Nutrition Recommendations for some Captive Amphibian Species (Anura and Caudata)

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2008

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Introduction

This document provides information on the nutrition of captive amphibians from the Orders Anura (frogs, bullfrogs and toads) and Caudata (newts and salamanders). Species in these Orders have very simple gastrointestinal tracts (GITs), but providing appropriate nutrition to captives can be extremely complex because of ontogenetic dietary shifts and environmental toxicities. Because of this complexity, this document can only address general factors in captive amphibian nutrition. In addition, with some exceptions, dietary provision usually must include live prey under specific environmental conditions that include the appropriate temperature, humidity and light.

Adding to the complexity of providing the appropriate nutrition to captive amphibian species is the lack of research on amphibian dietary needs. The academic and popular press often equates amphibian physiological needs with reptile species based on the similarity of environmental conditions because amphibians and reptiles are both ectotherms. This reasoning is both deductive and intuitive, but we lack reliable and valid research to affirm the belief.

The preparation of this document and the focus on Anura (frogs and toads) and Caudata (salamanders, mud puppies and newts) were chosen based on those species included in a survey requested by the Canadian Association of Zoos and Aquariums (CAZA) Amphibian Taxon Advisory Group (TAG) of amphibian species held by CAZA member institutions as of fall 2007.

The Amphibian Digestive System

An amphibian digestive system starts with a mouth tongue with taste buds capable of tasting bitter, salty, sour and sweet. The tongue of most anurans and terrestrial caudates is long, muscular and sticky (or sticky tongue pad) to capture prey such as adult insects, eggs (insect, fish and amphibian), insect larvae, small fish and, small rodents. Species such as Pipidae (clawed frogs), Sirenidae and, Proteidae (neotenic salamanders) that are fully aquatic do not have tongues and use negative pressure created by opening the mouth to pull prey in (buccal pump). Teeth are usually homodont and polyphodont and, when present, are used to hold prey that is usually swallowed whole although forelimbs may be used to push food into their mouth.

The amphibian GIT is simple (short and without a cecum), digestion is enzymatic (e.g., pepsinogen) and, peristalsis (muscular action) and ciliary action moves food through the system. The stomach of amphibians is low in pH (acidic) and the enzyme pepsinogen is converted to pepsin to breakdown proteins into amino acids. Amino acids are digested by trypsin and carbohydrates are converted to simple sugars by amylase. Bile salts and further enzyme action break down fats in the small intestine where lipase converts fats to fatty acids and glycerides to glycerol. Amphibian livers change in size seasonally because it is the storage organ for glycogen and fat. The GIT ends in the colon and the cloaca (feces and urine). Nitrogen is excreted in ammonia by aquatic tadpoles and adult aquatic frogs excrete nitrogen in urine (urea). Most terrestrial frog species excrete nitrogen as uric acid.

Factors in Captive Amphibian Nutrition

Several factors, because amphibians are ectotherms, other than the digestive system must be considered for captive amphibian nutrition. These factors are the **integument**, **vision**, **olfaction**, **thermoregulation**, **metabolism** and, **immune functioning**.

The Integument

The diet of an amphibian affects the health of its skin. In turn, the functioning of its skin can also affect the health of the amphibian. The skin of amphibians has a multitude of functions important in obtaining and absorbing nutrients: it absorbs and secretes electrolytes and water; it has a respiratory function; it has a role in thermoregulation; it has a sensory function; and, it can be a source of nutrients.

It absorbs and secretes electrolytes and water. The permeability of amphibian skin allows cutaneous water exchange and it is essentially the mechanism by how they "drink". Specifically, the "pelvic patch" is highly vascularized and it is the site where most of the cutaneous water uptake occurs. The pelvic patch can efficiently absorb water from pools, droplets or soil. Terrestrial amphibians, however, are best adapted to obtain water from soil with lower water potential than can aquatic amphibian species. The exchange of water and electrolytes makes the integument also a part of the kidney system. Amphibian species in arid climates use lipids and waxes to coat their skin and reduce water loss.

It has a respiratory function. Adult amphibians have lungs and larvae respire via internal gills. Both, however, obtain and discharge significant amounts of oxygen and carbon dioxide via cutaneous gas exchange.

It has a role in thermoregulation. The skin of amphibians can reflect or absorb heat. Adequate thermoregulation is important for GIT function and digestion.

It has a sensory function. The high sensitivity of amphibian skin includes detecting air currents, chemicals and electrodetection if the animal has a lateral line. These senses may be important in detecting prey and avoiding predators.

It can be a source of nutrients. Many amphibian species will eat their shed skin and/or feed young with skin sheddings.

Vision

The large eyes and binocular vision of anurans are essential for detecting prey, but amphibians also use olfaction when hunting. Tadpoles have smaller eyes, less binocular vision and rely more on olfaction for hunting. Anurans have colour vision equal to human ability.

Vision is also used to detect light and light of some level is needed to detect prey. For example, nocturnal amphibian species use low-intensity light for hunting.

Olfaction

Olfaction for prey detection is most important in tadpoles and in caudates who have smaller (or no) eyes and less binocular vision. However, all amphibian species have a Jacobson's organ (vomeronasal organ) and use it to differentiate hormonal states, individuals, eggs, breeding ponds, detect prey and, detect predators.

Thermoregulation

Amphibians are ectothermic and dependent upon the environmental temperature for physiological functioning including digestion and elimination. Extremes of temperatures (too high or too low) cause physiological stress and the animal will often stop eating and become inactive.

All amphibian species, however, will attempt to moderate the environmental temperature in some manner if possible. The integument has mucous glands that cause evaporative cooling and can decrease body temperature. Using behavioural thermoregulation can also reduce or increase body temperature as needed by basking, seeking shade, using substrates, hibernating, estivation or delaying activity until daylight or night fall.

The limited nature of captive environments does present a challenge to provide amphibian species with living areas that offer a selection of thermal areas. Pough (2007) states that individual temperature preferences and tolerance can fluctuate daily, seasonally, by social interaction, with age and/or is dependent on the thermal history of an individual. Even aquatic species (larvae and adults) whose body temperature is close to the ambient water temperature will use behavioural thermoregulation.

In general, when in doubt regarding the appropriate environmental temperature, recommendations are to start at a lower temperature and gradually increase the ambient temperature. Animals kept at temperatures too low for optimum physiological functioning may appear darker in color, they will be inactive and, they may not defecate. Exhibits should include areas of temperature gradients so animals may thermoregulate.

Metabolism

There is currently only minimal information available on amphibian metabolism. Both Donoghue (1998) and Huang et al. (2003) state that the equation for estimating daily kilocalorie provision for amphibians is 33(weight in kg)$^{.75}$. Donoghue, however, says the equation is estimating standard metabolic rate whereas Huang says the equation estimates metabolizable energy (ME). Therefore, the equation should be used as a starting point for daily kilocalorie provision then monitor for food intake, food refusals and body condition (e.g., weight loss or gain). In general, amphibians will eat more food if they are active and they will eat less or stop eating if inactive (assuming other factors such as illness, breeding, etc. are not present).

Specifically for salamander species, those who are paedomorphic (have external gills for breathing in water and do not have metamorphosis) and metamorphic (use cutaneous gas exchange when in water and undergo metamorphosis) have metabolic rates that increase as the temperature increases. In water, paedomorphs and metamorphs have similar metabolic rates but metamorphs have a higher metabolic rate in water than on land. As well, male and female salamanders may have different preferences for temperature and basking schedules (factors directly related to metabolic function) and these preferences may also be related to phenotype.

Immune Functioning

Immune functioning can be a factor in amphibian nutrition because of the complexity of environmental factors in physiological functioning. The environment and husbandry will directly affect food intake, digestion, metabolism and production of endogenous hormones. A healthy frog is one who eagerly hunts prey and eats adequate amounts of food to maintain its physiology. For example, healthy skin can protect against pathogens by producing peptide antibiotics in some species. Skin also contains pigments for camouflage and pheromone production for territorial issues and reproduction. This allows adequate to optimal communication with the environment and conspecifics to reduce stressors that can compromise immune functioning.

