

# Chapter 2

## Husbandry of *Xenopus tropicalis*

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### Abstract

*Xenopus tropicalis* combine the advantages of *X. laevis*, for example using explants and targeted gain of function, with the ability to take classical genetics approaches to answering cell and developmental biology questions making it arguably the most versatile of the model organisms. Against this background, husbandry of *X. tropicalis* is less well developed than for its larger, more robust relative. Here we describe the methods used to keep and breed these frogs successfully.

**Key words:** *Xenopus*, *tropicalis*, Husbandry, In vitro fertilization, *Xenopus* health, Re-circulating aquaria

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### 1. Introduction

*Xenopus* (*Silurana*) *tropicalis*, the Western clawed frog, is a small relative of the established laboratory model *X. laevis*. *X. tropicalis* is the only known diploid in the genus *Xenopus*, and at  $1.5 \times 10^9$  bp, has one of the smallest genomes of any tetrapod. These species are sufficiently similar that viable hybrids can be produced, albeit only using *tropicalis* sperm on *laevis* eggs and not vice versa (1). Early studies of *X. tropicalis* mainly concerned phylogenetic analyses and suggested that *X. tropicalis* and the morphologically similar, tetraploid *X. epitropicalis* form an outgroup (sometimes denoted *Silurana*) with respect to other *Xenopus* species (2). It was not until the late 1990s that *X. tropicalis*' potential for adding genomics and genetics to *Xenopus*' traditional strengths in developmental and cell biology began to be exploited. Its small, sequenced genome (3), together with a genetic map (4), abundant embryo production, and relatively short generation time, are making genetic approaches increasingly practical (5–7). A number of mutations are now cloned (8–10). The simplicity of its genome has also encouraged genome-scale epigenetic

studies (11, 12) and morpholino oligonucleotide knockdown screens (13). The shorter generation time makes possible multi-generation transgenic strategies such as Gal4/UAS (14, 15), Cre/lox (16), and insertional mutagenesis (17).

The enormous toolkit of protocols developed in *X. laevis* for embryological, biochemical, and gain-of-function studies are also readily transferred to the smaller *X. tropicalis*, although not necessarily without modifications. The ability to combine the established strengths of *Xenopus* with *X. tropicalis*' robust genomics and emerging genetics makes it arguably the most versatile of all vertebrate model organisms.

Despite this versatility, *X. tropicalis* has not replaced laboratory use of the extraordinarily tough *X. laevis*, which has evolved to cope with a wide range of habitat extremes and whose rate of embryonic development can be conveniently regulated by temperature. Nevertheless, if maintained under appropriate conditions, *X. tropicalis* are also very robust frogs that can produce abundant embryos for more than a decade. Several things are likely to have contributed to problems in establishing *X. tropicalis* in some laboratories: (1) Cross-species contamination from *X. laevis* (microorganisms that are tolerated by one species are frequently pathogenic in another) or from wild-caught frogs. (2) Temperature stress, which can have effects on egg quality and disease susceptibility for months after an event such as heat- or cold-shock. (3) Diet during early life, as stocks that were undernourished or overcrowded, may never recover good fertility. Optimal husbandry regimes leading to good egg quality, rapid maturation, and low susceptibility to disease are particularly critical for the multi-generation genetic and transgenic strategies, which are less feasible in *X. laevis*. This chapter provides husbandry and breeding protocols which have been successful for large colonies over the course of >8 generations of *X. tropicalis*. Continuing to improve and share husbandry techniques for *X. tropicalis* should enable many more researchers to take advantage of the genomic resources and genetic applications available for this model.

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## 2. Materials

### 2.1. *Xenopus tropicalis* Animals

*X. tropicalis* occur naturally in many countries of equatorial West Africa, but most laboratory stocks originate from Nigeria and Ivory Coast. Wild-caught animals are occasionally available, but extreme care must be taken to prevent disease introduction and taxonomic misidentification, as morphologically identical tetraploid species are known to occur in overlapping ranges. Most laboratories maintain outbred frogs; however, a few inbred strains of *X. tropicalis*

**Table 1**  
**Suppliers of *Xenopus tropicalis* adult frogs**

Supplier	Webaddress	Distribution area
NASCO	<a href="http://www.enasco.com/c/xenopus/Xenopus+tropicalis">http://www.enasco.com/c/xenopus/Xenopus+tropicalis</a>	Worldwide
<i>Xenopus</i> express	<a href="http://www.xenopus.com/products.htm">http://www.xenopus.com/products.htm</a>	Worldwide
US <i>Xenopus</i> resource center	<a href="http://www.mbl.edu/xenopus/index.html">http://www.mbl.edu/xenopus/index.html</a>	North America etc.
European <i>Xenopus</i> resource centre	<a href="http://www.port.ac.uk/research/exrc/">http://www.port.ac.uk/research/exrc/</a>	Europe etc.

