How frogs and humans interact: Influences beyond habitat destruction, epidemics and global warming

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Abstract. We review various ways that anurans have been of service to mankind, as well as threats to frog species from human activity beyond habitat destruction, global warming, and epidemic diseases. Over the centuries frogs have been a subject of fascination and entertainment, food, sources of medicinal preparations, and model organisms in biological research. For years many species were used in teaching anatomy, physiology and pharmacology, and in pregnancy testing. Current research has revealed antibiotic peptides, anti-tumour agents, analgesics and adhesive compounds in frog skin. There are also volatile compounds released from their skin; these chemicals repel various predators and may prove useful to humans. The global decline of amphibian populations is a major concern. Habitat destruction, global warming, and pandemic diseases are increasingly suspect in the decline of frog populations, but difficult to control. Restrictions in the food and pet trade are areas in which better enforcement could benefit anurans. However, not all human interactions have been deleterious to all species. The mechanics of highway building in North America commonly has created areas of run-off that provide breeding sites for select species. Similarly, in arid northern Australia, frogs aggregate in large numbers at artificial sites where human activity has provided stable water sources.

Key words: Anurans; beneficial interactions; deleterious interactions; food; frogs; knowledge; medicinal applications; peptides; pets; roads.

Introduction

Frogs (here defined broadly to include all anurans) are a conspicuous component of many ecosystems. They are found on every continent except Antarctica, and in virtually every habitat that provides access to water. It is estimated that there are more than 5,200 species of frogs (Frost et al., 2006), with substantially more to be discovered. This worldwide distribution, along with our (human) common need for

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water, has resulted in a close association of humans and frogs. In fact, nowadays, just about anyone can recognize a frog. Frogs have even made the phenomenal leap into human popular culture, for there is hardly a puppet more popular and better recognized than Kermit.

Since antiquity, when Aristotle was enthralled that frogs and humans share similar organ systems and biological needs, humans have been studying the lives of our amphibian neighbours (Holmes, 1993; Nussbaum and Oksenberg Rorty, 1995). Be they haute cuisine or a source of new medicinal drugs, it is clear that frogs have had an impact on our way of living (Tyler, 1997; Grenard, 1994, Adler, 2003).

For the last 25 years, evidence has accumulated demonstrating extinctions and major declines of many frog species (e.g., Stuart et al., 2004; Lips et al., 2006; Pounds et al., 2006). Although there is increasing evidence that there are several causes of these demographic changes (Johnson, 2006), the role of humans in this process is evident in terms of both direct habitat destruction and the introduction of exotics. Global warming is increasingly suspect as a problem on a planetary scale, but not one that can be easily or quickly managed. Here we attempt to review some of the specific ways that humans have an impact on frogs beyond the major factors of international concern such as habitat destruction, global warming, and pandemic diseases. We, in turn, present some solutions to alleviate the immediate risks to frogs brought on by excessive human use for food and interaction.

We also highlight some of the achievements humans have made off the frog's back and the knowledge we have learnt from studying frogs. In light of frog declines, we mention a few specific situations in which human activity has benefitted select frog populations. We work on the pragmatic premise that if benefits to the human race can be demonstrated from frog conservation, there is a better chance of influencing politicians and the general public to undertake steps to conserve populations and species.

Use in Drug Development

A wide range of novel chemical compounds occurs in the granular glands of anuran skin. Their isolation, identification, and characterisation have led to the development of drugs for human and veterinary use. This focus will increase. These glands are dispersed throughout the dorsal surface (rarely on the ventral) and often aggregated to form prominent structures which Duellman and Trueb (1986) term "macroglands". The parotoid glands of *Bufo* species and the tibial glands of some *Limnodynastes* species are examples.

Early (and some current) investigations sacrificed the donor and removed and homogenised the skin to extract the granular gland secretions. It remains essential to inactivate skin proteases, because degradation of peptides can commence in 10 minutes. However, sacrifice is no longer required because it is possible to obtain the secretions by electrically stimulating the skin, using a square-wave stimulator and a bipolar electrode (Tyler et al., 1992). However, it is imperative that investigators do not contribute to the further decline of frog populations. "Fixing" isolated skins is no better for extracting peptides than adding methanol to the wash-off of secretions obtained by electrical stimulation. Grant and Land (2002) have described the circuitry and use of an economically assembled stimulator.

Peptides and alkaloids

Caerulein (a case study). In the decade following 1960, many international drug companies directed particular attention to the source and development of anti-hypertensive compounds for human use. Professor Erspamer and his colleagues in Italy, in conjunction with the drug company Farmitalia, became involved in natural products pharmacology, and particularly the examination of peptides derived from the skin of frogs. In collaboration with Dr R. Endean of the University of Queensland, they focussed particular attention upon the large green tree frog *Litoria* (at that time *Hyla*) *caerulea*. The species was selected because Endean had noticed that his cat used to eat most frogs with impunity, but that it always vomited after eating *L. caerulea*. He reasoned that the frog must have unusual pharmacological activity (R. Endean, pers. comm. to M.T.). To further this research, the skins of hundreds of frogs were sun-dried and the secretions then extracted in methanol.

