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Amphibian water quality: approaches to an essential environmental parameter

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Appropriate water quality is essential for maintaining and breeding amphibians in captivity. Aquatic systems that maintain water quality have been employed for many years in the aquaculture and aquarium industries. These techniques are now more commonly being utilized for amphibians. Using information from the work of the authors and published literature on amphibians and fish, benchmarks are provided for common water-quality parameters for amphibians.

Key-words: amphibian; benchmark; breeding; water-quality parameters.

INTRODUCTION

Long relegated to a footnote in many reptile facilities, it is only recently that amphibians have been receiving the attention they deserve from zoos and aquariums. Unfortunately, this attention was slow in coming and was only in response to the urgency of the current extinction crisis facing the entire class. In reaction to the crisis, the zoo community, IUCN – The World Conservation Union and other conservation organizations have inaugurated a response to create captive survival-assurance populations to preserve threatened species and to allow the option of future reintroductions, if necessary (Pavajeau *et al*., in press).

Regrettably, it has quickly become apparent that there are many more taxa in need of help than there are facilities capable of providing assistance. Although zoos have had a great deal of experience with the amniotic terrestrial vertebrates, few have had a long history with amphibians. Most taxa have never been maintained in captivity and, of those that have, most have not been bred. Dendrobatidae are the most common captive-bred anurans in zoos (ISIS, 2007).

There are critical differences between the husbandry of amphibians, which have the most diverse reproductive strategies, and the other tetrapod vertebrates (reptiles, mammals) (Duellman & Trueb, 1986). Their skins are highly permeable, making them prone to desiccation and absorption of environmental pollutants directly through their skin. Perhaps the most descriptive metaphor that best describes the amphibians is, 'Think of these animals as fish with legs'. Fish are managed in captivity by providing them with a clean and appropriate water environment; this is exactly the same way in which amphibians should be managed.

Any supply of water for amphibians must meet certain minimal requirements to maintain the health and normal physiology of the animals (Schmuck *et al*., 1994). 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 might be required to maintain the organism, might have no effect or might be detrimental. Amphibians have invaded many different niches, and the individual water requirements for a given species and its tolerance to specific toxins vary. It should be noted that different life stages of an amphibian may also have different requirements. The overall concentrations of these substances and suspended material 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).

This article is a brief overview of water quality as it relates to amphibian husbandry. Most of the techniques presented have been developed by the aquarium and aquaculture communities to maintain and reproduce fish (Stickney, 1979). These have been successfully adapted by some institutions to maintain and breed amphibians. The key to these successes is in part owing to an appreciation and knowledge of water quality and the systems necessary to maintain high-quality water (as well as an understanding of the biology of the animals we maintain). Clearly, water quality is far more important for those animals that spend most or all of their time in it (e.g. larvae larviforms and other aquatic adults), but even for those who might only rely on terrestrial substrate moisture, water quality is an essential factor for health of the animal.

PARAMETERS FOR WATER QUALITY

Extensive work has been carried out in the field of aquaculture to quantify the relationship between water quality and the health of fish and some aquatic invertebrates (Environmental Protection Agency, 1976). In contrast, the published literature for amphibians is relativity scant.

Table 1 shows some common parameters for the water quality for amphibians. These parameters were developed from the published literature for amphibians and fish, as well as the direct experience of the authors. These are only guidelines. The tolerance to common toxins and the requirements for each species are still unknown for the majority of amphibian taxa. Therefore, these suggested levels should be considered only for guidance in evaluating system performance.

Water sources

Water is available from many sources for amphibian husbandry, and water quality of these sources varies extensively. A first step in evaluating a water supply is to perform appropriate tests for dissolved substances, pH and hardness. At least initially, it is very useful to have your water source tested by a laboratory qualified for this purpose. Most US counties or states provide water-testing services for water supplies intended for human consumption, and there are also many commercial laboratories that provide these services at a reasonable cost. The results of these tests will help to identify any pretreatment that is necessary for the water before it can be utilized for amphibians. The human potable water standards (less disinfectants) are a good start for evaluating water supply. If you would not drink it, then it is probably not the best water for your animals.

