WATER QUALITY

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INTRODUCTION

With its dual life cycle and anamniotic egg, the amphibian is more closely bound to a continuous supply of water than most other terrestrial vertebrate groups. This supply of water must meet certain minimal requirements to maintain the health and normal behavior of the organism. Water from either a natural source or a treated source (e.g., municipal water supply) is not a pure substance, but a suspension and solution of various organic and inorganic components. These additional substances in the water supply might be required to maintain the organism, might have no effect upon organism, or might be detrimental to the organism. The amphibian has invaded many different niches, and the individual water requirements for a specific organism are species dependent in many cases, perhaps subspecific in some cases. The overall concentrations of these substances in a supply of water are conveniently grouped together under the term "water quality." This includes all aspects of the water (e.g., pH, inorganic salts, organic compounds, metabolic waste products, dissolved gases, and bacterial suspensions).

Extensive work has been done in the field of aquaculture to quantify the relationship of water quality and health of fishes and some invertebrates (U.S. E.P.A., 1976). With the recognition of declining amphibian populations over the last decade, there has been a greater concentration on water quality and the Amphibia. The purpose of this monograph is to provide a basic understanding of water quality for amphibian keeper. This paper is not a complete representation of the exact state-of-the-art of aquaculture; instead, the authors hope to convey the principles that govern water quality management. A quick reference table is also provided that includes some generally accepted values for common water quality parameters. These values can be used to help evaluate water quality for animals in your charges.

Water, the miracle molecule

Water is the most abundant compound found on the surface of Earth. Its commonness often overshadows its uniqueness as a principal component of life. Without water, and its special physical and chemical properties, life could not exist. What are some these properties that make water so important for life?

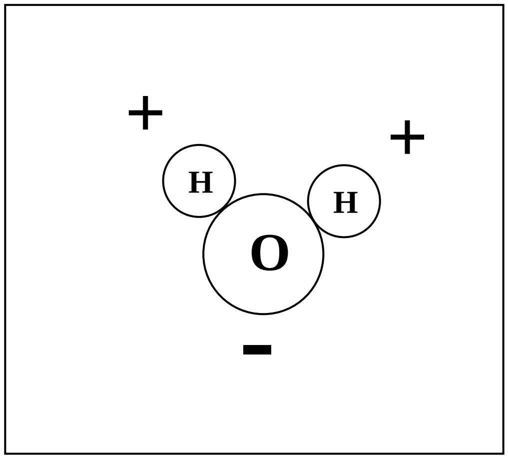
First, water is an excellent solvent, but not too excellent. Almost all of the chemical reactions associated with life take place in water, which constitutes over 90% of protoplasm. Many compounds and elements dissolve readily in water while many other molecules do not. This allows for both the chemistry and the structure of life. It also facilitates the transfer of other chemicals from the outside environment to the inner workings of organisms.

Water is a clear, colorless, tasteless liquid that freezes at 0^{o}C and boils at 100^{o}C in one atmosphere of pressure. It is most dense at 4^{o}C, a property that allows ice to float. This is an unusual property for any substance. In aquatic ecosystems such as ponds, the less dense ice forms on the top surface, protecting many species in the liquid medium below. Pure water is a very poor conductor of electricity, but when other substances are dissolved in water, its conductivity increases markedly. This property is used to quickly determine the amount of dissolved ions by measuring conductivity.

One liter of water has a mass of one kilogram (=1g/ml). This may appear to be a small amount, but animal enclosures can contain substantial quantities of water that add up to considerable mass. For example, one gallon of water weighs about eight pounds, so a 100 gallon tank contains ~800 pounds of water. A thousand gallon tank contains 4 tons of water! When designing amphibian enclosures, these great weights must be considered in both the structure of the enclosure, and the base and foundation that the enclosure rests upon.

It is common knowledge that water is made up of two hydrogen atoms and one oxygen atom, represented by the formula H_2O. This simplified formula conveys only part of the story. Water is structured in a way that the oxygen has a negative charge and the two hydrogens are positively charged creating a polar molecule. A much better representation of water is seen in Figure 1.

Figure 1 - The structure of the water molecule. Two hydrogen atoms are bound to one oxygen atom by covalent bonds. The resulting molecule has positive and negative electrical poles that attract other atoms.



The positive and negative areas attract oppositely charged atoms and molecules, making water an excellent solvent for other polar molecules such as alcohols, aldehydes, etc. It also is excellent solvent for many inorganic salts such as sodium chloride, calcium chloride, sodium carbonate, etc. Alternatively, molecules that are not polar and have no charged areas such as oils, hydrocarbons, etc., are not readily dissolved in water. Very large molecules, such as proteins, are also insoluble in water, but still interact with water at specific sites of the molecule.

Solutions and Suspensions

Before we continue our discussion of water quality, it would be advantageous to discuss the differences between materials that are in true solution and those that are in suspension. In particular, we are interested in solutions and suspensions where water is the dissolving medium or solvent (referred to as aqueous). Protoplasm, the substance of living organisms, is a mixture of both aqueous suspensions and aqueous solutions.

True solutions are homogenous mixtures on the molecular level. Individual molecules of a dissolved substance (solute) loosely bond to the solvent molecules, creating a uniform mixture. Solvent and solute molecules intermingle and move freely about together. In some cases such as dissolved salts in water, the solute molecules may break up into positive and negative components called ions. These ions are attracted to the positive and negative areas of the polar water molecule. When ions are present, water becomes more conductive of electricity. Molecules in solution cannot be removed by mechanical filtration; a chemical filter is necessary.

Suspensions are a mixture of larger particles in a fluid medium. When you place dirt into water and mix it vigorously, it forms a suspension of particles. These particles can be separated out by gravitational pull or mechanical filtering. There is no direct bonding between the molecules of the solvent and the suspended material. An easy test to see if a mixture is a true solution is to shine a thin beam of light through the liquid. If the beam is visible in the liquid from the reflection off the particles, it is a suspension. The smaller molecules do not reflect light in a solution, thus the beam is invisible.

Criteria for Water Quality

The following is a brief account of the most common parameters of water quality that affect the health of organisms in captivity. Each account will include information concerning the overall effect that these parameters will have upon the environment within an enclosure. Each of these parameters can be easily monitored by commercially available test kits that are sold by aquarium stores and scientific supply houses at a reasonable cost. Other more advanced instrumentation, such as spectrophotometers and pH meters, is also available. These provide more accurate and precise assays of water quality parameters

General hardness

General hardness (GH) is a measure of the amount of minerals dissolved in the water, primarily calcium and magnesium. It is measured in degrees of hardness (dGH). Many amphibians prefer moderately soft water (with little dissolved calcium and magnesium); however, it is always best to consider the natural habitat of the species in question. Is the animal from a river fed primarily by rainwater (soft), or from lakes in limestone bedrock (hard)? Water can be hardened by adding calcium and magnesium salts: about one gram of mixed calcium chloride and magnesium sulfate (mixed 6:7 by weight) will raise 10 gallons of water by 1 dGH and provide an ideal calcium to magnesium ratio (3:1). Water can be softened by diluting with distilled, reverse osmosis, or deionized water. Changes in hardness occur linearly, so diluting 6 dGH water with pure water by 50% gives 3 dGH. Changing water hardness can also affect pH, and vice versa. Make changes slowly and test often. Beware when using household water softeners – they replace hard water minerals with salt (sodium chloride), which can, in high concentrations, dehydrate amphibians.

Dissolved Oxygen as O_2

The concentration of dissolved oxygen (hereafter DO) should be great enough to maintain an overall aerobic environment for aquatic vertebrates, invertebrates, and bacteria. DO is particularly important for gill breathing animals such as fish, larval amphibians, and aquatic paedomorphic adult forms. Important bacteria for maintaining a suitable environment for amphibians include several groups that are commonly present and metabolize organic matter using the dissolved oxygen. If the level of DO falls too low, anaerobic decomposition of organic matter will take place resulting in the unwanted production of noxious gasses (e.g., hydrogen sulfide, a very toxic compound with the characteristic smell of rotten eggs) as well as carbon dioxide and methane (EPA, 1976). The removal of organic metabolic waste products by oxidation must also take place in aerobic conditions (e.g., nitrifying - refer to biofilters) (Stickney, 1979). Dissolved oxygen therefore has a double function; it is necessary for respiration of the amphibian in the enclosure and for maintaining a clean, unpolluted environment.

Oxygen enters water primarily through two methods: the direct dissolving of oxygen from the air via aeration or as a biological by-product of photosynthesis from bacteria, algae, and plants. One method of promoting gas exchange between water and air is by bubbling air through the water (aeration). This helps break the surface film between the water and the air allowing the exchange of gases. The majority of the exchange of gases does not take place at the interface of the bubble and the water, but at the water surface where these bubbles break the film that covers the water. Any method of breaking this surface film will aid in the exchange of gases between air and water (e.g., any type of surface agitation). The amount of surface area in a tank is thus important for promoting gas exchange. High narrow tanks have less area at the water/air interface, and thus less oxygen absorption. Low wide tanks have better gas exchange.

The biological introduction of oxygen into water by green plants and algae is cyclic due to the nature of the light and dark functions of photosynthesis. Oxygen is produced in the presence of light during the day through the reduction of carbon dioxide. At night, oxygen is consumed by the plants for metabolic functions. For this reason, oxygen levels often peak at the end of the day, then drop through the night, until the next morning when the light cycle starts again. The best time to check DO levels is early in the morning (before photosynthesis replenishes the depleted oxygen supply) to determine if there are sufficient levels of oxygen to maintain aerobic conditions throughout the night (Stickney, 1979).

