 2016, 2018) can elicit an appropriate response in some reptiles.

Reptiles are olfactory animals and providing olfactory experiences can increase exploration (Clark and King, 2008). For example, prey-scented items stimulate the exploratory behaviour of rattlesnakes for an extended period (Kuppert, 2013), and providing rat snakes with enrichment appears to result in animals that are 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 can increase exploration and behavioural diversity (increased movement, manipulation of the object, and tongue-touching) (Bashaw et al., 2016).

Food enrichment allows reptiles, which are natural hunters and browsers, to engage in active food searches (Kuppert, 2013). Staggering and randomizing food availability can provide enrichment by increasing the amount of natural behaviour due to the unpredictability of the food source. Scattering feed in multiple locations also promotes activity and increases hunting difficulty, particularly in an enclosure with a complex physical environment (Januszczak et al., 2016).

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

When providing live insects as food, they must be in moderate quantities so that animals are not overwhelmed, and they must be provided sufficiently early in the day to ensure basking and digestion can occur. For insectivores, such as lizards, feeding devices containing crickets are a preferred enrichment option (Bashaw et al., 2016).

As noted in Section 6.5, "Substrate", digging is an important behaviour for some reptiles, and they must be provided with a substrate. For these species, providing a variety of substrates, either as the enclosure substrate or in "dig boxes" that can be added to enclosures, may provide enrichment and encourage digging behaviour.

6.6.1 Social Enrichment

Reptiles are frequently considered non-social animals; however, that is not the case for all, and even those considered non-social may still use social cues and information. A review by Doody et al. (2013) indicates that some reptiles engage in some social interactions, particularly in relation to parental care.

As noted in Section 1.1.2, "Social Interactions", social cognition has been demonstrated in some species of reptiles. While single housing avoids potential aggression or harassment, it removes the possibility of social enrichment. When single housing of social species is justified, alternative forms of social enrichment should be provided, such as images or videos of other individuals, and the reaction of the animal should be monitored to ensure there are no aversive reactions or other detriments to welfare. Images should be placed outside the enclosure to allow animals to choose to approach or avoid them. Alternatively, additional physical enrichment could be provided to help compensate for reduced cognitive challenges that result from single housing.

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. 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 and enrichment practices.

6.7 HUMAN CONTACT AND HANDLING

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

Handling should be kept to a minimum, and precautions should be taken to avoid stress or injury to the animals. For some reptiles that are handled on a regular basis, stress may be reduced through gradual habituation. The benefit will depend on the species and purpose for handling the animal, and a species expert should be consulted.

Reptiles do not require human contact, although some species and individuals may tolerate it. Therefore, hands-on interactions should be minimized unless required (e.g., health checks, biomedical procedures).

Only personnel trained and competent in handling the species should directly interact with a reptile (CCAC, 2015). Specific training is required to care for venomous reptiles (see Section 13.1, "Working with Venomous Reptiles"). Facilities should have SOPs for handling species that are being maintained at the institution. An SOP for the emergency response to envenomation must be in place before receiving venomous 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 7.2.1, "Restraint Devices", can be utilized to transfer animals when escape or bite injury is a risk.

Many lizard species can spontaneously self-detach or drop a portion of their tail (caudal autotomy) when they feel threatened. For these species, care must be taken to ensure that the tail is not grasped or pinned. Small species of lizards, including various geckos, must be handled with extreme care as their skin is often delicate and can easily tear (Cooper, 2010).

Large lizards (iguanas and monitors) have powerful bites and can cause injury with their claws and tails. Leather gloves should be worn when handling these animals (Cooper, 2010).

Turtles can 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 not be lifted by the tail or inverted.

Training or behaviour modification allows complex cognitive functions to be exercised (Kis et al., 2015; Manrod et al., 2008) and may facilitate husbandry procedures (Augustine and Baumer, 2012; Augustine et al., 2013) while reducing stress for the reptiles (Hellmuth et al., 2012). For snakes, feeding can be used to remove a snake for the maintenance or cleaning of their enclosure.

6.8 CLEANING AND SANITATION

Reptile enclosures should be cleaned by designated animal care personnel to remove dirt and organic material. Disinfection (the killing of microbes and spores) should always be preceded by thorough cleaning, as all disinfectants have reduced functionality in the presence of organic material and proteins. Knowledgeable individuals such as biosafety officers or veterinarians should be consulted on disinfectant selection (Rzadkowska et al., 2016; Hemby et al., 2019). The type of disinfectant must be carefully selected to ensure it is not toxic to the animals (particularly for small reptiles (Cooper, 2010)) and can kill identified and anticipated organisms in the facility and in enclosures. Activated peroxides are broad-spectrum antimicrobials and are commonly used in reptile husbandry, but alternative disinfectants may be required, depending on the pathogen in question. No disinfectant should come into contact with the animals.

Cleaning schedules 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. In some herbivorous species, such as green iguanas (Iguana iguana) and some tortoises, juveniles require the ingestion of adult feces to achieve appropriate gut flora (Morafka et al., 2000). For these species, standard enclosure cleaning should involve removing feces, spot cleaning the cage floor, and cleaning enrichment items of feces and debris, but enclosures and items should not be sterilized unless there are disease concerns, or it is required by scientific activities. In contrast, other species completely avoid contact with their feces, and full cage and enrichment item sterilization have no adverse effects. Waste removal and the quantity of residual waste left behind are species-specific and should be stated in SOPs. Particular care should be taken when removing waste substrate from venomous enclosures, as shed fangs passed through fecal matter pose a significant health and safety risk.

Bioactive substrates are available that include isopods and other organisms that reduce the need for bedding changes; however, they should not be used for reptiles in laboratories as the bedding is not sterile.

6.9 RECORD KEEPING

Records must be maintained as described in Section 12, "Record Keeping", of the *CCAC guidelines: Husbandry of animals in science* (2017). Individual records are required for animals that undergo treatment, scientific procedures, or breeding. Specific requirements for breeding records are detailed in Section 5, "Breeding", of this document.

7 HANDLING AND RESTRAINT

To prevent injury to the animal, appropriate handling and restraint techniques must be used. The appropriate time and method of handling will depend on the species, as different species have different vulnerabilities that must be accounted for.