Many amphibian facilities use a local municipal water source for their operations, but some preconditioning of the water is almost always necessary. Municipal water is usually disinfected with free chlorine (Cl_2) or chloramines. Eliminating these disinfectants is a first step in pretreating water. If free chlorine is the disinfectant, simply ageing and aerating tap water for 24 hours is all that is required to condition it for use with amphibians. Aeration will also drive off other harmful gases (carbon dioxide, nitrogen, hydrogen sulphide) and bring desired gases (oxygen) into equilibrium between the water and the atmosphere.

If chloramines are used as a disinfectant in the water supply, this ageing process is ineffective. The chloramines need to be removed through chemical filtration or through chemical treatment. Activated carbon is less effective at removing chloramines than free chlorine from water. There are commercially available filters specifically designed to remove chloramines that contain carbon and additional media. To remove chlorine chemically, create a saturated solution of sodium thiosulphate ($Na_2S_2O_3$) in water by adding it to a small volume of water until no more chemical will dissolve (note that $Na_2S_2O_3$ is usually available as a pentahydrate $Na_2S_2O_3 \cdot 5H_2O$, which is suitable for this application). This solution can then be used to dechlorinate water by adding one drop of

WATER QUALITY PARAMETER	EFFECT ON AMPHIBIANS	ACCEPTABLE LEVELS	METHODS OF ALLEVIATION	COMMENTS	REFERENCES
Water hardness (dissolved Ca and Mg salts)	Hard water can cause skin problems in some species by disrupting normal osmotic regulation of the amphibian. Most show a preference for 'soft water' but this can be species dependant	< 75 mg litre^{-1} (ppm) of $CaCO_3$ for animals that require soft water > 100 mg litre^{-1} 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)		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); Carlson & Siefert (1974); Siefert & Spoor (1974); Siefert *et al.* (1975); Odum *et al.* (1984); 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	$< 0 \cdot 2$ mg litre^{-1}, N as unionized ammonia	Biological filtration, chemical filtration with appropriate medium, or water changes	Metabolic waste product Ammonia/ammonium ratio is pH and temperature dependant (see Table 2)	Tabata (1962); Herbert & Shurben (1965); Ball (1967); Jofre & Karasov (1999); Rouse *et al.* (1999); Whitaker (2001)
Nitrites NO_2^-	Toxic	$< 1 \cdot 0$ mg litre^{-1}, but ideally zero	Biological filtration, chemical filtration with appropriate medium or water changes	A product of aerobic biological action on ammonia NH_3/NH_4^+	Klingler (1957); Russo *et al.* (1974); Westin (1974); Marco *et al.* (1999); Whitaker (2001)
Nitrates NO_3^-	Slightly toxic	$< 50 \cdot 0$ mg litre^{-1}	Remove by photosynthetic action of green plants and by water changes	This is the end product of biological filtration	Westin (1974); Whitaker (2001)

pH	Can cause metabolic problems if not within acceptable range for species, disrupts ion exchange	Species dependant, but usually near neutral. pH below 6 and above 8 are potentially a problem	Change water source or add appropriate buffers		Cummins (1989); Warner *et al*. (1991); Whitaker (2001)
Chlorine Cl_2	Very toxic	0	Aerate for 24 hours or add chemical dechlorinator (e.g. sodium thoisulphate)	Some adult forms seem to be able to tolerate chlorinated water: *Ceratophrys ornata*, *Rana catesbeiana*, *Ambystoma texanum*, *Ambystoma tigrinum*, *Litoria caerulea*, *Ichthyophis kohtaoensis*	Arthur & Eaton (1971); Culley (1992); R.A. Odum, pers. obs
Chloramines ($ClNH_2$, ClN_2H, ClN_3)	Very toxic	$<0 \cdot 01$ mg litre^{-1} as Cl	Use chemical treatment specific for chloramines (e.g. Prime)	Similar to Cl_2 in toxicity, but also releases ammonia	Environmental Protection Agency (1976)
Copper (Cu)	Toxic	$<0 \cdot 05$ mg litre^{-1}	Carbon filtering and carbonate precipitation Do not use copper pipes	Copper water supply pipes can be flushed before collecting water	Pritchard-Landé & 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 10 mg litre^{-1}. Applications of 1 mg litre^{-1} are considered effective for preventing pipe corrosion	Phosphate sponges and filters are available to absorb phosphates	*Atelopus* spp adults seem to be particularly sensitive to phosphate toxicity	

Table 1. Water-quality parameters for maintenance of amphibians in captivity: DI, de-ionized; DO, dissolved oxygen; EPA, Environmental Protection Agency; RO, reverse osmosis.

the saturated thiosulphate solution for every 4 litres of water. Care must be taken not to use too much sodium thiosulphate (beyond the saturated solution dose as described here) because it can be toxic. Also, the chemical reaction between thiosulphate and chloramines leaves free ammonia in the water, which is a significant toxin that needs to be removed (Smith, 1982).