Dissolved oxygen is in dynamic equilibrium between the rate that oxygen enters the water and the rate it is removed. The major factors that affect oxygen entering the water are the rate of dissolving at the water/air interface and organic oxygen production. The factors that remove oxygen from the water are consumption by aerobic organisms through respiration and removal of oxygen by certain inorganic compounds. The sum of the removal of oxygen by respiration and these inorganic compounds is considered together as the biochemical oxygen demand (BOD). To maintain aerobic conditions in the water, the BOD must equal the oxygen entering the water in a certain period of time (e.g., 24 hours). This does not mean that oxygen input and output must be continuously equal; one is likely to exceed the other at any time without consequence, as long as the level of DO does not drop to a critical level.

Another factor that affects oxygen levels in water is the overall solubility of O_2 in water (measured as the saturation concentration when no more oxygen will dissolve). As the temperature of water increases, oxygen solubility decreases (11.3 mg/l at 10^{o}C and 7.3 mg/1 at 32^{o}C at one atmosphere pressure). Changes in temperature can dramatically change the DO in a system. If the temperature in a system rises, the equilibrium between metabolic use of oxygen, the biological production of oxygen, and the dissolving of oxygen at the water surface may destabilize, resulting in the depletion of the oxygen resource and an anaerobic environment.

Information regarding the minimum levels of DO for amphibian species is scant, with most work being performed with fish species. Wheaton (1977) concluded that aquaculture animals showed no signs of stress with DO levels greater than or equal to 5 mg/1 although previous workers have shown increased mortality or retarded development in fish embryos with reduced DO levels of 5 to 6 mg/1 (Seifert and Spoor, 1974; Seifert et al., 1975; (Brungs, 1971; Carlson and Siefert, 1974); Guligov, 1969). This information suggests that an adequate level of DO for an adult form may be insufficient for the larval form of the same species. This may be important for breeding some aquatic spawning amphibians (e.g., many species of anurans or salamanders) where the adult animals appear to be healthy, but their newly deposited eggs die in early development. In later developmental stages, some anuran larvae appear to be less susceptible to low oxygen conditions than many fish by directly forcing air over the gills at the water surface (Odum et al., 1984). In a more recent water quality study of the glass frog *Cochranella granulosa* (Hoffmann, 2010) that examined the effects of waste nitrogen in water relative to levels of dissolve oxygen, it was discovered that larvae developed quicker and used food resources more efficiently in anaerobic conditions. This may be a special adaptation to exploit low oxygen refugia in stream bottoms. Dissolve oxygen can be measured by a meter or spectrophotometer.

Gas Supersaturation

Tap water and well water can be supersaturated with dissolved gases (especially nitrogen and carbon dioxide, but sometimes even oxygen), which when exposed to aquatic animals, can cause gas-bubble disease, a condition similar to the bends in divers. Supersaturation is generally problematic only in the winter when the water is especially cold and capable of holding higher levels of dissolved gases. This condition is exacerbated when water is pressurized for transfer in piping. The treatment for supersaturation is degassing. This can be accomplished by aerating the water, heating the water, or just allowing the water to stand until it reaches equilibrium with the surrounding air. Aerating water to remove gas might seem counterintuitive, but remember that the water is supersaturated from being under pressure, and aeration at atmospheric pressure will bring the water back to equilibrium at atmospheric pressure quickly. Heating the water to room temperature will lower its ability to hold dissolved gases. Anyone who has allowed a tank full of cold tap water to heat-up and observed the formation of tiny air bubbles on the glass is familiar with this process. Similarly, water heated for boiling first releases the dissolved gas as tiny bubbles on the vessel walls before reaching the boiling point.

Nitrogen as Ammonia/Ammonium NH_3/NH_4^+

Ammonia generally enters the aquatic environment as a metabolic waste product of respiration (the majority of metabolic wastes from amphibian larvae and gill breathing adult amphibia are in the form of ammonia) and from the bacterial decomposition of organic matter. Urea and uric acid wastes excreted by organism are also readily reduced to ammonia by bacteria. This is obvious to the reptile keeper who has cleaned uric acid from a water bowl and has noticed the distinct ammonia smell. Ammonia may also be present in municipal water supplies, especially if chloramines are used as an antibacterial agent. Chloramines will be discussed in detail in the section on chlorine.

Experimentation on fish has shown ammonia to be an extremely toxic compound with lethal concentration as low as 0.2 mg/1 for rainbow trout fry (*Salmo gairdmeri*) (Liebmann, 1962) and only slightly higher concentrations have been noted to kill Atlantic salmon (*Salmo solar*) (Herbert and Shurben, 1965) and adult rainbow trout (Ball, 1967). There is evidence that suggest that many amphibian species may be more tolerant to ammonia than fish. Leopard frog larvae (*Rana pipiens*) exposed to ammonia concentrations as high as 1.5mg/l showed little evidence of reduced growth or increased developmental problems. In comparison, toxic effects in green frogs (*Rana clamitans*) were only seen at concentrations over 0.6mg/l, while American toads (*Bufo americanus*) were tolerant of concentrations up to 0.9mg/l (Jofre and Karasov, 1999). There may be a relationship between the environment of the tadpoles and the tolerance to NH_3. Species such *R. pipiens* and *B. americanus* that often breed in ephemeral pools, which may start to dry and become more polluted during the development of the larvae, seem more tolerant to ammonia toxicity. In one interesting study of African reedfrog (hyperoliids) larvae, high ammonia levels during development was correlated to increased tolerance to dryer harsher conditions at metamorphosis, resulting in higher survivorship (Schmuck et al., 1994).

In an aqueous solution, ammonia has two forms: the unionized ammonia molecule NH_3 that is extremely toxic and the notably less but still dangerously toxic ionized ammonium in NH_4^+. Tabata (1962) reported that NH_3 was 50 times more toxic to fish than the ionized form ammonium. These two forms of ammonia are in equilibrium in aqueous solutions that is both temperature and pH dependent.

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^-$$

As the temperature increases and the water becomes more basic (pH rises above 7), the left side of the equation is favored, increasing the concentration of the toxic ammonia molecule. High temperatures further exacerbate the situation because of the increased metabolism of animals held in the enclosure. Conversely, as temperatures and pH fall (more acidic), the toxic ammonia is transformed into the less toxic ammonium ion (NH_4^+). Emerson, et al (1975) experimentally established this relationship (see Table 1). Most assay methods measure total nitrogen of both ammonia and ammonium together. To determine the true potential toxicity of the test results, pH and temperature must also be measured. Table 1 can then be used to establish the amount of the more toxic ammonia in the water environment.

Ammonia is removed from the aquatic environment efficiently by certain bacteria. This is the basis of biological filtration and will be discussed in detail in the section dealing with methods and types of filtration. In an emergency, it can be removed with large water changes or chemical resins like AmLock or AmRid or similar products.

TABLE 1

Percentage un-ionized (i.e., more toxic) ammonia in aqueous ammonia solutions as a function of pH and temperature

More Acid **pH** *More Basic*

°C	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10
5	0.013	0.040	0.12	0.39	1.2	3.8	11.	28.	56.
10	0.019	0.059	0.19	0.59	1.8	5.6	16.	37.	65.
15	0.027	0.087	0.27	0.86	2.7	8.0	21.	46.	73.
20	0.040	0.13	0.40	1.2	3.8	11.	28.	56.	80.
25	0.057	0.18	0.57	1.8	5.4	15.	36.	64.	85.
30	0.080	0.25	0.80	2.5	7.5	20.	45.	72.	89.

(Emerson et al., 1975)

Nitrogen as Nitrite NO_2^-

Nitrites (NO_2^-) are formed in the aquatic environment by the oxidation of ammonia/ammonium by nitrifying bacteria (the first step of biological filtration). The toxicity of nitrites in the aquatic environment is similar to that of free molecular ammonia; however, the mechanism of action upon an organism is extremely different. The nitrite ion (NO_2^-) bonds with hemoglobin in the blood to form methemoglobin, which impairs oxygen transport by erythrocytes (EPA, 1976). There has been little work on nitrite toxicity in amphibians, so we have drawn some insight from the numerous fish studies published. Russo et al. (1974) experimented extensively with rainbow trout (*Salmo gairdneri*) and found minimum lethal levels at 0.14 mg/1 over a 10 day period. Salmonid fish seem to be especially sensitive to nitrite poisoning as established by Klingler (1957) who showed that the minnow (*Phoxinum laevis*) could tolerate many times these concentrations (animals could tolerate 10 mg/1 for 10 days).

Species specific toxicity of nitrites has also been observed in amphibians. Nitrite concentrations of 0.88mg/l were seven times more toxic to the spotted frog (*Rana pretiosa)* than the red-legged frog *(Rana aurora*) and pacific treefrog (*Hyla regilla*) (Marco et al., 1999). A recent study performed on the glass frog *Cochranella granulosa* larvae suggests that ammonia is much less toxic to this species than nitrites. Larvae reared in an anaerobic environment, which prevented the oxidation of ammonia into nitrites, had significantly lower levels of blood methemoglobin (2.3%) than animals reared in an oxygenated aerobic environment (19.3%) (Hoffmann, 2010). In a fully functional biological filtration system, the nitrites would be immediately oxidized into nitrates (see biological filtration section). This suggests that a partial functional biological filtration system may be more detrimental than no biological filtration at all. It also suggests that anaerobic conditions are not necessarily undesirable for the rearing of larvae adapted to these conditions.

