

Amphibian Nutrition

AZA Professional Training Program:

Amphibian Biology, Conservation and Management

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Introduction

Basic knowledge of amphibian nutrition lags far behind that of reptiles and even fish. Worldwide declines and extinctions of amphibian populations and the increased importance of *ex-situ* and *in-situ* captive propagation programs have highlighted the need for scientifically-based approaches for the nutritional management of these animals. Because it is usually impractical to duplicate the diets of wild amphibians in captivity, diets have usually been formulated with readily available prey items and improvised dietary supplements. This convenient “one size fits all” (crickets and more crickets) approach towards dietary husbandry is not always satisfactory in view of the rich species diversity of the Amphibia. This is especially true for modern zoo collections that house critically endangered species and strive to salvage and reproduce these species for conservation efforts rather than simply maintaining animals for display.

Improvement of captive amphibian diets will come from careful observations of species natural history (e.g. What do they eat in the wild) and experimental feeding trials that determine dietary requirements of specific nutrients for different species and for different life stages. Other feeding trials should be designed that examine optimal methods for providing nutritional supplementation to captive diets (gut loading vs. dusting). Knowledge of the nutritional content (e.g. levels of vitamins, minerals, fat content etc.) of wild amphibian diets is very helpful in determining appropriate captive diets and nutritional supplementation. Yet, while stomach contents for some species have been documented, very few dry weight nutritional analyses of the contents have been performed in order to scientifically formulate diets for use in captive programs. Research in these areas represents an opportunity for zoo staff including keepers, curators, nutritionists and veterinarians to make a ENORMOUS contribution to captive amphibian welfare and conservation.

Why is nutrition important? (Or...We've always fed them this way and never had any problems!)

Animals can seem to survive and even reproduce for long periods of time on imbalanced or otherwise improper diets. However, this rarely occurs without consequences and a great example is the high incidence of coronary heart disease, diabetes and obesity associated with the high fat, high calorie, low fiber diets favored by humans in Western societies. Some nutritional problems in captive amphibians will be really obvious (e.g. tetany or deformed bones with calcium/phosphorus imbalances), but others are considerably subtler and in fact, won't be initially recognized as a nutritional issue. Some very common problems in maintenance and breeding of captive amphibians that could have a nutritional basis include:

- Failure to thrive ("Just ain't doing right...")
- Reproductive failure
- High incidence of infectious diseases in an amphibian colony in the face of good water quality and husbandry
- Edema syndromes
- Limb deformities such as spindly leg syndrome

Sensitivity to specific nutritional deficiencies will vary between species and sometimes between individuals of the same species. So, the bullfrogs at your institution might do great on Diet X with Supplement B, but the recently imported Harlequin frogs might waste away on the same diet. Similarly, Wyoming toad Y out of a large group of might show clinical signs of "short tongue syndrome" (vitamin A deficiency) while other deficient toads in the group may appear to be healthy.

Nutritional Management of Insect-Based Diets

Dietary mainstays for most amphibians in captivity are cultured insects and other invertebrates including the domestic cricket, waxworms, mealworms, fruit flies and earthworms, among others. Cultured invertebrates are convenient, practical and cost-effective for use in diverse animal collections with busy (overworked) animal care staffs. In addition, with new emphasis on biosecurity to minimize the risk of introducing infectious agents to amphibian collections, cultured insects can be raised in protected circumstances that reduce their potential to be mechanical vectors of disease.

Despite these advantages, cultured invertebrates differ from wild invertebrates that automatically provide amphibians with the added nutritional benefits of their stomach contents. These stomach contents may contain a variety of nutrients not present in the prey item itself or may serve to balance the proportions of nutrients found in a prey item. As an example cultured invertebrates by

themselves are known to be deficient in calcium and vitamin A and deficiencies of these nutrients are common problems in captive amphibians (see below). To compensate for the dietary shortcomings of cultured insects it is necessary to use vitamin and mineral supplements.

