

## Reproduction technology key document

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There three critical targets of conservation breeding programs are reproduction, maintenance of genetic variation, and disease management; resulting in the provision of competent individuals for rehabilitation programs.

Reproduction technologies not only provide a convenient method for reproducing amphibians. They are essential for the reproduction of many threatened species, and in combination with gene banking greatly facilitate the maintenance of genetic variation. Conservation breeding programs for the flagship species, the Wyoming toad (*Bufo baxteri*), Boreal toad (*Bufo boreas boreas*), Puerto Rican crested toad (*Peltophryne lemur*), and Chinese giant salamander (*Andrias davidianus*) are either completely or significantly dependent on hormonal induction.

*Right. Studies improving hormonal induction of the common Fowler toad (*Bufo fowleri*) developed new methods used for the conservation of the critically endangered Wyoming toad (*Bufo baxteri*). Image Robert Browne.*



**Hormone preparation and use** - This method is used for a wide variety of amphibians in both commercial and conservation programs. Hormonal induction is the use of various hormones to bring the amphibians into breeding condition- called priming - and then to induce spawning and spermiation. Hormonal induction is a valuable technique; however, it is somewhat dependent on the **Reproduction conditioning** (> **Reproduction and larval rearing**) of the amphibians particularly females. The use of hormonal induction should also be viewed in light of the **Amphibian hormone cycle** (> **Reproduction technology**).

**In vitro fertilisation** - the fertilisation of eggs using sampled sperm and oocytes. This method enables the; 1) fertilisation of one female by many males, 2) the use of sperm and eggs from amphibian based in different location, 3) where sperm or eggs are of poor quality, and when the males and females produce gametes at different times. The viable nuclei from sperm or cells can also be used to fertilise oocytes using nuclear transfer (see

**Maintenance of genetic variation** > **Gene banking**).

*Right. The development of a suite of reproduction technologies for tomato frogs *Dyscophus antongilli* will enable efficient management and breeding of this and other amphibians from Madagascar. Image Robert Browne.*



**Short term storage of sperm and oocytes** – the storage of sperm or oocytes for hours to weeks. This is most frequently accomplished using saline solutions or low temperatures or these combined. These gametes may then be used for *In vitro* fertilisation (> **Reproduction technology**) or for **Gene banking (Maintenance of genetic variation)**.

**Gel removal and extract** – the manipulation and fertilisation of oocytes, eggs and embryos often requires the removal of the gel coat.

*Right. Red-eyed green tree frog *Agalychnis callidryas* eggs about 20hrs after fertilisation in their 4<sup>th</sup> division with 16 cells. Image Photographie Artman.*

**Handling and injecting amphibians** – good handling methods for amphibians are essential for their well being and health. They also enable the efficient and relaxed administration of medicines, hormones, and markings and tags.

#### **Glossary:**

*In vitro* – directly translated as in glass, means in a container.

Gamete – the male or female genetic component as a sperm or oocyte

Spermiation – the production of sperm and movement from the testes.

Spawning – the laying of oocytes.

Ovulation – the production of oocytes from the ovaries.

Oocyte – the unfertilised egg.

Egg – fertilised oocyte before the first division into two cells.

Zygote – the egg after first division into two cells.

Blastocyst – the developing egg before it starts to form organs.

Gene banking – the long term storage of amphibian sperm or cells to perpetuate their genetic variation.