Huey, D. and T. Beitinger (1980) determined that there was a relationship between the nitrite toxicity in *Ambystoma texanum* larvae and chloride (Cl^-) concentrations. The 96 hour LC50 was 1.09 mg/l nitrite in the absence of chlorides. Nitrite toxicity was greatly reduced with the addition of chlorides at 300mg/l. These larvae tolerated 10mg/l nitrite without any mortality. These findings suggest a potential treatment for water treatment to reduce, or eliminate the toxic effects of nitrites in amphibian tanks.

As with ammonia, nitrites are oxidized by bacterial action. In a balanced environment, these toxins can be safely removed without detriment to the housed organism. This is the second step in biological filtration (see Filtration section). The presence nitrite warrants corrective action and further monitoring of aquatic systems.

Nitrogen as Nitrates NO_3^-

Nitrates (NO_3^-), the final product of ammonia nitrogen oxidation in biological filtration, are formed by the action of nitrifying bacteria on nitrites. Unlike ammonia and the nitrite ion, nitrates are substantially less toxic. Lethal concentration that kill 50% (LC_{50}) of a study group within a certain period of time (in this case 96 hours) have been determined for several species and range from 900 to 2000 mg/1 (Trama, 1954; Westin, 1974), considerably lower than the fraction of a milligram per liter LC_{50} observed for ammonia and nitrites. For this reason, the presence of low to moderate levels (<1.5mg/l) of nitrates is not considered a great problem in an aquatic system; as the final product of nitrifying, their presence can be used as a gauge to determine the effectiveness of biofiltration. Still, concentrations above 2.5mg/l have produced sublethal effects on amphibians (Rouse et al., 1999) and should be considered a problem that needs immediate attention. The buildup of nitrates over time is an indication of insufficient water changes, which should be addressed by increasing water-change frequency.

Nitrogen as nitrates is in a form that can be utilized by the primary producers of an ecosystem, green algae and plants. Nitrates are a necessity for the growth of photosynthesizing plants and are commonly employed as part of many commercial fertilizers. This growth of primary producers has several benefits in an aquatic system including the additional biologically produced oxygen that is added to the water (DO) and as a possible food resource for some species. One drawback to the growth of these primary producers is aesthetic. Algae (the most common green plant that grows in aquatic enclosures) are considered unsightly by some individuals. The excessive growth of algae can become more than an aesthetic problem when algae production is great enough to clog filters, or if the mass of the algae is large enough to deplete DO during night time consumption of the algae. To control excessive algae growth, the amphibian keeper can take several steps. First, reduce lighting. Light is a necessity for photosynthesis and lower levels will slow algae growth. Secondly, make frequent water changes to reduce the concentration of nitrates. A final step is to introduce other species of green plants to compete with the algae for nutrients and increase the general aesthetic qualities of the enclosure. The presence of moderate amounts of algae is one of many indicators of a balanced, stable aquatic system, but its removal from viewing glass can be time consuming. A sudden rapid bloom or die off of algae in a well-established system can be an indication of a water quality problem and appropriate tests should be made.

pH

The concentration of the hydrogen ion H^+ (also common referred to as a proton) in an aqueous solution is called pH (measured as an inverse logarithm). This statement may sound complicated to those not well versed in chemistry and mathematics but, in reality, it is a simple concept. The more hydrogen ions present, the more acidic is a solution, and the lower the pH. The opposite counterpart of the hydrogen ion is the hydroxide ion OH^- that determines how basic or alkaline a solution is (bases are the opposite of acids). These two ions combine with each other to form water, thus canceling their individual effects on the solution.

$$H^+ + OH^- \longleftrightarrow H_2O$$

When the concentration of hydroxide and hydrogen ions is equal, the solution is neutral (neither acidic nor basic). This occurs at the medial point of the pH scale (0 through 14) at 7. As the pH drops below 7, the solution becomes more acidic until the pH reaches zero when the solution contains the maximum concentration of hydrogen ions. When the pH rises above 7, the number of hydroxide ions out numbers the hydrogen ions and the solution is basic. When the pH reaches 14, the concentration of the hydroxide is at its maximum.

In the wild, most unpolluted fresh water is at a pH between 6.5 and 8.5 (Stickney, 1979). The pH must remain fairly constant to preserve aquatic life even though there are continuous amounts of both hydrogen and hydroxide ions entering the aquatic system. The natural aquatic system has a pH stabilizing chemicals called buffers; of which the carbonic acid/carbonate buffer is one of the most important in fresh water systems (I will not go into the actual mechanism of buffers here. This information is readily available in a freshman chemistry text).

The pH requirements differ from species to species with the majority favoring slightly basic water. This will not be true of all species. Some species are selected to breed in acidic water (e.g., *Hyla andersoni* which breeds in acidic bogs) and for successful reproduction to occur, this environmental criteria must be met (Conant and Collins, 1991). The amphibian keeper should research pH requirements for each individual species to establish a suitable environment in captivity.

The effects of changing pH are becoming well documented in wild populations. Acid rain, caused by sulfur and nitrogen oxides dissolved in cloud water, has significantly impacted some populations of amphibians in North America and Europe. Cummins (1989)found that a pH of 4 alone was not sufficient to reduce growth rates and increase mortality in the common frog (*Rana temporaria*) larvae, but when tadpole densities were increased, detrimental effects were observed on subordinate individuals. Similar results have been seen for the barking tree frog (*Hyla gratiosa*). The pine woods treefrog, *Hyla femoralis*, showed higher mortality and lower weight at metamorphosis in lower pH, but there was no relationship between density and pH (Warner et al., 1991). There are many other references available showing the effects of acid water on amphibian larvae survival.

As discussed earlier, pH also profoundly affects ammonia toxicity. At a ‘lower’ pH, ammonia binds a hydrogen ion and exists in its ionic form, NH4+. This ammonium ion is much less toxic than the form that occurs at a ‘higher’ pH, NH3. The concentration of the two forms is equal at approximately pH = 9.3. Above this, the more toxic form predominates. Fortunately, most aquatic systems function best at a pH well below 9.5. However, even at a lower pH, NH3 is still present in some concentration.

Because of the interaction between pH and ammonia, a concentration of ammonia that is relatively harmless at pH 6.5 can be deadly if the pH rises to 7.5. The idealist will say ‘keep the ammonia concentration at zero and pH/ammonia interactions will never be a problem’ but the pragmatic knows that ammonia spikes do sometimes occur and tries to keep the pH near or slightly below neutral to minimize their impact. Remember this interaction when receiving a shipment of aquatic animals. The CO_2 and ammonia concentrations in the shipping water are likely high and the pH low. Float the bag to achieve a common temperature, but do not mix bag and tank waters. The higher pH of the tank water can cause the ammonium in the bag to convert to more toxic ammonia and kill the new arrivals. Simply pour the temperature-acclimated animals into a net over a bucket or sink and add only the animals to the tank.

Changing the pH of tap water is possible, but maintaining new levels can be labor intensive with much monitoring and tweaking. Perhaps changes are best reserved for particularly sensitive species or in areas where tap-water pH is far from neutral. pH can be lowered by adding small amounts of dilute acid to the system. The safest way to do this is to add a nylon bag of peat or sphagnum moss to the filter or tank. The waterlogged moss will release tannic and humic acids into the system, gradually and naturally lowering the pH, and also the hardness. Bubbling carbon dioxide into the water will also lower the pH and greatly enhance the growth of plants. Diluting tap water with distilled, deionized, or reverse osmosis water will lower pH, but it will also lower pH stability (carbonate hardness). pH can be raised by adding small amounts of dilute base to the system. Sodium bicarbonate (baking soda) is inexpensive and very effective. Start with a small dose (1/8 level teaspoon per 20 gallons) and wait 24 hours for the pH to stabilize before testing/tweaking again. Avoid commercial pH buffers, which can be high in phosphates and cause algae blooms.

The inexperienced must proceed with caution: always change the pH in small increments in a tank with living animals to minimize physiologic disturbance. pH changes on a logarithmic scale, so changes do not lend themselves to 'common sense.' A pH of 6 is not just a little lower than a pH of 7, it is 10 times lower, and 100 times lower than a pH of 8. Consequently, it will take a lot more acid to drop from 8 to 7 than from 7 to 6.

The pH in most closed aquatic systems will naturally fall over time. Decomposition of detritus (uneaten food, feces, shed skin, and dead plant material), respiration of the animals, and biological filtration (bacterial respiration) all bring down the pH. A good mechanical filter that is cleaned regularly, weekly water changes, and good aeration and circulation will help keep 'pH fallout' in check.

Chlorine as Cl_2 and Chloramines

Free chlorine (Cl_2) is a greenish gas that is well known for its highly toxic properties as can be attested to by the thousands of soldiers that died and were severely injured from chlorine exposure during World War I. In water, chlorine is the most toxic substance that we will discuss. Ironically for the amphibian keeper, it is this toxic nature of chlorine and its ability to denature proteins, which makes its encounter inevitable. Chlorine is generally used as an antibacterial agent in municipal water supplies and may be present in concentrations of over 9 mg/1 in some tap water (measured in Houston, Texas as an example, although levels were generally lower). The concentration of chlorine in municipal water supplies can vary greatly from day to day, or even hour to hour, depending on conditions at the water treatment facilities. Concentrations as low as 0.0034 mg/1 have been noted to reduce reproduction in fathead minnows with 72 hour LC_{100} (lethal concentration for 100% kill) at 0.15 mg/1 (Arthur and Eaton, 1971). LC_{50} (96 hour) for the shiners (*Notemigonus chrysoleucas*) was as low as 0.19 mg/1 (Esvelt et al., 1971). The concentrations found in municipal water supplies are many times greater than the minimum lethal concentrations for many aquatic life forms. For this reason, chlorine must be removed from the aquatic environment of gill breathing animals. It appears (from personal experience) that many adult forms of amphibians show no outward signs of chlorine poisoning from short term exposures (e.g., *Ceratophrys ornata*, *Ambystoma texanum, A. tigrinum, Litoria caerulea*, *Ichthyophis kohtaoensis*). Alternately, larval bufonids exposed to municipal water containing chlorine died within a few hours.