Current information about resource center stocks is provided by Xenbase (<http://www.xenbase.org/other/obtain.do>), but *X. tropicalis* are almost always readily available

exist (see Notes 1–3). *X. tropicalis* from different locations are kept under the same conditions and no significant differences in robustness or embryo quality have been reported (for some established suppliers, see Table 1).

## 2.2. Re-circulating Systems

There are two major suppliers that we have used successfully, Tecniplast and Aquatic Habitats. Systems from both suppliers are available either as large numbers of tanks linked to single water treatment units or as smaller stand alone systems. Which is best for a specific colony depends on the balance needed between the time taken to clean filters, a process which is much more efficient with a single treatment unit, and the risk of disease spreading between tanks.

The tanks in these systems can be chosen from a range of sizes all of which have the critical, secure lids and are of a plastic designed to be transparent to allow observation by care staff whilst minimizing the disturbance of frogs by movement around the tanks.

The re-circulated water is treated by a coarse filter, biofiltration, fine filter, activated charcoal, and ultraviolet light prior to being returned to the tanks. Around 10 % of water is changed automatically each day whilst conductivity, temperature, and pH are monitored and controlled automatically. Care staff can be called automatically on their mobile telephones if system parameters deviate from their norms.

## 2.3. Fill and Dump Systems

Most of these systems are home made and may be as simple as plastic baths with mesh lids or can be custom built. In the latter case, people have incorporated sloping floors to the tanks and special valves to aid drainage. Whatever the details of the tanks, it is vital that the lids are secure to prevent escape and that the water temperature is carefully regulated, particularly that the input water is free of chlorine and chloramine and that it is at the temperature of the dumped water.

**Table 2**  
**Marc's Modified Ringer's (MMR) solution**

Chemical	1× Concentrations	20× Concentrations	For 20× stock solutions
KCl	2 mM	40 mM	40 mL of 1 M solution
MgSO <sub>4</sub>	1 mM	20 mM	40 mL of 0.5 M solution
CaCl <sub>2</sub>	2 mM	40 mM	40 mL of 1 M solution
NaCl	0.1 M	2 M	116.8 g
HEPES	5 mM	100 mM	23.832 g

Dissolve in 800 mL, adjust pH to 7.4 and take the volume to 1 L. Autoclave and store at room temperature

- 2.4. Food

1. Horizon XP 23 pellets (Skretting, Wincham, UK).

2. Reptomin (Tetra, Southampton, UK).

3. Sera Micron (Sera GmbH, Heisenberg, Germany).

4. Tropical flake (Aquarian, Marts Fishcare Europe, West Drayton, UK).
- 2.5. Sea Salt

Tropic Marine. Available from almost all Marine Aquarium specialist pet shops, we use Tropical Marine Centre (<http://www.tmc-ltd.co.uk>).
- 2.6. Bicarbonate

Sodium bicarbonate (Mistral Chemicals, Antrin, Northern Ireland).
- 2.7. Marc's Modified Ringer's (MMR) (18)

0.1 M NaCl, 2 mM KCl, 1 mM MgSO<sub>4</sub>, 2 mM CaCl<sub>2</sub>, 5 mM HEPES (pH 7.8), 0.1 mM EDTA—most current formulations of MMR omit EDTA and are adjusted to pH 7.4 (see Table 2)

1× MMR—50 mL 20× MMR in 1,000 mL, pH 7.2–7.4, add 1 mL gentamycin (50 mg/mL). Gentamycin is added to a final concentration of 50 micrograms/mL to retard microbial growth. We keep a gentamycin 1,000× stock at 4 °C.

0.4× MMR—20 mL 20× MMR in 1,000 mL, pH 7.2–7.4, add 1 mL gentamycin (50 mg/mL).

