Labile water quality parameters in amphibian holding systems

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The maintenance of good water quality is essential for superior health and growth of aquatic organisms such as amphibians (Nace et al., 1974; Fox, 1981) and fishes (Snieszko et al., 1974). While this is recognized, the dimensions of water quality are not well defined for branchiate amphibians for which water quality standards established for fish have been taken as the guideline.

Among the water quality parameters of special interest to those who culture or maintain aquatic amphibians are dissolved oxygen and nitrogenous metabolites. Other parameters such as heavy metals, chlorine, and pH are also critical, but since these can be brought under control before the water enters the holding system they are not our topic here. Dissolved oxygen and nitrogenous metabolites, however, change within the system and levels that are stressful or toxic to amphibians may develop. The purpose of this paper is to review literature relevant to these labile parameters of amphibian holding systems and to summarize our observations.

Oxygen

Dissolved oxygen below 3.0 mg/l can result in reduced growth and vigor of warmwater fishes and concentrations below 1.0 mg/l may cause mortality (Andrews et al., 1973; Colt et al., 1975; Thorston et al., 1979). Similar information for branchiate amphibians is scant (Nace et al., 1974), however tadpoles of leopard frogs, Rana pipiens, maintained in dechlorinated tap water (pH=6.6 to 7.4; alkalinity=41 to 100 mg/l) have exhibited no mortality when oxygen fell below 1.0 mg/l for several hours or below 3.0 mg/l for several days (oxygen measured with a YSI Model 54 polarigraphic meter). Sublethal effects of reduced oxygen were not examined, although most tadpoles were motionless and clustered at or near the surface when oxygen fell below approximately 1.5 mg/l. These observations indicate that these amphibians are more tolerant of reduced oxygen than fishes, but until critical levels are defined researchers and culturists should maintain oxygen above 3.0 mg/l. As an index of this, distilled water at sea level and 21 C contains 8.7 mg/l of oxygen at saturation.

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Nitrogenous Metabolites

Background: Colt and Armstrong (1981) have considered the chemical and physiological mechanisms involved in the toxicity of nitrogenous metabolites to fishes and shellfishes. A major portion of the initial accumulation is as ammonia in either the ionized (NH_4^+) or toxic molecular (NH_3) form, the latter increasing with pH and temperature (Trussell, 1972; Emerson et al., 1975). Subsequently nitrites (NO_2^-) and nitrates (NO_3^-) increase as ammonia is oxidized by *Nitrosomas* and *Nitrobacter* bacteria (4.2 mg O_2 are required to oxidize 1.0 mg of NH_3 to NO_3^-).

While the toxicity of ammonia for aquatic amphibians is poorly known (Nace et al., 1974; Fox, 1980), data on fish show that at ammonia-nitrogen (NH_3-N) concentrations of 0.2 to 0.4 mg/l mortalities occur and levels as low as 0.05 mg/l result in reduced growth, histological abnormalities, and physiological changes (Robinette, 1976; Burkhalter and Kaza, 1977; Colt and Armstrong, 1981; Wellborn and Schwedler, 1982a). Consequently, it has been recommended that NH_3-N levels in fish culture systems be maintained below 0.02 mg/l (Thurston et al., 1979). The ammonium ion (NH_4^+) is relatively non-toxic for fishes (Trussell, 1972, Emerson et al., 1975; Roseboom and Richey, 1977; Thurston et al., 1979), but in excess of 25 mg/l NH_4^+-N can inhibit growth in shellfish (Colt and Armstrong, 1981) and at 14 mg/l interfere with cation exchange across frog skin (Huf and Wills, 1951).

Nitrites are considerably less toxic than molecular ammonia (NH_3) to fish and several species tolerate values greater than 5 mg NO_2^--N/l (Colt et al., 1975; Konikoff, 1975; Thurston et al., 1979). However, levels of NO_2^--N as low as 1.0 mg/l for channel catfish (*Ictalurus punctatus*) and 0.004 mg/l for rainbow trout (*Salmo gairdneri*) increase levels of methemoglobin, which is reduced hemoglobin characterized by its brown color (Smith and Russo, 1975; Huey et al., 1980). Under experimental conditions of increased nitrites, levels of methemoglobin increase in tadpoles of *Rana catesbeiana* and larvae of *Ambystoma texanum* (Huey and Beitinger, 1980a; 1980b). We have observed that adult *Xenopus laevis* develop "brown blood" disease if water quality is poor; a response which may also occur in post metamorphic forms of other aquatic amphibians. Nitrates are essentially nontoxic with fishes tolerating levels up to several hundred mg NO_3^--N/l (Colt et al., 1975; Lewis and Buynak, 1976).