Methods and schedules for providing supplementation of vitamins and minerals to invertebrate-based diets are not standardized between different zoos and other institutions that maintain amphibians. This lack of standardization (and scientific validation) of diets makes providing a specific or "cookbook" recommendation for these supplements very difficult or impossible. At a minimum regular (at least several times a week) vitamin-mineral supplementation of invertebrate diets should be standard practice at institutions that house captive amphibians. It is unfortunately still very common for cultured invertebrates to be fed without any supplementation, even at experienced institutions. For species that are part of a Species Survival Plan or other organized conservation effort the species husbandry manual or the species coordinator/nutrition advisor/veterinary advisor may have recommendations on diets that have been used successfully. For whatever diet plan is used, it is important to respond to indications of nutritional deficiencies from clinical signs or from necropsy reports and make adjustments to diets or diet supplements as needed. As mentioned earlier, research into amphibian nutrition with the goal of creating specific recommendations for creating and supplementing amphibian diets (including formulation of different diets for different species of amphibian) should be a top priority for zoos.

The most common ways to supplement insect-based diets are dusting prey items with vitamin-mineral mixes and/or the use of gut-loading in which prey items are fed a nutrient-rich diet that is often supplemented with a vitamin-mineral mix. For dusting, prey items are placed into a container immediately prior to use and are covered with a powdered vitamin-mineral supplement. For domestic crickets, gut loading is accomplished by feeding a variety of fruits and vegetables (e.g. leafy greens, sweet potatoes, carrots), using a commercially available cricket diet or by use of layer mash diets designed for domestic poultry. Crickets are gut-loaded 24-48 hours immediately prior to use.

There are several common errors in selecting and using vitamin-mineral supplements for dusting and gut-loading of invertebrate prey items. These include:

- Supplementing diets with calcium only. Remember that invertebrate diets may also be deficient in other nutrients such as vitamin A.
- Use of outdated (expired) supplements. Many nutrients degrade over time and any supplements more than 3-4 months old have probably lost a significant degree of potency. Although it may be less

expensive to buy supplements in bulk, there is no advantage to doing this if the nutritional value is lost over time.

- Improper storage of vitamin-mineral supplements. Many nutrients will degrade when exposed to light, heat or excessive humidity. Consider storing your vitamin-mineral supplements in the refrigerator with protection from light.
- When dusting prey items remember that the supplement powder will fall or wash off within minutes to hours.

Water is essential for good amphibian nutrition

Amphibians do not drink water, but instead absorb water and electrolytes through highly permeable skin. Plasma osmolality is an important factor in water absorption and balance. In aquatic species plasma is hyperosmolar to freshwater and hence animals can easily absorb water in excess of their requirements and must produce large volumes of urine in order to compensate. Non-aquatic species can re-absorb solutes such as urea from the urinary bladder and easily tolerate the resulting increases in plasma osmolality that enhance the ability to absorb water from substrates. Interestingly, some species have a cutaneous chemosensory ability that detects osmotic concentrations on potential hydration sources and therefore helps to avoid selecting locations with an unfavorable profile for hydration (e.g. hyperosmotic locations would result in water loss instead of gain). The electrolytes sodium and chloride are actively transported (processes use "energy" or ATP) through the skin by membrane bound pumps in cutaneous epithelial cells.

This fundamental difference in method for obtaining water and electrolytes between the Amphibia and other types of animals maintained in captivity makes attention to water composition and quality essential for good amphibian husbandry. And, just as one type of diet may not be adequate for all amphibian species, differences in water composition may be necessary to successfully maintain some specialized species in the the captive environment. Therefore when preparing to bring species into captivity it may be helpful to obtain analyses of water from the native environment to include factors such as pH, hardness and conductivity.

Some examples of the relationship between water and amphibian nutrition and health include:

- Terrestrial and arboreal anurans are less able than aquatic anurans to tolerate water with low levels of solute (sodium, potassium ect.). When these animals are placed in distilled or reverse osmosis water that has not been reconstituted they can be placed under osmotic stress (e.g.

excessive amounts of water flow into the frog) with subsequent fluid accumulation (edema).

