# **Reproduction conditioning**

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### General

The health and condition of anurans influences their reproductive potential. Amphibians should be in good reproductive condition before either natural or hormonally induced reproduction is attempted. The techniques used for reproductive conditioning of any species will depend on the environmental and physiological cues that determine reproduction. The main factors that affect reproductive condition in amphibians are nutrition, temperature, humidity and light cycles.

Reproductive conditioning of amphibians will also depend on the species reproductive strategies. For example in some species that live in environments with high seasonal variations in temperature, or variations in the in the availability of water, maturity can be seasonally entrained with one reproduction event a year. In contrast species may reproduce repeatedly and opportunistically when favorable conditions occur, such as heavy rains, and these species often mature at a certain size and almost independently of age.

In some species the females do not reproduce in every spawning event and sometimes not for several years. This behavior is a bet hedging strategy to a avoid loss of complete clutches from a female due to pond drying or exceptional predation. In these species the males often return to ponds during most breeding opportunities.

Females generally take much longer to mature than males and are larger at maturity. This particularly applies to pond or stream breeding species that lay numerous eggs. Breeding events at ponds can result in a high predation levels. If some males are predated there are still others to fertilise eggs. However, each gravid female is very important and when risking reproduction needs to spawn the maximum numbers of oocytes.

Environmental cues such as temperature and light promote the production of hormones that result in reproduction (see



*Right*: The green and golden bell frog (*Litoria aurea*) lives with cool winters and hot summers. However, this species reproduces without any temperature cycling. *Image Robert Browne*.

<u>Amphibian hormone cycle</u>). Sometimes even with environmental conditioning induction through hormones (see <u>Amphibian hormonal induction</u>) still are needed to manage reproduction. This can be simply because some species don not otherwise reproduce in captivity or to achieve spawning at will. Even when using hormones to induce amphibians to spawn it is very important to use the optimum temperatures for reproduction. Temperatures will also vary the response time to



induction, which may enable better management of spawning times and organisation of staff.

*Left*: The Mikudu caecilian (*Boulengerula uluguruensis*) has never reproduced in captivity. The amphibian conservation crisis has created the need for reproduction conditioning for a great diversity of amphibians. Image Ben Van Dyke. For temperate-zone species the stimulation consists mainly of an artificial hibernation and for tropical species the alternation of wet and dry periods.

There is no universal method to determine reproductive condition. However, once in condition with stage 6 oocytes females may retain oocytes for long periods until hormonal induction. In some species it is easy to identify the stages of maturation as the full-grown oocytes can be seen through the semitransparent ventral surface. In others the judgment must be made on less precise factors, like the 'fatness'. With some large amphibians such as giant salamanders ultrasound is used to determine oocyte development. Males can be sexed by a variety of secondary sexual characteristics.

#### Nutrition:

Amphibians should be fed an adequate amount of highly nutritional food. In the past diet has probably been a major cause of poor health and reproduction in captive amphibians. This food should have a nutritional profile particularly rich in vitamin A, other vitamins, and poly- and highly unsaturated fatty acids, energy, calcium and phosphorus. This food should be *ad libartum* for the last 2-3 weeks before reproduction, particularly with females (see <u>Amphibian diet and nutrition</u>).

#### Temperature:

In some species a seasonal cycling of temperature is essential for the maturation of oocytes in the ovaries. This cycling of temperature can include slow or rapid temperature changes. In different species either high or low temperatures may affect the maturation of testes. The optimal temperatures and cycles will be species specific. However, generalizations may be made for suites of species from some regions with regular annual climatic cycles, including Mediterranean climates, or those with slight but regular daily cycles such as tropical climates.

With all amphibians there are optimal temperatures to prepare for and to achieve reproduction. Seasonal temperature cycles may be necessary to mature oocytes. In some species simulating the natural temperature cycle can benefit reproduction. In other species found in climates with long periods without surface water significant rainfall may be paramount to temperature in predicating reproduction.