Free chlorine can be removed chemically or by "aging" the water for several days (aging allows time for the chlorine to dissipate out of the water). (NOTE: aging proceeds more rapidly when the water is well aerated and warmed.) Unfortunately, many municipal water supplies no longer use simple chlorine as a bactericidal agent. Instead, they are now using chloramines (NH_2Cl, $NHCI_2$, NCl_3) a more stable group of compounds that do not readily dissipate from water, greatly increasing the aging time required. The action and toxicity of chloramines is virtually the same as those of free chlorine (EPA, 1976). Both chlorine and chloramines can be removed from water with a variety of chemicals; the most commonly used is hypo, sodium thiosulfate ($Na_2S_2O_3$). These compounds reduce the free chlorine to the nontoxic chloride ion. When thiosulfate reacts with chloramines, toxic ammonia is also released in small quantities, which may present separate problem.

To remove chlorine chemically from tap water, create a saturated solution of sodium thiosulfate in water by adding it to water until no more chemical will dissolve (to assure saturation, leave a little undissolved chemical in the bottom of the container). To dechlorinate tap water, add one drop of the saturated thiosulfate solution for each gallon of water. Care must be made not to use too much sodium thiosulfate because it can be toxic.

Most tests for chlorine will also accurately assay the concentration of chloramines. As a rule, any chlorine is too much chlorine.

Phosphates

High phosphate levels (PO_4^{-3}) have recently become a significant problem in some amphibian facility water supplies. Many older cities have added phosphates to their municipal water to chelate (bind) lead found in older plumbing systems, thus prevent it from dissolving into the drinking water. Unfortunately, excess phosphates are bad for amphibians as they bind calcium. A high phosphorus:calcium ratio can lead to serious neurological and osteological problems (e.g., paralysis and metabolic bone disease) and even death. The phosphate ion is not effectively removed via reverse osmosis (RO) filtration, but it can be removed via commercially available phosphate sponges[1]. For larger amounts of water an arsenic filter (Arsenic/phosphate filter manufactured by BASF and distributed by Aquasana in Houston, Texas) has proven effective in removing phosphates from water supplied to an amphibian facility (J. Pramuk, pers. com.).

Copper

Copper is a very common component of potable water plumbing systems. It is used extensively in household and industrial water piping, heating coils, tanks, and many electrical devices as a conductor and as a protective covering. Copper is a reddish metal that oxidizes in air into a green patina. It dissolves in many acids and forms chemical complexes with ammonia in water. It is also very toxic to many aquatic organisms.

When water is allowed to stand in copper piping for any length of time (i.e., over night), some of the copper will become dissolve. When the water is then used in an amphibian enclosure, copper is added to the system, which may have detrimental effects on the inhabitants. Studies with *Rana pipiens* have shown that concentrations as low as 0.15 mg/l will kill 50% of newly hatched tadpoles in 72 hours (Lande and Guttman, 1973). Copper may also be found in some well water supplies. To assure that this is not a problem, copper should be monitored in amphibian water supplies. If copper piping is present, water should be allowed to run to clear the stagnant contents of the pipes prior to its use in amphibian enclosures. Copper tubing should also be avoided in filter and other plumbing systems used for amphibian and fish enclosures.

Water Sources

Municipal Tap Water

Water quality obviously begins with the water used to fill the tank initially. In most regions, simply aging and aerating tap water for 24 hours will be all that is required to condition it for use with amphibians. This treatment will drive off harmful gases (carbon dioxide, nitrogen, hydrogen sulfide) and bring desired gases (oxygen) into equilibrium. However, one will also want to buy kits or meters to test levels of pH and general hardness to be sure that these parameters are within acceptable limits. If chloramines or phosphates have been added to the municipal water supplies, pretreatment will be necessary (see sections on Chlorine as Cl2 and Chloramines and

[1] Phos-Zorb®, Aquatic Eco-systems, Inc. phosphate sponges, and Poly Filters® work well for phosphate removal. Another option would be to use a Tide Pool® sump filter and to place a phosphate pad in it. Cycling the water with the same pad (i.e., having a closed system) will remove more phosphate from your water source than a single-pass system.

Phosphates)

Well Water

Well water can be an acceptable source for use with amphibians, but again, one must test pH, hardness, and in coastal areas, salinity. In some regions, especially where water is pumped up from limestone bedrock, well water can be too hard and the pH too high -- test and treat accordingly. In agricultural areas, well water can also be high in phosphates and nitrates from fertilizers that seep into the water table. These substances cause algae blooms and at higher concentrations are toxic to animals, so testing well water for these ions is also recommended. Carbon filters can help keep pollutants in check, but see the caveats in the section on chemical filtration.

Well water can also be saturated with nitrogen and carbon dioxide, devoid of oxygen, and can even contain lethal quantities of hydrogen sulfide. Vigorously aerating the water for at least a day before use will drive off the nitrogen, carbon dioxide, and hydrogen sulfide, as well as raise the oxygen content. Well water can also contain unoxidized ferric (iron) compounds, which react with oxygen when exposed and precipitate from solution. If the precipitate settles on the gills or skin of fish and amphibians, it can cause irritation, excess mucus production, and even suffocation. Again, aerating the water before use will cause this reaction to occur away from the animals; the precipitate can then be filtered out or allowed to settle.

Rain Water

Rainwater is naturally soft, perhaps too soft for some species. Test hardness and, if near a city where pollution and acidification are problematic, pH. Also, one must consider how the rain is collected. Do not collect rain from a galvanized metal roof or one that has otherwise been treated chemically. A large tarp tied to four posts and angled at one end to drain into a plastic 50-gallon drum is effective, albeit not very attractive.

Similarly, water that collects in natural basins, such as ponds, streams, and lakes, can be a good source of acceptable water. One must check where the water is coming from – is it draining from a large parking lot covered with oil spills, or from a commercial farmer's field where it might have picked up fertilizers, herbicides, or insecticides? Another thing to consider is that this water might be contaminated with diseases or parasites from wild animals. Alternatively, the 'stuff' living in the water could be what makes it great. Natural pools teeming with invertebrate life offer more diversity and nutrition than could ever be cultured indoors.

Bottled Water

If tap water is not acceptable and a reliable outdoor supply is unavailable, bottled water might be an acceptable alternative. Again, the pH and hardness, and even the chlorine level, must be tested. Bottled spring water pumped up through bedrock can be unacceptably hard and basic. Furthermore, purity-testing requirements for bottled water are not as strict as for tap water. A recent survey by the Natural Resources Defense Council showed that 1 in 3 samples of bottled water contained contaminants, including synthetic organic chemicals, coliform bacteria, or even arsenic. In some cases, bottled 'spring' water was shown to be simply filtered bottled tap water. Consult the NRDC website or write/call NRDC Headquarters, 40 West 20th St., New York, NY 10011, 212-727-2700, to get the results for a particular bottled water source.

Bottled distilled water is another option. These products are essentially pure water and are similar to RO water discussed in the next section. In many cases, distilled water will need to be reconstituted prior to its use as the initial source of water in an enclosure (see below). Like RO water, distilled water can be employed as replacement water in a system.

Reverse Osmosis Water Supplies

If the water supply in a facility has high levels of copper or other contaminants that can not be addressed by other means, reverse osmosis water should be considered as a possible solution. This can be a safe and consistent way to ensure a constant supply of very pure, which in itself creates other problems. Reverse osmosis filters use high pressure to force water through a semipermeable membrane, leaving practically everything that was suspended and dissolved in the water behind. Osmosis is the tendency for water to move from areas where the ion concentrations are relatively low to where they are relatively high. This natural law brings solutions into equilibrium by making them equally concentrated. The process of reverse osmosis uses high pressure to make water do just the opposite, i.e., flow away from its dissolved ions. RO filters are now commonly available as affordable models that fit under a sink and produce modest amounts of purified water in a day. SpectraPure and Kent Marine are popular manufacturers among zookeepers and hobbyists. Larger expensive industrial units are also available that can produce large quantities of purified water. RO water is essentially pure, too pure in fact to be used as is in many cases. It may be used for species that normally live in pure rain water, such as some dendrobatids, but is not a necessity. For many other species, it must be 'reconstituted' by adding back a few beneficial trace elements, otherwise this ultra pure water will literally "blow up" your amphibian from osmotic pressure, and remove essential ions necessary to sustain life. The RO water moves across an amphibian's skin and into its body in an attempt to 'dilute' the concentrated ions there (again, osmosis). As a result, the animals might not be able to excrete all the excess water and they swell up (a condition called edema). Moreover, it is taxing to the kidneys. They are unable to keep up with the high filtration demand, and many ions are lost in the excess urine. Kidney failure is not an uncommon outcome of keeping amphibians in pure water.