Immunologically-challenged animals may also have less resources for reproduction. For example, immune compromised northern cricket frogs (*Acris crepitans*) had reduced spermatic cyst diameter and germinal epithelium depth and these factors limited hatching rates (McCallum & Trauth 2007).

Adult Anuran and Caudata Nutrition

Adult amphibians, as carnivores, hunt prey relative to their physical size. Smaller adult amphibians, for example, may be limited to hunting insects whereas larger amphibians will be able to hunt and eat fish, other amphibians, reptiles, birds, and small mammals.

This section will provide an overview of adult anuran and caudate nutrition. In addition, the provision of **dietary protein**, **fat**, **carbohydrates**, **vitamins** and **minerals** and **water quality** will be discussed as well as a brief note on **cannibalism**.

Adult Anura

The invertebrates commonly fed to captive amphibians are fruit flies (*Drosophila hydei* and *Drosophila melanogaster*), ants (various genera), crickets (*Gryllus* spp, *Acheta* spp), locusts (*Melanoptus* spp), springtails (*Collembola* spp), and blackflies (*Musca* spp). Beetle larvae such as superworms (*Zophobas* spp), mealworms (*Tenebrio* spp), and waxworms (*Galleria* spp or *Achroia* spp), brine shrimp (*Artemia* spp), water fleas (*Daphnia* spp), glass shrimp (*Palaemonectes* spp), various crayfish and, earthworms (*Lumbricus* spp), redworms (earthworm larvae), silkworms (*Bombyx* spp larvae), bloodworms (*Chironomidae* midge larvae), whiteworms (*Enchytraeus* spp), blackworms (*Lumbriculus* spp), and tubifex worms (*Tubifex* spp) are also fed. Vertebrate prey species are commonly used such as freshwater feeder fish (e.g., guppies, mollies, goldfish, smelt), and rats or mice (neonates to adults). Captive feeding often does not develop beyond insectivory using a few species. Feeding a variety of prey not only increases the activity and welfare of the captives, it will provide optimum nutrition.

Despite a classification as insectivores (carnivores), there is anecdotal evidence of some adults of amphibian species ingesting plant material. The Brazilian tree frog (*Xenohyla truncata*) is reported to have a diet of up to 40% fruit material (dry matter (DM) basis) (Silva, Britto-Pereira & Caramaschi, 1989). The adult Indian green frog (*Rana hexadactyla*) has been reported to eat plant material (*Das 1996*). Previously, there was lack of evidence from amphibian specie of enzymes needed to digest chitin, cellulose, or keratin (e.g., chitinase). However, Oshima et al. (2002) found chitanase in the pancreas of a toad (*Bufo japonicus*) that is a homologue to chitanases of other vertebrate species.

Most captive feeding programs of amphibians do not feed amphibian species every day and prey is provided in limited quantities on feed days. This may or may not be appropriate given the sedentary lifestyles of most captive amphibians. However, given that wild species will often eat 100's to 1000's of prey daily, we may have to re-think our amphibian feeding programs and the environments we provide for them. This document cannot answer these – and other questions – but, the information presented in this document may assist change in a positive direction.

Adult Caudata

Aquatic salamanders in the wild (larval salamanders, adult salamanders and neotenic species) prey on leeches, snails, crustaceans, insect larvae, small fish and other amphibians and many species are cannibalistic. Brine shrimp (frozen and dried), whole minnows and fish fillet pieces are often fed in captivity.

Terrestrial salamanders in the wild prey on invertebrates (earthworms, slugs, insect larvae and nymphs, adult insects, arthropods). In captivity, earthworms and beetle larvae (*Tenebrio molitor*) are fed, especially to Ambystoma and Plethodon species.

Dietary Protein

As insectivores (carnivores), amphibian diets will naturally be 30% to 60% protein (metabolizable energy – ME). Or, in other words, protein is 30% to 60% of the calories taken daily. For these species, dietary protein (approximately 9 kcal ME/g) is used as an energy source.

Dietary Fat

As insectivores (carnivores), the dietary fat fraction of amphibian diets will naturally range from 40% to 70% of calories and fat is also used for energy. Dietary fat provides approximately 9 kcal ME/g.

Carbohydrates

With a diet consisting of 30% to 60% protein and 40% to 70% fat, there is only a negligible amount of carbohydrates (all types) in insectivore (carnivore) diets. This applies to amphibian diets also. Captive amphibians fed diets with excessive carbohydrates (e.g., fibre) have developed intestinal blockage.

Vitamins and Minerals

The available information on the dietary vitamin and mineral needs of amphibians is limited to calcium metabolism and some of that information is presented in this section. While we do not know the vitamin and mineral needs of amphibians, experience in the captive care of these species has taught us about the apparent lack of some nutrients. Some of the information on some dietary deficits and excesses of nutrients is presented in the section titled "Nutritional Pathology".

It is natural to focus on the oral ingestion of nutrients. With amphibians, however, some nutrients are obtained from the environment passively via exchanges between the integument and the environment. Calcium, for example, is one mineral that can be absorbed via the integument in adult amphibians (Kingsbury and Fenwick, 1989) and via the gill surface in anuran tadpoles (Baldwin and Bentley, 1980). In addition, meeting the vitamin and mineral needs of an animal should be done in view of the environment the animal's physiology has evolved from and/or the animal has survived in. For example, neotropical amphibians may have evolved in environments with comparatively low levels of minerals. This may indicate that their physiologies are efficient at obtaining, using and storing micronutrients. Such efficiency may predispose these species to o developing vitamin and mineral toxicities.

In general, in captivity, amphibians are fed prey dusted immediately before feeding with a vitamin and mineral dust that includes calcium and vitamins A, B1, D3, and E. Commonly used brands are RepCal calcium with vitamin D3 and Herptivite (RepCal Research Laboratory, Los Gatos, CA USA), Dendrocare (Holland), Frog and Toad Cricket Dust or Frog and Toad Fruit Fly Dust (Rock Solid Herpetoculture, Thaxton, VA USA), or Nutrobal (VetArk, Winchester, UK). Frozen fish, if used, should be supplemented with vitamin B1, vitamin E and thiamine.

The timing of feeding appears critical for most captive amphibians. Diurnal species should be fed in the morning and nocturnal species should be fed at dusk. For some terrestrial species, misting immediately before feeding may stimulate feeding because some species (e.g., Dendrobatidae) are more active after a rainfall. Food that is not eaten within a reasonable time (usually 1-2 hours if fed appropriately) should be removed to prevent spoiling, loss of nutrients, and potential trauma to the amphibian (e.g., attack by prey). Most small captive amphibian species are fed twice daily with various other feeding regimes used depending on the species life stage and size.

Calcium. Stiffler (1993) presented an extensive review of calcium metabolism in amphibians. For amphibians, calcium is assumed to function similar as in other vertebrates for normal membrane function, muscle contraction, nervous system function and for regulation of enzymes. Plasma calcium (free calcium) levels in amphibian blood is similar to other vertebrates but it is unknown if available calcium is similar. For example, available plasma calcium appears to be least in Amphibia tetrapods. In amphibian species, there are seasonal changes in plasma cakcium as demonstrated in *Rana pipiens* with plasma levels falling in winter and an increase in spring and summer (Robertson, 1977).

Plasma calcium levels also change during metamorphosis as demonstrated in *Rana catesbeiana*. These tadpoles had < 8 mgd/dL (tadpoles were at 66% of the adult plasma calcium level) and this level increased to 12 mgd/dL when they became adults (Oguro et al. 1975). There are, however, species differences in calcium levels during metamorphosis and as adults. For example, *A. tigrinum* tadpoles were at 40% of the adult plasma calcium level (Stiffler et al. 1987; Stiffler, 1991) compared to the 66% found in the *Rana catesbeiana* tadpoles. The consensus is that the increase in plasma calcium during metamorphosis is needed for the ossification process from a cartilaginous skeleton to a bony skeleton.