- Some municipal water supplies have elevated levels of phosphorus. In theory, this could interfere with the balance of dietary calcium and phosphorus (usually a 1.5: 1 ratio of calcium to phosphorus is suggested). If the Ca: P ratio is altered, metabolic bone disease may occur (see discussion of MBD below). Phosphate binding resins or sponges may be helpful if high P levels are known to occur in your local water supply.
- Exposure to acidic conditions (less than pH 4 or 5) can interfere with sodium transport across the skin and result in hyponatremia (low blood sodium levels).

Minerals: Balance is Important

The minerals are a large group of nutrients that are often separated into 2 large groups based on the amounts needed in the diet. The macroelements such as calcium, phosphorus, potassium, sodium and magnesium are involved in activities such as osmoregulation, acid-base balance, body structural support (bone) and formation of electrical potentials (e.g. nerve and muscle function). The microelements or trace elements include iron, zinc, copper, manganese and iodine, among others. Some such as copper are involved as co-factors in a variety of metalloenzymes for metabolic pathways. Iron is well-known as a component of hemoglobin and myoglobin that are involved in oxygen transport. Iodine is a key component of thyroid hormone synthesis (and for amphibians, thyroid hormone is an important for the process of metamorphosis).

Nutritional requirements for minerals can be very complicated because balance of any one mineral is often dependant on the amount of other minerals in the diet. If any one mineral is present in excess then another mineral is likely to become deficient. For instance, molybdenum, inorganic sulfates, iron, zinc, calcium and cadmium can all influence the absorption of dietary copper. On paper, the dietary copper may seem to be adequate but in actuality, the animal is copper deficient because of another dietary excess.

The best recognized dietary deficiency of minerals in captive amphibians is metabolic bone disease:

Metabolic Bone Disease

Metabolic bone disease (MBD) is a generic term encompassing a spectrum of disease conditions or terminology (e.g. rickets; fibrous osteodystrophy; nutritional

secondary hyperparathyroidism, among others) usually referring to disorders of calcium and phosphorus metabolism. MBD in captive amphibians usually has a nutritional basis and is commonly associated with unsupplemented insect based diets. Unsupplemented insects have low total calcium content and inverse calcium to phosphorus ratio (C: P ratio). Other factors that influence or change the C: P ratio can also result in MBD. Examples that have been documented in amphibians include tadpoles or froglets raised in water with low calcium hardness or animals maintained in municipal tap water containing excess phosphorus.

Some aspects of normal calcium metabolism can be helpful in understanding metabolic bone diseases:

- Calcium is important not only for bone structure, but also for nerve and muscle function.
- The suggested dietary ratio of calcium to phosphorus in the diet is 1.5 to 1. Excess dietary phosphorus (inverse C:P ratio) interferes with calcium absorption. Similarly, kidney disease can increase blood phosphorus levels with subsequent effects on calcium metabolism.
- One of the major functions of Vitamin D is facilitation of calcium absorption from the intestine. There are 2 forms of vitamin D precursors. The vitamin D2 precursor comes from plant sources whereas the vitamin D3 precursor comes from animal sources. The vitamin D3 precursor requires exposure to UV light in the skin to become active. Animals such as New World primates and some birds are known to have an absolute requirement for vitamin D3. *It is unknown if amphibians (or some amphibian species) have a similar requirement.*
- Low blood calcium stimulates the release of parathyroid hormone which increases resorption of calcium from the bones. Another hormone calcitonin has the opposite effect in response to increasing calcium levels.

Clinical signs of MBD can include:

- Tetany. This refers to forceful muscle contractions/fasciculations that are sometimes interpreted as seizures. Episodes of tetany are often induced by handling, stress or exercise.
- Edema. This refers to fluid accumulation in the subcutaneous lymphatic sacs. This most likely occurs because of lymph heart failure associated with low calcium levels.
- Bloating: Low calcium levels can slow movement of food and gas through the intestinal tract.
- Loss of bone density and fractures. This can be observed on radiographs especially in the pelvis and vertebral bodies. Bone

deformities of the mandible (lower jaw), spinal column and legs may be seen.