Another common source used in facilities is well water. Well water can be an acceptable, consistent source of water for use with amphibians but again one must test for pH, hardness, metal content 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 (PO_4^{-3}) and nitrates (NO_3^{-1}) from fertilizers that seep into the aquifer. These substances cause algal blooms, and at higher concentrations are toxic to animals.

Well water can also be supersaturated with nitrogen and carbon dioxide, devoid of oxygen and can even contain lethal quantities of hydrogen sulphide. Vigorously aerating the water for at least a day before use will drive off the nitrogen, carbon dioxide and hydrogen sulphide, as well as raise the oxygen content. Aeration can also help precipitate some compounds (i.e. iron) before the water enters the animal enclosure.

Rainwater has also been used for captive amphibians. This resource can be a solution in isolated facilities that do not have other water supplies available. Rain water is naturally soft, perhaps too soft for some species (see reconstituting water below). Test for pH if air pollution is a consideration (acid rain). Also, one must consider how the rain is collected. Do not collect rain from a galvanized steel roof or one that has otherwise been treated chemically.

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; for example, is it draining from a large parking area covered with oil spills, or from a 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. If the source is in an area where chytrid fungus *Batrachochytrium dendrobatidis* is present, the water source should be screened for its presence. Alternatively, the 'stuff' living in the water could be beneficial for the care of the animals, particularly small larvae. Natural pools teeming with invertebrate life offer more diversity and nutrition than could ever be cultured artificially. One of the authors (R.A.O.) was successful only after multiple attempts to rear filter-feeding Banded rubber frog *Phrynomantis bifasciatus* larvae by using pond water containing large amounts of green algae and protozoa. Of course, an unfiltered natural water source is not appropriate for biosecure situations. Such sources would have to be filtered and disinfected to assure that no pathogens are introduced into a biosecure colony of amphibians.

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 (NRDC) showed that one in three 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 Street, New York, NY 10011, USA (Tel: +1-212-727-2700), to get the results for a particular bottled water source.

If the water supply in a facility has high levels of copper or other contaminants that cannot be addressed by other means, reverse osmosis (RO) water should be considered as a possible solution. This can be a safe and consistent way to ensure a constant supply of very pure water, which in itself creates other problems. Like rain water and distilled water, RO water is essentially pure. In fact RO water is, in many cases, too pure to be used as it is. It may be used for species that normally live in pure rain water, such as some dendrobatids and other phytotelm (water accumulated in tree holes and plants such as bromeliads) dwellers. For many other species, chemicals (salts for osmotic issues and trace elements for health considerations) need to be added. Common symptoms of osmotic imbalance created by too pure water include bloating and kidney dysfunction.

Commercial additives containing the requisite trace elements are available. A simple preparation to reconstitute RO water is listed below. This mix was developed largely by fish and aquatic plant hobbyists, and fine-tuned for amphibians.

In 100 litres of RO water dissolve:

- 4·0 g calcium chloride $CaCl_2$
- 4·6 g magnesium sulphate $MgSO_4 \cdot 7H_2O$
- 3·6 g potassium bicarbonate $KHCO_3$
- 3·0 g sodium bicarbonate $NaHCO_3$
- 0·13 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, with roughly 3° general hardness and 2° 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. The trace-element mix provides small quantities of elements that are usually present in low concentrations in most bodies of water. Trace-element mixes are available through hydroponics suppliers (e.g. Homegrown Six Pack, 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 algal blooms. A de-ionizing (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.

Designing water systems for amphibians

Two general types of aquatic system are currently used to house amphibians in collections – the open system and the semi-closed system. The open system allows fresh, clean water to enter the enclosure, at a flow rate whereby the water remains within the enclosure for a short period of time and is then discharged. This flow may be established by a variety of methods, including misting, spraying and direct influx of liquid water. It may be continuous or intermittently added (i.e. timer). 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.