As a result of the above studies, it was demonstrated that the predominant polypeptide (which they named caerulein) could produce a significant and sustained fall in blood pressure when introduced intravenously in pentobarbitone-sedated dogs at concentrations of 10-100 ng/kg (Bertaccini et al., 1968). In terms of its anti-hypertensive potential, it was unfortunate that not all smooth muscle reacted similarly and caerulein was found to cause a potent stimulation on the musculature of the gastrointestinal tract in situ. They noted, "... vomiting and diarrhoea occur in the dog, abdominal discomfort, borborygmi and awareness of intestinal movements in man, and pseudo-antidiuresis due to pylorospasm in the rat". Although these side effects precluded the possibility of the polypeptide being used as an oral anti-hypertensive drug, it has been used as a stimulant to restore gut motility following surgical induced atony and to dilate the gall bladder prior to cholecystography. It is now available in a synthetic form under the trade names of Ceruletid, Takus, Ceosunin, Cerulex and Tymtran. More recently, the effects of caerulein as a cholecystokin octapeptide (CCCK-8) receptor has been examined by Harro et al. (1990). This work led to the conclusion that endogenous CCCK-8 and CCK-8 receptors are involved in the neurochemical basis of anxiety. There are also indications that caerulein can provide relief for sufferers of chronic schizophrenia (Maroji et al., 1982; Watanabe et al., 1984). The implication of this finding is that caerulein or an analogue is to be found in mammals, probably in the intestinal wall, but it has yet to be detected.

Caerulein is estimated to have an analgesic property several thousand times more potent that morphine. However, there is no available evidence that it has any affinity for the receptors of opioids, despite the fact that the analgesic effect is blocked by the morphine antagonist Naloxone (de Castiglione, 1982; Erspamer and Melchiorri,

(a) (b)

Figure 1. Chinese pharmaceuticals. (a) Skinned and eviscerated *Bufo* species for preparation of a tonic. (b) Modern tonic tablets.

1973). Erspamer (1994) has prepared a major review of the variety of bioactive compounds in frog skin.

Antibiotic compounds. The use of topical preparations derived from frog skin for antibiotic purposes probably antedates researched history, and is perpetuated in current, traditional Chinese medicine. Samples of ancient and modern preparations are shown in fig. 1. Studies during the past 40 years, on frogs and toads from many continents, have revealed the existence of antibiotic peptides and alkaloids (Preusser et al., 1975; Cevikbas, 1978; Suzuki et al., 1995; Clarke, 1997; Nicholas and Mor, 1995; Stone et al., 1992; Clarke et al., 1994). Amongst the most promising antibiotics are the Magainins derived from *Xenopus laevis* and reported by Zasloff (1987), and the antiviral action of the Caerins from Australian frogs of the genus *Litoria* (Van Compernolle et al., 2005).

Although the future applications for antibiotic skin secretions look promising, many compounds prove to be haemolytic. It is the exceptions that are potentially applicable for medical and veterinary purposes.

Hallucinogens. Bufotenine, a compound found in the parotoid glands of some *Bufo* species has powerful hallucinogenic properties. Users express the secretions upon glass in the sun and scrape it off when it is dry. It is then smoked. Legislation to outlaw the practice varies from declaring Bufotenine a prohibited substance in New York (Chamakura, 1994), to prohibiting the possession of a *Bufo* species (*marinus*) in Queensland, Australia.

Anti-tumour agents. One of the traditional Chinese medicines is Ch'an Su, which is produced from the skin secretions of local toads, such as *Bufo gargarizans* and *B. melanostictus*. Ch'an Su is available in large quantities. Recently it has been shown to include several novel bufadienolides, which are variously active against nine cancer cell lines (Nogawa et al., 2001). Currently deep frozen *B. marinus* are being exported from Australia to China for similar pharmaceutical applications.

Analgesics. In addition to the reported analgesic attributes of caerulein, another potent analgesic is Epibatidine, representing a new class of alkaloids and derived from the skin of the Ecuadorian frog *Epipedobates tricolor* (Spande et al., 1992; Elguero et al., 1996). Curiously, the morphine antagonist Naloxone did not restore pain sensitivity, contrary to that of caerulein. Problems associated with its toxicity may limit its systemic use (Shen, 1995).

Anti-inflammatory compounds. Steroids are commonly found in anuran skin secretions. Their use for anti-inflammatory purposes has a long history in folk medicine. An example was observed in the former Czechoslovakia where a woman suffered multiple bee stings to her face. A doctor directed local children to collect frogs. The frogs were killed and their dorsal surface was laid upon the patient's face; this relieved the pain and swelling.

There has been only limited research upon anuran steroids and they have not been subjected to any modern review.

Natural adhesives. Several species of frogs produce skin exudates that have considerable shear and tensile strength. The adhesive strengths of secretions of five species were reported by Evans and Brodie (1994). More recently Graham et al. (2005, 2006) described the composition of the glue and its strength in the Australian species *Notaden bennetti*. Almost as strong as polyacrylate glues but non-toxic, the *N. bennetti* secretion has considerable potential for use in surgery.

Volatiles. In addition to non-volatile compounds, many frog species produce odorous compounds (Smith, 2001; Smith et al., 2003, 2004a, 2004b). Although more work is needed to characterise the biological function of these volatiles, the odorous secretion of *Litoria caerulea* is repellent to mosquitoes (Williams et al., 2006). The volatile secretion of *L. ewingi* has antimicrobial action and is repellent against a range of potential predators and parasites, including snakes, rats, and mosquitoes (Smith, 2001; Tyler and Smith, 2001).

Spermicidal compounds. In contrast to their use as a means of diagnosing pregnancy (see below), anurans have been investigated for an opposing purpose; the synthetic Magainin peptides have been found to have spermicidal properties (Edelstein et al., 1991).

Teaching

The use of frogs in teaching basic anatomy to high school and tertiary students has been a standard practice for more than 150 years. By far the best-known and complete dissection guide is that of Ecker (1864) on European *Rana* species, a work translated into English by Haslam (1889). A second and equally well illustrated work was produced by Hoffman (1873-1878), differing in having a more comparative approach.