0.05× MMR—2.5 mL 20× MMR in 1,000 mL, pH 7.2–7.4, add 1 mL gentamycin (50 mg/mL).
- 2.8. Human Chorionic Gonadotrophin

Chorulon (Intervet/Schering-Plough Animal Health, Milton Keynes, UK).
- 2.9. Software

Filemaker Pro (Filemaker, Santa Clara, California, USA).

### 3. Methods

#### 3.1. Transporting and Delivering *X. tropicalis*

Moving *X. tropicalis* between labs is straightforward providing careful planning takes place. The necessary health checks and paperwork preparation must be arranged in advance, packaging obtained, and travel arrangements made with an experienced animal shipper. For short journeys (less than 10 h), we normally keep the frogs in water from their tanks in 25 L buckets ~1/3 full. These buckets have firmly clipped-on lids with 7 mm holes drilled both in the lid and in the sides of the bucket just below the lid to allow the frogs to breathe when the buckets are stacked. For longer journeys or those that include air travel, we pack the adults in wet moss in plastic containers (27 cm × 14 cm × 17 cm, max 10 frogs) with small breathing holes. It is critical not to recycle packaging between different species of *Xenopus*, as many microorganisms are benign in one species but infectious and pathogenic in the other. These plastic containers are themselves packed in a polystyrene box and cardboard box, both having breathing holes. To maintain the temperature, pre-warmed (or cooled) gel packs are added inside the polystyrene box. Packed like this, *X. tropicalis* can arrive in good condition even after journeys lasting 3 days in weather conditions that are not extreme. We check the predicted weather for the journey and occasionally postpone sending frogs if extremes of heat or cold are predicted at either transit or destination airports. Your shipper may have specific requirements for labeling of the boxes and this too should be checked well in advance. We have found that it is always advisable to arrange the shipment for the beginning of the week so that staff are on site to receive the frogs even if delays occur.

When frogs arrive it is important to know what feeding regime they had been kept under until that point. The supplier will normally be happy to provide a sample of the food that they are using and keeping to the same frequency of feeding as the frogs had previously experienced seems to prevent the occasional loss of embryo quality that can occur when frogs are moved. Changing to a new feeding regime should be done over a period of several weeks. Sperm and embryos may also be sent to avoid these problems (see Notes 4 and 5).

Since *X. tropicalis* may harbor asymptomatic infections which bloom after shipping stress, it is sensible to quarantine newly arrived stocks for a period of 3 months. A common approach is to segregate new arrivals from the main colony in either a separate room or at least rack of tanks with entirely separate nets, buckets, etc. to avoid transfer of contaminated water. People operate in this area only at the end of the working day so that there is no chance of pathogens being re-introduced to the main colony. Whilst in quarantine, the frogs need to be observed frequently for loss of weight, appetite, or skin condition. As more is discovered about the diseases

to which *X. tropicalis* are prone and tests for them are developed, it may in time be possible to test the animals for specific diseases during this period. We currently do this routinely for *Batrachochytrium dendrobatidis* (below), but have not found it in *X. tropicalis*, only *X. laevis*. Frogs that show any sign of disease should be culled humanely or, if they are very valuable, then embryos should be produced by in vitro fertilization and the resulting adults added to the main colony, at least minimizing the chances of horizontal transmission of disease.

### **3.2. Keeping Adult Frogs in Re-circulating Systems**

The systems we have are made either by Tecniplast or Marine Biotech. The parameters that we use for systems containing *X. tropicalis* are listed below:

- Temperature: 25.5 °C (range 24–26 °C). It is important that the temperature for adult frogs does not exceed 26 °C, since we and others have found that this causes a long-term decrease in egg/embryo quality without affecting the health of the adult frog. Temperatures of 21–24 °C are tolerated but are likely to result in depressed immune function. Larval growth may be more rapid at temperatures up to 30 °C. Sharp temperature fluctuations, e.g., when replenishing dump-and-fill systems with water more than 1 °C different from the tank content, can be extremely stressful to both adults and tadpoles and must be avoided.
- pH: 7.9 (range 7.5–9.0).
- Conductivity: 500–1,200 µS (conductivity as high as 1,500 µS is used by some laboratories), controlled by addition of aquarium sea salt.
- Water: Mains water (naturally hard in Portsmouth) or micro-filtered water.
- Tank volume: 2, 5, 10, 27, 40, or 400 L.
- Stocking Density: ~1–2 adults per liter (in the EU directive animals of snout to vent length <6 cm must have a minimum water depth of 6 cm with the first animal having >160 cm<sup>2</sup> of surface area and an additional >40 cm<sup>2</sup> for each other animal in the tank; for frogs of 6–9 cm in length the depth must be at least 8 cm, the first animal must have >300 cm<sup>2</sup> of surface area and each additional animal >75 cm<sup>2</sup>).
- Flow rate: 1.33 L per minute.
- Lighting: fluorescent lights on a 13 h light/11 h dark cycle with night-light.
- 10 % of the water is exchanged every 24 h.
- Environmental enrichment: Dark-colored plastic guttering and downpipe tubes cut into approximately 20 cm lengths provide shelter that some frogs take advantage of. Artificial weed made from plastic gardening bags cut up and secured around a pebble