Exchange sites for calciumin amphibians are the gills in larvae, the integument in adults, and, the small intestine (duodenum) in both. For tadpoles, as much as 75% of their dietary calcium needs are taken from their water environment via the gill epthelia (Baldwin and Bentley, 1980) but there are also speciated differences in this mechanism. Tadpoles of *A. tigrinum* and *N. maculosus* do not appear to absorb calcium in this manner (Baldwin and Bentley, 1981).

As vertebrates, anurans store calcium in bone but they also store calcium in the endolymphatic sac located in the inner ear (Pilkington and Simkiss, 1966). The endolymphatic sac in healthy anurans enlarges until it surrounds the brain and the spinal canal with protusions between the vertebrae as lime sacs (calcium carbonate crystals).

The recommended dietary Ca:P ratio for vertebrate insectivores is 1.5:1 (Eidhof, Venema & Huisman, 2006).).

Vitamin D_3. Vitamin D_3, or cholecalciferol, is essential for calcium metabolism in vertebrates. This was demonstrated in *Rana pipiens* (Robertson, 1975) when 2.5mg of vitamin D_3 produced a significant increase in plasma calcium. The intestines and kidney of adult *Rana pipiens* and the gills of tadpole *Rana catesbeiana* increased their uptake of calcium when stimulated by vitamin D_3 (Baldwin and Bentley, 1980).

Water

Amphibian urinate or lose moisture via the integument at about 33% of their body weight daily (excepting amphibians of dry climates) and maintaining an osmotic balance with the environment is an imperative. Cells of the integument can actively pump salt for maintaining an osmotic balance.

Whether an amphibian is an aquatic or terrestrial species, hydration is important to maintain health and physiological functioning. The use of municipal and well water for captive amphibians requires some water management to assure the well-being of the animals. Recommendations include:

1. Let water stand for 24 to 48 hours before use to allow chlorine to dissipate.

2. Cleaners containing ammonia, chlorine and phosphates should not be used in the exhibit and/or on items to be used in the exhibit.
3. Limit nitrite levels to <0.1 parts per million (ppm). (Whitaker, 2001; Banks et al., 2008)
4. Limit nitrate levels to (<1.5 mg/L (Banks et al., 2008).
5. If soil is used in the exhibit for terrestrial amphibians, the animals should be able to absorb water from the soil (Pough, 2007). This means maintaining a high (-100 to -200 kPa) matrix water potential level of the soil ("soil that can be squeezed into a ball that does not crumble when the pressure is removed").
6. Flush the water system to remove standing water that may have leached chlorinated biphenyls or metal into the water.
7. Metals known to be toxic to amphibians include aluminum, antimony, arsenic, cadmium, copper, lead, manganese, mercury, molybdenum, silver and zinc (Blaustein et al., 2003; Browne et al., 2007).
8. Carbon dioxide levels should be < 6 mg/L.
9. Uneaten food should be removed from the water immediately after feeding is complete.
10. Unionized ammonia levels should be < 0.02 ppm (Diana et al., 2001).
11. Latex gloves can be lethal to tadpoles (Sobotka & Rahwan, 1994).

A potential problem during winter with municipal and well water is a build-up of gases (gas supersaturation) that can cause "gas-bubble disease" (the bends) in aquatic amphibians because cold water can hold higher levels of dissolved gas. The build-up of gases usually include nitrogen, carbon dioxide and/or oxygen. The problem is easily solved by aeration allowing the gases to dissipate and/or warming the water to decrease its ability to hold high levels of gas.

McRobert (2003) typically maintained tadpoles used in his research in water kept at 22 to 30°C. The research found that relatively minor increases in tadpole water temperature (e.g, 1°C increase) significantly lowered survival rates and increased the length of time until metamorphosis.

Cannibalism

Cannabilism does occur among adult, carnivorous amphibian species. In general, housing by size (e.g., small with the small) at all life stages will reduce or eliminate most cannibalism.

Nutrition and Reproduction

In general, about two months prior to breeding season, captive amphibians should be fed ad lib with high quality feed (Browne and Zippel 2007). Consuming large amounts of prey are common in the wild prior to breeding season and during seasonal increases of invertebrate populations. However, the provision of food should return to normal levels after breeding to prevent obesity.

Nutrition of Larvae

In general, even less is known about the dietary needs of larval amphibians than of adults and the focus of research has been on environmental conditions. Adequate and appropriate food and temperature (water and/or air) appear to be the two major factors for larval survival (Alvarez

& Nicieza, 2002). In addition, other factors include housing density, light, water pH level and, dissolved oxygen (McDiarmid and Altig 1999).

Egg mortality in captivity can be high (e.g., 50% to 90%) and the rate of tadpole mortality can be even higher (36.1% to 80%) (Browne and Zippel 2007; Banks 2008). Some factors in egg and larval mortalities include the use of latex gloves (Sobotka and Rahwan 1994) and lethal bacterial blooms that result from decomposing eggs, gel or food.

The anamniotic eggs of amphibians contain an ovum with one egg membrane and the yolk provides energy for some development to metamorphosis. In general, anurans that hatch as a tadpole have less yolk reserve than those anurans that hatch as froglets (Mitchell 2001). The eggs of salamander species take longer to develop and require more nutrients than eggs of anuran species. Both anuran and caudate larvae may not start feeding immediately after hatching.

Anuran Larvae

Larval anurans (tadpoles) can be herbivorous (algae and bacteria), omnivorous, macrophagous (zooplankton and small crustaceans), carnivorous or oophagous (obligate egg-feeders) and cannibalism – when the opportunity exists - is common. Oophagous tadpoles usually develop in the leaf axils of plants (e.g., bromeliads) and feed on the infertile eggs left by the female for the developing tadpole (Pough 2007).

Anuran tadpoles have a wide mouth with or without keratinous denticles and are filter filters. The tadpole mouth cavity has mucus to entrap small particles and larger particles are swept by papillae to the esophagus. The mucus breaks off in pieces and the pieces are swept into the esophagus. Aquatic anuran larvae have internal gills and terrestrial larvae have large, external gills and/or respiratory tissue (expanded tails or lateral folds) for breathing. Their stomach is underdeveloped and their intestine is much longer than the adult intestine to facilitate the absorption of nutrients.

Caudata Larvae

The larvae of caudates have external gills, a rudimentary tongue, true teeth (both jaws), functional jaws and their digestive system is similar to adult caudates. All caudate larvae are carnivorous and most are cannibalistic. Caudate larvae feed on various life-stages of invertebrates and smaller amphibian larvae. Some aquatic newts species will feed on algae. Prey size is comparable to developmental stage: younger larvae take smaller prey such as zooplankton and prey size will increase with larval size.

Many caudate species have direct development meaning larval and juvenile life stages are concurrent. Parental caudates (pairs) will attend eggs (e.g., prod or fan eggs) and it is recommended that parents of these species attend their eggs (Pough 2007

Housing Density

Browne and Zippel (2007) provide extensive recommendations for three levels of housing densities for rearing larvae: low-density, medium-density, and high-density larval rearing. In a low-density system, larvae are predictably cannibalistic and have individual units. Low-density systems are work intensive because they require individual monitoring and water changes for many units. The advantages of this method include less competition for food, no cannibalism and, larger tadpoles and froglets. Medium-density systems have three to ten larvae per liter and the advantages of this system include less cost. Disadvantages of medium-density housing include longer larval life stage, smaller tadpoles and, the requirement of more water changes. High-density housing puts 40 to 80 larvae per liter and results in more larvae at less cost. However, water changes must be frequent, there is competition for food and, a flow-though water system is usually needed. Feeding in high-density housing should be with a fine, particulate, high-quality feed such as ground fish pellets with spirulina powder to ensure distribution and satiation (Browne et al. 2003). Greens such as spinach, cabbage, and kale have been toxic for amphibian larvae (probably due to their high oxalate content).

Growth

As stated above, diet and environmental temperature are the major determinants of (Alvarez & Nicieza, 2002). However, the effect of diet depends on temperature and the interactive effect of diet and temperature affect the animals' size at metamorphosis. In general, larvae fed a diet high in protein and lipids are larger than larvae fed a diet high in carbohydrates at 17 °C but not at 12 or 22 °C. This research used the Iberian painted frog (*Discoglossus galganoi*) and it has not been repeated using other species.