The open system is one in which water and other matter, food and energy, are continually entering (influent) and leaving (effluent) the enclosure. Waste products, organic toxins, decaying organic matter, dead food items, inorganic compounds, etc, are flushed from the enclosure, and the water quality is maintained as 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. In theory, open systems can be the least complicated, most maintenance-free type of system. Another benefit to these systems is that potentially pathogenic organisms do not build up within the enclosure because they are continually removed with the effluent. The main problem with such a system is having a continuous sufficient supply of appropriate quality (and temperature) water. Open systems are commonly employed in large aquaculture operations, such as fish hatcheries, where large quantities of water are pretreated before they are used.

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 within amphibian enclosures at many institutions. Incorporating mechanical, chemical and biological filtration with the occasional partial water change in amphibian enclosures has greatly reduced mortality and has facilitated successful breeding of several species. However, the open system has many benefits for maintaining water quality and reducing pathogens, while reducing system complexity, over semi-closed systems.

The materials used to construct any water system are important. Metallic containers, vessels and piping should be avoided. In particular, copper pipes leach copper into the water and should be avoided, in both the supply lines and filter systems. Inexpensive PVC piping is easy to install and has few of the negative aspects associated with metal piping.

There are a variety of specialized plumbing fittings that simplify the construction of an aquatic system, as well as adding flexibility. In particular, bulkhead fittings have a lot of potential uses because they connect the plumbing to the inside of an enclosure. This piping can be used to provide influent, filtration or drainage to the tank. Pipes come in a variety of nominal sizes and can be adapted to small or large systems.

Filtration

In semi-closed systems, water quality is maintained by continually treating the water with a filter system. There are three basic types of filters employed for aquatic husbandry: mechanical, chemical and biological. It is a good idea, in semi-closed systems, to incorporate all three filter types to maintain appropriate water quality. It should be noted that although each filter type has its specific function, it is not uncommon for a filter to perform several functions simultaneously.

For small volumes of water, wad-type and canister filters are commonly employed for mechanical filtration. These small units can be very effective in maintaining water clarity and may also provide other types of filtration functions. The medium used in wad filters is a clump or a pad of polyester wool, which is inexpensive and easily obtained. Many types of cartridge mechanical filters have become available commercially for aquatic systems (see Plate 1). These filters employ a manufactured cartridge element that is available in

Plate 1. A common design for a canister filter. Depending on the media installed, these filters can provide mechanical, chemical and even biological filtration. *R. A. Odum, Toledo Zoological Society.*

different grades that correspond to the smallest particle size they will remove. Pressurized water is forced through the cartridge where the particulate is trapped. Both these types of filters will clarify the water effectively but fail to remove micro-organisms that could be pathogenic. At best, this type of filter will remove small filter-feeding organisms but not their food (Wickins & Helm, 1981).

It should be noted that 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 these other types of filters to remove particulate inhibits their primary function and greatly reduces their efficiency. To prevent this problem, a mechanical filter should be employed to remove the particulate so that the operation of the other types of filters is uninhibited. Such mechanical filters are commonly incorporated into filter-system designs.

The earliest mechanical-filtration systems developed 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 *c.* 6 μm with a sand diameter of 0·3 mm (manufacturer's data). The slow sand filter functions by gravitational flow through the filter and is limited to a slow flow rate. Slow sand filters are generally very large structures installed in large aquatic systems.

The rapid sand filter forces water through the sand under pressure and has a greater flow rate and, therefore, a larger capacity per size than the slow sand filter. Commercially manufactured rapid sand filters are generally too large to be used in a small enclosure containing below 750 litres of water. For larger enclosures, small swimming pools or hot tubs, rapid sand filters are available commercially. There are also small to very large-size units developed specifically for the aquaculture industry (see Plate 2).

With all mechanical filters, suspended particles are merely concentrated but not removed from the system. These filters must be cleaned regularly to remove the physical particulates or the organic components will decompose and chemically corrupt the water quality.

Filters that can remove dissolved substances from water are considered chemical filters. A common type of chemical filter that is currently in many household is the water purifier for drinking water. These filters contain cartridges with mechanical prefilters and activated carbon chemical filters. Larger

Plate 2. A simple rapid sand filter designed for whirlpool baths and small swimming pools is excellent for filtering larger volumes of water. These are readily available from many sources. *R. A. Odum, Toledo Zoological Society.*

versions of these activated carbon filters have been in use for many years to assist in maintaining water quality in aquariums.