Rana species continued to be the most popular subjects and Marshall (1882, and numerous revisions) and Whitehouse and Grove (1930) were the most popular guides in the U.K. In contrast, *Xenopus* was used in South Africa (Millard and Robinson, 1945-1955: three editions). More detailed studies of *Xenopus* anatomy are provided by Deuchar (1975), and Nieuwkoop and Faber (1994). Studies using *Xenopus* have become increasingly popular, to the point at which it has largely replaced *Rana* as the laboratory anuran.

Frogs and toads have also been the most popular subjects for teaching physiology and pharmacology. Two preparations have been used most commonly: one is the isolated, perfused, rectus abdominis muscle, which responds to the neurotransmitter acetylcholine with a slow contractile response. The second is the isolated, perfused, sciatic nerve/gastrocnemius muscle preparation. Stimulation of the sciatic nerve with a square-wave stimulator causes the gastrocnemius muscle to contract. Nerve-muscle transmission was demonstrated by adding atropine to the perfusate, so preventing transmission at the muscle endplate. These are classical experiments that originally contributed to an understanding of basic physiology in vertebrates.

Pregnancy Testing

Galli-Mainini (1948) of Argentina described a human pregnancy test, which involved injecting male *Bufo arenarum* subcutaneously with a small quantity of urine from the patient. Pregnancy could be confirmed by the release of spermatozoa into the frog's bladder and cloaca. A pipette was introduced into the cloaca to obtain a sample. The test was accurate, rapid (taking 3-4 hours for a result), and inexpensive. Previously there were two tests available: the Ascheim-Zondek and Friedman. Both required the use of mammals, there was a delay of many days for a response, and they were relatively expensive because of the need to maintain laboratory colonies of the animals.

Throughout the world the amphibian test was adopted; many other anuran species were substituted for *B. arenarum*, and were found to respond similarly: *Rana* species globally, *Xenopus laevis* in Europe and Africa and, in Australia the introduced cane toad, *B. marinus* (Bettinger and O'Loughlin, 1950; McDonald and Taft, 1953).

The use of anurans for pregnancy testing was a standard technique for 20 years. Figures to indicate the extent of their use are generally unavailable, but 11,000 were known to have been imported into South Australia predominantly for this purpose in 1962, which at that time had a human population of only 987,000 (Australian Bureau of Statistics). Extrapolating globally suggests that the worldwide use must have been huge.

Advancing Science: Nobel (and Ig Nobel) Pursuits

As described previously, frogs have provided and continue to provide an important learning platform for man. In fact, a number of significant scientific breakthroughs can be attributed to studies with frogs. To date, approximately 10% of the Nobel prizes in physiology and medicine have resulted from investigations using frogs (see http://nobelprize.org/).

Two aspects of amphibian biology have been particularly important in advancing biology and medicine. Because development in most anuran species is external and their eggs are large (compared to the more common aquatic vertebrates; i.e., fish), they have been model organisms in experimental embryology (extensively reviewed in Callery, 2006). Hans Spemann's experimental manipulations, which won him the Nobel Prize in 1935, would not have been possible without the large eggs of the amphibians he worked on. Those studies established the organizer effect in embryonic development.

Because it is easier to do transgenic manipulations with anuran eggs than mammalian eggs, anurans, particularly *Xenopus* and its close diploid cogener *Silurana*, have continued to be important model organisms for developmental biology research in the modern era of molecular biology (Sparrow et al., 2000).

The other biomedical area to which amphibians have contributed significantly (and Nobel winning science) is neuromuscular physiology. The fact that anurans are ectothermic means that when their tissue is cool, they can survive for hours to days as in vitro preparations. Thus, amphibians are ideal organisms for certain studies in physiology. Although fish are similarly cold blooded, they do not have muscles in the extremities that can be so easily isolated. Nor are fish as similar to humans in their basic body plan. The ability both to maintain alive and to isolate tissues and organs has clearly been a critical factor in the research of John Eccles, Alan Hodgkin, and Andrew Huxley. Their work on ionic involvement in excitation and inhibition in neural transmission won them the Nobel Prize in 1963.

The study of frog neuromuscular tissue has also played an important role in physics and our understanding of electricity. A chance observation by the famous anatomist Luigi Galvani (1737-1798) led him to discover "animal electricity" in

1786. Galvani found that when the leg of a dead frog was touched with a metal knife, the leg twitched violently. Galvani thought that the muscles of the frog must contain electricity. Although slightly misguided in his hypothesis, Galvani's observation was the cornerstone for the study of neuromuscular physiology and ultimately the understanding of electrical (ionic) conductance in nerve and muscle fibres.

By 1792 another Italian scientist, Alessandro Volta, disagreed with Galvani's hypothesis that muscles contained electricity. Volta realised that the main factors in Galvani's discovery were the two different metals — the steel knife and the tin plate — upon which the frog was lying. Volta showed that when certain solutions come between two different metals, electricity is created. This led him to invent the first electric battery, the voltaic pile, which he made from thin sheets of copper and zinc separated by moist pasteboard (Pera, 1992).

Galvani's observation and Volta's invention of the battery were fundamental to the study of electricity and magnetism. Experiments dealing with these two phenomena were described by philosophers hundreds of years before Christ. However, for nearly 2000 years those experiments dealt mainly with static electricity. The absence of a source of continuous electrical energy posed a severe limitation in the progress of understanding the underlying physics of the observed electrical and magnetic phenomena (Visser, 2005).