Other growth tendencies:

1. The effects of food shortage depend on when the lack of food occurs. If the lack of food occurs before the minimum size for metamorphosis, a food shortage results in extension of the larval period. If a food shortage occurs later in development (e.g., maximum size for metamorphosis), the larval period is shortened (Alvarez & Nicieza, 2002).
2. Metamorphosis will begin at an earlier age if food is available only during the daytime than compared with metamorphosis when food is available ad lib (Nicieza 2000).
3. Larval size affects maximum tadpole size and, tadpole size correlates with size at metamorphosis. However, size at metamorphosis is not related to larval size (Alvarez & Nicieza, 2002).
4. Large larvae metamorphose earlier than small larvae (Alvarez & Nicieza, 2002).
5. Oophagous tadpoles grow faster and become larger adults when fed more frequently (everyday versus every 4 days) (Liang, 2002).
6. Herbivorous tadpoles must have food available ad lib because they have shorter gut transit times than carnivorous species (1 to 6 hours for herbivores vs 8 days for carnivorous species) (Liang, 2002). (**Please Note**: Feeding only after gut emptying (e.g., once every eight days) is not an optimum nor a humane feeding regime to preserve healthy physiological functioning).

Diet and Fatty Acids

Liang (2002) fed tadpoles either chicken egg yolk or the eggs of the Taiwanese tree frog (*Chirixalus eiffingeri*. Tadpoles fed chicken egg yolk grew slower than tadpoles fed eggs of conspecifics. It is hypothesized that the lipid differences between the eggs accounted for the growth differences. The eggs of conspecifics, for example, most likely have the correct fatty acid composition needed for the developing tadpoles versus the fatty acid composition of the chicken egg yolks (Liang, 2002; Huang et al., 2003).

The fatty acid profiles of amphibian eggs resemble those of insectivorous lizards: the eggs contain 20:5n-3; they are high in 20:4n-6; and, they are low in 22:6n-3 (Speake and Thompson, 2000). There are differences in the fatty composition of aquatic amphibians versus terrestrial amphibians. Aquatic amphibian eggs have higher fractions of non-polar lipids than terrestrial amphibian eggs (Huang et al., 2003).

Cannibalism

Cannibalism does not occur among larval herbivore amphibians but it is a normative behaviour for carnivorous amphibian species. As a normative behaviour, however, cannibalism may undermine captive breeding programs. In general, housing by size (e.g., small larvae with the small) at all life stages will reduce or eliminate most cannibalism.

When possible, however, surplus larvae could be used as food for conspecifics or other carnivorous amphibian species. Larvae fed conspecifics grew faster than larvae fed heterospecifics or commercial food (Huang et al., 2003) and the faster growth may be due to the availability of appropriate essential fatty acids. For example, *Chirixalus eiffingeri* tadpoles fed eggs of their own species and chicken egg yolk had faster growth and development for the tadpoles feeding on conspecific eggs versus the group feeding on chicken yolk (Liang, 2002). The faster growth was despite the chicken egg yolk offering more lipid. In addition, *Chirixalus eiffingeri* have higher levels of n-3 fatty acids (e.g. 18:3n-3 and 20:5n-3) which may be important for the growth of tadpoles.

Nutrition of Juveniles (Froglets)

At metamorphosis, larvae develop into juveniles and a corresponding diet change occurs because the juveniles are capable of eating different prey. In general, when the front legs of an anuran larva begin to emerge, they will stop eating (McDiarmid& Altig, 1999; Browne et al., 2003; Browne & Zippel, 2007) and continued feeding at this stage will waste food and foul the water from decomposing food. For terrestrial species, once metamorphosis begins, their habitat should be changed to a sloped enclosure of 2/3 transitional water (< 3 mm) and 1/3 terrestrial habitat (Fenolio, 1996; Browne & Zippel, 2007).

After metamorphosis, small insects sized relative to the size of the juveniles can be fed. Feeding should be ad lib. The mortality rate of captive amphibian juveniles can range from 31–71% (Banks et al., 2008).

Providing Prey to Captive Amphibians

Most amphibian species will only take moving, live prey although there is some anecdotal evidence that an amphibian may eat dead prey if it is wiggled and dangled. Captive amphibians must be supplied with appropriate prey. “Appropriate prey” means prey species that provide complete nutrition in a size, number and manner suitable for the life stage and size of the predator. For example, the predator may become the prey if they are fed prey that is larger and stronger. Or, live prey left for extended periods with a predator may injure the predator. A large population of ants, for example, killed juvenile amphibians overnight (Banks et al., 2008) and there have been numerous reports of amphibians injured by uneaten rodents left in the exhibit.

Feeding Invertebrate Prey. Invertebrate prey offer complete nutrition in the wild but this is not necessarily the case for captive-raised invertebrates. Captive invertebrate prey may be deficient in some nutrients unless they are raised and maintained on a vitamin and mineral rich diet. Appendix C offers a complete diet for raising and maintaining cricket feeder insects and there are various commercial diets available.

Finely ground calcium carbonate can be used to quickly increase the Ca level of crickets for feeding to amphibians. Feed the crickets a 10% Ca diet and, if the crickets will be maintained longer on a calcium diet, reduce their Ca dietary level to 8% (Eidhof, Venema & 1997).

The importance of provisioning the correct diet for prey animals is illustrated in Table 1 and Table 2. In Table 1, feeding silkworm larvae (*Bombyx mori*) on a natural diet of mulberry leaves results in an increase in fat (and, possibly, a correct fatty acid composition) and, an increase in vitamin E and Ca. The decrease in P, in this instance, is a positive result because it corrects the skewed Ca:P ratio of 0.10:1.37 to a more desirable ratio of 0.91 to 0.75 (from a ratio of 1:13.7 to a ratio of 1.2:1). As stated previously, a Ca:P ratio of 1.5:1 is recommended for insectivores (Eidhof, Venema & Huisman, 2006).

Table 1. Comparison of the Nutrient Composition of Silkworm Larvae (*Bombyx mori*) Fed a Natural Diet of Mulberry Leaves Compared to a Greens Diet (Dierenfeld and Fidgett, 2006)

Diet	% Protein	% Fat	Vitamin A IU/g	Vitamin E IU/g	% Ca	% P
Silkworm larvae Mulberry diet (*Bombyx mori*)	53.0	20.2	-	0.47	0.91	0.75
Silkworm larvae (*Bombyx mori*)	53.7	8.1	0.67	0.004	0.10	1.37

Note: All nutrients % dry matter (DM)

Table 2 shows changes in the Ca:P ratio of adult Jamaican crickets (*Gryllus assimulus*) fed diets with varying levels of Ca. In addition, Table 2 shows the progression of the change in Ca:P ratio from before eating the diet, to 24 hours after eating, 48 hours after eating and, 72 hours after eating. This table demonstrates that the dietary level of Ca for the prey is as important as the timing of presenting the prey to the predator if the prey is not maintained on an appropriate diet.

Table 2. The Calcium:Phosphorus (Ca:P) Ratio in Adult Jamaican Crickets (*Gryllus assimulus*) Fed Diets with Varying Levels of Calcium (Ca) at 24 Hours, 48 Hours and 72 Hours Post-feeding (based on Eidhof, Venema & Huisman, 2006)

%Ca in feed	Ca:P ratio in Cricket Prey			
	Before Feeding	**24 hours**	**48 hours**	**72 hours**
7.15	0.33:1	1.14:1	0.99:1	0.83:1
7.69	0.14:1	0.58:1	0.95:1	0.75:1
8.92	0.22:1	0.69:1	1.42:1	1.76:1
10.57	0.14:1	1.00:1	1.00:1	1.64:1
11.07	0.22:1	1.44:1	1.70:1	2.49:1
12.14	0.33:1	1.11:1	1.04:1	1.00:1
11.44	0.14:1	1.40:1	1.80:1	1.82:1
12.77	0.22:1	1.49:1	2.26:1	2.58:1

Invertebrate prey vary in the amount and types of amino and fatty acids they provide. Therefore, a large variety of invertebrate species should be fed to ensure complete, balanced nutrition. For example, beetle larvae (superworms (*Zophobas* spp**)**, mealworms (*Tenebrio* spp), waxworms (*Galleria* spp or *Achroia* spp)), other larvae (silkworms (*Bombyx* spp), bloodworms (Chironomidae midge larvae)) worms(blackworms (*Lumbriculus* spp), tubifex (*Tubifex* spp), whiteworms (*Enchytraeus* spp) earthworms/nightcrawlers (Lumbricus spp), redworms (earthworm larvae), are high in fat, have a deficient Ca:P ratio and are low in sulfur and amino acids. The nutrient deficiencies of these foods mean they should be used as complementary feed with other prey species to ensure a balanced diet.