Handling and restraint of snakes and lizards should be minimized during peri-ecdysis to reduce the risk of dysecdysis, and it should be 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). A regenerated tail skeleton is composed of cartilage (rather than bony vertebrae) and may differ in morphology and function compared to the original appendage.

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 the posterior weight with the other hand. Simple handling and physical restraint should have minimal negative impacts on the animal's welfare, but if this is not 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. However, due to the inherent risks and side effects of chemical restraint, it should not be used routinely in place of appropriate handling or physical restraint by competent personnel.

7.1 HANDLING

Guideline 14

Reptiles should only be handled when necessary, and handling time should be minimized.

Animal handling involves approaching and manipulating an animal in a calm and safe manner. The method and expected approximate duration of handling must be justified.

Anyone handling reptiles must be competent or work under qualified supervision. Specific training and care must be taken when handling venomous species, and appropriate tools must be available.

For some animals and situations, operant conditioning can be used to facilitate handling for purposes such as blood collection, weighing, and moving animals. Operant conditioning can be used to desensitize animals to tactile interactions. The animals should be made aware when 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 the animal and help establish reliable tactile interactions between the trainer and the animal (Hellmuth et al., 2012).

7.1.1 Free Handling

Free handling refers to handling and manipulating an animal without the use of tools; however, it should be combined with the use of tools when necessary. 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 negative impact on their behaviour. The handler must always maintain awareness and control of the animal's movements and prevent animals from moving in ways that can cause harm to themselves or the handler (e.g., maintain distance between the animal and the handler's head and neck, and monitor tail movements in large lizards, which can deliver an injurious blow). Protective gloves should be selected based on the physiological features of the animal, the context for handling, and any anatomical features of the animal that may harm the handler (e.g., fangs) or may be harmed by the handler (e.g., epithelium of the animal). When protective gloves are worn, handlers must still be able to perform appropriate handling techniques.

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

- Lizards should have their body weight supported while being handled. For many species, the limbs should be restrained. Precautions should be taken to ensure the animal's claws cannot become entangled in the handler's clothes. Pressure on the tail should be avoided to reduce the risk of autotomy if applicable to the species.
- 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, with the head also 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, with the handler's hands placed far enough away from the head to prevent bites. 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, providing the turtle's weight is not supported by the tail.

7.1.2 Snake Hooks

A snake hook is a C-shaped blunt hook at the end of a handle. The size of the hook should be appropriate for the size of the animal, and the length of the handle should be matched to the context of handling. 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.

7.2 PHYSICAL RESTRAINT

Animal restraint skills are a subset of animal handling that involve the restriction of the animal's movement for a period of time.

7.2.1 Restraint Devices

All restraint devices should allow visualization of the animal. There must be enough designated instruments and they must be adequately disinfected between uses to prevent cross-contamination.

7.2.1.1 Transfer Boxes

Transfer boxes are typically small, ventilated boxes resistant to animal strikes and forces (e.g., digging) that can be used to transport animals relatively short distances over a relatively short period of time. Transfer boxes should be designed to be comfortable to the animals (e.g., 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 a transfer box to their enclosure.

7.2.1.2 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 bag. It may be necessary to place the bag in a secondary, hard-sided container to limit the animal's movement or prevent 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.

7.2.1.3 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.

7.2.1.4 Tongs or Forceps

Tongs or forceps should be used with care as the animal's weight and the pinching mechanisms of the tool can cause injury. The tips of tongs or forceps should be padded 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 for venomous snakes. Tongs and forceps must not be used to pick up an animal by the tail.

7.2.1.5 Snake Tubes

Snake tubes must be made of clear plastic or acrylic, open on one end, and completely or partially open on the other end to allow for unimpaired breathing. Tubes 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 their head 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 open end and 50-75% of the way through the tube. The tube and snake should be grasped together to prevent the snake from moving either forward or backward.

Venomous species may deposit venom in the tube, and the snake tube must be cleaned with species-specific venom neutralization protocols at the end of every use.

Snake tubes can be used for anesthetic induction (see Section 7.4, "Chemical Restraint").

7.2.1.6 Body Socks

Mildly elastic cloth tubes, such as casting stockinettes, can be used to restrain small 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 so that the claws do not snag in the material and lead to broken toes. Body socks can be removed by cutting them open with bandage scissors.

7.2.1.7 Boards

Larger crocodilians can be secured to wooden boards or placed inside tubular structures (culverts) for transportation. Their 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, and their head must be controlled to prevent this.

7.3 VENOMOUS ANIMALS

Venomous animals should only be handled or restrained by designated personnel, and additional training that is specific to the venomous species is required for all 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 if envenomation occurs. 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 the use of appropriate restraint and transfer devices must be implemented unless sufficient justification is provided otherwise. It is helpful for handlers to clearly display a written description of the species in use (e.g., on an ID badge) in case the handler becomes non-verbal in an emergency.

All appropriate tools for handling and restraint should be prepared before starting any procedure involving a venomous reptile. Cages should remain locked until they are ready to be opened. An announcement should be made that the cage is about to be opened 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, the snake should be directed into a snake tube. This can be done on a worktable, the floor, in a bucket, or directly from a snake bag. If a snake appears dead, tongs or a snake hook should be used to test for movement. Once confirmed dead, the mouth of a dead animal should be carefully taped closed to avoid unintentional contact with the fangs (Lock, 2008). Venom may be deposited on tools and in enclosures, and they should be considered contaminated with venom until appropriately cleaned.

7.4 CHEMICAL RESTRAINT

This section focuses on the use of drugs to restrain an animal. For information on anesthetic use for painful procedures, see Section 10.9, "Anesthesia and Analgesia".

Chemical restraint protocols for reptiles are challenging due to the wide variety of species involved; different species and even different individuals respond differently to sedatives, hypnotics, tranquilizers, and anesthetics. Chemical restraint protocols for mammals are difficult to extrapolate to reptiles, and published studies of sedative protocols for reptiles should be consulted. Drugs used for the chemical restraint of reptiles are off-label, and a veterinarian experienced with the chemical restraint of reptiles should be consulted when planning for their use in a new scientific activity 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.

For non-painful procedures, alfaxalone and propofol are commonly used sedative agents in reptiles. Body temperature should be maintained throughout the procedure and recovery.