Commercial additives containing the requisite trace elements are available, but seem to provide little pH buffering capacity (carbonate hardness or KH, sometimes called alkalinity). Test the reconstituted water for carbonate hardness and add sodium bicarbonate (baking soda) to raise the KH and stabilize pH. One teaspoon of baking soda will give 4 degrees (72 mg/l) carbonate hardness to 13 gallons, but will also raise the pH significantly. Allow the pH several hours to equilibrate, and adjust the amount of sodium carbonate to give the desired pH and stability.

The following do-it-yourself reconstitution formula allows finer control and can be tailored to meet individual needs. This mix was developed largely by fish and aquatic plant hobbyists but fine-tuned for amphibians.

In 100 gallons of RO water dissolve:

- 15.0 g calcium chloride $CaCl_2$
- 17.6 g magnesium sulfate $MgSO_4 \cdot 7H_2O$
- 13.6 g potassium bicarbonate $KHCO_3$
- 11.3 g sodium bicarbonate $NaHCO_3$
- 0.5 g commercial trace element mix.

Dissolving the crystals in a jar of water first and then adding the solution to the storage tank will ensure proper mixing. The final composition is similar to moderately soft fresh river water (Appendix B), with roughly 3 degrees general hardness and 2 degrees carbonate hardness, ideal Ca:Mg (3:1) and Na:(Ca+Mg+K) (1:4) ratios, and depending on aeration levels, a pH around 7.4. Reducing the calcium and magnesium will soften the water, and reducing the bicarbonates will reduce the pH (and unfortunately the pH stability), and the product will be a better approximation of Amazon water. For smaller volumes, the formula can be cut proportionally to suit individual needs.

The trace element mix provides small quantities of elements that are usually present in low concentrations (hence trace) in most bodies of water. Although deadly at higher concentrations, they seem to be necessary in small amounts for normal metabolic function, growth, and development, and they greatly enhance plant growth. The quantity recommended yields 0.1 mg/l iron, the ideal for aquatic plants. Trace element mixes are available through hydroponics suppliers (e.g., #6 chelate trace element from Homegrown Hydroponics).

RO filters do not remove everything. Some nitrates, phosphates, and silicates, which can be present in tap water at low concentrations, can pass through. Although not toxic at low levels, these substances can cause unsightly algae blooms. A deionizing (DI) filter cartridge used in conjunction with the RO filter will help eliminate nitrates and phosphates, should they prove problematic. A DI filter uses chemical resins that must be periodically regenerated or replaced. Special silica-removing RO membranes are available, but this substance is generally only a problem in salt-water aquaria.

Reconstituted water should be used to fill tanks initially and for water changes. In primarily aquatic systems that (a) do not receive regular, large water changes, and/or (b) have a high rate of evaporation due to powerful lights, splashing, overhead air circulation, etc., only pure RO water should be used to top-off when water evaporates.

RO filter membranes do eventually go bad, so test the product water periodically with a conductivity meter, or purchase a model with a built-in purity meter. Using a water softener inline before the RO unit will extend the life of the (expensive) filter membrane: removing salt is easier on the membrane than removing hard-water minerals. RO membranes are also highly sensitive to chlorine, and most manufacturers offer in-line carbon filters to be placed upstream from the RO filter. Using carbon in this fashion will remove chlorine from the water supply and reduce or eliminate the need for chemical filtration in the individual tanks, but one must regularly replace the carbon cartridge according to the manufacturer's recommendations.

Temperature and plumbing

We recommend that you always use water from the cold tap and heat it (or allow it to warm to room temperature) in the aging tank. Water that passes through a hot water heater absorbs toxic heavy metals and minerals, which concentrate there like scale in a teapot. Also, hot water is more likely to leach toxins from metal pipes. Lead pipes are problematic at any temperature, for animals and humans, and should be replaced. Low-dose lead poisoning causes long-term neurologic problems. As noted earlier, water that sits in copper pipes overnight can absorb enough copper to cause acute copper toxicity and death in amphibians. Chronic copper exposure in some animals has been found to cause development of copper crystals in the liver. Old copper pipes, which might form an oxidized scale on the inside, seem less likely to leach than new ones. Running the water for several minutes before use to flush the system reduces copper levels in the water. Using plastic (PVC or CPVC) pipes eliminates the problem of leached heavy metals and is ideal for cold water applications, but running hot water through them might cause the leaching of toxic vinyl chlorides. CPVC is more heat-resistant than PVC; flexible PVC (rubber hose) is worst.

Overall System Design Considerations

Two types of aquatic system are currently used to house amphibians in the zoo or private collection - the open system and the semi-closed system. The open system allows new, clean water to enter the enclosure in a continuous flow, where the water remains within the enclosure for a short period of time and is then discharged. In semi-closed systems, a quantity of water is added and removed periodically (e.g., weekly or monthly) as a percentage of the total water volume in the system. A completely closed system is possible but not recommended because the long term buildup of inorganic and some organic substances that are not easily removed by normal methods of controlling water quality.

The Open Systems

The open system is one in which matter, in this case water, is continually entering (influent) and leaving (effluent) the system. Waste products, organic toxins, decaying organic matter, dead food items, inorganic compounds, etc., are continually flushed from the enclosure and the water quality is maintained so long as the rate of influent flow is sufficient. No type of enclosure filtration is needed because the water is never in the enclosure very long. This is the ideal system for large animals in a relatively small enclosure, which would require large exterior biofilters to remove the extensive waste products and large mechanical filters to help limit particulate and bacteria. In theory, it is also the least complicated; most maintenance free type of system for any amphibian enclosure, but it requires suitable and continuous supply. The problem is having a sufficient supply of water.

The Semi-Closed Water Systems

The most common type of aquatic system used by the aquarist to maintain water quality is the semi-closed system. What has been learned in the aquarium field has been successfully adapted for use with the amphibian enclosure at many institutions. Incorporating mechanical, chemical, and biological filtration with the occasional partial water change in our amphibian enclosures has greatly reduced mortality and has aided in the successful breeding of several species of amphibians.

Filtration

Mechanical Filters

Filters that remove organic and inorganic particles suspended in water are called mechanical filters. While other types of filters (e.g., chemical and biological) can also remove particulate, this is not their primary function. In many cases, the ability of other types of filters to remove particulate inhibits their primary function and greatly reduces efficiency. To prevent this problem, the mechanical filter should be employed to remove the particulate so that the other types of filters can operate uninhibitedly and are commonly incorporated in filter system designs.

Mechanical filter efficiencies are measured by the percentage of removal of particles of a minimum size. As an example, a filter might remove 100% of 10um diameter particles, 25% of particles that are 4um in diameter and only 7% of particles that are 2.5um. Another parameter of mechanical filter system design is amount of time between filter cleanings or changes. The smaller the particulate removed, the shorter time between cleanings. The smaller the filter size, the shorter time between cleanings. If it is desired to remove very small size particles, a series of filters can be employed, each filter removing a smaller size particle than the preceding, until the ultimately desired fine filtration can be achieved. This will greatly reduce maintenance time by increasing running time between cleaning.

One of the common types of mechanical filter employed in the small enclosure or aquaria is the wadding type filter. The medium used in these filters is a wad of polyester wool, which is inexpensive and easily obtained. The efficiency of these filters is dependent upon the depth and packing of the wool and the length of time that it has been in operation. These types of filters will clarify the water effectively, but fail to remove microorganisms that could be pathogenic. At best, this type of filter will remove small filter-feeding organisms, but not their food (Wickins and Helm, 1981).

One of the earliest developed filtration systems are the slow and rapid sand filters. These filters utilize fine sand as the filter medium, are more efficient than the wad type filters, and can remove particles effectively down to ~6um with a sand diameter of 0.3mm (manufacturer's data). The slow sand filter functions by gravitational flow through the filter and is limited to a slow flow rate. The cleaning procedure involves swirling the top layer of water covered sand and siphoning off the residue. Slow sand filters are generally very large structures installed in facilities during construction.

The rapid sand filter forces water through the sand under pressure and have a greater flow rate and, therefore, a larger capacity per size than the slow type sand filter. Rapid sand filters are cleaned by forcing water under pressure through the filter in a reverse direction (backwashing). Commercially manufactured rapid sand filters are generally too large to be used on the small enclosure below several hundred gallons of water, although it is possible to build a smaller version with PVC pipe and fittings. For larger zoo enclosures, small swimming pool or hot-tub rapid sand filters are available commercially, as well as small to very large size units developed for the aquaculture industry.

Many types of cartridge mechanical filters have become available commercially for managing water quality in animal enclosures (see Figure 3). These filters employ a manufactured cartridge element that is available in different grades that correspond to their ability to remove particulates. Pressurized water is forced through the cartridge where the particulate is trapped. Cleaning is accomplished by removing the cartridge element and, depending upon the type of element, the cartridge is washed or replaced. In many cases, manufactures offer different cartridges that can be used in the same housing, including chemical filter options (see below). Mechanical filter cartridges are available to remove particulate between 20 and 0.2um. This lower extreme would remove most types of bacteria. As mentioned in our introduction to mechanical filters, when employing a very fine filter it is recommended to use two or more filters in series to prevent the rapid clogging of the fine filter medium. Cartridges of 0.2um retention are very expensive and tend to prematurely clog from larger particulate.

Figure 2
A cartridge type mechanical filter commonly used for amphibians

Note that all mechanical filters only trap waste particles – they do not remove them from the water system. Unless the filter medium is changed or cleaned regularly, the filtered material will decompose in the filter and release toxins back into the circulating water. These may be eliminated by biological filtration, but do create additional load (see below). The longer the solids are in the water, the more they will liquefy and degrade water quality. It is much easier to deal with mechanical waste than chemical waste.