Experimental: To examine further these matters in amphibians we measured total ammonia-nitrogen (NH_{tot}-N) and molecular ammonia-nitrogen (NH_3-N), in cultures of *R. pipiens* and *X. laevis* tadpoles. Twenty recently hatched *R. pipiens* tadpoles were cultured in trays or jugs with 2.5 liters of water (pH=6.6 to 7.4; alkalinity=41 to 100 mg/l) which was replaced once every 24 h. Additionally, the jugs were provided with a continuous flow of water at 18 to 50 ml/min. Total ammonia-nitrogen was measured by Nesslerization and NH_3-N was computed from the pH and temperature conditions (Trussell, 1972; Emmerson et al., 1975).

Both NH_{tot}-N and NH_3-N were higher in the trays than in the jugs and within the 24 h accumulation interval levels of molecular ammonia stressful to fish were frequently observed in the trays, but only rarely in the jugs (Fig. 1 and 2). Note that the accumulation of metabolites increased as the tadpoles grew and dropped as the number of animals decreased and that metamorphorsis was earliest in the jugs. At the metabolite levels which developed, no effects on *R. pipiens* tadpole condition or behavior were observed. In a static system with several hundred *X. laevis* tadpoles at 5 to 7 per liter, however, higher levels of metabolites accumulated and the incidence of metamorphic failure was greater when NH_{tot}-N exceeded 20 mg/l and NH_3-N exceeded 0.75 mg/l than when NH_{tot}-N was below 10 mg/l and NH_3-N was below 0.1 mg/l. Nevertheless, in both cases these tadpoles showed a greater tolerance for nitrogenous metabolites than do fishes.

Appendix: Monitoring and maintenance of water quality

Although quite tolerant in comparison with fish, it is desirable that for amphibians commonly used in research and teaching labile water parameters be defined. However, until stressful levels are more closely defined, water quality for amphibians should probably be set at levels acceptable to fish as the data presented here indicate these levels are safe for *R. pipiens* and *X. laevis* tadpoles.

Dissolved oxygen can be monitored adequately with a portable polarigraphic meter (e.g. Yellow Springs Instrument Model 54 or 57). For quick quantification of nitrogenous metabolites several types of water analysis kits which are both economical and effective exist (Boyd, 1977; 1979; 1980), e.g. Bausch and Lomb SpectroKits™, Ecological test kits, CHEMetrics test kits, Hach Chemical Co. Water Ecology Kits model AL-36B and Hach Chemical Co. Direct Reading Engineer Laboratory Model DR-EL/2.

In general for the labile parameters, aeration through an airstone or addition of freshwater will maintain oxygen at acceptable levels (>3 mg/l). Control of nitrogenous metabolites, however, requires special treatment as volatilization does not suffice. Water can be changed on a continuous or daily basis if an ample supply is available. If water conservation is necessary, ammonia can be removed by an ion exchange resin which replaces the ammonia ion with another such as sodium (Bower and Turner, 1982), or by biofiltration which utilizes bacteria to convert molecular ammonia to less toxic nitrites and essentially nontoxic nitrates (Lewis and

Buynak, 1976; Lewis et al., 1981; Sutton and Lewis, 1982). Alternatively the percentage of ammonia in the molecular form can be reduced dramatically by lowering the pH to below 7.0 to 7.5 (Trussell, 1972; Emerson et al., 1975). Nitrite toxicity can be reduced markedly if the water contains 3 parts chlorides for each part nitrite (Wellborn and Schwedler, 1982b), a level which would be exceeded in many municipal tap water systems (our system normally holds chlorides at 30 to 40 mg/l, but water from Lake Michigan has less than 5 mg/l).