Other common prey fed in captivity are wingless fruit flies (*Drosophila hydei* and *Drosophila melanogaster*), ants (various genera), pinhead (nymph) and adult crickets (*Gryllus* spp, *Acheta* spp), locusts (*Melanoptus* spp), springtails (*Collembola* spp), and blackflies (*Musca* spp). These prey have a poor calcium content, an inverse Ca:P, and low vitamin A content. Other invertebrate prey commonly fed in captivity are brine shrimp (*Artemia* spp), water fleas (*Daphnia* spp), glass shrimp (*Palaemonectes* spp) and, various crayfish.

Common recommendations for increasing the available nutrition of invertebrate species is to dust the prey with a supplement powder (a vitamin and mineral dust that includes calcium and vitamins A, B_1, D_3 , and E) prior to feeding to the predator. The deficits of this method include an unknown dosage ingested by the predator for several reasons: a dose size for dusting is not presecribed, the powder may not adhere to the prey, the predator may ingest a large dosage of the supplement or may get a very small dose if there are multiple, more dominant predators.

Supplements commonly used by researchers for dusting invertebrate prey include RepCal calcium with vitamin D_3 and Herptivite (RepCal Research Laboratory, Los Gatos, CA USA), Dendrocare (Holland), Frog and Toad Cricket Dust or Frog and Toad Fruit Fly Dust (Rock Solid Herpetoculture, Thaxton, VA USA), or Nutrobal (VetArk, Winchester, UK).

An alternative method to dusting is to raise and maintain prey on a diet that provides them with complete nutrition. In addition, this method must be used with designing a diet that utilizes complementary prey species to ensure the correct range of protein (amino acids), fats (fatty acids) and vitamin and minerals is available for the predator.

Feeding Vertebrate Prey. Adult vertebrate prey provides bone, most trace minerals and vitamins (via the liver and kidney), iodine (via the thyroid) and, zinc (via the pancreas). However, most adult vertebrate prey raised in captivity are obese and probably provide a high level of saturated fat and may cause the predator to develop a hypervitaminous of fat-soluble vitamins. Neonatal and juvenile vertebrate prey does not provide as much fat, but they may be deficient in calcium and fat-soluble vitamins. The most common vertebrate prey fed in captivity are fish (e.g., guppies, mollies, goldfish, smelt) and various life stages of rats or mice

NUTRITIONAL PATHOLOGY

Anecdotal information and some empirical information is available regarding the pathology suffered by captive amphibians by inappropriate nutrition. This pathology is often treated as a disease process rather than a result of inappropriate nutrition.

Dietary Deficiencies

Apparent inappetence is a common problem when feeding some captive amphibian species. Usually, this develops from offering inappropriate prey species, the wrong size of prey and/or, an incorrect feeding schedule. In addition, some prey may be deficient in the nutrition they provide especially when a variety of prey is not available. Other causes of inappetence, however, can be attributed to disease processes including oral lesions, trauma and neoplasia.

Husbandry practices can contribute to inappetence. These practices include inappropriate humidity, housing, temperature, humidity, group social issues (dominance, breeding, stress), poor water quality and, an incorrect frequency and timing of feeding (Hadfield et al., 2006).

Temporary anorexia may be a normal fasting during estivation in Sirenidae (sirens), Leptodactylidae (e.g., tropical frogs: Budgett's frog, *Lepidobatrachus asper*), Bufonidae (e.g., Sonoran desert toad, *Bufo alvarius*), and Ranidae (e.g., true frogs: ornate-horned frog, *Ceratophrys ornata*)(Secor 2005). Some species may also be anorexic during the breeding season (Guha et al., 1980; Laming & Cairns, 1998)

The signs of malnutrition in captive amphibians include inappetence, weight loss, dehydration, lethargy and, in aquatic species, edema. With some exceptions, physical signs include a concave abdomen and lack of muscle over the limbs and the vertebral column, creating a prominent urostyle and transverse processes. An exception to this are stubfoot toads (*Atelopus spp*) who have prominent transverse processes (Donoghue 1998).

Dietary Excess

Obesity is commonly found in some captive amphibians because of calorie excess and inactivity. Excessive calories are easily provided when beetle larvae (superworms (Zophobas spp), mealworms (Tenebrio spp), and waxworms (Galleria spp or Achroia spp), whiteworms and tubifex are the main or only prey item. These invertebrates have a high lipid content. Also, if older, obese rodents are fed, these prey animals are also high in lipids.

Obesity in many amphibians may only be found at post mortem because, in most amphibians, fat is stored in coelomic bodies (celom; the body cavity) and around the heart. An exception are some Bufonidae (true toads) who can develop inguinal fat bodies In the groin area.

Captive amphibians who have eaten high levels of dietary fat for an extended period can develop corneal lipidosis (lipid keratopathy). Corneal lipidosis develops as haziness in the cornea and progresses to blindness. Although corneal lipidosis may be considered a disease of aged amphibians, it is correlated with high levels of dietary cholesterol and high serum cholesterol level (Shilton et al., 2001; Keller & Shilton, 2002). Corneal disease results in reduced food intake and/or anorexia because the animal cannot hunt prey.

Consuming an excessive number of prey can result in gastric overload. Gastric overload is an acute (medical emergency) and a result of dietary excess. Distention of the stomach from excessive consumption impairs respiration and circulation and the animal can develop hypovolemic shock.

Metabolic Bone Disease

Pathology related to improper dietary provision for normal calcium metabolism is a primary problem of captive amphibians. For example, Yoshimi et al. (1996) fed frogs crickets raised on Ziegler cricket diet and dusted with 1:1 Pervinal (canine vitamin and mineral supplement) and Osteofrom (human chelated mineral supplement). The frogs developed hypercalcemia and tissue mineralization from ingesting excess dietary calcium and vitamin D_3.

Metabolic bone disease (MBD) can be caused by deficiencies in dietary calcium; improper calcium:phosphorus (Ca:P) ratios; lack of the appropriate ultraviolet (UV) light spectrum; low calcium content of prey; and, deficienciesand excesses of vitamin D. The clinical signs of MBD include: lack of bone mineralization; bone deformities (vertrebrate, limbs, mandible); abnormal posture; abnormal movement; tetany; edema; fractures of long bones; and, lack of radio-opaque calcium carbonate in the endolymphatic sacs.

Hypovitaminosis A

A deficiency of vitamin A develops in captive amphibians if they do not have a source of dietary vitamin A because they cannot synthesize carotinoids, including vitamin A (retinal) (Wright 2006). It is unknown if vitamin A precursors (e.g., carotene, lycopene, phytoene) are dietary essentials for amphibians.

Vitamin A is important for a healthy integument; it is incorporated into the xanthophore (yellow pigment) chromatophore cells in the amphibian skin as carotiniods (Frost-Mason et al. 1994); and, it is important in calcium metabolism.

Signs of hypovitaminosis A occur in postmetamorphic animals and include lethargy, weight loss, wasting and, “short tongue syndrome” (reduced ability to capture live prey with the tongue)(Pessier et al., 2005; Wright 2006).