Chemical Filters

Filters that can remove dissolved substances from water are considered chemical filters. An example of a chemical filters is the de-ionizers that are used to remove ionic compounds from a water supply (see the source above). Another type of chemical filter that is currently common in the American household is the water purifier for drinking water. These filters contain cartridges with mechanical pre-filters and activated carbon chemical filters. Larger versions of these activated carbon filters have been in use for many years to assist in maintaining water quality in animal enclosures. The water de-ionizer would remove important buffering ions well as other trace elements from the water environment, making it less stale and unsuitable for housing animals.

Activated carbon is a very special substance with very unique properties that make it an ideal material for amphibian enclosure chemical filters. It is a highly adsorptive and porous material that readily removes dissolved organic compounds, micro-particulate, and certain reactive nonionic chemicals (e.g., free chlorine). It will even remove some ions, such as copper. The numerous pores create an effective surface area exceeding10,000m^2 per kilogram of carbon (Kinne, 1976). As water passes through this porous matrix of activated carbon, organic compounds (which include organic solvents, organic metabolites, some proteins, lipids, etc.) loosely bond with the carbon and are effectively eliminated from the water. This removes raw materials from the water that might otherwise be used by potentially pathogenic bacteria. The carbon also removes organic chemicals that form films at the water and air interfaces, which could reduce oxygen absorption. The porous matrix catches very small micro-particulate (e.g., some bacteria) thus acting as a fine mechanical filter. As mentioned in the introduction to mechanical filters, the action of activated carbon as a mechanical filter is secondary and, in many cases, contradictory to its primary function as a chemical filter. If no mechanical pre-filtration is used, the activated carbon granules will become coated with particulate, sealing the porous surface and effectively halting chemical filtration. Activated carbon filters should always have a mechanical pre-filter to remove most of the particulate prior to the chemical and fine mechanical filtration by the carbon filter.

Another use of activated carbon filters is as a filter for a primary water source. These filters are commercially available and are effective as long as their flow ratings are not exceeded. It is possible to construct a primary water source filter inexpensively (as an alternative to the commercial models) utilizing simple plastic plumbing fittings and pipe. These filters can remove free chlorine from domestic water as well as trace organic solvents that have been found in many municipal water supplies. The efficiency of each of these filters will depend upon pipe size, length and design as well as the quality of the activated carbon used. The maximum effective flow rate and functional operating time must be determined experimentally by monitoring the chlorine content of the filter discharge. Activated carbon is less effective in removing chloramines from water than it is free chlorine. The result is that the filter medium must be changed more frequently if the antibacterial agents used in the water supply are chloramines.

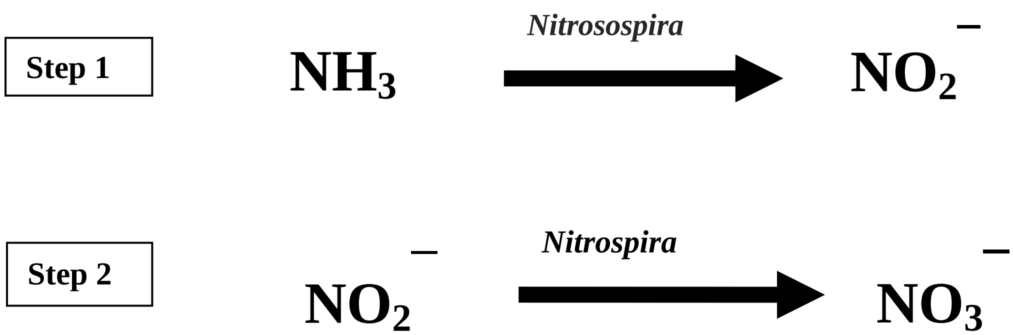
Ammonia is not removed from water by activated carbon, but other filter chemicals can be employed. Zeolite or Ammo-Chips® are readily available from aquarium supplies, but these substances must be replaced regularly to assure effectiveness. Generally, these ammonia chemical filters are used only in crisis situations, when biological filtration capacity has been exceeded in a system, or the biological filter has been badly damaged. These filters can buy time to correct these problems.

However, chemical media can become saturated with toxins, and if they are not changed regularly they will begin releasing those toxins back into the water. Unlike mechanical filters, one can't see when a chemical filter needs changing. It is generally recommended that chemical media be changed every 2-4 weeks, but this will vary widely depending on the amount of media in the filter and the chemical load in the water. Some experts recommend using only a small amount of carbon every few weeks for a couple days at a time, discarding the carbon after each use.

Biological Filters

I have briefly mentioned biological filtration in the preceding sections of this paper. Biological filtration is perhaps the most important and the most complex type of filtration in an enclosure. Its action is neither mechanical nor chemical. Its function is the accumulative effects of a community of millions of living bacteria. Once this community is established in an enclosure, its actions appear unified, as if the community was in itself a single separate organism. A biological filter possesses many basic characteristics of life itself and, for the purpose of this discussion, should be considered as both a community of organisms and as a separate life form that lives in symbiosis with the animals housed in the enclosure. For this reason, in the enclosure that depends upon biological filtration to maintain water quality, the health and well being of animals in the enclosure is directly related with the health of the biological filter.

Biological filters remove the toxic nitrogenous metabolic waste products of the animals and other organisms (e.g., decomposing bacteria) from the water in an enclosure. Most totally aquatic vertebrates excrete ammonia as a metabolic waste product. More terrestrial forms excrete urea or uric acid as metabolic waste, which is quickly reduced to ammonia by certain bacteria. The primary function of a biological filer is to oxidize toxic ammonia/ammonium (NH_3/NH_4^+) into a less toxic form, ultimately the nitrate ion (NO_3^-). This process of bacterial oxidation of ammonia is called nitrifying. The nitrifying process involves two steps - each performed by different groups of bacteria: Those that oxidize ammonia and those that oxidize nitrites. Until recent work, it was generally believed that a *Nitrosomonas* and *Nitrobacter* were the primary bacteria involved in these processes. Genetic studies have shown that other forms of bacteria are involved in freshwater systems. Ammonia is primarily oxidized by *Nitrosospira* (Burrell et al., 2001) and perhaps *Nitrosomonas,* while nitrites are oxidized *Nitrospira* (Hovanec et al., 1998). These bacteria consume the nitrogenous waste products, utilize these wastes as a source of food and excrete nitrogen in a more oxidized form. Oxygen is crucial for nitrifying to take place and conditions in a biofilter must always be aerobic. The overall process follows the sequence below:



We have already discussed the toxicity of ammonia, nitrites, and nitrates in the earlier sections. Both ammonia and nitrites are very toxic, while the end product of the nitrifying process, nitrates, is not toxic in low concentrations. In a balanced symbiotic relationship between the animals that excrete waste products in the water and the biofilter that feeds from these wastes, both animal and bacteria benefit -- the animal by having a clean, unpolluted water supply and the bacteria for their very subsistence. Once this relationship is established in an enclosure, both animal and biofilter cannot exist without the other. If the filter dies, the water will rapidly become polluted and toxic substances will accumulate, threatening the life of the animal utilizing the water. If the animal is removed from the enclosure, the biofilter's food supply is removed and the biofilter will slowly die of starvation.

The biofilter has three separate parts. First, there must be a suitable substrate for the bacteria to colonize. The second part is the mechanics of maintaining sufficient circulation through the substrate to provide enough oxygenated water for aerobic activities. The final part is the actual bacterial colony. Many different materials have been used as a substrate for biofilters. The most commonly used substrate in small enclosures is aquarium type gravel. Both silica and calcium carbonate based gravels are employed (the calcium carbonate has the extra benefit of acting as a source of carbonate ions for pH buffering). Other substances that have been employed for biofilter substrates include sand, porous plastics, porous ceramics, etc. Almost any nontoxic insoluble substance that has sufficient places for the *Nitrosospira and Nitrospira* to attach is suitable as a substrate. For this reason, the presence of nitrifying bacteria is not limited to the biofilter proper; they are found in every part of the aquatic system, attached to any structure that has a suitable micro-structure (e.g., activated carbon, tubing, rocks, decorations, walls, gravel, etc.) and is in a continuous aerobic environment. The biofilter is designed to give the maximum micro-sites for nitrifying bacteria to attach in the smallest volume possible, while maintaining an aerobic environment.

Circulation through the biofilter is normally accomplished through the use of pumps and airlifts. Water must flow through the filter medium slowly in order for the bacteria to be able to adsorb the nitrogenous wastes (Hawkins and Anthony, 1981; Wickens and Helm, 1981). The minimum flow rate is determined as the slowest flow rate that maintains aerobic conditions throughout the entire biofilter medium. If the flow rate is too slow or ceases entirely, the filter will become anaerobic and will start producing ammonia rather than adsorbing it (Stickney, 1979). The nitrifying bacteria will quickly die and be replaced by species that are favored to live in an oxygen deficient environment. Many of the species of anaerobic bacteria produce toxic by-products, both inorganic (e.g., hydrogen sulfide) and organic (e.g., *Clostridium*). The resulting buildup of toxins could kill the animals that are in the enclosure.