Isoflurane or sevoflurane are occasionally used inhalation anesthetics. While these gases induce general anesthesia, they are commonly used for anesthesia maintenance in reptiles when injection is not practical. The benefits of using inhaled volatile gases are that they are very safe, reversible, easily titratable, and can be used in a contactless chamber in non-apneic reptiles. However, these gases require specialized equipment, including oxygen, a 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 stand-alone 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. The use of small-volume syringes and small-gauge needles is advised.

Guideline 15

Reptiles must be continually monitored during chemical restraint, 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.

To improve the animal's survival in the event of an adverse reaction to anesthesia, a method of artificial respiration, including manual bagging and the ability to quickly adjust the body temperature, should be available.

For more information on the use of anesthetics, see Section 10.9.1, "Anesthesia".

7.5 SAFETY OF PERSONNEL AND ANIMALS

The safety of both the handler and the animal must be considered during 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 should be present. Animals can potentially be injured by inappropriate handling techniques and by inappropriate contact with elements in their environment. Handling and restraint of venomous animals requires extensive training in addition to specific procedural and infrastructure requirements (see Section 7.3, "Venomous Animals").

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 handler with a compromised immune system or other health concerns should consult the institutional health and safety officer or equivalent individual to identify appropriate personal protective equipment.

8 HEALTH AND DISEASE CONTROL

Guideline 16

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* (CCAC, 2017)
- adherence to the Canadian Association of Laboratory Animal Medicine (CALAM) *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
- a response plan for a potential infectious disease outbreak; execution of the plan should limit disease propagation and spread until the outbreak is confirmed, the pathogen is identified, and a veterinarian can determine pathogen-specific measures (see Section 8.3, "Disease Management in the Event of an Infectious Outbreak")

8.1 DISEASE PREVENTION

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.

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. A veterinarian should also 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 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 a 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”, and Section 3, “Facility Management and Personnel”).
- Husbandry – Reptiles should be fed a high-quality diet, and practices should be in place for the effective sanitation and prevention of overcrowding (see Section 6, “Husbandry”).
- Biosecurity for the animals – SOPs should limit access to the animal facilities (see Section 3, “Location”, and Section 10.3, “Security System”, of the *CCAC guidelines: Laboratory animal facilities* (CCAC, 2024).
- 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, with a disease prevention strategy.

All of these components should be included in the disease prevention and control program. Incorrect husbandry accounts for the majority of diseases in captive reptiles (Suedmeyer, 1995).

8.2 HEALTH MONITORING AND DISEASE DETECTION

Guideline 18

Standard operating procedures 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 (see Section 9, “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 length of time the animals are held; the type of scientific activities; and the potential effects on other animals in the facility and on scientific activities.

The selection of agents to test and evaluation procedures (including test intervals and verification) 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 to ensure testing methods and samples are appropriate. Health monitoring programs may include the use of environmental monitoring of both the room (e.g., temperature and humidity) and the enclosure. 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 associated SOPs should be updated regularly and in response to any increase in the prevalence of diseases for particular species or new information on reptile health. The literature should be reviewed by the veterinarian 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. 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 to expedite 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. Quarantine or isolation procedures must be implemented when an infectious condition is suspected, to prevent pathogen spread while diagnostic testing occurs.

Any animal health concerns or other potential animal welfare issues must be 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 Divers and Stahl (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).

Several reptile species will commonly harbour opportunistically pathogenic organisms (e.g., a small number of pinworms 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 them that may result in suppression of their immune systems and increase the risk of infection (Cooper, 2010). Immune responses of reptiles are temperature-dependent and function more effectively at the higher range of the preferred optimum temperature zone, underlying the importance of providing an appropriate temperature and temperature gradient in enclosures. Some species 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 (e.g., 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 intervening. Signs of a potentially sick reptile include:

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

Common categories of reptile diseases or conditions include:

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

Common lizard-specific diseases or conditions include:

- dysecdysis
- hepatic lipidosis
- periodontal disease
- thermal burns

Common turtle-specific diseases or conditions include:

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

Common snake-specific diseases or conditions include:

- blister disease (related to excessive humidity, with subsequent infection by bacteria)
- cloacal scent gland adenitis
- dysecdysis

- ophidiomycosis (snake fungal disease)
- snake cryptosporidiosis
- 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 reoccurrence. This plan should be developed in anticipation of an outbreak, with flexibility for modification based on the nature of the disease or outbreak. The plan should include a communication strategy involving veterinarians, veterinary and animal care personnel, investigators, the facility manager, and the animal care committee. Restrictions on the use of animals in scientific activities and animal movement and contact during a disease outbreak should be described in the plan. Access to quarantine facilities or a means of isolating animals must be available (see 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 or depopulation, will depend on the nature and extent of the outbreak, the health status of the animals, and the types of scientific activities. If infected animals are to be euthanized, proper containment measures must be in place for handling and disposing of the animals and the contents of the enclosure, and for decontaminating 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 the 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 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 scientific activities. Reptiles must be observed regularly; however, some animal-based measures can cause disturbance (e.g., for species that hide, digging the animals up or removing cover to facilitate observation). The welfare assessment plan must balance regular observation with the frequency of such disturbance. Combining animal observation with resource-based and management-based measures can help minimize disturbance of the animal while obtaining the necessary information to assess their welfare. Generally, animal-based measures are the best for identifying the actual welfare status of the animals but are less useful than resource-based measures and management-based measures in identifying specific causes of poor welfare.

Resource-based measures evaluate the suitability of the enclosure for the particular animal being housed. Criteria for the development of resource-based 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). Resource-based 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 on animals. Like resource-based measures, management-based measures 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, therefore, be based on the species and individual animal (for example, see Moszuti et al. (2017), Stockley et al. (2020), and Hoehfurtner et al. (2021)). The literature and experts should be consulted on potential behavioural indicators for the species involved. Preference tests can also be used to determine situations and resources that provide positive experiences; for example, see Hoehfurtner et al. (2021).

Investigators must have a good understanding of the biology and behaviour of the species they are working with in order to make appropriate assessments of their welfare. They should also be able to recognize how different factors could affect behaviour and physiological parameters in the 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 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 unrelated 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 observe behavioural nuances.

