

Full-spectrum lighting and heat in the Sandy and John Binns vivaria are controlled electronically and equipped with safeguard alarms. The system above simulates three periods of light: morning sunrise, day, and sunset (moonlight is optional). Temperature is electronically controlled to maintain two periods of a diurnal cycle that correspond to day and night. A ventilation system continually refreshes air quality. Should the system fail and go into thermal-overload, a sensor kills lighting and heating and activates a fan to ventilate the enclosure. Any system failure activates an audible alarm, and sends a coded message to a beeper or telephone.

# HUSBANDRY

# Aspects of Light and Reptilian Immunity

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E ffective reptilian husbandry is important to hobbyists and Zoos, where many species are raised in captivity as a component of conservation projects, but also because reptiles are used as laboratory models in bioscience research and even in farming ventures that raise them for pets, food, and raw materials. Reptilian biology and physiology are very different than those of endothermic vertebrates (birds and mammals). Preventive reptilian medicine means improving human-reptile relationships and supporting a well-regulated reptilian immune system. Light is an important factor in this effort, and light means something different to reptiles than to us humans. To illuminate the effects of light on reptilian immunity, we must examine the reptilian immune system, the properties of light, and reptilian light perception.

# The Reptilian Immune System

Reptiles are positioned phylogenetically between higher and lower vertebrates and, in terms of evolutionary development, their immune systems are similarly intermediate. Immunologists distinguish between nonspecific defense mechanisms, such as barriers (skin and gut mucosa), protective bacterial flora, and natural resistance, and the complex immune system (IS), within which we distinguish between paraspecific (innate) and specific components. The cellular/molecular agents of the paraspecific IS include macrophages, cytokines, and toll-like receptors (which facilitate the recognition of a wide range of microbial molecules), whereas the specific IS utilizes lymphocytes, immunoglobulins, and immunoglobulin-superfamily-receptors. "Immunity" is much more than just antibodies (= immunoglobulins). The modern specific IS is functionally based on the ancient paraspecific IS; life without the paraspecific IS is impossible. The reptilian paraspecific IS is very well-developed, whereas their specific IS is handicapped by the lack of lymph nodes and germinal centers, and by having a reduced variety of immunoglobulins that, in turn, have some functional limitations. Furthermore, constraints imposed by reptilian ectothermy places a heavy reliance on the paraspecific IS alone.

In conclusion, the reptilian IS is heavily paraspecific. Overall, the reptilian IS acts to permanently monitor the body's integrity. It communicates with and regulates other functions, such as metabolism and neural and endocrine activities, and is affected by them in turn. These systems together form the immuno-neuro-endocrine network. For example, immune-cell cytokines trigger the hypothalamus, which responds by releasing hormones that regulate the immune cells. This scenario provides some idea of how light may influence the reptilian IS.

# Light

Light on earth is a continuous spectrum of electromagnetic radiation from the sun that has been filtered through kilometers of atmosphere. Conceptually, we divide the "light" portion of this spectrum (= the photo-environment) into bands of different wavelengths (measured in nanometers = nm) that range from infrared to ultraviolet, with the portion of the spectrum visible to humans limited to red, green, blue, and the wavelengths in between (i.e., we see only red, green, and blue, and all other colors are mixed in our brain depending on wavelength composition and intensity). This view is heavily anthropocentric and based solely on human photoreceptor design (eyes, retina) and human information processing (brain). The infrared wavelengths that are invisible to us are perceived as heat, and the equally invisible UV-A spectrum tans our skin, while UV-B helps synthesize vitamin D3 within our bodies. The portions of the spectrum visible to humans supply color and contrast for habitat orientation. The hue that humans perceive depends on



