Why ban the use of frog in laboratory?

The frog has been used regularly as an experimental animal for more than a century and has served as an important model organism throughout the history of life sciences. For instance, atrioventricular conduction was first understood from an experiment conducted on frogs[1]. Eighteenth-century biologist Luigi Galvani discovered the link between electricity and the nervous system through a famous experiment using the limbs of frogs[2,3]. The African clawed frog (*Xenopus laevis*) was first widely used in laboratories in pregnancy assays in the first half of the 20th century[4,5]. Human chorionic gonadotropin (hCG), a hormone found in the urine of pregnant women, was found to induce ovulation in *X. laevis*. The idea of somatic cell nuclear transfer originated with an experiment on adult intestinal cells from frogs, cloned in 1958 (ref. 6). The same technique was later used in mammals to create Dolly. Thus frogs have historical importance in the development of biology.

Selection of an experimental animal depends on three factors, viz. sensitivity, reproducibility and availability. When we consider these aspects, the frog is certainly one of the most suitable animals for training students in biology, physiology, pharmacology and toxicology. Before the ban on the use of frogs was imposed in 1999, our laboratory used frog experiments for demonstrating potentiation and antagonism of drugs on rectus abdominis muscle preparation. The Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA) has instructed laboratories to procure frogs only from licensed breeders, but there are no licensed frog-breeders in India. This has now forced many laboratories to switch from frogs to mammals (e.g. use of the rat ileum for demonstration of drug actions). However, for such demonstrations, the use of mammals is inappropriate not only because mammalian tissues are less robust, but also because mammals are more demonstrably sentient. Thus restrictions in the use of frogs have only added to the cruelty in experiments.

Shortly after the ban, some animal welfare groups and activists swung into action and destroyed the tanks used for housing frogs captured from the fields. This was followed by 'street-fight' tactics employed by some animal welfare groups which forced their way into laboratories and let animals loose. Such unfortunate incidents have diminished public confidence in statutory bodies such as the CPCSEA[7].

Catching frogs from the wild was prohibited since 1972 (under Wildlife Protection Act 1972) because of widespread export of frog meat[8]. The large scale capture of frogs from agricultural fields and supply to laboratories has led to an ecological imbalance[9]. However, there seems to be no reason for the simultaneous restriction on the breeding of frogs. The licensing authority, CPCSEA, has not been able to give any details about the availability of frog breeders in this country. In a country that pays scant attention to the welfare of animals, it is surprising that rules that serve no purpose become very popular with law enforcement authorities.

Industrial pollution, pesticides in agriculture, deforestation, global warming and infection are the major causes for decline in the number of amphibians, and not only because of catching frogs from nature[10–12]. Breeding and supply of frogs constitute a very large enterprise (http://www.frogbreeders.com/). There is a heavy demand for frog meat in South-East Asia, and China is the biggest producer and exporter of frogs in Asia. Brazil is the largest supplier of frogs to USA for experimental and research purposes (http://www.fao.org/fishery/culturedspecies/Rana_catesbeiana/en). Breeding frogs on a commercial scale can be a profitable opportunity in India.

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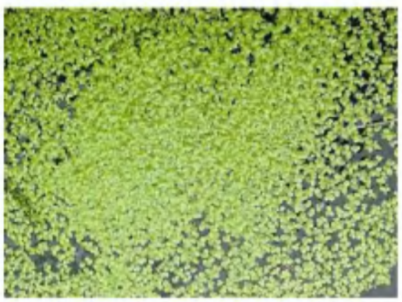
Bioethanol production from *Lemna gibba* L.

World ethanol demand has been on a rapid rise as a result of the environmental impact associated with the use of methyl tert-butyl ether (MTBE) as a gasoline additive for improved octane rating. Bioethanol now a preferred fuel due to its lower impact on the environment and its high octane rating. Modern automobiles are being designed to use higher proportions of bioethanol (flexi fuel) or only bioethanol as fuel. Industrial growth and increase in automobiles have led to high ethanol demand in the world especially in Asian countries like China and India. Thus bioethanol production has become a worthy investment.

World bioethanol production in 2004 was estimated to be 40 giga litres (GL)[1]. Brazil and US are world leaders in bioethanol production and together account for about 60% of the world ethanol production exploiting sugarcane and corn respectively. In India, in spite of having several agricultural and domestic products for the extraction of bioethanol and biodiesel we are far behind our energy requirement that can decrease our energy dependence on fossil fuel. There is need for sustainable bioresources that can be harnessed in large quantity and at faster rate having higher efficiency for biofuel production.

Several agricultural products and wastes as well as few aquatic weeds have been exploited for bioethanol production with different degrees of efficiency but only a few have gained industrial importance due to several biochemical and physiological limitations. *Lemna gibba* L., commonly known as duckweed, can be established as an alternative source of bioethanol because of the following attributes: it is an extensive aquatic weed in India and other parts of the world, its growth rate is very fast, it has less economic value, it contains high amount of carbohydrate, cellulose and hemicellulose compared to several other aquatic plants. When effectively managed, *L. gibba* may yield 10–30 tonnes DM/ha/year.



Aquatic body showing mat of *Lemna gibba* L.

It is a small, floating perennial aquatic weed found worldwide and often seen growing in thick, blanket-like mats on still or slow moving, nutrient-rich waters. They are monocotyledons of the botanical family Lemnaceae and are higher macrophytes. *L. gibba* has widespread distribution ranging from tropical humid climate to the temperate areas of Europe, Asia, Africa, South America and North America. In India, *L. gibba* is distributed in most of the static and slow moving water bodies in eastern India mainly north-eastern region and northern India, from tropical to temperate climate. This aquatic weed can also grow on mud or damp rocks. It prefers a sunny position in still water that is rich in nitrates and carbonate.

Saccharomyces cereviceae (yeast) efficiently utilizes carbohydrate, cellulose and hemicellulose in *L. gibba* and can convert it to simple sugars which ultimately gets converted to ethanol under oxygen limiting conditions. Due to the presence of high amount of minerals, amino acids and protein[2], the hydrolysate slurry of *L. gibba* is found as a suitable medium for the growth of yeast.

In our preliminary study, when the hydrolysed product of *L. gibba* was fermented with *S. cereviceae* (by adding 10 ml of broth culture per 500 ml of *Lemna* hydrolysate, OD – 0.50 at 610 nm) for 10 days, starch production was found to be about 22.78 mg/ml. From one kg dry weight of *L. gibba* (powder), we could produce an average of 330 ml of ethanol. Ethanol yield was about 20% and the efficiency of ethanol production was as high as 25%. Further research on its suitability for bioethanol production at industrial level needs to be evaluated.

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