Treatment of MBD begins with correction of the diet by adding appropriate supplements to insect based diets. Pharmaceutical grade calcium carbonate, calcium citrate or calcium lactate are good choices. If the affected animals are fed high vitamin A diets (e.g. diet frequently contains rodents) consider at least temporarily removing these items because large amounts of vitamin A can interfere with absorption of vitamin D (Bruce 1950). Do not remove vitamin A supplements from insect-based diets because these are usually considered to be vitamin A deficient. Animals should be provided with a full-spectrum UV light source in order to make adequate amounts of vitamin D3. Check water parameters such as calcium hardness and phosphorus content.

Individual treatment of animals with severe MBD is by (Wright 2001):

- Calcium supplementation such as oral calcium gluconate (Neo-Calglucon®) at 1 ml/kg body weight daily or a bath in 2.3% calcium gluconate daily for 1-2 hours. Supplementation should continue for a minimum of 30 days. For animals with tetany calcium gluconate can be provided as an emergency measure at 100mg/kg body weight injected intramuscularly or intracoelomically.
- Supplementation with vitamin D3 at 100-400 units/kg body weight given orally once a day or as a continuous bath at 3 units per ml of water. Injectable vitamin D3 at 1000 IU/kg body weight intramuscularly may be helpful when given 24h after an episode of tetany and emergency calcium supplementation.

It is important to recognize that a variety of other vitamins and minerals can influence bone health including vitamins C, K and A, magnesium, copper, manganese, iron, zinc and fluoride. These potential interactions should be kept in mind especially if an institution has recurrent metabolic bone disease problems that do not appear to resolve by dietary manipulation/improvement.

Vitamins

Vitamins are unrelated organic compounds that are found in foods and are necessary for a wide variety of metabolic processes. For purposes of classification and diet formulation they are often placed into the very broad categories of fat-soluble vitamins and water-soluble vitamins.

Fat-Soluble Vitamins

The fat-soluble vitamins are designated by the letters A, D, E and K and are involved in physiologic processes ranging from calcium metabolism (D) to blood clotting (K). The fat-soluble vitamins can be stored for long periods of time in adipose tissue (fat) and the liver. Caution should be used when supplementing diets or individual animals with fat-soluble vitamins because they can reach toxic levels in the body. The ratio of different fat-soluble vitamins in the diet can be important because too much of any one vitamin can interfere with the absorption of the other fat-soluble vitamins. One rule of thumb has suggested a ratio of 100 IU vitamin A to 10 IU vitamin D to 1 IU vitamin E. Specific nutritional problems related to fat-soluble vitamins in amphibians are noted below.

Vitamin A Deficiency ("Short Tongue Syndrome")

Vitamin A deficiency was first recognized in captive Wyoming toads (Pessier 2005), but has subsequently been identified in other anuran species, especially bufonids. Other species of conservation concern that have had signs of vitamin A deficiency are the Panamanian golden frog and the Kihansi spray toad (Poole 2006; Lee 2006). A common manifestation of vitamin A deficiency is the so-called short tongue syndrome (STS). Animals with STS gradually become unable to capture prey, sometimes despite vigorous effort. Some affected animals eventually require hand feeding. In ranid and dendrobatid frogs suspected vitamin deficiency has manifested as swellings centered on the lower eyelids.

The basis for the poor ability to capture prey in animals with STS is a process called "squamous metaplasia" of the cells that line the surface of the tongue. The tongue of amphibians is usually lined by cells that produce vast quantities of mucus that promotes sticking of prey items to the surface. With squamous metaplasia, the lining cells of the tongue are replaced by keratinizing squamous cells. With reduced or no mucus the tongue becomes less sticky with the end result being an inability to capture prey. Squamous metaplasia occurs because vitamin A is required for cells to differentiate into mucus producing cells. Without vitamin A to stimulate mucus differentiation, the lining cells of the tongue go to a "default" mode of squamous epithelium.