A variety of environmental and biological factors modulate an effective reptilian immune system. Barriers include structures like skin and gut mucosa that prevent entry and retention of invasive pathogens. In addition to barriers, natural resistance against specific pathogens (an alligator, for example, is resistant to tetanus toxins) and defensive surface flora comprise the three parts of the unspecific defense system. The body's paraspecific (innate) and specific (acquired) immune systems enable it to attack various dangerous substances, infectious pathogens, toxins, and transformed cells of the organism itself. These components form what we call the complex immune system. Innate, inherited paraspecific components include macrophages (a type of white blood cell that consumes foreign material and releases substances that stimulate other cells of the immune system), their toll-like receptors (proteins sensitive to exposure to molecular substances that are associated with some pathogens), and cytokines (small proteins released by cells that affect interactions between cells; cytokines include a variety of agents that, among other things, trigger inflammation and respond to infections). Specific components are cloned or activated only after exposure to a foreign dangerous substance (an antigen); these include lymphocytes (small white blood cells that produce antibodies [= immunoglobulins] that recognize and attach to bacteria and toxins or body cells that have been taken over by viruses or have become cancerous). These customized receptors are part of the body's immunoglobulin-superfamily-receptors and enable lymphocytes to attack dangerous materials. Hormones are chemical messengers secreted into blood by endocrine organs and transported to specific target cells, the functions of which are regulated in various ways. Hormonal (endocrine) activities and neural and immune functions correspond closely and interact within the immuno-neuro-endocrine network. Ectothermy is the ability to regulate internal body temperature behaviorally by exploiting environmental sources of heat.



Light visible by humans is but a very small region of the entire spectrum. Note that humans see only three colors (red, green, blue) and all other visible colors are mixed in our brain depending on wavelength composition and intensity. However, reptiles apparently see four colors, the same three that we see plus at least some wavelengths of ultraviolet (UV) light, and pitvipers and at least some boas and pythons can see five, those seen by other reptiles plus infrared (IR) light. Consequently, the reptilian perception of the visible world is quite different from our own, something that must be considered when designing lighting systems capable of sustaining healthy animals.

the "color temperature" of light (usually expressed in degrees Kelvin [° K]), and that, in turn, depends on which wavelengths are filtered out by the atmosphere. "Warm" reddish hues dominate when blue is filtered (about 3000° K) and reds are emphasized, such as at sunrise and sunset, when the sun is low on the horizon and light must travel a greater distance through the atmosphere. "Cool' bluish hues prevail when the red spectrum is filtered (to ~10000° K) by, for instance, cloud cover, and the blue spectrum is favored. Sunny daylight lies at ~6000° K. Another aspect of light is the intensity received (illuminance). Illuminance depends on latitude, season, time of day, and degree of cloud cover, and is measured in lux, calculated as "adjusted watts"/m<sup>2</sup> = lumens/m<sup>2</sup> (watts weighted at human spectral sensitivity).

Is this the same for reptiles? How do reptiles see the world? What do reptiles perceive of the electromagnetic spectrum, the photo-environment? Reptiles see hues and lux that we hardly can imagine. Let's take a "reptocentric" look at the "visible" spectrum to clarify some anthropocentric misunderstandings.

### **Reptilian Light Perception**

Microanatomical studies of lateral eyes show that most mammals are dichromats (have two different types of cones [= receptor cells that respond to different wavelengths of light]). Humans and Old World primates are trichromats (three cone types with peak sensitivities in red, green, and blue, covering 400–700 nm). Reptiles are tetrachromats with a fourth cone type for UV-A below 400 nm. Additionally, the peak sensitivities of the red, green, and blue cones are shifted slightly when compared to those of humans. Reptiles see in the UV-A range and use spectra differently. Behavioral studies show that UVspectra and reptile-correct color rendering of artificial light settings is not only necessary for conspecific, interspecific, and intersexual recognition, but that brightness and contrast also are critical for motion perception and foraging, and probably also for maintaining a sense of well-being ("happiness") in reptiles.



The problem of providing an optimal reptilian photo-habitat with artificial light (non-continuous spectrum or lacking UV-A) is that they are optimized for human sensitivities rather than for those of reptiles. To illustrate what insufficient light might be doing to reptiles, notice the difference in the quality of this image (even in grayscale) when one of the three primary colors (in this case, blue) is deleted from digitized photographic images.



The parietal eye is a photosensory organ connected to the pineal body, active in triggering hormone production (including reproductive hormones) and thermoregulatory behaviors. It is sensitive to changes in light and dark, but does not form images, having only a rudimentary retina and lens. It is visible as an opalescent gray spot on the top of some lizard's heads; the parietal eye also is referred to as a "pineal eye" or "third eye." Although the parietal eye of this Grenada Bush Anole (*Anolis aeneus*) is quite prominent, those of many lizards are difficult to see.

This illustrates the difficulty of providing an optimal reptilian photo-habitat with artificial light. Light fixtures providing a non-continuous spectrum or one lacking UV-A do not bother humans, but may dramatically affect reptiles. Artificial lighting has been optimized for human sensitivities rather than reptilian demands.