Hypervitaminosis A

Hypervitaminosis A usually develops in captive amphibian animals fed prey who have been fed high concentrations of vitamin A and/or an excessive dietary intake of vitamin A. For example, amphibians fed mammalian livers and/or whole immature rodents (rich in vitamin A) are at risk for developing hypervitaminosis A. Clinical signs of hypervitaminosis A include MBD, anemia, liver disease and, weight loss (Crawshaw 2003).

Hypervitaminosis D

Hypervitaminosis D usually develops in captive amphibian animals fed prey who have been fed high concentrations of vitamin D_3 and/or excessive dietary intake of vitamin D_3. For example, captive amphibians fed diets of goldfish (high in vitamin D_3) are at risk for developing hypervitaminosis D (Frye 1992).

Hypovitaminosis D

A deficiency of vitamin D_3 can be related to a dietary deficiency and/or to lack of UVB light. Clinical signs of a deficiency include: bone deformities, seizures, edema, poor growth, reproductive problems, muscle weakness, anorexia, gut stasis and, constipation. Hatching failure can occur even in females without sufficient exposure to UVB on an adequate diet with adequate vitamin D_3 and calcium.

Hypovitaminosis B’s

Some neurological and musculoskeletal disorders (peripheral nerve demyelination, paralyses, scoliosis, spindly leg syndrome) are correlated with a deficiency of B vitamins, especially a thiamine deficiency (Wright & Whitaker, 2001; Crawshaw, 2003). In general, thiamine deficiency is caused by the ingestion of prey high in the thiaminase enzyme.

Secondary Nutritional Hyperparathyroidism (SNHP)

There are several potential causes of secondary nutritional hyperparathyroidism (SNHP): calcium and phosphorus deficiencies and excesses; vitamin D_3 deficiencies and excesses; dietary fatty acid imbalance; hypervitaminosis A; and, renal disease (Wright & Whitaker 2001).

Captive raised rodents are high in vitamin A and excessive use as a prey is implicated in the development SNHP.

Gout

Amphibian species that excrete nitrogen as uric acid are predisposed to develop gout (deposition of urate crystals in soft tissues and joints). Gout develops in these species because of a lack of hydration. Those animals that develop gout need to be monitored for hydration status and the maintenance of adequate hydration is the most important factor in prevention.

Beta-carotene Deficiency

Ocular disease, skin disease and lack of color in captive amphibians have been attributed to a deficiency of dietary beta-carotene. Prey species should be fed beta-carotene supplements. Carotenoid pigments can be found in the crude fat of insect prey (Dierenfeld and Fidgett, 2006) but the carotenoids may need to be fed to the prey.

Iodine Deficiency

Tadpoles may not begin metamorphosis if they have an iodine deficiency (spindly leg).

Oxalate Toxicity

Oxalate toxicity in amphibians (e.g., herbivorous aquatic life stages) develops in animals fed plant diets (e.g., spinach and kale) that are high in oxalates (salt of oxalic acid) (Wright and Whitaker 2001). These animals develop renal calculi (renal disease). Oxalate toxicity can also develop in adult amphibians that are fed insects raised on diets high in plants rich in oxalates.

Environmental Light and Ultraviolet Light Factors Related to Nutrition

The light requirements of captive amphibians can be complex depending on life stage, season, specie and the quality of light (spectrum, intensity, and photoperiod). For example, most amphibian species appear to prefer a luminance (light without heat) of >90 lux, but there are some species that prefer as little as < 0.01 lux.

Appropriately providing the ultraviolet (UV) spectrum for captive amphibian species can be a challenge if only because we lack specific information about the appropriate spectrums. The UV spectrum has three bandwidths: UVA, UVB, and UVC. UVA (long-wave) is from 320 – 400 nanometres (nm); UVB (medium wavelength) is from 280 – 320 nm; and, UVC (short wavelength) is from 100 – 280 nm. UVC is filtered by the earth's atmosphere and is not a factor in this discussion. UVB light is the spectrum of concern for captive amphibian species because UVB light is necessary for the biosynthesis of vitamin D_3 which is necessary for normal calcium metabolism (Allen & Oftedal, 1989; Carman et al., 2000). It is possible, however, that nocturnal species of amphibians may not require UVB light.

Frogs raised without UV light developed femur curvatures and the curvatures were reversed by provisioning UV light 30 cm above the holding tanks on timers for 30 minutes each morning and 30 minutes each afternoon (Banks et al., 2008). MBD was prevented from developing by provisioning tadpoles with full-spectrum fluorescent lights (12:12 light/dark cycle) (McRobert, 2003). When possible, exposure to natural sunlight is the most efficient means of providing UVB light. For example, Komodo dragons at the Louisville Zoo were housed in an exhibit providing 150 days of access to direct sunlight. These animals were able to maintain normal circulating levels of the vitamin D metabolite, 25-hydroxyvitamin D, for the remaining year when they were housed indoors (Gyimesi and Burns, 2002).

Of course, problematic with amphibian species, is a lack of specific and comparative information on physiological correlates for healthy calcium homeostasis. It is possible that some amphibian species may need UVB gradients (Adkins et al., 2003). However, appropriate housing for any animal will allow behavioral methods of selecting microhabitats and this should be the same for UV light provision.

UV light does not penetrate through standard glass or Plexiglas® (100% filtered), and it is partially filtered (30% - 50%) by wire mesh. UV light can penetrate some UV-permeable acrylics, but these acrylics are expensive and the permeability will decline as the acrylic ages. Products that produce artificial UVA and UVB light are marketed as "full spectrum", but the products vary in spectrum, effective life-span and cost. In general, it is recommended that products that emit UV light and heat are the most effective both in cost and prevention of MBD. Most ectotherm species will seek heat rather than UV light and an exhibit with separate units – one that provides UV and one that provides heat – is a waste of the UV light because the ectotherm will choose to bask under the heat lamp and not under the UV lamp. In addition, cutaneous conversion of pre-vitamin D_3 to vitamin D_3 is a temperature dependant process and it is best to ensure the animal is sufficiently heated when exposed to UVB. Some of the newer self-ballasted bulbs emit both UV light and heat.

Full-spectrum lights (fluorescent and screw-base), mercury vapour lamps and blacklights are available. All lamps other than the mercury lose their effective wavelengths after approximately six months of use. Mercury vapour lamps must be replaced only when they burn out ,but the initial cost is more than full-spectrum lights. Another difference is that full-spectrum fluorescents and bulbs must be positioned about 12 cm from the animal for an effective wavelength. The backlight bulbs and mercury vapor bulbs can emit UV at a greater distance (see product monograph). Generally, UV emission is highest closest to the light source and output will decrease with distance away from the source. Bulbs tend to degrade at about the same rate, therefore higher output bulbs have a longer useful lifespan than lower output bulbs. Some commercial UV products used by researchers are:

ESU Reptile D Light®
NECs 40 W black-light fluorescent tubes (BLB 40)
Reptisun® products by ZooMed® 10 - 20 cm
Sylvania® BL350 blacklight 10 - 20 cm
Sylvania®40 W black-light fluorescent tubes
Westron® Active UVHeat (screw-base, self-ballasted mercury vapor lamp)
ZooMed Power Sun® UV (screw-base, self-ballasted mercury vapor lamp)

Deleterious Effects of Ultraviolet Light

Global warming and the increase in UVB light have led researchers to conclude that UVB light is a factor in the decline of amphibian populations. UVB light is associated with abnormal DNA, immunosuppression, cancers and, decreased and/or lack of survivorship in all life stages of smphibian species. It is recommended that - in meeting the physiological need for UV light to captive amphibians - we should also provide microhabitants for protecting themselves and their offspring from the deleterious effects of UV light.

Eggs. Amphibian eggs are naturally protected from normal UV light by colour and a jelly coating. Although some amphibian eggs are of a light colour, those eggs that are darker in colours are protected by UVB resistant melanin. In addition, many species lay their eggs under debris, in leaves or in foam nests that protect from UVB radiation.

The jelly capsules of aquatic amphibian eggs absorbs UVB (up to 99%) andprevent penetration to the egg. The absorbance capability of jelly capsules can differ between species. Most jelly will also accumulate debris that also protects the ova from UVB light. In addition, the photolyase in amphibian eggs activates photorecovery of damaged DNA when embryos are exposed to UVA, UVB and visible light (> 400 nm) (Grant and Licht 1995; Crump et al. 1999). This protection, however, is not effective with artificially high doses of any type of UV radiation.