The potential adverse effects of vitamin A deficiency are not limited to STS. Vitamin A is known to be involved in a wide variety of physiologic functions including:

- Reproductive performance. This may be a consideration for problems such as reduced egg hatchability or reduced tadpole survival

- Immune function. Some amphibian species show a high incidence of infectious disease problems that are not easily explained by poor husbandry.
- Vision
- Bone Development

The occurrence of vitamin A deficiency in captive amphibians may be explained by several factors.

- Insects by themselves are a poor source of pre-formed (active) vitamin A and require supplementation (e.g. dusting and gut loading) to reach adequate levels in the overall diet (Pennino 1991; Barker 1998; Finke 2002; Finke 2003). If supplementation is inadequate, vitamin A deficiency may occur, especially in apparently sensitive groups such as the bufonids.
- Some groups of amphibians may have greater dietary requirements for pre-formed vitamin A or for vitamin A precursors called carotenoids. The carotenoids are plant-based forms of vitamin A that are converted to active vitamin A in the intestine. A common carotenoid is beta-carotene. Some species may only be able to convert some of the different types of carotenoids into active vitamin A. For example, it is unknown which (if any) of the amphibians can convert beta-carotene and therefore supplements which only contain beta-carotene as a source of vitamin A may be inadequate.
- Nutrients such as the other fat soluble vitamins or minerals such as zinc may interfere with vitamin A absorption or metabolism. The relative balances of these nutrients required by amphibians are unknown.

Diagnosis of vitamin A deficiency is by:

- Clinical signs of difficulty in catching prey items (bufonids especially). In ranids or dendrobatids swelling of the lower eyelids has been observed.
- A histopathology report of "squamous metaplasia" in the tongue, oral cavity or sometimes the urinary bladder or cloaca in one or more animals that have died should raise suspicion for the possibility of vitamin A deficiency as a "herd health" problem.
- If fresh liver can be obtained at necropsy from an animal with suspected vitamin A deficiency an analysis for active vitamin A can be performed at a nutrition or toxicology laboratory. Liver retinol (vitamin A) values less than 20-40 µg/g (ppm) are suspicious for vitamin A deficiency.

Treatment of suspected vitamin A deficiency is by:

- Correction of problems in the diet and diet supplementation. Check for expired supplements (more than 3-6 months old) and supplements that have been stored improperly (heat, humidity ect are bad). Make sure that vitamin supplementation has been in regular use. If supplements that use only carotenoids (e.g. beta-carotene) as a source of vitamin A are being used consider switching to a brand that uses pre-formed vitamin A (retinol). Guidelines for concentrations of vitamin A supplementation of cricket gut loading formulas have been suggested (Finke 2003).
- If signs of vitamin A deficiency are severe consider treating animals directly with a vitamin A supplement. Oral administration of 1 IU (international unit) vitamin A/gram body weight is given daily for two weeks or until resolution of clinical signs. Vitamin A for oral use can be prepared from commercially available liquid gel caps--usually 10, 000 IU vitamin A each--marketed for human use with capsule contents diluted in vegetable oil to achieve a concentration that allows accurate administration to small patients. Alternatively, topical administration of a water miscible injectable formulation of vitamin A marketed for use in humans can be tried (Aquasol A Parenteral, Mayne Pharma (USA), Paramus, NJ 07652) (Dr. D. Armstrong, personal communication). The 50, 000 IU/ml solution is diluted with sterile water to 5, 000 IU/ml and the dilution applied directly to the skin. For 15-25 gram body weight toads one drop of the diluted solution is dispensed from a tuberculin syringe without a needle and for smaller 1-5 gram body weight animals one drop from a tuberculin syringe with a 27 gauge needle attached is applied. Treatments are given daily for up to 2 weeks for severely affected animals and as little as twice weekly for less severely affected individuals.