Activated carbon has unique properties that make it an ideal material for amphibian enclosure chemical filtration. It is a highly adsorptive and porous material that readily removes dissolved organic compounds, micro-particulate and certain reactive non-ionic chemicals (e.g. free chlorine). It will even remove some ions, such as copper (Periasamy & Namasivayam, 1996; Seco *et al.*, 1999), although it is not as effective as other means. The numerous pores create an effective surface area exceeding 10 000 $m^2 kg^{-1}$ of carbon (Kinne, 1976). As water passes through this porous matrix of activated carbon, organic compounds loosely bond with the carbon and are effectively eliminated from the water. The porous matrix catches very small micro-particulate (e.g. some bacteria), thus acting as a fine mechanical filter, which can reduce its capacity as a chemical filter if flow is inhibited. Activated carbon filters should always have a mechanical prefilter to remove most of the particulate before the chemical and fine mechanical filtration by the carbon filter.

It is important to remember that chemical filter media have a finite capacity to absorb toxins and chemicals. The chemical media will ultimately become saturated with toxins and, if they are not changed regularly, they will begin releasing those toxins back into the water. Most chemical filters give no visible signs of when this occurs. 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. We recommend the use of chemical filtration in new systems or in systems with a known problem that the chemical medium will address.

The last type of filter is the biological filter. It is perhaps the most important and the most complex type of filtration in any system. 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 both as a community of organisms (Hovanec *et al*., 1998; Burrell *et al*., 2001) and as a separate life form that lives in symbiosis with the animals housed in the enclosure.

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. The primary function of a biological filter is to oxidize toxic ammonia/ammonium (NH_3/NH_4^+) into a less toxic form, ultimately producing the nitrate ion (NO_3^-). It should be noted that free ammonia (NH_3) is the most toxic form of ammonia/ammonium. The form of ammonia present depends on temperature and pH (see Table 2).

This process of bacterial oxidation of ammonia is called nitrifying. It is not the intention here to discuss the details of the biological processes that occur in biological filtration. Below is a basic equation of the overall nitrifying process (Lees, 1952):

$$\underset{\text{Ammonia/Ammonium}}{NH_3/NH_4^{+1}} \Longrightarrow \underset{\text{Nitrite}}{NO_2^{-1}} \Longrightarrow \underset{\text{Nitrate}}{NO_3^{-1}}$$

Circulation through a biofilter is normally accomplished using pumps and airlifts. Water must flow through the filter at a medium-slow rate in order for the bacteria to be able to adsorb the nitrogenous wastes (Hawkins & Anthony, 1981; Wickins & 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). If this occurs, the nitrifying bacteria will quickly die and be replaced by species that favour living in an oxygen-deficient environment. Many species of anaerobic bacteria produce toxic by-products, both inorganic (e.g. hydrogen sulphide) and organic (e.g. *Clostridium*), which could result in a build-up of toxins that could kill the animals that are maintained in the enclosure.

	PH								
	MORE ACIDIC								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	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

Table 2. Percentage un-ionized (i.e. more toxic) ammonia in aqueous ammonia solutions as a function of pH and temperature (Florida Department of Environmental Protection, 2001).

Common types of biofilters that have been used for many years include the under-gravel filter, the reverse-flow under-gravel filter, trickle filters and sponge filters. All these have demonstrated their effectiveness and information on their function and setup is readily available.

One of the newer advances in biofilters is the fluidized bed filter. These compact filters utilize the same basic technology as an under-gravel filter with several major improvements. A fluidized bed filter is usually in the form of a clear plastic column 2·5–12·5 cm in diameter and 0·3–1·0 m long, which usually hangs on the outside of the tank. A small pump, with a mechanical prefilter to remove particulates, injects water to the bottom of the column. The water flows upwards through the column and overflows back into the enclosure. The flow of water is just great enough to keep the sand suspended in the water column (fluidized) 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, the entire medium is aerobic and provides an excellent base for nitrifying bacteria. These filters are rapidly gaining in popularity and replacing the more conventional wet/dry trickle filters. One of the authors has used Quicksand® fluidized bed filters for years with excellent results. However, fluidized beds deteriorate rapidly when water ceases to flow through them (e.g. during a power failure).