Warwick et al. (2013) provide a list of behavioural signs that may indicate captivity-related stress in reptiles, such as anorexia, freezing, death-feigning, pigmentation changes, and prolonged retraction of the head, limbs, or tail. Indicators of positive welfare are more difficult to ascertain and are often focused on evaluating enrichment (Benn et al., 2019; Eagan, 2019); however, behaviours such as exploration and normal levels of feeding activity indicate a non-stressed reptile.

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 furniture, hides, and enrichment (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 such as feeding)

    - social interactions, such as the presence or absence of aggression in a colony
- 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
    - 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 scientific activities being conducted.

10 EXPERIMENTAL PROCEDURES

Guideline 21

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

In addition to minimizing direct impacts on animals, measures should also be taken to minimize the potential impacts of procedures on other reptiles in the room, as they may be affected by the production of alarm pheromones (e.g., Mason and Parker, 2010) (see Section 2.1, "Animal Rooms and Procedure Rooms").

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 a subject matter expert, 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, the research team, and the animal care committee.

Institutions should have a policy or SOP for repeated procedures on all 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 their lifetime must be described, taking into account the invasiveness and negative welfare impacts associated with the procedures and the short- and long-term impacts on the welfare of the reptile (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

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

All procedures have the potential to cause negative welfare states. However, it can be difficult to assess these states in reptiles, and it should be assumed that a procedure that would cause these states in a mammal is likely to cause the same states in 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 who 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 procedures.

If animals are to be used for handling, they should be carefully chosen based on species, individual temperament, health, age, and sex, where pertinent.

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 used in scientific activities should be made of non-porous materials and thoroughly cleaned and disinfected between uses to minimize the possibility of cross-contamination, especially when being shared.

Guideline 22

Endpoints must be developed and must be approved by the animal care committee before the commencement of a scientific activity to minimize any negative impacts of procedures on the animals.

The *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022) states that protocol authors must establish appropriate endpoints and intervention points that are specific to the scientific activity (e.g., initiation of treatment, termination of a procedure, and euthanasia) and plans for monitoring. Investigators should consult both the veterinarian and experts on the species when determining endpoints and intervention points. Key references relevant to the scientific activity should also be consulted to determine the earliest practical endpoints.

Defining humane intervention points (i.e., clinical signs that indicate an intervention is required to humanely treat an animal by reducing negative welfare impacts) 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, personnel should be familiar with normal or expected behaviour so that they can recognize deviations. When an animal model is in development or new to an investigator, pilot studies should be performed to establish scientific endpoints and humane intervention points.

Monitoring frequency must be established based on the protocol's level of invasiveness, expected clinical (or other) signs, 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 joint endeavour involving investigators, veterinarians, and veterinary and animal care personnel. Monitoring score sheets that incorporate assessment parameters can be helpful in monitoring for endpoints.

Animals should be removed from the scientific activity when endpoints are met or when advised by the veterinarian. Animals experiencing negative welfare states that are not part of the animal use protocol and cannot be relieved 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), evolutionary development (Nomura et al., 2013; Woolley et al., 2004), and clinical veterinary research (Balko and Chinnadura, 2017; Carsia et al., 2018; Skovgaard et al., 2018).

Protocol authors should determine whether reptiles are required for the scientific activity, and if so, which species, strain, and life stage provide the best model of the biological processes involved in their work, taking into account the specific needs of each and the welfare considerations of working with those animals for the scientific activity. Animal availability is also a consideration. Animals must not be obtained until measures are in place to care for them appropriately to ensure their health and welfare. Measures to protect the animal's welfare (e.g., providing special or additional technical expertise and highly trained personnel) should be intensified as necessary, based on the procedures to be conducted.

10.2 ADMINISTRATION OF SUBSTANCES

General information concerning the administration of substances can be found in the *CCAC guidelines: Scientific procedures (Part A – Administration of substances and biological sampling)* (CCAC, 2025). This section provides additional information for reptiles.

The route of administration depends on the chemical characteristics of the substance to be administered (including absorption rate), the site of action, the potential for tissue irritation, and the practicalities of administration (e.g., Knotek, 2019; Divers and Stahl, 2019). Substances are commonly delivered intramuscularly, intracoelomically, subcutaneously, intravenously, or orally. For systemic distribution of a substance, parenteral injections are often preferred over an oral route.

For all injection routes, administration sites caudal to the kidney (e.g., hind limb) may reduce the bioavailability of substances, depending on the compound, due to first-pass metabolism (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.

The dose of substances to be administered must be appropriately scaled for the individual animal, considering their metabolic rate at the holding temperature (see Mayer, 2019).

Before the intramuscular administration of any compounds, there must be assurance that the animal is adequately hydrated and has an appropriate body temperature. There must also be assurance that the environmental temperature will be kept stable.

Intramuscular and subcutaneous injections are frequently selected because of injection site access and rapid compound uptake and distribution. However, subcutaneous injections may be technically difficult to administer to small reptiles. 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). Repeated intramuscular injections should not be carried out at the same site, especially into small muscles, as they may cause muscle damage. 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, slow administration of a small volume using a small gauge needle is advised. Subcutaneous injections can be spread over multiple sites to reduce the volume per site. Due to the low elasticity of reptile skin, injection sites should be held off by the fingers of the handler as the needle is withdrawn to prevent backflow of the administered compound.

Intracoelomic injections deliver compounds to the highly absorptive coelomic cavity. Due to the absence of a diaphragm in reptiles, volumes injected into the coelomic cavity should be limited to avoid lung compression. Compounds that cause tissue irritation should not be administered intracoelomically, as they can cause long-term damage to the viscera and fluid accumulation in the coelomic cavity.

Intravenous administration is only feasible on medium-sized and large reptiles; some intravenous catheters may require incisions and sutures for placement.

Intraosseous catheters may be beneficial in smaller reptiles and for delivering large volumes of fluid, but their use should be discussed with a veterinarian. Placement of an intraosseous catheter requires surgical aseptic technique and requires that the animal be anesthetized unless they are in shock or otherwise unable to handle anesthesia. Drug diffusion may be slower than for intravenous administration, but it is usually faster than intracoelomic administration (Young et al., 2012).

Some species may discolour their skin after any type of injection and personnel administering substances should differentiate this response from an adverse reaction.