with a cable-tie also provide shelter and somewhere to sit partially out of the water, which again is behavior exhibited by some animals. The “weeds” are disposable and the pipes can readily be sterilized by autoclaving. Some plastics may leach endocrine disruptors, which are likely to affect gender ratios.

- Frogs in a re-circulating system are best fed small amounts of food frequently to minimize the uneaten food that the filters have to clear.

### **3.3. Keeping Frogs in Fill and Dump Systems**

- Water temperature (as above) can be maintained by placing tanks in a heated room, by placing thermostatically controlled heat mats under each tank or by placing combined heater thermostats directly in the water. Place mesh around immersed heaters to prevent frogs from touching it directly and burning themselves.
- A central reservoir of water is needed to provide a continuous supply of water at the right temperature, pH, and conductivity from which chlorine and chloramine have been removed, either by standing or by using commercially available products.
- The frequency of water changes needs to be a compromise between minimizing the disturbance to the frogs and maximizing water quality. We test water quality weekly (nitrogen products and pH) using commercial aquarium kits in order to optimize the timing of water changes. The time between changes varies significantly depending on stocking density and feeding frequency.
- Frogs kept in fill and dump systems are best fed to satiety some little time prior to a water change.

### **3.4. Feeding**

The regime below applies to animals in re-circulating systems. In fill and dump aquaria, the frogs are fed to satiety using the same food shortly before a water change. Frogs tend to ignore food that has been in the water for more than a few minutes.

- Adults: Horizon XP 23 pellets-fed 2–3 times per day, two pellets for each frog per feeding (5 days each week) or ~3 Reptomin sticks/frog (3 days each week), supplemented with whole fish flake 1×/week.
- Froglets: Horizon XP 23 pellets-fed 2–3 times each day, two pellets each and fish food (tropical flake; 7 days each week).
- All frogs may be given occasional supplements of lamb’s heart or similar, cut to the appropriate size. A varied diet is always chosen for frogs with weight loss, or those that are observed to fail to compete for food. It has recently been reported that *X. tropicalis* tadpoles can fix on the food that they are fed after metamorphosis and so become difficult to feed thereafter. This suggests that a widely available food source should be chosen by frog users and suppliers to standardize on.



### 3.5. Health

One of the main health issues for *X. tropicalis* users is having frogs of very high quality so that the embryos they produce are of an excellent standard. This requires close monitoring of the recovery of female frogs between egg production cycles. Females can be ovulated up to six times each year, with a minimum of 6 weeks between ovulations depending on local regulations; males can be used more frequently. We ask ourselves the questions below when deciding if a frog is suitable for re-use:

- General appearance: with your experience do you consider it likely to lay well?
- Skin texture: is it smooth and free from abrasions and redness?
- Body shape: is this consistent with this frog's history (known to be a slim or plump frog?).
- Body weight: if it is possible to record body weight then this is a useful additional indicator of recovery; has this increased since the last use, has it returned to this animal's normal range?

### 3.6. *X. tropicalis* Diseases in Laboratory Colonies

Most health issues with lab-bred *tropicalis* stem from suboptimal husbandry regimes, with stress resulting in decreased resistance to normally benign microorganisms. In low-metabolism animals such as frogs, symptoms may appear months after a stress or infection has occurred, and secondary opportunistic infections are often present in dead or morbid specimens, further complicating diagnosis. It is extremely important to keep *tropicalis* and *laevis* colonies completely isolated, as many microorganisms that are benign in one species may be acutely pathogenic in the other.