*Left*: The critically endangered blackeyed tree frog (*Agalychnis moreleti*) lives in a warm climate with fairly constant temperature. *Agalychnis moreleti* will breed all year around in captivity as will its cogener the red-eyed tree frog *A.callidryas*. *Image: Fotographie Artman*.

Many species of anurans from temperate or cold climates with distinct seasons require a period of cooler temperature or even 'hibernation' to maturate oocytes. Keeping amphibians at lower temperatures for 4 to 6 weeks has been shown to increase oocyte production in some amphibians. For example in the case of female Puerto Rican crested toads temperatures may be slowly lowered from 26°C to 17°C for 30 days.

The European newts *Triturus*, for example, need to be maintained at a low temperature before the breeding period; this has been shown with *T. cristatus*, *T. vulgaris*, *T. vittatus* and *T. montandoni*. Other species such as *Bufo marinus*, *B. viridis*, *Discophus antongilii* and *Kaloula pulchra*, all reach maturity under stable environmental conditions.

*Right*: The common marbled newt (*Triturus marmoratus*) from southern Europe has a terrestrial morph. *Triturus* newts need a cool period to mature their ovaries. *Image Robert Browne*.

In some species, a hibernation period in conjunction with adequate fat reserves is essential to mature oocytes. A problem of hibernating anurans is unacceptable mortality. The immune system of anurans tends to become less responsive at lower than physiologically optimum temperatures.





*Left*: The ribbed newt (*Pleurodeles watle*) comes from a similar area to the marbled newt but will reproduce without temperature cycling. However, a slight warming of water can stimulate reproduction. Image Robert Browne.

Immunity can also take weeks to recover after low temperatures. In sensitive species where cool adapted pathogens are present, to avoid mortalities hormonal priming may be the preferred for reproduction. Some pathogens including chytridiomycosis also prefer lower temperatures. Independently or together, in some hibernating anurans these responses to low temperature can result in increased pathology and unacceptable mortality.

#### Aestivation:

Amphibians from regions that have dry periods seek shelter in deep shelter to retain moisture. When these dry periods are regular and seasonal, or when they are irregular but generally of long duration, many amphibians aestivate. Aestivation is slowing of metabolism in some animals during hot or dry periods. Some amphibians that reproduce after these long dry periods may be stimulated in captivity to reproduce after simulation of aestivation. However, in some species it has been difficult to simulate aestivation without mortality from desiccation.

#### Rain and humidity

Similar to the extreme of aestivation some amphibians respond to dry periods followed by wet periods to simulate natural seasonal cycles and breeding conditions.

## Light cycles:

For most species of amphibians the effects of light cycles are not known. In many species reproduction is dependent on rainfall or other season factors that have no apparent relationship to light. Other species during reproductive maturation are buried or species like caecilians active underground.

Most studies have shown that the effect of temperature is paramount to the effect of lighting on amphibian reproduction. However, with some temperate amphibians lighting cycles have profound effects on the reproductive cycles. For example, bullfrogs and the skipping frog (*Rana cyanophlyctis*) need long photoperiods for full ovarian maturation without excessive atresia of oocytes. In contrast for the large-scale reproduction of *Xenopus* spp. 12/12 hr light/dark cycles maximize reproduction success and fecundity.

In conservation breeding programs where species are housed individually reproduction may be facilitated by the use of natural lighting. However, multiple species in the same room will need to come from similar regions for the use of light to facilitate reproduction. These species are likely to come from mid- to high latitudes.

#### Age:

Before attempting to reproduce anurans – and particularly in the case of hormonal induction – females should be of breeding age and in good condition. In some species females may take a year or two to reach maturity independent of their size or condition. However, good nutritional regimes and temperatures can mature the males of some species over periods of less than six months.

However, many species of amphibians can take years to reach maturity. In their first breeding season Puerto Rican crested toad males are 1-2 years old and females 2-3 years old. Many salamanders take 3-5 years to reach maturity, and Chinese giant salamanders may take at least 5 years in captivity and much longer in the wild.