Oral administration includes incorporating compounds into food items and manual administration of a compound or treated food items in the buccal cavity, pharynx, esophagus, or stomach (gavage). When compounds are incorporated into food items, eating must be monitored. Manual administration can be accomplished by opening the animal's mouth with rounded implements and gentle pressure, followed by compound administration with a rounded blunt object such as a blunt syringe. Care must be taken to avoid damage to the oral mucosa, as this can lead to stomatitis. A veterinarian must be consulted for the administration of compounds directly to the stomach (gavage). In all manual administration situations, the glottis must be avoided to prevent accidental respiratory administration.

For more information on routes of drug administration for reptiles, see Coutant et al. (2018).

10.2.1 Lizards

Intramuscular injections should be given in the cranial epaxial muscle or the forelimb muscles (e.g., triceps or biceps). Substances that are nephrotoxic or subject to active clearance by the kidney should not be injected at the pelvic limb. Injection at the base of the tail should be avoided for those species that demonstrate autotomy or use their 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 large lizards. The ventral coccygeal (tail) vein is the preferred site for intravenous injections for medium-sized and large lizards that do not demonstrate autotomy (Knotek, 2019). Alternative intravenous injection sites include the jugular vein and cranial vena cava.

Personnel administering compounds orally must be aware that some lizards have a reflexive increase in bite pressure when objects are inserted into their 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° angle into the middle of the muscle. Snakes should be firmly restrained to prevent them from shifting when injected. Injections in more caudal locations might be necessary when using a snake tube (James et al., 2018).

Unlike 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 the 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. Repeated intramuscular injections at the same site, especially into small muscles, should be performed with care as they may cause muscle damage and abscesses.

Compounds are commonly delivered subcutaneously at the loose skin between the neck and thoracic limbs. Non-nephrotoxic compounds and compounds with reduced bioavailability due to the kidneys may also be administered in front of the pelvic limbs.

For intracoelomic administration, turtles should be placed on their side so that the urinary bladder falls 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 should not be used for intravenous injection 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, but dorsal recumbency should be avoided as it is very stressful for the animal. One method of accessing the ventral tail vein involves 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 ventral 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 one-third down the length of the tail along 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. An alternative site for intravenous injection is the occipital venous sinus.

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

10.3 COLLECTION OF BODY FLUIDS OR TISSUE

General information concerning biological sampling can be found in the *CCAC guidelines: Scientific procedures (Part A – Administration of substances and biological sampling)* (CCAC, 2025). This section provides additional information for reptiles. Methods for sampling depend on the temperament, size, and anatomy of the reptile (see Divers and Stahl, 2019; Girling and Raiti, 2019).

10.3.1 Blood Collection

Anesthetics may be needed to restrain the animal and minimize stress. Appropriately sized needles must be selected to minimize tissue trauma, and methods for the proper visualization of blood vessels may be needed.

The impact of the puncture site on blood parameters 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. Ethylenediaminetetraacetic acid (EDTA) generally provides superior blood samples with fewer white blood cells and platelet clumping (Divers, 2019). However, blood from some species of chelonians, boas, skinks, and monitors may develop hemolysis with EDTA, and thus lithium heparin should be used as the anticoagulant.

10.3.1.1 Survival Blood Collection

Those drawing blood from reptiles must know how much blood can be drawn (Sykes and Klaphaké, 2008), as the total blood volume 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). A smaller volume of blood should be taken if the reptile is stressed, as these animals can partition body fluids and reduce 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).

Gross contaminants should be removed from reptiles' skin, and the site should be disinfected before venipuncture, as the skin's inelasticity creates the potential for pathogens to enter (Eatwell et al., 2014).

10.3.1.1.1 Lizards

The ventral coccygeal 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). 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 small lizards such as geckos, in lizards with short tails, or when the ventral tail vein does not provide a sample. Lizards have large abdominal veins just under the skin, along the ventral midline. These veins are easily visualized but are easily damaged by venipuncture (Divers, 2019). Care should be taken not to puncture any underlying coelomic organs.

The jugular vein may be used for venipuncture in lizards such as iguanas, monitor lizards, or chameleons (Eshar et al., 2018). 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 is 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 a safe venipuncture site in geckos when it is applied in a shallow manner (Mayer et al., 2011; Cojean et al., 2020). A 27-29-gauge needle may be inserted at a 45° 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 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; 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 scale widths 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, depending on the competence and preference of the personnel (Isaza et al., 2004; Brown, 2010; McFadden et al., 2011). For cardiac puncture, the snake should be restrained on their back. The heart is located in the cranial third of the body. It 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° 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 for blood sampling, particularly in 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 than for other sampling methods. The vein can generally be occluded at the base of the neck or by restraining the animal in a 30°-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).

In large turtles, the brachial vein (or brachial venous sinus) in the forearm can be used; however, lymph fluid contamination is common (Divers, 2019). This site is located near the shoulder joint caudal aspect of the humerus.

In large species, the dorsal coccygeal vein can be sampled from the dorsal midline of the tail, but the position of this vessel varies between species. There is a significant risk of contamination with lymphatic or cerebrospinal fluid (Divers, 2019) and potential hemodilution at the coccygeal site; therefore, sampling from the brachial vein is preferred (López-Olvera et al., 2003). However, the dorsal midline may be advantageous in large tortoises (such as *Centrochelys sulcata*) as it is accessible in conscious tortoises, provided the tail can be withdrawn from under the carapace. This technique can also be useful for 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 along the midline, with the needle pointing dorsally.

The subcarapacial plexus should not be used due to common adverse effects, including hind limb paralysis and accidental intrapulmonary injection (Innis et al., 2010; Coutant et al., 2018). If its use is justified, the animal must be monitored, with prompt investigation of any changes in respiration, and adequate antinociceptive drugs 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", and 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 must 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 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. See Divers (2019) for a description of the method.

10.3.3 Tissue Biopsy

Tissue biopsy should be treated as a surgical procedure using aseptic technique, analgesia, and wound closure as appropriate. Certain types of research, especially those 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 adopted 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). If the implant is surgically attached to the body wall to prevent expulsion via the alimentary tract (Bryant et al., 2010), aseptic technique, analgesia, and wound closure should be used as appropriate.