Vitamin A Excess

As noted above excess dietary vitamin A in African clawed frogs was reported to interfere with vitamin D absorption with subsequent metabolic bone disease. Other potential signs of vitamin A excess include problems with the skin (increased shedding; vesicles; ulcers); liver (hepatocellular degeneration; fibrosis); and bone (osteoporosis; deformities).

Vitamin E Deficiency

Vitamin E is important for scavenging chemical free radicals formed from metabolic processes. These free radicals can damage cell membranes and result in necrosis (cell death) in tissues such as the fat or skeletal muscles. Vitamin E deficiency has not been well described in amphibians, but Wright 2001 mentions steatitis (inflammation of fat) in animals fed rancid fish or rodents.

These items contain large numbers of free radicals that deplete the body stores of vitamin E.

Vitamin D Deficiency and Excess

See the discussion on Metabolic Bone Disease above for more information on vitamin D and vitamin D deficiency. Excess vitamin D in the diet can cause very high elevations in blood calcium levels with deposition of the excess calcium into the tissues and subsequent tissue injury. In most cases excess vitamin D in amphibians will come from over supplementation of the diet.

Water Soluble Vitamins

The water-soluble vitamins are not stored in the body to any great degree and include the large family of B-vitamins and vitamin C. Although there are very few confirmed deficiencies of water-soluble vitamins in amphibians, it is possible that occurrences have gone unrecognized. The signs of many water-soluble vitamin deficiencies are non-specific (e.g. weakness or weight loss) and might also include problems not often easily identified in amphibian patients such as anemia (low red blood cell count).

B-Vitamins

These include nutrients such as thiamine, riboflavin, biotin and folic acid, among others. In general, they are involved in metabolic processes related to energy, cell metabolism and cell division. Among the conditions that might involve B-vitamin deficiencies:

- Thiamine (vitamin B1) deficiency can be observed in aquatic amphibians that are fed previously killed (frozen) fish. Some fish species naturally contain thiaminase enzymes that rapidly degrade thiamine after the death of the fish. If frozen fish are a regular or substantial part of the diet consideration should be given to adding a thiamine supplement to the diet. Symptoms of a thiamine deficiency are usually neurological and include seizures and tremors.
- The “spindly leg syndrome” and other musculoskeletal abnormalities such as scoliosis (curvature in spine) in tadpoles and metamorphs of various anuran species (especially dendrobatids) have been postulated to have an association with B-vitamin deficiencies. These deficiencies were attributed to possible leaching of B-vitamins from flaked fish foods (Wright 2001).

Vitamin C

Vitamin C is essential in the synthesis of collagen, which is the backbone of connective tissues (including bone) in the body. Signs of deficiency include bleeding (from weak blood vessel walls) or bone deformities (poor bone structure). Some animals (such as humans, guinea pigs and some fish) are unable to synthesize vitamin C and must obtain this nutrient from the diet. The dietary requirements for vitamin C in amphibians are unknown.

Lipids

Lipids or fats are utilized as energy sources and also are a major component of cell membranes. There are essential and non-essential fatty acids. Essential fatty acids must be obtained from the diet because the body cannot produce them. Of importance is that species can differ in the types of fatty acids that are essential or non-essential. Balances of different fatty acids can be important for processes such as absorption of other nutrients like vitamin A or for regulation of inflammatory processes. Little is known about fatty acid requirements for amphibians; however, because of their importance studies are needed in order to better understand nutritional needs of amphibians in captivity.