Establishing a new biofilter is almost a nurturing process that can take several weeks to several months. The filter must be fed ammonia and monitored through the process. When fully established, a properly sized filter is capable of oxidizing the ammonia load to nitrates quickly, and there are no ammonia or nitrites detectable in the system. Feeding the filter can be accomplished by using natural (animals) or artificial (dilute ammonia or an ammonium salt) sources of ammonia.

Nitrifying bacteria will also invade a 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.

Maintaining a biofilter

Again, the biofilter should be thought of as a living entity in an 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 every day. Also, attention must be paid to 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.

Plate 3. A biofilter cycling tank. Different types of biofilters, including trickle, sponge and fluidized bed filters, are cycled in this tank using an artificial source of wastes (ammonia). These filters can then be transferred to other tanks containing animals, without cause for concern about transferring pathogens between groups. Note the instructions to the keeper for daily maintenance on the left side of the tank. *R. A. Odum, Toledo Zoological Society.*

It is a good idea to always keep a few extra biofilters going in tanks with heavy simulated bioloads (see Plate 3). The filters can be attached to a disinfected tank and liquid household ammonium (with no detergent or perfume), added daily at a rate of four to five drops per *c.* 38 litres of water. The water must be daily monitored for ammonia, and the amount of household ammonia added can be adjusted as appropriate. In this way, when a new tank is set up, an established biofilter is available without having to wait for a new one to cycle. This filter should be free of potential pathogens because it has not been in contact with a system that contained animals. This is vital for that unexpected batch of larvae. Also, if appropriate precautions are taken, these filters could be used in a biosecure situation.

Plants

Another often-overlooked form of filtration (bio and chemical) comes with the addition of living plants to the system. Plants help to 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. Plant-based filter systems are so effective that they can even be used for treating human sewerage (Levy, 2007). Furthermore, plants greatly enhance the attractiveness of an aquarium and provide oviposition sites for many amphibians. If the inhabitants of the tank are large or active and tend to tear up rooted plants, it is possible to culture plants in a separate tank adjacent to the animal tank, and use filters to pump water from one tank to the other. Just letting the tendrils of a potted plant, like Pothos *Epipremnum aureum*, dangle into a tank can significantly reduce nitrogenous wastes, especially nitrates.

Water testing

Water testing kits and devices are readily available from many sources. They vary from simple colorimetric systems (e.g. dip sticks, cells) to highly sophisticated and accurate spectrophotometers (i.e. like those manufactured by Hach). In most cases, the simple colorimetric systems are adequate for the amphibian keeper to monitor water quality and diagnose problems. When a system is initialized, the water should be tested frequently. Once established, it can be monitored less frequently. Ammonia/ammonium, nitrites, nitrates, pH, hardness and phosphates are tests that should be performed, at least initially. It is highly recommended that the primary water supply be monitored occasionally. Municipal water supplies frequently change their chemical composition depending on the situation of their supply or for maintenance (i.e. water-line repairs usually are followed by higher concentrations of chlorine to disinfect the lines).

Without water testing, the amphibian keeper cannot know the quality of the water they are providing for their animals, making them oblivious to this most important aspect of amphibian husbandry. Many of the significant and commonly encountered toxins in aquatic systems are in such low concentrations they cannot be seen nor do they have a smell. Testing is a better strategy than mere faith that the water is 'good'.

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.

Waste-water management

With the realization that diseases spread by human activity have caused declines, and in some cases outright extinction of amphibian populations, considerable attention should be given to how waste water from amphibian facilities is disposed of (see also Robertson *et al.*, in press).

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. It is often the case that the damage is done before an 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. If eggs or larvae die, water quality should be one of the first areas examined to find a possible cause. If mortality in adult amphibians is a problem, 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 and answers are only found by applying the principles of water management.

Checklist for a healthy aquatic system

- 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 overfeed the animals: uneaten food and excessive faeces 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.
- Monitor water quality (not discussed in this paper).