Another photoreceptor present in reptiles is the so-called "third" or pineal eye and the associated pineal gland. Pineal cells show the highest sensitivity to wavelengths of 600–750 nm, and are an effective light dosimeter with resulting thermal, immune, and reproductive regulatory consequences.

Additionally, some reptiles can see precisely even at night by exploiting the infrared portion of the spectrum. Pit vipers and boid and pythonid snakes use infrared sensors and sophisticated signal processing that operates like an image-improvement algorithm to precisely monitor their surroundings from emitted infrared in the dark.

Humans use rods (receptor cells that are very sensitive to low light intensities) rather than cones for black and white vision under dim conditions. Surprisingly, geckos, most of which are nocturnal, lack rods, but have instead very sensitive cones for night color vision.

We do not know precisely nor can we really appreciate how reptiles see their environments, but we definitely know that the



Most geckos are nocturnal, but surprisingly lack rods (which are most active in mammalian night vision), instead having extremely sensitive cones that also allow color vision. The series of pinholes visible in the eye of a Turnip-tailed Gecko (*Thecadactylus rapicauda*) from St. Vincent come into play only when the vertically slit pupil constricts when exposed to bright light. Slit pupils, found in many nocturnally active animals, allow a greater range of light entry than a round pupil, because they are able to expand greatly in dim light. Slit pupils are particularly useful in combination with aspherical lenses, as they allow an image to be focused properly regardless of width. When light is bright, the pinholes block out more light than even a very slender simple pupil, while still allowing the eye to focus effectively.



Pitvipers, such as this Timber Rattlesnake (*Crotalus horridus*, top), and boas and pythons, such as this Emerald Treeboa (*Corallus caninus*), have heat-sensitive pits that extend vision into the infrared spectrum. Although usually associated with targeting homeothermic prey, they also may function in precise predator shape detection and evaluation of habitats suitable for ambushing prey or use as refugia.

reptilian immune system can "see" the world. Where is the connection between light and the immune system?

#### Light and Its Immune Effects

Whole-body-mediated light immune effects.—Reptiles are solar powered. As ectotherms, reptiles maintain their optimal body temperatures behaviorally. They depend directly on the infrared portion of the light spectrum as a source of heat (via basking) and indirectly on convection (from warm substrates and warm or cool burrows). Warming light is a pivotal factor in regulating reptile microclimates and microhabitats. This solar energy is a critical underpinning for well-functioning reptilian immune metabolism. The specific IS is more susceptible to temperature changes than the paraspecific IS, due to simple chemical and more complex biochemical reasons (the hypothalamo-pituitaryadrenal axis and the hormone corticosterone). During winter and in other unfavorable conditions, when specific antibodies are depleted, reptiles have to rely solely on the paraspecific IS.

Light also serves to dry the skin, providing a less comfortable surface for the growth of bacterial pathogens while promoting the growth of defensive bacterial flora. Finally, UV light itself has a direct disinfectant action. Body movements and gut peristalsis to prevent bacterial overgrowth are dependant on appropriate body temperatures and, last but not least, in combination with vision, light allows foraging and digestion to take place in order to supply the reptilian immune system with nutrients.

*Eye-brain-mediated light immune effects.*—In addition to the nonspecific radiation support to the reptilian defense system, other more sophisticated light effects influence reptiles' complex immune systems. The lateral eyes transduce light signals to the brain vision center and, via an alternate pathway, to the hypo-



PATHWAYS BY WHICH LIGHT MODULATES REPTILIAN IMMUNE SYSTEMS

Full-spectrum light, monitored by eyes (paired and parietal) and infrared sensors (pit organs and heat receptors on the surface and in the interior of the body), can dramatically affect the efficacy of reptilian immune systems.

thalamo-pituitary axis. From there, modified hormone patterns are created. These have a dramatic influence on reptilian immune systems. The pineal eye regulates serotonin and melatonin synthesis in the pineal gland depending on the degree and rhythm (daily or seasonal) of light exposure. Both neuro-hormones modulate the reptilian immune system.