The effects of exposure of eggs to UVB may not be visible until metamorphosis and the length of exposure is correlated with increased growth problems (Pahkala et al, 2001; Pahkala et al, 2003) such as lack of limb development and/or limb deformity (Ankley et al, 2000). Exposed eggs also grow slower (Belden and Blaustein, 2002; Blaustein and Belden, 2003) and adult reproductive success can be decreased (Goater, 1994; Scott, 1994).

Larval stage and metamorphosis. After hatching, larvae are protected from UV light by melanin, skin secretions and behaviour (avoidance of intense sunlight and thermoregulation) (Hofer and Mokri 2000). There is a high mortality, however, in several species for the first few days after hatching. This high mortality is suspected to be due to loss of the normal protecants of embryos (Grant & Licht, 1995; Crump et al., 1999; Hofer & Mokri, 2000; Licht, 2003). Baud & Beck (2005) found an interactive effect between UVB light and copper (Cu) and this affect significantly reduced the survival of spring peeper tadpoles (*Pseudacris crucifer*).

Adults. Poison dart frog adults chose a low level UVB condition (Han et al. 2007). Sunburn may be a possibility under high intensity lights or if an animal cannot escape the light by choosing a protected location. “Sunburn” has clinical signs and these include increased mucous production, erythema (inflammatory redness of the skin), blistering, ulceration, and skin sloughing of affected skin (Wright 2001).

Note: Investigators used acetate filters underwater to cover eggs during a UVB trial (Berrill & Lean, 1998; Crump et al., 1999). These investigators discovered that acetate filters were highly toxic to hatchlings (but not to eggs).

Environmental Enrichment

Despite classification as a “lower level” vertebrate, amphibian species thrive in environments that provide variety, activity and challenges (Seidensticker & Forthman, 1998). Dietary provision can also meet this need for variety, activity and challenge. Feeding live prey by placing it in, around and under leaf debris, sand, wood, rocks, flower pots, live plants and flowing water provides hunting opportunities. Different types of foliage covered with aphids can be placed at varying times and places within the exhibit (Campbell-Palmer et al., 2006). Varying the environmental conditions of the exhibit at feeding times (e.g., “rain”) improve the environmental complexity and increase the exhibit humidity. Such activities encourage display of natural behaviors, improve health and, promote colorful displays in the animals.

Plants successfully used in amphibian exhibits include mosses (aquatic and terrestrial) rushes, aquatic plants (*Elodea*, *Vallisneria*), peppermint, wild strawberries, deer grass, seep willow and, grape vines (McRobert 2003). The warm, humid environs of amphibian exhibits should be excellent areas to grow even tropical plants. "Rainy seasons" (e.g., a 3 hours misting) can be used to promote breeding in some species and can increase appetitive behaviors (Campbell-Palmer et al., 2006).

Environmental Toxicities Related to Nutrition

The physiology of amphibian species such as cutaneous gas exchange due to the permeability of the integument makes them vulnerable to toxic environmental substances. These substances may or may not come in contact with captives as part of dietary provision. This section will present some of those environmental substances relative to captive environments. Common items and substances that may be toxic to captive amphibian species include: plastics, metals, chlorine, ammonia, nitrates, carbon dioxide and food toxicity.

Plastics

The use of plastic in our society is so widespread that we probably do not realize the extent of plastics (phenolic, acrylics, polyvinyl chloride) that may affect captive amphibian environments. The main concern is the leaching of substances such as polychlorinated biphenyls (PCBs2) and other toxicants even when human grade plastics are used. For example, many water systems use plastic piping and standing water from this piping may have levels of chlorinated biphenyls (Browne et al 2007). Flushing the water system each morning or ensuring an appropriate pipe rating for water used in amphibian habitants is essential.

Metals

Metal toxicity can affect the reproductive, nervous, endocrine, and immune system function (Sanchez-Dardon et al., 1999; Zapata et al., 2001). These toxicities can also interefere with metamorphosis and growth rates (Lefcort et al., 1999). Tadpoles are especially susceptible to metal toxicity because of their ravenous appetites to meet their high nutritional requirements (Hopkins et al. 2000). Species adapted to water with a low pH are especially at risk for metal toxicity because a low pH increases the risk for metal leaching (Beattie & Tyler-Jones 1992; Glooschenko et al., 1992).

There are numerous metals known to be toxic to amphibians: aluminum, antimony, arsenic, cadmium, copper, lead, manganese, mercury, molybdenum, silver and zinc (Blaustein et al., 2003; Browne et al., 2007). The metals, if used in exhibits and cages, in plumbing or – especially – in contact with water where leaching is prominent. Copper, for example, is commonly used in potable water plumbing systems and in well water systems. Copper will leach into water that stands in copper pipes (e.g., overnight) and a concentration of only 0.15 mg/L killed 50% of newly hatched *Rana pipien* tadpoles in 72 hours (Landé and Guttman 1973).

Chlorine

Captive amphibians may come into contact with chlorine in water supplies or from cleaners. Municipal water may have concentrations of > 9 mg/L chlorine (used as an antibacterial) and this concentration probably varies according to the water treatment facility. Such concentration of chlorine is lethal to aquatic species and species largely dependent on a captive water source. Chlorine is an unstable chemical and it easily dissipates from water by letting the water stand for several days, keeping it aerated and warm. Municipal water treatment may use other bactericidal agents (e.g., chloramines) that are stable in water and do not easily dissipate from water. These bactericidal agents are equally as toxic to aquatic life as chlorine.

Chlorine is a common element in many cleaners and residues on dishes and surfaces can be toxic to amphibians. Essentially, it is recommended that any cleaner containing chlorine is.

Ammonia

Metabolic wastes, municipal water and cleaning products are the main sources of ammonia in the captive environment. Ammonia can be toxic to aquatic life at minimal levels of 0.5 mg/L (Jofre & Karasov, 1999). Signs of ammonia toxicity include abnormal behaviour, changes in skin pigmentation, increased mucous production and, death.

(Diana et al., 2001). If chronic toxicity develops from low levels of ammonia, animals will develop immunosuppression and susceptibility to diseases (Whitaker 2001).

Ammonia from protein respiration (especially from larvae and gill-breathing adult) and bacterial decomposition of organic matter will commonly enter captive aquatic systems. In addition, municipalities often use ammonia as an antibacterial agent and ammonia is in municipal water if chloramines are used. Cleaning agents containing ammonia should not be used to clean any item that may come in contact with captive amphibians at any life stage.

Nitrites and Nitrates

Nitrites and nitrates can be lethal and can also cause failure to thrive and deformity with nitrite toxicity more severe than nitrate toxicity. Nitrite toxicity is similar to the effects of ammonia, although it appears that the effects are species specific. Nitrite concentrations of 0.88 mg/L, for example, did not affect the red-legged frog (*Rana aurora*) and the Pacific treefrog (*Hyla regilla*) as severely as it did the spotted frog (*Rana pretiosa*) (Blaustein et al. 1999). Nitrites form when nitrifying bacteria oxidize ammonia and ammonium. Nitrite toxicity causes methemoglobinemia (methemoglobin in the blood) that prevents blood oxygen delivery to tissue.

Nitrates are less toxic to aquatic life when compared to ammonia and nitrites. For example, a sublethal concentration is > 2.5 mg/L. However, (<1.5 mg/L is recommended for aquatic systems. Nitrate concentration is an indicator of lack of water changes.

Phosphate Toxicity

Phosphates in some municipal water systems (minimizes leaching from lead pipes) are toxic to amphibians. Even a low-level of phosphates will interfere with calcium metabolism, cause tetanic seizures and, death (Stiffler, 1993).

Carbon Dioxide

Carbon dioxide concentrations in water can increase due to lack of aeration and the level should be < 6 mg/L.

Food Toxicity

Captive aquatic amphibians can develop a toxic syndrome caused by partially decomposed food. This toxic syndrome can develop efrom small amounts of decomposed food in aquatic environments with high water flow rates.