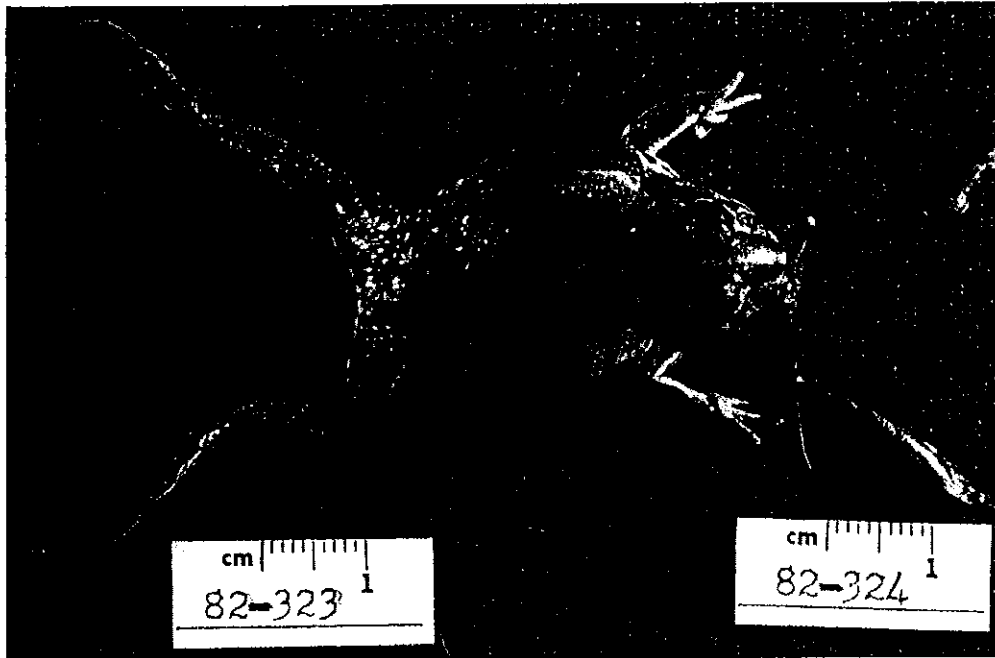
There is one well-recognized disease of lipid metabolism that is recognized in captive amphibians:

Lipid Keratopathy or "Corneal Lipidosis"

Lipid keratopathy is a disease in which cholesterol (a lipid component of cell membranes) is deposited within the cornea of the eye. Affected animals develop a white opacity which usually begins at the periphery of the eye and then extends centrally. Animals with severe corneal lipidosis are blind and may have difficulty prehending food. The cause is unknown, but affected animals have been shown to have elevated blood cholesterol levels. It is likely that this is a dietary issue associated with high cholesterol or inappropriate fatty acid composition in food items such as the domestic cricket (Shilton 2001; Wright 2003). Treatment is by reduction of dietary fat (e.g. no rodents or fatty fish and feeding of low-fat diets to domestic crickets) and feeding of a varied diet that may allow for more normal dietary fatty acid assimilation.

Gastric Overload and Impaction

Many amphibians will strike at and attempt to consume foreign materials that are not part of their "approved diet" (e.g. rocks, cagemates). In most instances these items will pass without difficulty through the digestive tract. In some instances large objects can cause intestinal tract obstructions resulting in death or the need for emergency surgery. I have seen at least 2 instances in which the acrylamide water cubes used for raising domestic crickets have been inadvertently ingested (these wiggle like prey items!) and caused obstruction and death.



Urinary Bladder Stones

Most amphibians will not develop bladder stones because the urine is normally very dilute and nitrogenous waste products are comprised of ammonia or urea. An exception is the monkey tree frogs (*Phyllomedusa* sp.). These frogs are unusual in that they excrete uric acid as the primary nitrogenous waste product and dehydration can lead to precipitation of waste products and subsequent bladder stone formation. Treatment is by surgical removal and attention given to adequate hydration/humidity.

Poor Overall Nutritional Condition/Cachexia

The non-specific findings of weight loss and muscle atrophy are common in captive amphibians. At necropsy examination there is depletion of the coelomic (gonadal) fat bodies, which represent the primary site of fat storage. Cachexia can be associated with a variety of systemic diseases, but more often is seen with improper husbandry (e.g. inappropriate food items; stress associated with shipment etc.). Treatment is by providing caloric support either by hand-feeding or by tube feeding.

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