10.5 PROCEDURES FOR GENETICALLY MODIFIED REPTILES

Genetically and phenotypically unique reptiles can be created through genetic modification or specialized breeding programs. The introduction of technology that permits gene editing directly in the embryo has led to an interest in the genetic modification of a wide variety of taxa, including reptiles (Nomura et al., 2015; Rasys et al., 2019). The selection of methods to generate new genetically modified strains must include the application of 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 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, in 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 continually developing programs for specific phenotypes, including colouration or pattern and scale variation (e.g., scaleless bearded dragons and snakes). Any special needs of the animals produced must be addressed (e.g., special husbandry needs), whether the altered phenotype is acquired through genetic modification or manipulated breeding (e.g., bearded dragons with reduced scalation lose water faster (Sakich and Tattersall, 2021)).

Reptiles used 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 genotyping method. For example, shed skin can frequently supply sufficient genetic material for genotyping snakes and lizards, with no stress to the animal; however, the shed material must be compatible with the genotyping method employed (e.g., 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 should 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 information related to their welfare should be given to the animal care committee as soon as possible. Stable germ-line transmission does not 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 any problems with genetically modified strains.

Genetically modified reptiles may respond differently to drugs, food, and some experimental conditions compared to animals of the same species that have not undergone genetic modification. These changes 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 drug testing or toxicity studies.

10.6 IMAGING

Imaging includes 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, the procedures create numerous occasions for animals to be stressed. Factors to consider when imaging reptiles include repeated injections, anesthesia, handling, transportation, experimental conditions (e.g., tumour burden or surgery), and fasting (Cojean et al., 2018; Williams et al., 2019a). These factors should be addressed in relation to both the welfare of the animals and the validity of the imaging results, with particular attention to the number of times and frequency of imaging, given the significant impact of repeated anesthesia on the physiology of an animal. For serial imagery, animals must be 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, this must be balanced with the inherent risks 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

Behavioural studies on reptiles include mating and courtship, social behaviour, feeding behaviour, environmental preference, and predator/prey behaviour. To achieve valid and interpretable outcomes of any behavioural testing regime, animals should be healthy, have a good welfare status, and be well-acclimated to the housing environment. The literature should be consulted for guidance on behavioural studies (e.g., Martin and Bateson, 2007).

When possible, a reward strategy (e.g., highly preferred food) must 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 may be adapted for some scientific activities and species.

Aversive stimulation (e.g., shock) and deprivation or restriction of resources (e.g., food) must only be used when there is no alternative. Such procedures require strong scientific justification to be provided to the animal care committee. If aversive techniques are approved, they must be used in the least invasive fashion and for the shortest duration possible.

10.8 FOOD AND FLUID INTAKE REGULATION

Food and fluid regulation (e.g., for metabolic studies or as part of operant conditioning) requires in-depth knowledge of the species and individual animal physiology. Aggressive searching for food may cause distress to animals, and reptiles may stop eating if food restriction is too severe. Anorexic reptiles may not recover when they start eating again due to the metabolic demands of food digestion. In some cases, the provision of smaller food items more frequently is preferred to completely fasting animals.

Scientific activities involving food or fluid intake regulation require the establishment of humane intervention points (CCAC, 2022) such as body condition, skin tenting, or consistency of saliva, depending on the species, and close monitoring of the animals (see Section 9, "Welfare Assessment"). Given the large spectrum of requirements for reptiles in terms of hydration, feeding, and digestion, those using reptiles must know the individual animals they are working with. The digestive system of reptiles is considerably different from that of warm-blooded animals, and reptiles do not eat as regularly, 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. The rate at which an animal loses weight is highly variable among species. If consistent weight loss is observed, the frequency of monitoring should be increased.

10.9 ANESTHESIA AND ANALGESIA

10.9.1 Anesthesia

Guideline 23

Anesthetics must be used in procedures where there are expected to be noxious stimuli and in experiments requiring extensive handling or manipulation with a reasonable expectation of trauma and physiological damage to the animal.

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 between reptiles and mammals and affect drug action in reptiles during all phases of anesthesia.

A veterinarian must be consulted for the appropriate anesthetic regimen for the reptile of interest. Within and between species, the rate at which anesthetics take effect can vary greatly, depending on the animal's body temperature. Animals should be maintained within their preferred optimal temperature zone; within this zone, the effects of the anesthetics are better understood and managed (Mans et al., 2019). Anesthetic induction and recovery are frequently longer processes in reptiles than in mammals and must be appropriately planned for, particularly the recovery phase.

There is no evidence to support cold narcosis (via hypothermia) as a safe and humane method for anesthesia.

10.9.1.1 Inhalation Anesthetics

Volatile anesthetic gases are useful for prolonged procedures or if a rapid recovery is desired. Isoflurane with oxygen as a carrier gas is the most commonly used inhaled anesthetic for reptiles due to the availability of data and the safety of the animals and personnel. 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 anesthetics should not be used for species capable of prolonged breath-holding (e.g., aquatic chelonians and bearded dragons).

Intubation is easily achieved for most species of reptiles due to their highly pronounced glottis. Intubation is required for mechanical ventilation and may be required for prolonged procedures. A veterinarian should be consulted when considering intubation and mechanical ventilation. Due to the anatomical features of the reptile heart, 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 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, a gas vaporizer, delivery circuits, gas scavenging, and appropriate ventilation for human safety. Inhalation anesthetics should not be used without all the necessary equipment in place and should be maintained according to the manufacturer's specifications.

10.9.1.2 Injectable Induction of Anesthesia

Injectable induction of anesthesia is commonly performed with the surgical plane of sufficient duration 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 greater safety (Mans et al., 2019). Mans et al. (2019) provide a list of suggested anesthetic protocols for many species.

Propofol and alfaxalone are common injectable anesthetics for reptiles; however, neither should be used as the sole agent for painful procedures (Balko and Chinnadurai, 2017). They can be used in combination with inhaled anesthetics via a secured airway. Propofol must be administered intravenously or intraosseously. It provides rapid and secure induction, minimal accumulation from repeat injections, relatively long-lasting anesthesia, minimal excitatory side effects, and rapid recovery with little residual effect. Propofol is rapidly metabolized and is noncumulative but produces 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. Commercial preparations of alfaxalone require large volumes to be administered to obtain the desired concentration; this may make intramuscular injection challenging. Alfaxalone can be used for a brief period of sedation and restraint; for example, when blood sampling (Bertelsen and Sauer, 2011; Hansen and Bertelsen, 2013; Kischinovsky et al., 2013).