Many other notable experiments in physics, biology, and chemistry have been achieved through the study of frogs. Some have received recognition primarily because of a perceived humorous aspect of frogs. In the last decade, three Ig Nobel prizes have been awarded to work on frogs. Two of them were awarded in 2000; one for the taste of tadpoles, another for the magnetic levitation of a frog. The last was awarded in 2005 for the smell of adult anurans. The Ig Nobel prizes are awarded for research that first makes you laugh and then makes you think (see http://www.improb.com/ig/). The awards were instigated to help communicate science to the general public in both a fun and informative way.

In all these cases, the actual phenomena studied (taste, smell, and the physics of magnetic fields) are not so exotic to be worthy of an award, least of all, one with a humorous edge. The humour therefore lies in the organisms that were involved in the experiments and not the topic itself. Thus, it is worth asking why frogs are perceived in this way. Many factors may apply: the shortest, squattest bodies in the vertebrate world; a surprising saltatory gait; large eyes; tailless adults; a general harmlessness, etc. Collectively, the body plan of anurans is so distinctive as to make them exceptional among vertebrates (Handrigan and Wassersug, in press). Being both bizarre and benign helps build some intrinsic whimsy into these beasts.

Use in Taxonomy and Systematics

Unfortunately, the use of anurans for humankind in almost all cases requires some collecting of specimens from nature. The taking of adequate samples to be placed in museum collections is essential for the study of taxonomy and systematics, which in turn have a role in conservation. The same applies to tissue samples for molecular studies. Unfortunately it is clear that the ethical issue of limiting samples to numbers that do not affect the continued viability of a local population, has not always been observed.

An example of over-collecting brought to our attention was that of a collector who took 650 adult calling males of one species from an isolated locality. The following year he took 97 and expressed surprise that subsequently there was none at the site. The species no longer can be heard or seen there. This example may be exceptional but it demonstrates the need for herpetologists to minimise their impact on study animals. In the light of evidence of declining populations (Stuart et al., 2004; Pound et al., 2006), collection policy is an issue that has been addressed. In many jurisdictions, there are now regulations requiring permits for collecting. Unfortunately, whereas scientists are likely to abide by such judicial constraints, ecologically sound prohibitions on one's collecting are either not in place or not enforced in many regions. This problem is most severe in the poor tropical countries that have the highest diversity of anuran species.

The Pet Trade

There is no doubt that trade in frogs for pets (much of it illegal) is having a huge impact upon some natural populations. Whereas possession of exotic species is highly attractive to many keepers, the fundamental issue in some European countries is a ban on keeping local endemic species. Many leading European herpetologists approve this ban, which increases pressure upon the fauna of other continents. Hohn (2003) demonstrated that in New York, trade in local species included some that were of conservation concern. Elsewhere trade is almost entirely in exotic species. For example, in Europe, Martens and Jelden (1992) reported that 54% of anurans in the pet trade are *Dendrobates* species and 27% *Phyllobates* species.

Bizarre species, such as *Ceratobatrachus guentheri* of the Solomon Islands and several species in Madagascar, are threatened by their demand overseas as pets (Glaw and Vences, 1992). Local governments may perceive their export as valuable means of income, without any regard for their conservation in perpetuity.

The pet trade will continue. All that can be hoped for is an international approach to providing approved breeding establishments, where frogs can be bred to provide stock for private keepers and zoos. This action needs to be combined with increased penalties to deter the illegal trade that is rife at present.

Human Consumption

In most parts of the world, the consumption of the hind legs of frogs has been a traditional food source, but at such a level that it had little impact upon the viability of local frog populations (Cooke, 1989). However, the novelty of the food as a gourmet item created a demand in Europe (particularly France and Switzerland) that could only be satisfied by importations from Asia. Beebee (1996) stated that in the mid-1980s, Bangladesh, India, and Indonesia were each exporting 3,000 tons of legs every year, and calculated that the developed world consumed 6,500 tons of legs per year. Local extinctions have resulted from the practice, and several Asian countries have placed embargoes on further exports (see Oza, 1990). Kusrini and Alford (2006) reported that Indonesia exported around 5,600 tons of frog legs in 1992 and around 3,900 tons in 2002. Of these exports 83.2% went to Europe.

Frog farming probably started in the USA where it was promoted as a "get rich quick" activity (fig. 2). The reality was that it is not economically feasible on a small scale. Modern frog farms, such as the Jurong Frog Farm in Singapore breed tadpoles of the North American species *Rana catesbeiana* in large concrete ponds. Disease, cannibalism, and maintenance of adequate water quality remain significant problems (Lutz and Avery, 1999), but *Rana catesbeiana* has been introduced into several countries as a food source (Lever, 2003).

Indonesia, Singapore, Taiwan, Brazil, and Uruguay also are countries where frog farms are being developed. In Indonesia, local *Rana limnocharis* and *R. cancrivora* are farmed, and Susanto (1989) provided considerable detail on their husbandry. Food for frogs in a pelletised form is manufactured in Taiwan and exported to many countries.

It is clear that the provision of frogs for the restaurant trade is a major threat to frog populations on two accounts. One is the excessive hunting of frogs for human consumption. The other is the threat to local species by the escape of the American bullfrog, where it has been introduced for frog farming. Competitive pressure on native species from the introduced bullfrogs in California is well documented (Kupferberg, 1997). One of us (RW) collected tadpoles from several sites on both sides of Japan's main island where feral bullfrog larvae are the most common tadpoles encountered. Despite the lack of evidence that bullfrogs have helped either the economy or human nutritional status in Japan in any way, public demand and economic considerations are likely to cause bullfrog introductions to continue around the world.