Several species of Mycobacteria (which may be zoonotic and capable of producing skin lesions in human) have been identified in *tropicalis* colonies, often in healthy animals/water but in some instances resulting in catastrophic mortality, as no treatments are available. In one report, symptoms included a loss of diving reflex, bloating, and ulcerative skin lesions. The causative agent was initially characterized by Trott et al. (19) and named *Mycobacterium liflandii* (20); it has also been found in mainland Europe (21) in frogs imported from the US. Like many diseases of *Xenopus*, it is found in asymptomatic animals when they are housed in good conditions. A number of other mycobacterial species have been reported to infect *X. tropicalis* (e.g., (22)).

Much less dangerous to *X. tropicalis* is the chytrid fungus *B. dendrobatidis*. While this pathogen is fatal to many amphibian species, *X. tropicalis* can survive repeated high dose infections (23), although there are also cases where it has been pathogenic in colonies kept at high density (24). We have tested the prevalence of *B. dendrobatidis* in *X. tropicalis* colonies from many European labs and cannot detect it using qPCR, although parallel testing of *X. laevis* colonies found *B. dendrobatidis* in all but one lab



(Coxhead and Guille, unpublished). These data suggest that this fungus is not a significant risk to *X. tropicalis*, but it remains to be determined whether *tropicalis* can act as a reservoir for the pathogen. Since *B. dendrobatidis* is thought to be one of the primary causative agents in the worldwide decline of many wild amphibian populations, transportation and sale of potential carrier species such as *Xenopus* and *Rana* are expected to come under increasing regulatory pressure.

### **3.7. Producing Embryos**

Embryos can be produced either by natural mating or by in vitro fertilization; the latter is used when large numbers of 1 or 2-cell embryos are required synchronously, for example for transgenesis. Since injection of frogs and squeezing are procedures that require licenses in the UK and in many other countries, it is essential that prospective frog users check their local regulatory requirements. Our protocol is shown below:

- Females are primed by an injection of human chorionic gonadotrophin (HCG) (10 IU, 0.1 mL) into the dorsal lymph sac using a 30 g needle, 1–3 days before the eggs are required.
- A boosting dose of 100 IU of HCG (0.1 mL) is given into the dorsal lymph sac using a 30 g needle as early as possible on the day the eggs are required.
- Egg laying starts normally 3–5 h after the boosting dose and may last for several hours.
- Egg collection by squeezing (see below) should not be repeated until at least 45 min has passed.
- To provide testes for in vitro fertilisation males are sacrificed by terminal anesthesia using MS222.
- Testes are removed, blood vessels dissected away, and the testes stored in L15 supplemented with 10 % calf serum at 14 °C.
- Testes should be used as quickly as possible since there is a dramatic loss in fertility if the testes are stored for any length of time.
- Mash 1 testis in L15/10 % calf serum (0.2 mL) and add to squeezed eggs in a Petri dish using a pipette.
- Shake dish/or stir with pipette to ensure good mixing.
- After 5 min flood with 0.05× MMR.
- All solutions we use are pre-warmed to 25 °C.
- Some labs use solutions at 4 °C.

### **3.8. Squeezing Eggs from the Female Frog**

This procedure should only be performed by trained individuals, since these small frogs are relatively fragile and should be treated gently. Squeezing seems to result in higher mortality than natural mating. If possible, only squeeze the amount of eggs required for the experiment, rather than emptying the frog.

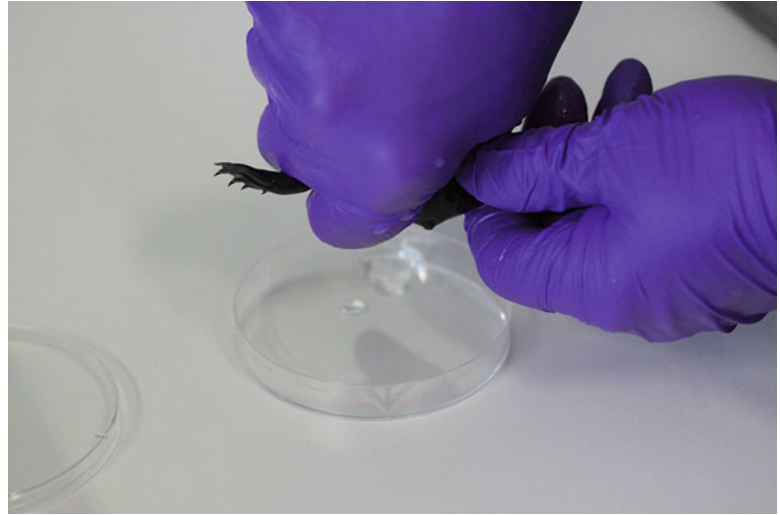


Fig. 1. The hold used to squeeze eggs from a female frog. The cloaca is placed above a Petri dish and the *left* back leg held forward gently using the *right* forefinger. Gentle pressure is applied to the abdomen with the *right* thumb and may be augmented with further gentle pressure with the *left* thumb and forefinger.