In the small enclosure, a commonly used biofilter is the under-gravel filter. In its simplest form, the under-gravel filter is the least expensive and is extremely dependable in operation. A filter plate is installed on the bottom of the enclosure in the area that will contain water. It is then covered with 50 to 75mm of gravel. The filter plate forms passageways so that water can flow in a down direction through the gravel, under the plate and be discharged back into the water above the gravel. Airlift tubes are used to maintain flow through the gravel. Under-gravel filters also function as slow sand and gravel mechanical filters, removing particles effectively. Unfortunately, the accumulation of these particles in the filter medium can decrease the filter's ability to function as a biological filter. The gravel must be swirled occasionally and the resulting suspended detritus siphoned off to prevent the accumulation of particulate from clogging the filter bed. If the filter bed is clogged, a cessation of water flow will occur and the filter will become anaerobic, causing the problems discussed earlier.

A modification of the under gravel filter is the reverse flow system. In this system, water is pumped under the filter plate so that the flow through the gravel is upward. The benefit to this modification is that the upward flow prevents the accumulation of detritus from entering the filter, thus preventing clogging. The particulate that does settle to the bottom lies on top of the filter bed, rather than being pulled into the gravel by the downward flow. If a reverse flow system is employed in an enclosure, there should always be a mechanical prefilter installed to remove particulate before the water is pumped under the gravel bed. This is important to prevent the accumulation of detritus under the filter plate, which could restrict flow. Experimentation established that the reverse flow system, when used in conjunction with a mechanical and activated carbon prefilter is more efficient in removing nitrogenous wastes than a direct flow system under unusually high biological loads (e.g. raising large numbers of amphibian larva (Odum et al., 1984).

Sponge filters can also act as biofilters, using rising air bubbles to draw water through the medium. A sponge has countless nooks and crannies and provides a large surface area for bacterial growth. Sponge filters are appropriate for smaller tanks with only a few, small animals. However, like the gravel bed, the sponge acts as a mechanical filter and can become clogged with detritus. It must be cleaned periodically to remain effective. When cleaning it is important to rinse the sponge free of detritus without wiping out the bacterial colony. Usually, wringing under a warm stream of water a few times will suffice. Remember to always keep the water level above the plastic air tube on the sponge filter or little water will flow through it.

Nitrifying bacteria will also invade any mechanical filter and function as a biofilter if the flow rate is not too rapid. Certain types of mechanical filters provide the appropriate conditions for biofiltration better than others. One of the best is the slow sand filter. Generally the rapid sand filter's flow rate is too fast to allow effective biological filtration (Wickins and Helm, 1981). The wad type filters usually have flow rates slow enough to provide adequate conditions for biofiltration. The main drawback in depending upon a mechanical filter to provide all or a large portion of the biological filtration in a system is that when the filter medium is replaced (to remove accumulated particulate) the bacterial colony is also removed. The results can be catastrophic with a sudden rapid increase of ammonia and nitrites in the water. To prevent this situation, it is best to keep the biological and mechanical filters as separate parts of an overall system.

Figure 3
A small fluidized bed filter

In some cases, it might be necessary to have more biological filtration than can be contained within the enclosure and the use of an auxiliary biofilter is needed. This can frequently occur in display enclosures, where in order to maintain an effective display of animals, the conditions may be crowded. To obtain extra biofiltration, a tank containing another biofilter can be used in conjunction with the display. Water is pumped from the display enclosure into the auxiliary biofilter and allowed to flow back to the display by gravity after it passes through the filter. Another external filter is the trickle filter. A medium of small plastic or ceramic balls or tubes is placed in a vertical tower structure several feet high. Water is sprayed on to the medium and allowed to trickle down the filter to a reservoir at the bottom. The medium is always wet and dripping, but never submersed. The water is then pumped back into the enclosure. These filters are very efficient because they provide a large surface area for the bacteria, as well as an oxygen saturated environment.

One of the newer and most efficient biofilters is the fluidized bed filter. These compact filters utilize the same basic technology as an undergravel filter with several major improvements. A fluidized bed filter is usually in the form of a clear plastic tube 2-5 inches in diameter and 1-3 feet long, and hangs on the outside of the tank. A small pump feeds the filter, with a sponge prefilter to remove particulate. Water is pumped from the tank to the base of the filter where it then rises up through a bed of sand, out a port at the top and back into the tank. The flow of water is just great enough to keep the sand suspended in the water column without forcing it out of the filter. This sand provides a huge surface area for bacterial growth, and because it is constantly suspended in the water (fluidized), there are no dead spots as with an undergravel filter. These filters are rapidly gaining in popularity and replacing the more conventional wet/dry trickle filters. One of the authors has used QuikSand® fluidized bed filters for years with excellent results. A problem in using fluidized beds is that they deteriorate rapidly when water ceases to flow through them, e.g., during a power outage.

Do not confuse fluidized bed filters with sand filters; they both use sand, but that is where the similarities end. Water passes through a sand filter from the top down, compacting the sand bed, which acts as a dense mechanical filter to trap suspended particulate. They are decent mechanical filters but only marginal biological filters because they tend to clog and often develop by-pass channels in the medium during backwashing. Fluidized bed filters flow from the bottom up. They provide absolutely no mechanical filtration, just a huge, easily accessible surface area for bacterial growth.

Starting a biofilter

The bacteria for biofiltration are present everywhere and one need only provide a suitable environment for them to grow and allow them ample time (usually 3-6 weeks at tropical temperatures) for them to colonize the filter. A source of food for the filter is mandatory to start a filter. This can be done by adding chemicals (see below), or the use of living aquatic organisms. Fish are commonly used by amphibian keepers to start a filter. The bio-load (mass of animals) should be kept low at first and the ammonia level frequently tested while the biofilter is becoming established. Seeding the new tank with some gravel or other biofilter medium (and the attached bacteria) from an established filter will usually accelerate the process, although considerations should be given to the possibility of transferring disease to the new tank from the seeding source.

In order for the biofilter to function it must be initially invaded by the appropriate bacteria, which in turn must reproduce to form a balanced colony, capable of keeping toxic nitrogenous wastes at a minimum. When an enclosure is first set up to house an amphibian, biofilter medium (e.g., gravel) is generally washed and disinfected. The best disinfectant is diluted household bleach. The residual chlorine can be neutralized to nontoxic chloride with sodium thiosulfate. At this time there is no biofilter. To establish the biofilter, food (in the form of ammonia) must be present. This can be from a natural source (e.g., an animal placed in the enclosure) or from a synthetic (usually ammonium chloride). When this food source for the bacteria is present, the organisms can invade the filter medium. The nitrifying bacteria can enter the system from the air, from the gut of an animal placed in the enclosure, or they can be seeded from an already established biofilter. Bacteria mixtures are also available as commercial products for the aquaculture industry, but it is questionable if these are really effective. The time it takes for the filter to become a balanced colony of bacteria can vary from a few days to several months, depending upon many factors, some of which are not fully understood. If animals are used to produce ammonia to initialize a biological filter, it is best to start with an absolute minimum biomass (e.g., one small amphibian or fish) and then increase the biomass in steps over a period of time while monitoring ammonia and nitrite concentrations.

The first bacteria that must become established are the *Nitrosospira*, which consume ammonia and produce nitrites. Once they are established, ammonia levels tend to fall rapidly. The growth of nitrite oxidizing bacteria may be inhibited in the presence of ammonia, which may cause a build up of nitrite until the concentration of ammonia has diminished (Lees, 1952). High levels of nitrites may also inhibit the *Nitrosospira*. This inhibited response of the nitrifying bacteria occasionally causes the ammonia and nitrite levels to fluctuate until the filter colony reaches equilibrium. We have personally observed the initialization of many biofilters, and as long as the biomass is not too great, the nitrogenous waste product buildup is normally minimal, with a balanced filter usually being established in a week or two. This is not always the case and problems can occur. For this reason, the initiation of any biofilter should be monitored carefully to prevent the possible buildup of toxic wastes that may result in adverse problems with the housed animal.

Although nitrites tend to cycle with ammonia and testing only for the more toxic ammonia is generally sufficient, nitrites can present problems of their own. The bacteria that convert nitrites to nitrates require some phosphate in the water; if ammonia has spiked and waned but nitrites continue to be high, adding some phosphate is often enough to bring things inline. Excess nitrites can enter fish and amphibians and bind to the hemoglobin, interfering with respiration (brown-blood disease). Such animals will increase buccal pumping, gill waving, and/or trips to the surface for air, even though the water may be well oxygenated.

Hydrogen ions are also a product of biological filtration, so one must regularly test pH. Under certain circumstances (e.g., soft water, high bioload), a good biofilter can bring down the pH in a matter of days.

Instead of waiting 3-6 weeks while gradually increasing the bioload, one can cycle the tank without animals so that the biofilter will be established when the animals arrive. This technique has the additional bonus that no animals are put at risk while the filter is becoming established and toxins are spiking. One method suggested includes adding 4-5 drops of pure household ammonia (without additives or perfumes) everyday for each 10 gallons of water. Ammonia concentration will quickly spike and then wane, usually by the end of the first week. As ammonia is converted, a nitrite spike closely follows. At that point, cut back to 2-3 drops of ammonia per 10 gallons per day until nitrites reach 0. This technique works great, producing robust bacterial colonies in biofilters in less than 2 weeks. Exercise caution: ammonia is deadly toxic to fish and amphibians; make sure it has been consumed completely before animals are added.

A more refined approach is to dose with ammonium chloride (NH_4Cl), which gives finer control over concentration and eliminates the risk of introducing secondary toxins (perfumes, detergents) sometimes found in household ammonia. To dose 4mg/l N per day, add 0.58g NH_4Cl for each 10 gallons of water. Again, follow the ammonia spike and wait for nitrites to wane before adding animals.