The toxic syndrome is due to the growth of fungal hyphae in uneaten food which may reduce water circulation and promote anerobic decay. The toxins affect all the aquatic life in the water and are quickly lethal. Tadpoles, for example, first become motionless on the water surface and may respond violently (uncoordinated, rotating swimming behaviour) to any intervention. They die within a few hours.

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Appendix A

Table 3. Percent Water Content, Crude Protein, Crude Fat, Ash and Gross Energy of some Common Prey Species for Captive Amphibians

Prey Species	%Water	Crude protein %	Crude Fat %	Ash %	Gross Energy Kcal/g
Black worm (*Tubifex sp*)	81.6[3]	47.8[3]	20.1[3]	0.7[3]	5.57[3]
Blood worm (*Chironomus sp*)	90.1[3]	52.8[3]	9.7[3]	4.5[3]	-
Cockroach (*Periplaneta Americana*)	61.3[3]	53.9[3]	28.4[3]	3.3[3]	6.07[3]
Cricket pinhead (nymph) (*Acheta domestica*)	77.0[4]	15.4[4]	3.3[4]	1.1[4]	1.0[4]
Cricket adult (*Acheta domestica*)	73.2[2] 69.0[3]	64.3[2] 64.9[3]	22.8[2] 13.8[3]	- 5.7[3]	- 5.3[3]
Cricket adult – hi Ca diet (*Acheta domestica*)	69.7[3]	65.2[3]	12.6[3]	9.8[3]	5.4[3]
Earthworm (*Allolobophora calignosa*)	80.0[3]	62.2[3]	17.7[3]	5.0[3]	4.65[3]
Fruit fly (*Drosophila melanogaster*)	70.4[3]	70.1[3]	12.6[3]	4.5[3]	5.12[3]
Fruit fly larvae (*Drosophila melanogaster*)	78.8[3]	40.3[3]	29.4[3]	9.8[3]	5.57[3]
Fruit fly pupae (*Drosophila melanogaster*)	67.6[3]	52.1[3]	10.5[3]	14.1[3]	4.84[3]
Locust – grass diet (*Locusta migratoria*)	40.5[2]	52.7[2]	32.6[2]	-	-
Mealworm (*Tenebrio molitor*)	62.9[2]	51.8[2]	31.1[2]	-	-
Mouse neonate	-	64.2[1]	17.0[1]	9.7[1]	4.87[1]
Mouse juvenile	-	44.2[1]	30.1[1]	8.5[1]	6.65[1]
Mouse adult	-	55.8[1]	23.6[1]	11.8[1]	5.25[1]
Night crawler (*Lumbricus terrestris*)	83.7[3]	60.7[3]	4.4[3]	11.4[3]	4.93[3]
Rat neonate	-	57.9[1]	23.7[1]	12.2[1]	5.30[1]
Rat juvenile	-	56.1[1]	27.5[1]	14.8[1]	5.55[1]
Rat adult	-	61.8[1]	32.6[1]	9.8[1]	6.37[1]
Silkworm larvae Mulberry diet (*Bombyx mori*)	81.6[2]	53.0[2]	20.2[2]	-	-
Silkworm larvae (*Bombyx mori*)	82.7[2]	53.7[2]	8.1[2]	-	-
Water flea dry (*Daphnia sp*)	8.3[3]	55.2[3]	6.6[3]	10.8[3]	
Wax moth larvae (*Galleria mellonella*)	61.9[2]	41.2[2]	51.4[2]	-	-

Note: All nutrient levels % dry matter (DM)
Ash = total mineral content

[1]Dierenfeld, Alcorn & Jacobsen, 2002
[2]Dierenfeld & Fidgett, 2006
[3]Bernard, 1997
[4]Finke, 2002

Appendix B

Table 4. Percent Water Content, and Vitamin A, Vitamin E, Calcium (Ca) and Phosphorus (P) Levels of some Common Prey Species for Captive Amphibians

Prey Species	% Water	Vitamin A IU/g	Vitamin E IU/g	Ca %	P %
Black worm (*Tubifex sp*)	81.6[3]	-	-	0.11[3]	0.85[3]
Blood worm (*Chironomus sp*)	90.1[3]	-	-	0.38[3]	0.90[3]
Cockroach (*Periplaneta Americana*)	61.3[3]	-	-	0.20[3]	0.50[3]
Cricket pinhead (nymph) (*Acheta domestica*)	77.0[4]	1.0[4]	0.01[4]	0.28[4]	2.5[4]
Cricket adult (*Acheta domestica*)	73.2[2]	0.81[2]	0.08[2]	0.21[2]	1.42[2]
Cricket adult – hi Ca diet (*Acheta domestica*)	69.7[3]	-	-	0.90[3]	0.92[3]
Earthworm (*Allolobophora calignosa*)	80.0[3]	-	-	1.72[3]	0.90[3]
Fruit fly (*Drosophila melanogaster*)	70.4[3]	-	-	0.10[3]	1.05[3]
Fruit fly larvae (*Drosophila melanogaster*)	78.8[3]	-	-	0.59[3]	2.30[3]
Fruit fly pupae (*Drosophila melanogaster*)	67.6[3]	-	-	0.77[3]	2.73[3]
Locust – grass diet (*Locusta migratoria*)	40.5[2]	2.9[2]	18.9[2]	0.04[2]	0.43[2]
Mealworm (*Tenebrio molitor*)	62.9[2]	0.81[2]	0.03[2]	0.12[2]	1.42[2]
Mouse neonate	-	35.53[1]	0.05[1]	1.17[1]	-
Mouse juvenile	-	30.88[1]	0.17[1]	1.47[1]	-
Mouse adult	-	578.27[1]	0.10[1]	2.98[1]	1.72[1]
Night crawler (*Lumbricus terrestris*)	83.7[3]	-	-	1.52[3]	0.96[3]
Rat neonate	-	21.33[1]	0.47[1]	1.85[1]	-
Rat juvenile	-	-	-	2.07[1]	-
Rat adult	-	151.38[1]	0.14[1]	2.62[1]	1.48[1]
Silkworm larvae Mulberry diet (*Bombyx mori*)	81.6[2]	-	0.47[2]	0.91[2]	0.75[2]
Silkworm larvae (*Bombyx mori*)	82.7[2]	.67[2]	0.004[2]	0.10[2]	1.37[2]
Water flea dry (*Daphnia sp*)	8.3[3]	-	-	0.10	1.17
Wax moth larvae (*Galleria mellonella*)	61.9[2]	0.15[2]	0.51[2]	0.06[2]	1.20[2]

Note: All nutrient levels % dry matter (DM)

Ca = calcium P = phosphorus

[1]Dierenfeld Alcorn & Jacobsen, 2002

[2]Dierenfeld & Fidgett, 2006

[3]Bernard, 1997

[4]Finke, 2002

Appendix C

Table 5. A High Calcium (8%) Diet Formulated for Crickets based on Bernard (1997)

Ingredient	% by Weight
Corn, grounddv	8.0
Alfalfa meal (17% CP)	10.0
Soybean meal (48% CP)	29.0
Wheat, ground	27.0
Calcium carbonate (38-40%Ca)	20.0
Dicalcium phosphate (21% Ca, 18% P)	2.0
Salt	0.5
Mineral Premix[1]	0.25
Vitamin premix[2]	0.25
Soybean Oil	3.0

[1]**Mineral Premix fractions per kilogram (kg):** 144 g Ca, 0.04 g P, 4.3g Mg, 0.6 g K, 84.2g Fe, 83.3g Zn, 81.1g Cu, 119g Mn, 0.32g I, 0.08g Se

[2]**Vitamin Premix fractions per kilogram (kg):** 28,000,000 IU vitamin D_3, 132,000 vitamin E, o.6g vitamin K_1, 7.1g thiamin, 2g riboflavin, 35.6 niacin, 9.5g D-pantothenic acid, 2g pyridoxine, 1.5g folic acid, 99mg biotin, 6 mg vitamin B12, 190g choline