*Skin-mediated light immune effects.*—Immune cells under the skin react directly to the deep-penetrating (red) portion of the spectrum and modify immune function. Further, UV-B regulates vitamin D3 endosynthesis in the skin (the primary source of D3 in many reptiles), which then is hydroxylated to bioactive calcitriol (1,25-hydroxy-cholecalciferol). Cytokines, such as interferons, trigger immune cells to D3 hydroxylation and immune cell populations (macrophages) are upregulated via cal-

citriol receptors. Calcitriol also attaches to immune cells to stimulate production of antibacterial agents. That might also explain the anticarcinogenic side effects of D3. Whereas calcitriol's regulating capacity on calcium metabolism is often mentioned, the direct D3 immune effects are overlooked. Also noteworthy is the fact that calcium itself plays an important role in long-distance immune cell communication via nanotubules.

#### **Conclusions and Summary**

For decades, herpetoculturalists have discussed the need for proper heating and lighting. Preventative reptilian medicine emphasizes the importance of a well-regulated immune system, and has found a strong link to proper lighting. Improving reptilian photo-environments (a combination of lamps, position, reflectors, control, and maintenance) is still and will continue to be a critical factor in the creation of appropriate habitats for captive reptiles. Proper lighting is a process not a bulb!

Veterinarians engaged in preventative reptilian medicine are aware and appreciate the technological advances in reptilian lighting. The movement has been from incandescent and halogen lamps to fluorescent full-spectrum tubes, compact lamps, metal halide lamps, self and external ballasted mercury vapor lamps, or combinations thereof. The latter are capable of providing reliable UV-B, UV-A, visible light intensities, and color temperatures. LED-lamps may hold promise for the future.

Properly controlled lighting systems and maintenance are necessary for reptilian enrichment, which should be a major concern for responsible herpetoculturists. Given that over 9,000 rep-



PROPER LIGHTING IS A PROCESS, not a bulb. Establishing lighting systems necessary for maintaining optimally regulated reptilian immune systems (= healthy reptiles) is a complex network of several processes involving initial choices, extensive planning, effective implementation, and ongoing monitoring, maintenance, adjustments, and improvements.



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Lighting necessary for the maintenance of healthy reptiles involves a sequence of processes that only begins with the choice of lights. Not only must lighting provide the proper spectral range for vision, but intensities and photoperiods play important roles in regulating reptilian immune and endocrine systems. Well-designed lighting systems, such as these in the Sandy and John Binns vivarium, require research, experimentation, and constant monitoring. Proper lighting is a process, not a bulb!

tilian species occupy almost as many varied microhabitats, no single form of illumination will address all needs. An upcoming topic of concern is the misinterpretation of geographic climate data versus microhabitat demands and the ensuing chronic overradiation.

Reptiles perceive light differently than humans and other mammals. They are tetrachromats with additional perception in the UV-A range below 400 nm and their red, green, and blue cones have shifted peak sensitivities when compared to those of humans. Due to ectothermy and a consequent heavy reliance on the paraspecific immune system, light not only modulates but also provides pivotal support for reptilian immuno-neuroendocrine networks. Endosynthesis of vitamin D3 via UV-B is important for mineral metabolism and also for immune regulation. Veterinarians, technicians, scientists, and hobbyists must be aware of the many different aspects of light-dependent reptilian physiology, and they and their reptiles will benefit tremendously from further investigations in this area. Proper lighting is a process not a bulb.

#### References

- Besedovsky, H.O. and A. Del Rey. 1996. Immune-neuro-endocrine interactions, facts and hypotheses. *Endocrine Review* 17:64–102.
- Carillo-Vico, A., J.M. Guerrero, P.J. Lardone, and R.J. Reiter. 2005. A review of the multiple actions of melatonin on the immune system. *Endocrine* 27:189–200

