SECTION 1: Husbandry

Xenopus Husbandry

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Adult frogs that are well-cared-for will give high-quality eggs and embryos for use in every *Xenopus* protocol. Thoughtful frog husbandry is thus pivotal to successful research using these organisms. Protocols for successfully raising tadpoles, establishing and maintaining water quality, and detecting specific pathogens are key to maintaining healthy frog populations.

A HISTORICAL VIEW

Although frogs have been popular laboratory animals from the beginnings of experimental science, the genus *Xenopus* has become the most-used laboratory amphibian for two reasons. First, ovulation can be induced throughout the year by hormone injection, and, second, the physical attributes of the embryos make them accessible to state-of-the-art technical approaches. Following extensive use in pregnancy tests, embryologists noted that they need not rely on production of embryos from seasonal frogs, and *Xenopus laevis* was popularized for experimental usage by Mikhail Fischberg and his student John Gurdon in Europe, and by Don Brown in the United States (Gurdon and Hopwood 2000).

In the natural state, *Xenopus* live in ponds, ditches, or rivers in Southern Africa. Wild-caught *Xenopus laevis* can yield outstanding numbers and quality of embryos. *X. laevis* can be shipped from South Africa or bred in Europe, Japan, or the United States and supplied to research groups. The frogs can be housed for many years of repeated ovulation and natural fertilization, although males are often harvested for testes and in vitro fertilization. In the case of *Xenopus tropicalis*, animals may be purchased or bred in individual research groups, especially in the case of transgenic animals.

Cursory assessment of the often muddy "natural" habitat of *Xenopus* suggested the animals were tolerant to a range of water conditions, but in the laboratory, optimal health and fecundity require careful attention to water chemistry, including optimal pH, ion levels, and temperature.

Historically, *X. laevis* were kept in bathtubs, artificial ponds or plastic tanks, with a "fill and dump" regimen of dechlorinated water and diets of offal and maggots. These frogs often picked up parasites from slaughterhouse offal, and between cleanings, offensive fouling of the water occurred. Furthermore, the water would spike in pH, causing free ammonia to damage the animals, so veterinarians came to require that the water be kept clean by constant flow of clean water. This brought its own problems, because the incoming water was often cold and saturated with atmospheric gas, leading to "gas bubble disease" (Green 2009). Animals directly from southern Africa were generally overtly

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healthy and vigorous, but brought with them endemic skin and liver parasites, and so importation of wild-sourced animals has declined.

IMPROVING *XENOPUS* HUSBANDRY

Although animals can be held in still tanks, with at least biweekly dump and refill, it is difficult to maintain constant and optimal water quality to ensure health. Opportunistic pathogens may thrive (Feazel et al. 2009) and still-water tanks fed by nonsterile dechlorinated water are therefore not a long-term mechanism for keeping *Xenopus* adults healthy.

The current best solution is to install recirculating systems, which provide optimal water and temperature parameters and can include dozens of tanks and hundreds of frogs (see Protocol 1: Animal Maintenance Systems: *Xenopus laevis* [Shaidani et al. 2020a]; Protocol 2: Animal Maintenance Systems: *Xenopus tropicalis* [Shaidani et al. 2020b]). In these systems, purified water is supplemented with salts, enters the tanks, and on drainage is filtered to remove solid waste. The water then passes through a biological filter, consisting of denitrifying bacteria on a solid support, where breakdown of nitrogenous waste prevents high levels of ammonia that can damage animals. The water is sterilized by UV illumination before reentering the system. In theory, little new water needs to be added to a recirculating system, but in practice a water change of ∼10% per day or 50% per week prevents buildup of potential toxins.

A diet of pellet food that includes all needed nutrients can keep frogs well-nourished and prevents fouling of the water that diets such as liver elicit.

Egg production is also affected by temperature fluctuation, and temperatures of >27°C can sterilize males or result in death. Seasonal changes can occur even in a recirculating system due to fluctuations in heating and air conditioning, so a chiller/heater should be installed to maintain the desired temperature.