10.9.1.3 Anesthesia Monitoring and Recovery

Guideline 24

Reptiles must be continually monitored during anesthesia, from induction to 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 physiology of reptiles, including the fact that many reptiles are hypoxia tolerant. Pulse oximetry readings 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 can influence monitoring requirements for 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 the 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 to prevent increasing 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 animal from escaping.

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

10.9.2 Analgesia and Anti-nociception

Guideline 25

Following the precautionary principle, reptiles should be provided with analgesia for procedures that are likely to be painful, based on the best available scientific evidence.

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., 2019b; Perry and Nevarez, 2018). While there is debate in the scientific community on whether "analgesia" or "anti-nociception" is more appropriate for reptiles, the term "analgesia" is 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 expected to be aversive stimuli for reptiles and should be relieved by analgesia.

There is limited information on analgesia in reptiles; however, information on some analgesics in particular species is provided by Sladky and Mans (2012), Chatigny et al. (2017), and Divers (2019). Long-term use of analgesics may impact feeding behaviours, and feeding routines should be adjusted accordingly.

The route of administration of an analgesic should be based on the size and temperament of the individual reptile. The route may impact the effectiveness of the analgesic; for example, 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.

The unique preferred optimum temperature zone for the species should be known. Since reptiles are ectothermic, assisted temperature regulation must be provided during surgery. Heating pads and lights can generate considerable heat, and the temperature of any fluids used on or administered to the animal can impact cooling.

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 the sterility of the field should be maintained until the procedure is complete. 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 species, being overly aggressive with harsh disinfectants and excessive removal of outer mucous and epidermis can increase the risk of secondary bacterial colonization of the skin.

Incisions should be made between, not through, scales in relevant species. Sutures should be made with a monofilament material using an everting pattern, as reptile skin tends to invert naturally (McFadden et al., 2011).

See Section 10.9, "Anesthesia and Analgesia", for guidance on the use of anesthetics and analgesics before, during, and after surgery. 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 scientific activity 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 that supports 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 the suture material and incision sites. The recovery area should be secured to prevent animals from escaping.

Post-procedural analgesic should be provided when pain is reasonably expected. Antibiotic therapy may be necessary for some types of surgery, but it should only be provided when advised by the veterinarian, based on current practices to limit antibiotic resistance. All monitoring and post-procedural care must be documented in 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* (CCAC, 2010) apply to the euthanasia of all animals in science. This section provides additional information that is specific to the euthanasia of reptiles in laboratory settings. For guidance on the euthanasia of reptiles in field settings, see the *CCAC guidelines: Wildlife* (CCAC, 2023).

> **Guideline 26**
>
> Euthanasia of reptiles must only be carried out by competent personnel using an approved method that is best suited to the species and life stage of the animal and to the objectives of the scientific activity.

For all methods of euthanasia:

- SOPs should be developed 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 species involved and their ability to confirm the death of the animal.
- Equipment must be appropriately maintained and cleaned before use or reuse.
- Animals must not be housed with unfamiliar animals before euthanasia.
- Stress caused by handling should be minimized.
- Death should be confirmed with a secondary method, such as destruction or removal of the brain or exsanguination; death may also be confirmed by freezing for particular species and life stages (see Section 11.6, "Verification of Death").

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

If the brain is needed for a scientific activity, brain destruction can be avoided if the method of euthanasia has been well-studied in the species and has been confirmed to be reliable at the temperature at which euthanasia will be taking place. If the method of euthanasia has not been studied in the species, persistent cardiac arrest should be confirmed for at least two hours with the reptile in their preferred optimal temperature zone.

11.1 INJECTION

The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) lists intravenous injection of barbiturates as an acceptable method of euthanasia for all reptiles. Nevarez (2019) indicates that a pentobarbital injection (60-100 mg/kg) via intravenous injection is suitable for most reptiles. While barbiturate injection is the preferred approach for the 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 acceptable when other routes are not accessible (Laferriere et al., 2020). For the injection of euthanasia solutions into the intracoelomic space, the irritating nature of the compound must be determined, and dilution or buffering may be required (Nevarez, 2019).

Intracardiac administration with barbiturates should be performed on animals that are 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 a suitable route (e.g., intravenous, intracoelomic, intrahepatic, intracardiac, or intracranial).

Injection of potassium chloride into fully anesthetized reptiles is 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 the 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). In this method, diluted, buffered TMS is administered intracoelomically, and once the reptile is anesthetized, a more concentrated solution of unbuffered 50% TMS is administered intracoelomically. There appear to be substantial species differences in sensitivity, and the use of this method must be supported by species-specific evidence (Conroy et al., 2009).

T-61 is a combination of paralytic, narcotic, and local anesthetic. When used alone, T-61 can cause involuntary vocalizations and movements, and it must be injected intravenously at a slow rate (AVMA, 2020). While T-61 is used in the field for reptiles, it should only be used in institutional environments when alternatives are not appropriate.

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, inhaled anesthesia may be appropriate when followed by another method 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* (CCAC, 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 the destruction or removal of the brain.

11.4 FREEZING

Freezing is a method of euthanasia that involves a rapid drop of the animal's internal temperature to an extreme low, typically through immersion in liquid nitrogen. The use of rapid freezing to euthanize reptiles must be conducted only on animals less than 4 g and when specific scientific justification is provided. Before freezing, animals should be rendered unconscious, either through induction of hypothermia (e.g., placed in a refrigerator) or by general anesthesia (AVMA, 2020). Once the primary method of euthanasia is complete, death should be confirmed by freezing the animal (regardless of size) to a body temperature of -15°C or lower for at least 24 hours. Freezing is not appropriate for species and developmental stages that are resistant to freezing (for examples, see Packard et al. (1999), Constanzo et al. (1995), Baker et al. (2003), and Paukstis et al. (1989)).