Trade in Brazil was described by Rocha-Miranda et al. (2006) as follows:

"Currently there are about eight large companies farming bullfrogs, with 600 establishments in Brazil.... The estimated meat production of 400 tons/year is destined almost entirely for the domestic market, but is still thought to be insufficient to meet demand ... 1500 to 4000 animals are slaughtered daily to provide meat, liver (in the form of paté), skin (for diverse products), oil (for the perfume industry); what is left is recycled as frog food."

Biological Control

Lever (2001) listed 137 introductions of the cane toad into different countries, principally for the purpose of biological control of insect pests of agricultural

Figure 2. Advertisement promoting frog farming. Circa 1925.

significance. By far the most disastrous of these was the entry of 102 *Bufo marinus* into Australia in 1935 in an attempt to control insect pests of sugar cane. To date A$7 million has been spent by the State and Federal governments in an attempt to control the introduced predator, which now occupies an area of more than 1,000,000 km^2. Currently, the toad is advancing across northwest Australia at a rate of more than 100 km each year, and desperate efforts are being made to halt its spread (fig. 3).

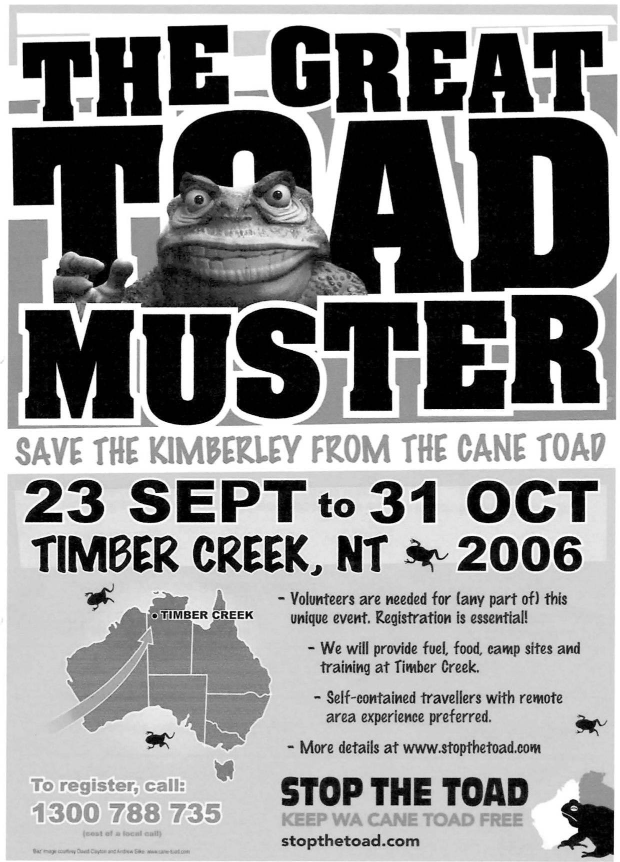

Figure 3. One of many Australian posters.

Beneficial Impacts

The impact of humans on frog populations might be interpreted as entirely deleterious. However, such an interpretation would be incorrect and should be challenged for several reasons.

Tyler and Watson (1998) examined the impact following observations at an isolated cattle station in the Northern Territory of Australia and the more populated farming communities of Victoria in the southeast of the continent. In the Northern Territory, they were able to demonstrate that the construction of out-houses, providing showers and toilet facilities for staff, created environments where vast numbers of frogs were able to survive during arid periods. Without these refuges the frog population would have been much lower. At one site, 24 *Litoria caerulea* were found in a single toilet cistern and more that 100 *L. rothii* in a shower block.

In retrospect, the invasion of *Litoria* at this site is not surprising, given how closely humans and frogs are in both time and space just because of their common dependence on fresh water. How they interact varies greatly, and often depends on the ways in which humans manage water flow and water bodies. Clearly, draining rivers and streams, introducing predatory fish, and agricultural chemicals have vastly restricted the ranges of species in areas where humans and frogs may otherwise co-exist. Environmental augmentations that have helped selected species on a large scale have occurred, however, and this has happened on a much greater scale in North America.

For example, spring peepers (*Pseudacris crucifer*) are doing fine despite the massive decline of anuran species from many tropical and montane regions. This species breeds in temporary pools overgrown with grass and cattails, but not otherwise canopied (Skelly et al., 2002, 2005; Halverson et al., 2003). In North America, this habitat is commonly found along the edges of roads, tens of thousands of kilometres of which have been built up and paved since the advent of the automobile.

In building highways in North America, earth from the side of the road is scooped up to elevate the roadbed. The resulting roadbed drains well, which makes it safer for auto traffic. The construction also creates ditches at the sides of the road, and the ditches become new seasonal pools in which frog species such as *P. crucifer* that are "open canopy specialists" (Skelly et al., 2005) can breed. In addition the roads have right-of-ways that usually include the ditches. These public lands are typically separated by fences from adjoining private land, particularly in areas where farm animals graze and could be killed if they wandered on to the road. Those fences also, however, help keep cattle and other farm animals out of the ditches.

It may not be obvious that the automobile has in any such indirect way helped any frogs; particularly considering the great mortality of anurans on highways (Hels and Buchwald, 2001). It should be acknowledged though, that the high rate of crushed frogs on roads in many areas is, in part, the result of the abundance of breeding sites for frogs produced by humans right along the margins of those roads.