- Chen, T.C., G.C. Schwartz, K.L. Burnstein, B.L. Lokeshwar, and M.F. Holick. 2000. The *in vitro* evaluation of 25-hydroxyvitamin D-3 and 19-nor-1 alpha, 25-dihydroxyvitamin D-2 as therapeutic agents for prostate cancer. *Clinical Cancer Research* 6:901–908
- Else, P.L. and A.J. Hulbert. 1981. Comparison of the "mammal machine" and the "reptile machine," energy production. *American Journal of Physiology* 241:350–356.
- Feske, S., H. Okamura, P. Hogan, and A. Rao. 2003. Calcium/calcineurin signalling in cells of the immune system. *Biochemical and Biophysical Research Communications* 311:1117–1132.
- Fleishman, L.J., W.J. McClintock, R.B. D'eath, D.H. Brainard, and J.A. Endler. 1998. Colour perception and the use of video. Playback experiments in animal behaviour. *Animal Behaviour* 56:1035–1040.
- Fleishman, L.J. and M. Persons. 2001. The influence of stimulus and background colour on signal visibility in the lizard *Anolis cristatellus. Journal* of Experimental Biology 204:1559–1575.
- Gombart, A.F., H. Chen, L. Brandi, K. Olgaard, N. Borregaard, J.S. Adams, and H.P. Koeffler. 2005. Vitamin D3-mediated regulation of the antimicrobial peptides CAMP and DEFB4 is evolutionarily important for innate immunity in humans and primates. *American Society of Hematology Annual Meeting Abstracts* 106:3079.
- Guillette, L.J., A. Cree, and A.A. Rooney. 1995. Biology of stress: Interactions with reproduction, immunology and intermediary metabolism. In: C. Warwick, F.L. Frye, and J.B. Murphy (eds.), *Health and Welfare of Captive Reptiles*. Chapman & Hall, London.
- Hamasaki, D.I. and E. Dodt. 1969. Light sensitivity of the lizard's epiphysis cerebri. *Pflügers Archives* 313:19–29.
- Honkavaara, J., M. Koivula. E. Korpimaki, H. Siitari, and J Viitala. 2002. Ultraviolet vision and foraging in terrestrial vertebrates. *Oikos* 98:505–511.
- Hunt, D.M., S.E. Wilkie, J.K. Bowmaker, and S.Poopalasundaram. 2001. Vision in the ultraviolet. *Cellular and Molecular Life Sciences* 58:583–598.
- Loew, E.R., L.J. Fleischman, R.G. Foster, and I. Provencio. 2002. Visual pigments and oil droplets in diurnal lizards, a comparative study of Caribbean anoles. *Journal of Experimental Biology* 205:927–938.
- Mayr, A. and B. Mayr. 1999. A new concept in prophylaxis and therapy: Paramunization by poxvirus inducers. *Pesquisa Veterinária Brasileira* 19:91–98 (http://snipurl.com/xrbq).
- Meissl, H. and M. Ueck. 1980. Extraocular photoreception of the pineal gland of the aquatic turtle *Pseudemys scripta elegans*. Journal of Comparative Physiology A, Neuroethology, Sensory, Neural, and Behavioral Physiology 140:173–179.
- Pichaud, F., A. Briscoe, and C. Desplan. 1999.Evolution of color vision. *Current Opinion in Neurobiology* 9:622–627.
- Pough, F.H., R.M. Andrews, J.E. Cadle, M.L. Crump, A.H. Savitzky, and K.D. Wells. 2003. *Herpetology*. Prentice-Hall, Upper Saddle River, New Jersey.
- Ralph, C.L., B.T. Firth, and J.S. Turner. 1979. The role of the pineal body in ectotherm thermoregulation. *American Zoologist* 19:273–293.
- Roberts, J.E. 2000. Light and immunomodulation. *Annals of the New York Academy of Sciences* 917:435–445.
- Roth, L.S.V. and A. Kelber. 2004. Nocturnal colour vision in geckos. Proceedings of the Royal Society of London B 6:485–487.
- Sillman, A.J., J.K. Carver, and E.R. Loew. 1999. The photoreceptors and visual pigments in the retina of a boid snake, the Ball Python (*Python regius*). *Journal of Experimental Biology* 202:1931–1938.
- Sillman, A.J., V.I. Govardovskii, P. Rohlich, J.A. Southard, and E.R. Loew. 1997. The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*), a microspectrophotometric, scanning electron microscopic and immunocytochemical study. *Journal of Comparative Physiology* A 181:89–101.
- Van Hemmen, J.L. 2006. Indeterminacy and image improvement in snake infrared "vision." American Physical Society 2006 March Meeting, Baltimore, Maryland.
- Warr, G.W., K.E. Magor, and D.A. Higgins. 1995. IgY: Clues to the origins of modern antibodies. *Immunology Today* 16:392–398.
- Warkins, S.C. and R.D. Salter. 2005. Functional connectivity between immune cells mediated by tunneling nanotubules. *Immunity* 23:309–318.
- Zapata, A.G. and C.T. Amemiya. 2000. Phylogeny of lower vertebrates and their immunological structures, pp. 67–110. In: L. Du Pasquier (ed.), *Origin and Evolution of the Vertebrate Immune System*. Springer-Verlag, Heidelberg.