PRODUCTS MENTIONED IN THE TEXT

Hach: integrated water-analysis system spectrophotometer, manufactured by Hach Company, Loveland, CO 80539, USA. http://www.hach.com

Homegrown Six Pack: trace-element mix, manufactured by Homegrown Hydroponics, http://www.homegrown-hydroponics.com/

Prime: aquatic conditioner to remove chlorine, chloramine and ammonia, manufactured by Seachem Laboratories Inc., Madison, GA 30650, USA. http://www.seachem.com

Quicksand®: fluidized bed filter, manufactured by Bio-Con Labs Inc., Gainsville, FL, USA. http://www.bioconlabs.com

REFERENCES

ARTHUR, J. W. & EATON, J. G. (1971): Chloramine toxicity to the amhipod, *Gammarus pseudolimnaeus*, and the fathead minnow, *Pimephales promelas*. *Journal of the Fisheries Research Board of Canada* **28:** 1841–1845.

BALL, I. R. (1967): The relative susceptibilities of some species of fresh-water fish to poisons. I. Ammonia. *Water Research* **1:** 767–775.

BRUNGS, W. A. (1971): Chronic effects of low dissolved oxygen concentrations on fathead minnow (*Pimephales promelas*). *Journal of the Fisheries Research Board of Canada* **31:** 1119–1123.

BURRELL, P. C., PHALEN, C. M. & HOVANEC, T. A. (2001): Identification of bacteria responsible for ammonia oxidation in freshwater aquaria. *Applied and Environmental Microbiology* **December:** 5791–5800.

CARLSON, A. R. & SIEFERT, R. E. (1974): Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). *Journal of the Fisheries Research Board of Canada* **31:** 1393–1396.

CULLEY, D. D. (1992): Managing a bullfrog research colony. In *The care and use of amphibians, reptiles, and fish in research*: 30–40. Schaeffer, D. O., Kleinow, K. M. & Krulisch, L. (Eds). Bethesda, MD: Science Center for Animal Welfare.

CUMMINS, C. P. (1989): Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles. *Functional Ecology* **3:** 45–52.

DUELLMAN, W. E. & TRUEB, L. (1986): *Biology of amphibians*. Baltimore, MD: Johns Hopkins University Press.

ENVIRONMENTAL PROTECTION AGENCY (1976): *Quality criteria for water, July 1976*. Washington, DC: U.S. Environmental Protection Agency.

FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION (2001): *Calculation of un-ionized ammonia in fresh water: Storet Parameter Code 00619*. Tallahassee, FL: Florida Department of Environmental Protection. ftp://ftp.dep.state.fl.us/publabs/assessment/guidance/unnh3sop.doc

GULIDOV, M. V. (1969): Embryonic development of the pike (*Esox lucius* L.) when incubated under different oxygen conditions. *Problems of Ichthyology* **9:** 841–851.

HAWKINS, A. D. & ANTHONY, P. D. (1981): Aquarium design. In *Aquarium systems*: 1–46. Hawkins, A. D. (Ed.). New York, NY: Academic Press.

HERBERT, D. W. M. & SHURBEN, D. S. (1965): The susceptibility of salmonid fish to poisons under estuarine conditions. II. Ammonium chloride. *International Journal of Air and Water Pollution* **9:** 89–91.

HOVANEC, T. A., TAYLOR, L. T., BLAKIS, A. & DELONG, E. F. (1998): *Nitrospira*-like bacteria associated with nitrite oxidation in freshwater aquaria. *Applied and Environmental Microbiology* **64**(1): 258–264.

ISIS (2007): *ISIS species holdings*. Minneapolis, MN: International Species Information Systems. http://app.isis.org/abstracts/abs.asp

JOFRE, M. B. & KARASOV, W. H. (1999): Direct effect of ammonia on three species of North American anuran amphibians. *Environmental Toxicology and Chemistry* **18**(8): 1806–1812.

KINNE, O. (1976): Cultivation of marine organisms: water quality management and technology. In *Marine ecology* **3**(1): 19–300. Kinne, O. (Ed.). London: Wiley.

KLINGLER, K. (1957): Sodium nitrate, a slow acting fish poison. *Schweizerische Zeitschrift fuer Hydrologie* **19**(2): 565–578.

LEES, H. (1952): The biochemistry of the nitrifying organisms. 1. The ammonia-oxidizing system of *Nitrosomonas*. *Biochemical Journal* **52:** 134–139.