This symbiotic association of humans, and their roads, with certain frogs and their breeding sites is far less common in Europe, where most roads pre-date the automobile. Two things in Europe work against frogs. For centuries, as Europeans have improved the farmland along river courses, they have drained temporary puddles and pools that otherwise would have been breeding sites for frogs. They have redirected the flow of just about every waterway that is near agricultural land in order to control flooding. Many of the major roads date back to the Romans and are built at the margins of floodplains, which means that runoff naturally drains back into neighbouring streams and rivers.

Neither the Romans nor the modern Europeans have elevated their highways to the extent that the North Americans have. Similarly, the typical open-herding of farm animals, i.e., moving them from one patch of grazing land to another, is more typical in Europe than North America. This is perhaps because the long history of land ownership in Europe results in many families owning multiple small and separate plots of land. Few people (even wealthy people) in Europe ever acquire large contiguous blocks of grazing land. Within this setting, fences are an obstacle to

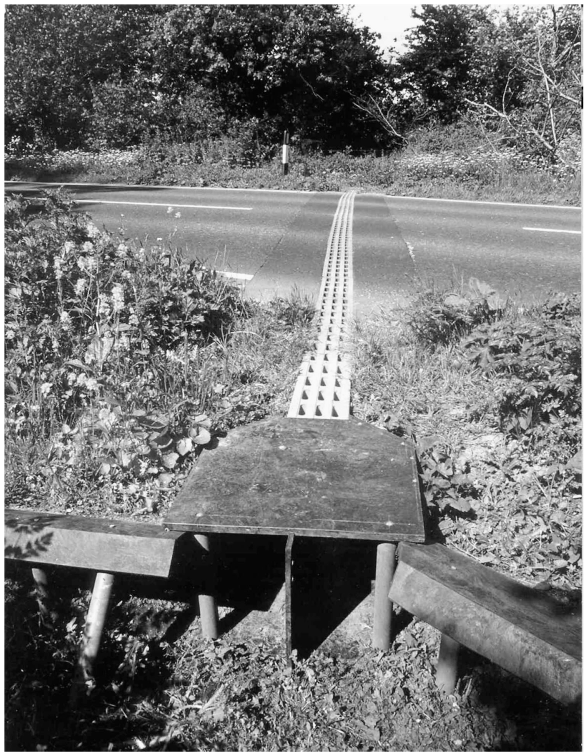

Figure 4. Toad tunnel at Newhaven, UK. Reproduced with permission of ACO Polymer Products.

efficient ranching rather than structures that might protect ponds and pools, in which anurans breed, from large grazing mammals. One simply sees far fewer fences in Europe than along the roads of North America.

In sum, without elevated roads, which would produce new roadside ditches, and fences to isolate cattle from the few ponds and pools that are left, European anurans have few safe places to breed. The few ponds that are left are on floodplains, in areas heavily used by humans.

It is thus not surprising that Europeans have been at the vanguard of promoting and installing toad tunnels under their roads (Langton, 1989). The need in Europe for these structures is most obvious in mountainous regions. In North America, where there are hills above roads there are often man-made aquatic sites between the hills and the roads proper. This is far less common in Europe, where amphibians coming down from the hills and heading to the few remaining ponds and pools in the floodplain almost always have to cross a major road. Toad tunnels can provide safe access to water for amphibians where they must cross highways to move from elevated feeding habitat to lower-lying breeding sites (fig. 4).

On a continental scale these tunnels are still, however, so rare as to be of greater symbolic than substantive significance to anurans. Nonetheless, in some regions of central Europe, toad tunnels may be the only hope that anurans have of surviving in the patchy landscapes that have been heavily modified for millennia by human farming and occupation.

As much as crossing roads is a hazard for most anurans, roads can also facilitate dispersal when species elect to follow rather than cross them. Recent data show that toads follow roads, and this is helping the current rapid westward dispersal of the cane toad in Australia (Brown et al., 2006).

Conclusions

There is considerable reciprocity in the impacts between anurans and humans. A few negative impacts by humans, such as their sacrifice for physiological or other scientific purposes, have diminished substantially in recent years. However, these pale in comparison to the numbers lost from habitat destruction and large-scale environmental degradation. In rare cases, such as with highway construction in North America, human habitat modification may help a few species. But most human-frog interactions continue to be detrimental to anurans.

Acknowledgements. RW's research is supported by the Natural Science and Engineering Research Council of Canada. We thank JoAnne Phillips and Ella Tyler for editorial assistance. ACO Polymer Products is thanked for permission to reproduce fig. 4.

References

Adler, K. (2003): Amphibians and humans. In: Grzimek's Animal Life Encyclopaedia, Vol. 6: Amphibians p. 51-55. Hutchins, M., Duellman W.E., Schlager, N., Eds. Farmington Hills, MI, Gale Group.

Beebee, T.J.C. (1996): Ecology and Conservation of Amphibians. London, Chapman and Hall.

Bertaccini, G., deCaro G., Endean, R., Erspamer, V., Impicciatore, M. (1968): The action of caerulein on the systemic arterial blood pressure of some experimental animals. Brit. J. Pharmacol. Chemotherap. **33**: 5971.

Bettinger, H.F., O'Loughlin, I. (1950): The use of the male toad, *Bufo marinus*, for pregnancy tests. Med. J. Aust. 1950 **1**: 40-42.

Brown, G.P., Phillips, B.L., Webb, J.K., Shine, R. (2006): Toad on the road: use of roads as dispersal corridors by cane toads (*Bufo marinus*) at an invasion front in tropical Australia. Biol. Conserv. **133**: 88-94.

Cevikbas, A. (1978): Antibacterial activity in the skin secretion of the frog *Rana ridibunda.* Toxicon **16**: 195-197.