Although some diseases remain problematic, particularly the chytrid fungus and the opportunistic pathogen *Mycobacteria liflandii*, the introduction of recirculating systems, with scheduled changes of UV sterilizing bulbs, has diminished outbreaks of disease. The incoming water is generally held in a reservoir, where it can degas, and because it only replaces a fraction of the water every day, gas bubble disease is not a problem, even with fairly vigorous recirculation (see Protocol 4: Defining the Specific Pathogen-Free State of *Xenopus* Using TaqMan Assays [Hensley et al. 2020]).

Recirculating systems have different tank sizes. Large tanks allow the frogs to swim, as in the wild, and should be considered optimal. Thus, tanks of several feet in length allow *X. laevis* to swim, and 10 inches of water depth allows them to hang with their nostrils and eyes above the water, as they prefer to do. However, keeping ovulated frogs in groups that can rest for a few months before reovulation can necessitate smaller tanks. For *X. tropicalis*, tanks are often smaller, commensurate with their size (two to three frogs per liter is optimal for adults). Finally, some investigators give their frogs places to hide or rest, providing a richer environment. Wide PVC pipe lengths allow this for both *X. laevis* and *X. tropicalis*, and for *X. tropicalis*, floating items such as plastic lids allow them to retreat from well-lit areas or sit partially submerged. Tanks should have a secure lid, although it is essential to maintain an air gap between the lid and the water surface to prevent the frogs from drowning.

FROM TADPOLES TO FROGS

In addition to maintenance of healthy adults, similar water parameters apply to raising tadpoles (see Sec. 2, Protocol 3: How to Grow *Xenopus laevis* Tadpole Stages to Adult [Ishibashi and Amaya 2021]; Protocol 3: Raising and Maintaining *Xenopus tropicalis* from Tadpole to Adult [Lane et al. 2021]). The tadpoles generally start life as embryos in Petri dishes where they develop into swimming tadpoles. Shortly thereafter, they can be seen pumping water through their gills, and this is when they start feeding on particulate food. The ideal time to transfer them to the aquarium is still a matter of research, but usually they start feeding in Petri dishes, before being gradually

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introduced to the aquarium water, and then to a gentle flow in the frog system (Ishibashi and Amaya 2021; Lane et al. 2021).

INSTITUTIONAL ANIMAL CARE COMMITTEES AND *XENOPUS* HUSBANDRY

Animal welfare is regulated by the local institutional animal care and use committee (IACUC), but ultimately overseen by federal agencies in the United States. The IACUC has jurisdiction over general issues such as when the animals should be transferred out of the laboratory, to rooms directly supervised by institutional staff or to a recirculating system. Most frequently tadpoles are under the care of the principal investigator's staff. The IACUC has considerable latitude to decide when the tadpoles transition from "prevertebrates," which under law are not considered to be "vertebrate animals," for the purposes of pain management or euthanasia according to the American Veterinary Association Guidelines for the Euthanasia of Animals (National Research Council 2020). Some IACUCs consider the hatching stage or feeding to be the transition to vertebrate animalhood, whereas other IACUCs consider development of a vertebral column at metamorphosis to define a vertebrate. Regardless of definition, it is good and humane practice to ensure that tadpoles are anesthetized before procedures such as dissection or fixation once they develop reflex responses (tailbud stage). It is easy to achieve this by immersion in solutions of tricaine or benzocaine buffered with sodium bicarbonate, which increase adsorption of the drug. Although both these have been shown to be efficacious, an additional criterion has been introduced in the Guide to Lab Animal Care (National Research Council 2011) that administration of anesthetics should use "pharmaceutical-grade" products. This has led to some confusion, because these may not be available other than as ointments or gels, which are impractical to deliver in the water. However, MS-222 from Western Chemical is considered pharmaceutical-grade by at least some veterinarians. Moreover, local IACUCs are empowered to approve conventions based on what is allowed at National Institutes of Health (NIH) laboratories or where U.S. Food and Drug Administration (FDA)-approved fish anesthetics from specific vendors are approved. Investigators should consult their local veterinarians for assistance.

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