While the female frog is still under water, grasp in one hand, the head facing the operator, the index finger between the hind legs, but more to the left of the frog, (therefore, the right of right-handed operator) so that the cloaca is exposed.

After this initial manipulation, the frog is positioned so it is facing upwards, so that the cloaca is the lowest point of the frog's body. With gentle pressure on the abdomen, eggs will be extruded from the cloaca into a receptacle, usually a Petri dish. It is important that the dish remains dry or contains high salt buffer to ensure that eggs are not prematurely activated. The production of eggs can be increased by gentle pressure from the index finger and thumb of the opposite hand (see Fig. 1).

### 3.9. Natural Mating

Male *X. tropicalis* are stimulated to mate by priming and boosting injections identical to those used to induce ovulation in females, the same doses at the same times (see above).

- Males and females are kept apart until after the boosting doses.
- A pair is isolated in the main tank system (in a tank where the water flow is reduced or stopped altogether), or placed in individual glass or plastic tanks.
- After sufficient eggs are laid, the frogs are removed to a fresh tank and the eggs either left in the tank to grow on or removed to Petri dishes using nets for experimental work.

### 3.10. Raising Tadpoles

The care of young tadpoles is, in our hands, the most difficult period of the *X. tropicalis* life cycle and tadpole survival can easily be compromised if conditions are not ideal.

Eggs are initially squeezed into 90 mm plastic Petri dishes and fertilized as described above. Gastrulation of dejellied embryos is easily affected by overcrowding/sticking. Plastic dishes should be coated by rinsing with 0.1 % BSA to reduce sticking of dejellied embryos. Fertilized embryos should be kept at no more than 100/dish and spread to minimize touching each other. From hatching to a few days old, the tadpoles are kept in Petri dishes in an incubator in  $0.05 \times \text{MMR}$  containing Gentamycin ( $100 \mu\text{g/mL}$ ) at  $25^\circ\text{C}$ . As the embryos become larger, they are moved to glass Petri dishes of 200 mm diameter. We try not to overcrowd them and spread embryos from the same squeeze between a few Petri dishes (*c.* 100 embryos per dish). Fresh media at the same temperature should be exchanged daily and any dead tadpoles removed, or live tadpoles transferred to a fresh dish.

Feeding tadpoles (5 days post-fertilization) are kept in standing water ( $0.75 \text{ g/L}$  sea salt (e.g., Tropic Marine for keeping marine fish) in distilled water) in 5 L tanks. It is important to remove tadpoles from antibiotic-containing media when they start feeding.  $\sim 50\%$  water is changed daily, taking care to equilibrate the temperature of the fresh water to that of the tank beforehand, to avoid sudden temperature shocks. For very young tadpoles, it is easier to start with a low water level and top up daily. After 2 weeks, an air bubbler is used to oxygenate the standing water gently.

Tadpoles are fed on Sera Micron (Sera; Heisenberg, Germany) powder, which is strongly recommended for standing water systems since it does not foul water quickly and can “bloom” in the presence of bright light, producing a healthy self-sustaining green plankton on which tadpoles can feed. During the first 2 weeks, very small amounts of Sera Micron are added several times a day, enough to maintain faintly green water. The amount of Sera Micron is increased for older tadpoles so the water maintains a deep green color. Frequent feeding (up to ten times each day) ensures that food is always available in the water column. Metamorphosing tadpoles and froglets are moved to flow-through systems and fed whole fish flake and crushed Reptomin sticks (pieces that are big enough for froglets to grasp with their forelimbs but small enough to swallow) daily. Metamorphs benefit from floating platforms in the tank on which they can rest out of the water. As the metamorphs become larger, they will eat the normal trout pellets/Reptomin.