Chamakura, R.P. (1994): Bufotenine — a hallucinogen in ancient snuff powders of South America and a drug of abuse on the streets of New York City. Forensic Sci. Rev. **6**: 1-18.

Clarke, B.T. (1997): The natural history of amphibian skin secretions, their normal functioning and potential medical applications. Biol. Rev. **72**: 365-379.

Clarke, D.P., Durrell, S., Maloy, W.L., Zasloff, M. (1994): A novel antimicrobial peptide from bullfrog (*Rana catesbeiana*) skin, structurally related to the bacterial antibiotic, Polymyxin. J. Biol. Chem. **269**: 10849-10855.

Cooke, R.G. (1989): Anurans as human food in tropical America: ethnographic, ethnohistoric and archaeological evidence. Archaeozoologia **3**:123-142.

de Castiglione, R. (1982): Dermorphin and ceruletide, prototypes of two families of analgesic peptides. Farmaco **37**: 305-313.

Deuchar, E.M. (1995): *Xenopus*: The South African Clawed Frog. London, John Wiley.

Duellman, W.E., Trueb, L. (1986): Biology of Amphibians. New York, McGraw-Hill.

Ecker, A. (1864): Die Anatomie des Frosches. Braunschweig, Friedrich Vieweg.

Edelstein, M.C., Gretz, J.E., Bauer, T.J., Fulgham, D.L., Alexander, N.J., Archer, D.F. (1991): Studies on the in vitro spermicidal activity of synthetic magainins. Fertil. Steril. **55**: 647-649.

Elguero, J., Campillo, N., Paez, J.A., Antonio, J. (1996): Non conventional analgesics: epibatidine, a powerful nicotinic analgesic. An. R. Acad. Farm. **62**: 303-321.

Erspamer, V., (1994): Bioactive secretions of the Amphibian integument. In: Amphibian Biology, Vol. 1: The Integument. Heatwole, H. and Barthalmus, G.T., Eds. Chipping Norton, New South Wales, Australia, Surrey Beatty and Sons.

Erspamer, V., Melchiorri, P. (1973): Active polypeptides of the amphibian skin and their synthetic analogues. Pure Appl. Chem. **35**: 463-494.

Evans, C.M., Brodie, E.D. (1994): Adhesive strength of amphibian skin secretions. J. Herpetol. **28**: 502–504.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., De Sa, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. Wheeler, W.C. (2006): The amphibian tree of life. Bull. Amer. Mus. Nat. Hist. **297**: 1-370.

Galli-Mainini, C. (1948): Pregnancy test using the male Batrachia. J. Amer. Med. Assoc. **138**: 121.

Glaw, F., Vences, M. (1992): A Fieldguide to the Amphibians and Reptiles of Madagascar. Leverkusen, Moos-Druck.

Graham, L.D., Glattauer, V., Peng, Y.Y., Vaughan, P.R., Werkmeister, J.A., Tyler, M.J., Ramshaw, J.A. (2006): An adhesive secreted by Australian frogs of the genus *Notaden*. In: Biological Adhesives, p. 207–223. Smith, A.M., Callow, J.A., Eds. Berlin, Springer-Verlag.

Graham, L.D., Glattauer, V., Huson, M.G., Maxwell, J.M., Knott, R.B., White, J.W., Vaughan, P.R., Peng, Y., Tyler, M.J., Werkmeister, J.A., Ramshaw, J.A. (2005): Characterization of a protein-based adhesive elastomer secreted by the Australian frog *Notaden bennetti*. Biomacromodules **6**: 3300-3312.

Grant, J.B., Land, B., (2002): Transcutaneous amphibian stimulator (TAS): a device for the collection of amphibian skin secretions. Herpetol. Rev. **33**: 38-41.

Grenard, S. (1994): Medical Herpetology. Pottsville, Reptile and Amphibian Magazine.

Halverson, M.A., Skelly, D.K., Kiesecker, J.M., Freidenburg, L.K. (2003): Forest mediated light regime linked to amphibian distribution and performance. Oecologia **134**: 360-364.

Handrigan, G.R., Wassersug, R.J. (2007): The anuran Bauplan: a review of the adaptive, developmental, and genetic underpinnings of frog and tadpole morphology. Biol. Rev. **82** (1).

Harro, J., Pold, M., Vasar, E. (1990): Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: influence of acute and subchronic diazepam treatment. Naunyn-Schmiedeberg's Archiv. Pharmakol. **342**: 62-67.

Haslam, G. (1889): The Anatomy of the Frog. Oxford, Clarendon Press.

Hels T.I., Buchwald, E. (2001): The effect of road kills on amphibian populations. Biol. Conserv. **99**: 331-340.

Hoffman, C.K. (1873-1878): Dr H.G. Bronn's Klassen und Ordnungen der Amphibien in Wost und Bild. Leipzig, C.F. Winter.

Hohn, S.M. (2003): A survey of New York State pet stores to investigate trade in native herpetofauna. Herpetol. Rev. **34**: 23-27.

Holmes, F.L., (1993): The old martyr of science: the frog in experimental physiology. J. Hist. Biol. **26**: 311-328.