11.5 EUTHANASIA OF EGGS

There appears to be emerging evidence that oviparous species are conscious at hatching and during the last few days before hatching (CCAC, 2010). Animals in late incubation (the latter third) and new hatchlings should be euthanized as described above. This may require opening the shell to access the animal.

Freezing of eggs is a common method of euthanasia during early and mid-incubation (i.e., the first two-thirds), and any eggs at this stage that will be disposed should first be frozen.

11.6 VERIFICATION OF DEATH

Animals must be verified as 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) and longer for large species.

11.7 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.

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 scientific activity, see Section 4, "Procurement", particularly regarding regulations, documentation, and transportation. 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 transfer 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.

12.2 REHOMING

Where permitted by regulatory authorities (e.g., federal, 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 for the animals. 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 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

Captive-bred reptiles should not be released into the wild. The release of captive wildlife, including reptiles, is discussed in the *CCAC guidelines: Wildlife* (CCAC, 2023). 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. Reptiles that have been subject to artificial genetic manipulation must not be released from research facilities.

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 scientific activity (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 reactions 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*, and *Mycobacterium fortuitum*). Handling protocols should include handwashing immediately after handling the animals and between handling different animals. While animals may be screened for 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 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.

Commonly used 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 scientific activities requires justification for the inherent risks of their use. Guidance on the safe handling of venomous reptiles is provided in Section 7.3, "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, institutional 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 species-specific training are 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 clearly posted. An SOP for 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 that is appropriate for the species, stored appropriately, and within the expiry date must be available, either at a local health provider or institutionally. Antivenin is a regulated biological product in Canada, and a 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.

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

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GLOSSARY

Acclimation – a persisting physiological, biochemical, or morphological change within an individual animal during their life as a result of prolonged exposure to an environmental condition such as a high or low temperature; generally, the changes are reversible.

Analgesia – decrease in response to noxious stimuli.

Anesthesia – a state caused by an external agent leading to loss of sensation and motor function.

Aseptic – the absence of living germs, free from septic or poisonous putrefactive products.

Brumation – dormancy experienced by ectothermic reptiles in response to low environmental temperatures and shortened day length; the animals typically cease eating and drinking and become more sedentary, with or without burrowing.

Carapace – the hard outer covering of a shell.

Chloramine – a chemical compound that contains chlorine and ammonia, often found in municipal water supplies.

Chromatophore – a cell containing pigment, which produces colour.

Dewlap – a flap of skin or similar loose flesh that hangs beneath the lower jaw or neck.

Dysecdysis – abnormal shedding of the skin.

Dystocia – a condition in which only a portion of the eggs are laid during oviposition or the fetuses are delivered during parturition.

Ecdysis – the process of shedding the old skin (in reptiles) or casting off the outer cuticle (in insects and other arthropods).

Ectothermic – an animal that assumes the temperature of their surroundings.

Endosymbiont – an organism that lives inside another organism in a symbiotic relationship.

Endpoint – predetermined criteria for intervening in a procedure to terminate, minimize, or reduce an animal's negative welfare state, which takes into account the welfare of the animal (welfare endpoint) and the goal of the scientific activity (scientific endpoint).

Environmental enrichment – enhancements to an animal's environment that go beyond meeting their basic species-specific needs and further improve overall quality of life.

Fomite – a non-living object that can carry disease organisms (e.g., mops).

Genetically modified – a deliberate modification of the genome (the material responsible for inherited characteristics).

Genotyping – a process used to determine differences in the genetic make-up (genotype) of an individual animal by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence.

Gut loading – providing a particular food to an insect prior to feeding the insect to a reptile to increase the nutritional value of that insect.

Hemipenes – paired male reproductive organs.

Hepatic – associated with the liver.

Hypothermia – lower than normal body temperature.

Intraosseous – within the bone.

Intraperitoneal (intracoelomic) – within the peritoneum or body cavity.

Microbiome – a community of microorganisms within a defined environment (e.g., the microorganisms inhabiting the skin of an animal).

Morbidity – visible manifestation of a diseased state.

Mortality – loss of life; death.

Nociception – the process of communication through the nervous system when noxious stimuli have been detected by particular receptors (nociceptors).

Noxious stimuli – stimuli that are damaging or potentially damaging to tissue.

Oocyte – an immature egg cell.

Operant conditioning – a process of modifying behaviour in which the desired behaviour is followed by a rewarding or reinforcing stimulus.

Oviparous – producing young by means of eggs that hatch after being laid by the parent.

Pain – an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage.

Parthenogenesis – asexual reproduction in which an embryo develops from an unfertilized egg.

Peri-ecdysis – the period around ecdysis or shedding of the skin.

Personal protective equipment (PPE) – garments or equipment designed to protect personnel from injury or infection when working with animals, including physical injury (e.g., bites, scratches), biohazards, and airborne particulate matter.

Phenotype – the observable physical properties of an organism which include the organism's appearance, development, and behaviour.

Plastron – the ventral surface of the shell.

Prandial status – relating to a meal.

Quarantine – confinement of animals that may carry an infectious disease for a specified period to allow for evaluation.

Sentinel animal – a specified-pathogen-free (SPF) animal known to be susceptible to an infectious agent that is placed in an area suspected of being contaminated (e.g., in a new shipment of laboratory animals under quarantine), with follow-up testing for infection or the development of antibodies to the infectious agent.

Standard operating procedure (SOP) – a written document that describes in detail how a procedure should be carried out.

Stomatitis – inflammation of the mucous membrane.

Stress – a state caused by factors external to an animal that displace homeostasis; stress can be beneficial (e.g., in triggering a flight response if the animal is threatened, helping them to cope with changes in their environment). However, prolonged stress can cause changes to an animal's endocrine system, leaving them less able to cope with their environment.

Three Rs – replacement, reduction and refinement in animal-based science, as first explained by W.M.S. Russell and R.L. Burch in 1959 in *Principles of Humane Experimental Technique*.

Torpor – an inactive state often associated with low body temperature and low metabolic rate in response to reduced food availability.

Vomeronasal system – a sensory system in the nasal cavity that includes the vomeronasal organ and detects chemical cues in the environment.

Viviparous – giving birth to live young.

Welfare – the physical and mental state of an individual animal and how this animal is experiencing the conditions in which it lives.

Zoonotic – relating to the transmission of a disease from a non-human species to humans.