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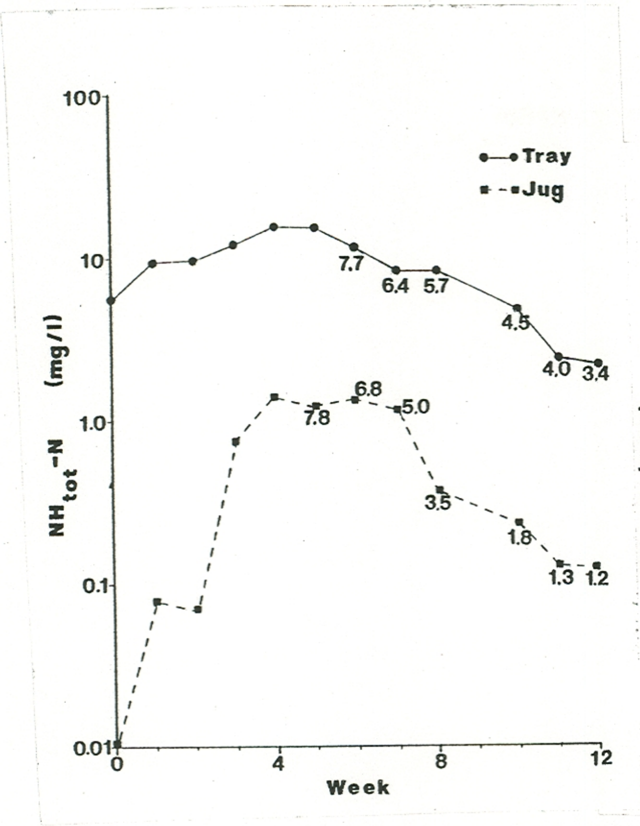

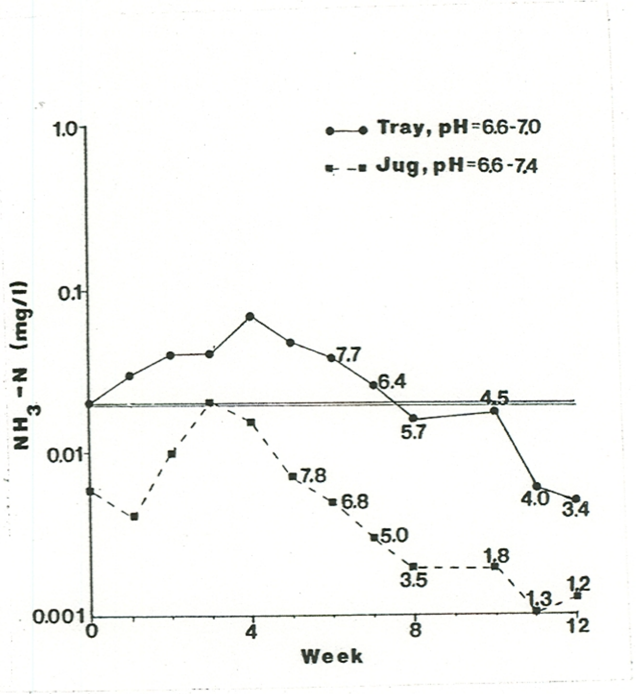

Figure 1 (Left). Weekly record of total ammonia-nitrogen (NH_{tot}-N) in jugs and trays stocked with recently hatched *Rana pipiens* tadpoles at 8 per liter. Water in all containers was changed daily and maintained at 20 to 23 C. Additionally, jugs were provided with a continuous flow of water at 18 to 50 ml/min. Measurements were made once per week 24 h after the entire water volume was replaced with fresh water. Samples from each of 20 jugs or 20 trays were pooled and each point is the average of triplicate measurements on the pool. Numbers adjacent to points indicate tadpoles per liter after removal of metamorphosed individuals.

Figure 2 (Right). Weekly record of molecular ammonia-nitrogen (NH_3-N). Conditions were as detailed in caption for figure 1. With one exception, values in jugs remained below the maximum level recommended for fish in culture systems (double line at 0.02 mg/l). This was not true for the trays.