LEVY, S. (2007): From effluence to affluence. *Audubon Magazine* **March-April.** http://audubonmagazine.org/solutions/solutions0703.html

MARCO, A., QUICHANO, C. & BLAUSTEIN, A. R. (1999): Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. *Environmental Toxicology and Chemistry* **18:** 2836–2839.

ODUM, R. A., MCCLAIN, J. M. & SHELY, T. C. (1984): Hormonally induced breeding and rearing of white's treefrog, *Litoria caerula* (Anura: Pelodryadidae). In *Proceedings 7th reptile symposium on captive propagation husbandry, July 26–29, 1983, Dallas, Texas*: 42–52. Tolson, P. J. (Ed.). Thurmont, MD: Zoological Consortium.

Pavajeau, L., Zippel, K. C., Gibson, R. & Johnson, K. (In press): Amphibian Ark and the *2008 Year of the Frog* Campaign. *International Zoo Yearbook* **42.** DOI:10.1111/j.1748-1090.2007.00038.x.

Periasamy, K. & Namasivayam, C. (1996): Removal of copper(II) by adsorption onto peanut hull carbon from water and copper plating industry wastewater. *Chemosphere* **32**(4): 769–789.

Pritchard-Landé, S. P. & Guttman, S. L. (1973): The effects of copper sulfate on the growth and mortality rate of *Rana pipiens* tadpoles. *Herpetologica* **29**(1): 22–27.

Robertson, H., Eden, P., Gaikhorst, G., Matson, P., Slattery, T. & Vitali, S. (In press): An automatic waste-water disinfection system for an amphibian captive-breeding and research facility. *International Zoo Yearbook* **42**. DOI:10.1111/j.1748-1090.2008.00048.x.

Rouse, J. D., Bishop, C. A. & Struger, J. (1999): Nitrogen pollution: an assessment of it threats to amphibian survival. *Environmental Health Perspectives* **107:** 799–803.

Russo, R. C., Smith, C. E. & Thurston, R. V. (1974): Acute toxicity of nitrite to rainbow trout (*Salmo gairdnieri*). *Journal of the Fisheries Research Board of Canada* **31:** 1653–1655.

Schmuck, R., Geise, W. & Linsenmair, K. E. (1994): Life cycle strategies and physiological adjustment of reedfrog tadpoles (Amphibia, Anura, Hyperoliidae) in relation to environmental conditions. *Copeia* **4:** 996–1007.

Seco, A., Gabaldón, C., Marzal, P. & Aucejo, A. (1999): Effect of pH, cation concentration and sorbent concentration on cadmium and copper removal by a granular activated carbon. *Journal of Chemical Technology and Biotechnology* **74:** 911–918.

Siefert, R. E. & Spoor, W. A. (1974): Effects of reduced oxygen on embryos and larvae of white sucker, coho salmon, brook trout and walleye. In *The early life history of fish. The proceedings of an international symposium. Oban, Scotland, May 17–23, 1973*: 487–495. Blaxter, J. H. S. (Ed.). Berlin: Springer-Verlag.

Siefert, R. E., Carlson, A. R. & Herman, L. J. (1975): Effects of reduced oxygen concentrations on the early life stages of mountain whitefish, smallmouth bass, and white bass. *Progressive Fish-Culturist* **36:** 186–190.

Smith, J. M. D. (1982): *Introduction to fish physiology.* Neptune, NJ: TFH Publications.

Stickney, R. S. (1979): *Principles of warmwater aquaculture*. New York, NY: John Wiley and Sons.

Tabata, K. (1962): Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide. *Bulletin of the Tokai Regional Fisheries Research Laboratory* **34:** 67–74.

Warner, S. C., Dunson, W. A. & Travis, J. (1991): Interaction of pH, density, and priority effects on the survivorship and growth of two species of hylid tadpoles. *Oecologia* **88:** 331–339.

Westin, D. T. (1974): Nitrate and nitrite toxicity to salmonid fishes. *Progressive Fish-Culturist* **36:** 86–89.

Whitaker, B. R. (2001): Water quality. In *Amphibian medicine and captive husbandry*; 147–157. Wright, K. M. & Whitaker, B. R. (Eds). Malabar, FL: Krieger Publishing.

Wickins, J. F. & Helm, M. M. (1981): Sea water treatment. In *Aquarium systems*: 63–128. Hawkins, A. D. (Ed.). New York, NY: Academic Press.

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