### 3.11. Sexing *X. tropicalis*

*X. tropicalis* are more difficult to sex than *X. laevis* and even trained staff can make errors especially with immature frogs. There are subtle differences in the frogs, as follows:

- (a) Mature male *X. tropicalis* usually have rough nuptial pads on the inner forelimb to aid the amplexus grip. This may appear as a darker (sometimes lighter) stripe, usually more distinct after HCG priming. The rough texture of the nuptial pad is the most reliable male feature.



Fig. 2. The smaller adult male (on the *right*) and female *Xenopus tropicalis*.

- (b) Males tend to appear slimmer and smaller than females, which have a characteristic triangular shape if healthy and well-fed.
- (c) The cloaca is more pronounced in females than in males, especially after hormone treatment.
- (d) Males (Fig. 2, right) tend to be considerably more skittish, jumpy, and difficult to catch.

### 3.12. Identifying Animals and Record Keeping

The use of *X. tropicalis* for multi-generation genetic or transgenic studies requires careful recording of the genetic history of each animal or group of siblings; in addition, good husbandry and legal requirements in some countries need similar records. The method below has been used to keep records for a complex multi-generation colony.

The offspring of a specific mating or pair of parents are assigned a unique stock number as well as a genotype describing the stock (which may not be unique). Information about each stock is kept in a centralized database (e.g., Filemaker Pro) and includes the genotype of the stock (including the generation, e.g., F2, F3), date of birth/fertilization (DOB), maternal and paternal stock number and genotype, any local regulatory information including procedure number in the UK, and the number of animals. Some of this information (stock number, genotype, DOB, and license information) may be printed on adhesive waterproof tank labels to identify the tanks in which the frogs are housed. Labels have space for additional information, for example, the mating date of the frogs, so that the animals can be rested for an appropriate period prior to re-use.

Occasionally, frogs from a particular stock need to be identified and isolated as individuals, e.g., when a clutch of offspring contains both non-carriers and carriers of a particular mutation. This information can be added to the stock number (e.g., T300-C.1 = mutation-carrying individual 1 of the T300 stock, ~half of whom are non-carriers). Sometimes stocks with the same genotype, e.g., F2 and F3 transgenics from the same founder, can be combined to save space in the frog room, but that information is added to the database so the genetic history of stocks is not lost. Transgenic offspring carrying the same construct, but from different founders, should not be mixed.

One of the challenges of working with *X. tropicalis* is the similarity in patterning between individuals limiting the scope for identifying frogs visually using a photographic database as for *X. laevis*. We have collaborated extensively with a machine vision company to test whether the hue and patterns on *X. tropicalis* could be identified using state-of-the-art technology but it proved impossible (P. Coxhead and M.J. Guille, unpublished data). As a result, we are investigating microchipping the animals. At least two labs (Harland and Mead) are using this technique routinely in *X. tropicalis* and have protocols on their lab websites (see *also* Chapter 6). Frogs can also be kept in smaller (2 L or 5 L) tanks as individuals or in pairs, but this may have negative effects on the welfare of the frogs, which seem to prefer living in larger groups.

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## 4. Notes

1. The *N* strain derives from wild-caught frogs obtained by Marc Kirschner from Nigeria and subsequently inbred to the F12 generation in the Grainger lab and elsewhere (see Chapter 1). DNA from an F7 *N* frog was used to produce the draft genomic assembly (3), making it the strain of choice when targeting specific sequences, e.g., with morpholino oligonucleotides, since *N* frogs are likely to have fewer variations from the published genomic sequence. The *IC* line originates from frogs collected near Adiopodoume, Ivory Coast, and inbred in the Grainger and Zimmerman laboratories to F12.
2. *IC* frogs and embryos are usually less pigmented than *N*. Other stocks originating from Nigeria and Ivory Coast are not necessarily related to the *N* and *IC* strains. Other inbred lines include *TGB* and *Golden*.
3. Crosses between different strains are useful to generate map-cross frogs rich in interstrain polymorphisms, which facilitate genomic and meiotic mapping studies.

4. The complexity, cost, and stress to adult frogs involved in sending them long distances have resulted in the recent adoption by a number of labs of the practice of sending frozen sperm (25), testes, or even fertilized eggs of *X. tropicalis* lines from one lab to another.
5. Frozen sperm are sent on dry ice whilst fresh testes can be sent in 1× MBS in 50 mL screw capped tubes on frozen gel packs in polystyrene boxes; a similar approach but with 25 °C gel packs can be taken for fertilized eggs.

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