Maintaining the biofilter

Again, think of the biofilter as a living entity in the enclosure. The bacteria must be supplied with a constant flow of oxygenated water at the appropriate temperature, which contains low levels of ammonia and nitrite as food. Without these necessities, the filter will suffocate and starve. If the tank must sit idle (without animals), move the biofilter to a tank with animals to keep it going, or simply feed it ammonia (see section above) every day. Also, be aware of the amount of time a biofilter is shut down during servicing. The longer it is down, the more bacteria suffocate and the less effective the filter will be until it recovers. Do not clean a biofilter excessively; just rinse the media if and when necessary. Never use chemical disinfectants on a biofilter unless you plan start the initialization process again. Antibiotics can also kill a biofilter, so always treat sick animals in a separate 'hospital' tank if possible.

It is a good idea to always keep a few extra biofilters going on tanks with heavy bioloads. That way, when a new tank is set up, a living biofilter is ready to transfer without having to wait for a new one to cycle. This is vital for that unexpected batch of tadpoles.

Plants

Another often-overlooked form of filtration (bio and chemical) comes with the addition of living plants to the system. Plants help remove organic as well as inorganic waste from the water and are a great source of oxygen. Some aquarists use only living plants for filtration. Furthermore, plants greatly enhance the attractiveness of an aquarium and provide an oviposition site for many amphibians and fish. If the inhabitants of the tank are large or active and tear up rooted plants, try culturing the plants in a separate tank adjacent to the animal tank. Use the filters to pump water from one tank to the other. Just letting the tendrils of a potted plant, like pothos, dangle into a tank can significantly reduce nitrogenous wastes, especially nitrates.

Other water quality technology

There are two other devices one might want to consider: UV sterilizers and ozone generators. UV sterilizers expose small amounts of tank water to intense short wavelength ultraviolet light, thereby killing viruses, bacteria, fungi, and algae. The light is entirely contained within the sterilizer, which sits outside the tank, and poses no threat to the tank inhabitants. The key to its operation is that the water and microorganisms must be exposed to the UV light for a sufficient amount of time (contact time), and this is controlled by the water flow rate through the sterilizer. Each unit comes with its own recommended maximum flow rate – exceed this and the unit will be much less effective useless at sterilizing. UV sterilizers are usually put inline somewhere after the mechanical filter, as suspended particulates can block the light from hitting the target organisms. Installing a valve on the mechanical filter's outflow line will allow a small portion of its effluent to be directed into the sterilizer tube and the majority of flow back into the tank. Like all UV lights, these bulbs are relatively short-lived and need to be replaced periodically (usually every 6 months). Using a sterilizer for only a couple hours per day is all that is required in some cases and extends bulb life. The amount of time the sterilizer needs to be on will depend on the tank size, sterilizer power, and flow rate through the sterilizer. The sterilizer vendor should be able to help with these calculations as they relate to each individual system. It is recommended not to use a UV sterilizer on a new tank until the biofilter is established. Also keep in mind that sterilizers heat the water as would a heater of equal wattage.

Understand that UV only sterilizes the water exposed to it, not all the water in the tank. While the effluent might be sterile, the tank water can still be teeming with microscopic organisms. A UV sterilizer does not kill everything in the tank, but by killing most of the organisms in the water passing through it, it helps keep microorganisms in check.

Ozone is a highly reactive form of oxygen containing three oxygen atoms (O_3) bound together (compared to normal oxygen O_2). It chemically reacts with and destroys most organic molecules, pesticides, colors, and microorganisms in the tank. It provides complete water sterilization, but it can be a dangerous gas if not handled and vented correctly. Ozone generators force ozone into the water inline with the filtration system, and because ozone is a relatively unstable compound, it quickly reverts to the O_2 state and so will not harm the animals. When using ozone, the oxidation potential of the water needs to be monitored closely. If too much ozone is supplied and an excess remains in the enclosure water, the inhabitants in the tank could become bleached and burned.

Water changes

Regular water changes are essential to rid a system of the minor toxins that are not managed (like nitrates and phosphates), and to replenish any nutrients that were absorbed by the plants and animals. A minimum of 10-20% water change every 1-2 weeks will generally suffice.

Conclusion

Understanding water quality is essential for the long term successful breeding, rearing, and maintaining of amphibians in captivity. Proper monitoring of water can establish negative trends in aquatic systems before problems arise. Many times, the damage is done before the increase in mortality and morbidity is observed.

When a problem is encountered, the water quality should be tested (along with other possibilities) to determine if there is a cause and effect relationship. As mentioned earlier in this paper, if eggs or larvae die, water quality should be one of the first areas examined to find a possible cause. If mortality is a problem in keeping adult amphibians, check the quality of the water supply. If a relationship between water quality and mortality or health problems is discovered, improve the quality of the aquatic environment. The solutions to water quality problems are many, answers are found by applying the principles of water management.

The checklist for healthy aquatic systems:

- Start with high quality water.
- Filter the water three different ways: mechanically, chemically, and biologically.
- Clean mechanical media at least weekly, replace chemical media regularly, and treat biological media as living organisms.
- Do not overcrowd a tank: keep the bioload reasonable.
- Do not over feed the animals: uneaten food and excessive feces will foul the water.
  Test the quality of the water regularly (at least ammonia and pH levels). Ask yourself, "Would I drink this water?"
- Where possible, incorporate live plants.
- Perform water changes often; if this is not possible or feasible, consider topping off with RO water to help maintain water chemistry in the interim.
- Monitor water quality

Quick Reference Table

WATER QUALITY PARAMETER	EFFECT ON AMPHIBIANS	ACCEPTABLE LEVELS	METHODS OF ALLEVIATION	COMMENTS	REFERENCES
Water hardness (dissolved Ca and Mg salts)	Hard waters can cause skin problems in some species osmotic regulation of the amphibian. Most show a preference for 'soft water', but this can be species dependent	<75mg/l (ppm) of $CaCO_3$ for animals that require soft water >100mg/l should be considered hard water for an amphibian	Diluting hard water with RO, DI or distilled water Hardening soft water with Ca and Mg salts (only recommended for reconstituting RO, DI distilled water)		(Wright and Whitaker, 2001)
Dissolved oxygen as O_2	Oxygen is needed for amphibian respiration and aerobic processes.	>80% Saturation	Aeration	Some anurans and salamanders may be able to tolerate very low levels of oxygen	(Gulidov, 1969; Brungs, 1971; Siefert and Spoor, 1973; Carlson and Siefert, 1974; Siefert et al., 1974; Odum et al., 1984; Wright and Whitaker, 2001)
Gas Supersaturation	Gas bubble disease	Gases should be at equilibrium with atmosphere	Aeration until equilibrium is achieved	Common in well water and pressurized municipal water sources, especially when cold	
Ammonia/Ammonium -NH_3/NH_4^+	Very toxic	<0.2 mg/l, N as un-ionized ammonia	Biological filtration, chemical filtration with appropriate medium, or water changes	Metabolic waste product Ammonia/ammoniu m ratio is pH and temperature dependent (see Table 2)	(Tabata, 1962; Herbert and Shurben, 1965; Ball, 1967; Jofre and Karasov, 1999; Rouse et al., 1999; Wright and Whitaker, 2001)
Nitrites NO_2^-	Toxic	<0.5 mg/l, but ideally zero	Biological filtration, chemical filtration with appropriate medium, or water changes	A product of aerobic biological action on ammonia $NH3/NH4^+$ Very Toxic to *A. tigrinum* larvae	(Klinger, 1957; Russo et al., 1974; Westin, 1974; Huey and Beitinger, 1980; Marco et al., 1999; Wright and Whitaker, 2001;

					Hoffmann, 2010)
Nitrates NO_3^-	Slightly toxic	<50.0 mg/l	Remove by photosynthetic action of green plants and by water changes	This is the end product of biological filtration	(Westin, 1974; Wright and Whitaker, 2001)
pH	Can cause metabolic problems if not within acceptable range for species, disrupts ion exchange	Species dependent, but usually near neutral. pH below 6 and above 8 are potential a problem	Change water source or add appropriate buffers		(Cummins, 1989; Warner et al., 1991; Wright and Whitaker, 2001)
Chlorine Cl_2	Very toxic	Undetectable	Aerate for 24 hours or add chemical dechlorinator (e.g. sodium thoisulfate)	Some adult forms seem to be able to tolerate chlorinated water: *Ceratophrys ornata*, *Rana catesbeiana*, *Ambystoma texanum*, *Ambystoma tigrinum*, *Litoria caerulea*, *Ichthyophis kohtaoensis*	(Arthur and Eaton, 1971; Culley, 1992) R. A. Odum, pers. obs
Chloramines ($ClNH_2$, ClN_2H, ClN_3)	Very toxic	<0.01 mg/l as Cl	Use chemical treatment specific for chloramines (e.g. Prime)	Similar to Cl_2 in toxicity, but also releases ammonia	(EPA, 1986)
Copper (Cu)	Toxic	<0.05mg/l	Carbon filtering and carbonate precipitation Don't use copper pipes	Copper water supply pipes can be flushed before collecting water	(Landé and Guttman, 1973)
Phosphates (PO_4^{-3})	Toxic to many animals, interferes with calcium metabolism	Toxicity may be species specific. EPA limits PO_4^{-3} to 10mg/l. Applications of 1-mg/l are considered effective for preventing pipe corrosion	Phosphate sponges and filters are available to absorb phosphates	*Atelopus* spp larvae seem to be particularly sensitive to phosphate toxicity	

DI. de-ionized; DO. dissolved oxygen; EPA. Environmental Protection Agency; RO. reverse osmosis.

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