Johnson, P.T.J. (2006): Amphibian diversity: decimation by disease. P.N.A.S. USA **103**: 3011-3012.
Kupferberg, S.J. (1997): Bullfrog (*Rana catesbeiana*) invasion of a California river: The role of larval competition. Ecology **78**: 1736-1751.
Kusrini, M.D., Alford, R.A. (2006): Indonesia's exports of frogs' legs. Traffic **21**: 13-24.
Langton, T.E.S. (1989): Reasons for preventing amphibian mortality on roads. In: Amphibians and roads. Proceedings of the Toad Tunnel Conference Rendsburg, Federal Republic of Germany, 7-8 January 1989, p. 75-80. Langton, T.E.S., Ed. Shefford, England, ACO Polymer Products.
Lever, C. (2001): The Cane Toad. The History and Ecology of a Successful Colonist. Otley, West Yorkshire, Westbury Publishing.
Lever, C. (2003): Naturalized Reptiles and Amphibians of the World. Oxford, Oxford Univ. Press.
Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J. Carey, C., Livo, L., Pessier, A.P., Collins, J.P. (2006): From the cover: emerging infections disease and the loss of biodiversity in a Neotropical amphibian community. P.N.A.S. USA **103**: 3165-3170.
Lutz, C.G., Avery, J.L. (1999): Bullfrog culture. South. Regional Aquaculture Center Publ. No.436.
Marshall, A.M. (1882): The Frog: An Introduction to Anatomy, Histology and Embryology. London, Macmillan.
Martens, H., Jelden, D. (1992): Proc. Sixth Ord. Gen. Meet. SEH Budapest 1991, p. 309-313. Korsos, Z., Kiss, I., Eds.
Millard, N.A.H., Robinson, J.T. (1945): The Dissection of the Spiny Dogfish and the Platanna. Cape Town, Maskew Miller.
McDonald, I.A.,Taft, H.P. (1953): Some clinical applications of a rapid pregnancy test with the use of the male toad (*Bufo marinus).* Med. J. Aust. **2**: 162-164.
Moroji, T., Watanabe, N., Aoki, N., Itoh, S. (1982): Antipsychotic effects of ceruletide (caerulein) on chronic schizophrenia. Archiv. Gen. Psychiatr. **39**: 485-486.
Nicholas, P., Mor, A. (1995): Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Ann. Rev. Microbiol. **49**: 277-304.
Nogawa, T., Kamano, Y., Yamashita, A., Pettit, G.R. (2001): Isolation and structure of five new cancer cell growth inhibitory Bufadienolides from the Chinese traditional drug Ch'an Su. J. Nat. Prods. **64**: 1148-1152.
Nussbaum, M.C., Oksenberg Rorty, A. (1995): Essays on Aristotle's De Anima. Oxford, Oxford University Press.
Oza, G.M. (1990): Ecological effects of the frog's legs trade. Environmentalist **10**: 39-42.
Pera, M. (1992): The Ambiguous Frog: The Galvani-Volta Controversy on Animal Electricity. Princeton, Princeton University Press.
Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E. Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sánchez-Azofeifa, Still, C.J., Young, B.E. (2006): Widespread amphibian extinctions from epidemic disease driven by global warming. Nature **439**: 161-167.
Pruesser, H.J., Habermehl, G., Sablofski, M., Schmall-Haury, D. (1975): Antimicrobial activity of alkaloids from amphibian venoms and effects on the ultrastructure of yeast cells. Toxicon **13**: 285-289.
Rocha-Miranda, F., Martins Silva, M.J., Mendonca, A.F. (2006): First occurrence of Bull Frogs (*Rana catesbeiana*) in Federal District, Central Brazil. Froglog **2006**: 1-4.
Shen, T.Y. (1995): Medicinal chemical studies of anti-inflammatory and analgesic natural products. J. Chin. Chem. Soc. **42**: 617-621.
Skelly, D.K., Freidenburg, L.K., Kieseckerc, J.M. (2002): Forest canopy and the performance of larval amphibians. Ecology **83**: 983-992.
Skelly, D.K., Halverson, M.A., Freidenburg, L.K., Urban, M.C. (2005): Canopy closure and amphibian diversity in forested wetlands. Wetlands Ecol. Mgmt. **13**: 261-268.
Smith, B.P.C. (2001): Characterisation and Function of Volatile 'Defensive' Secretions in Two Species of Australian Tree-frog. PhD Thesis, University of Adelaide.

Smith, B.P.C., Hayasaka, Y., Tyler, M.J., Williams, B.D. (2004a): β-caryophyllene in the skin secretion of the Australian Green Tree Frog, *Litoria caerulea* White: An investigation of dietary sources. Aust. J. Zool. **52**: 521-530.

Smith B.P.C., Tyler, M.J., Williams, B.D., Hayasaka, Y. (2003): Chemical and olfactory characterization of odorous compounds and their precursors in the parotoid gland secretion of the green tree-frog, *Litoria caerulea.* J. Chem. Ecol. **29**: 2085-2100.

Smith, B.P.C., Williams, C.R., Tyler, M.J., Williams, B.D. (2004b): A survey of frog odorous secretions, their possible functions and phylogenetic significance. Appl. Herpetol. **2**: 47-82.

Spande, T.F., Garraffo, H.M., Edwards, M.W., Yeh, H.J.C., Pannell, L., Daly, J.W. (1992): Epibatidine: a novel (chloropyridyl) azabicycloheptane with potent analgesic activity from an Ecuadoran poison frog. J. Amer. Chem. Soc. **114**: 3475-3478.

Sparrow, D.B., Latinkic, B., Mohun, T.J. (2000): A simplified method of generating transgenic *Xenopus*. Nucleic Acids Res. **28**: E12.

Stone, D.J.M., Bowie, J.H., Tyler, M.J., Wallace, J.C. (1992): The structure of caerin 1.1, a novel antibiotic peptide from Australian tree frogs. Chem. Commun. **17**: 1224-1225.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W. (2004): Status and trends of amphibian declines and extinctions worldwide. Science **306**: 1783-1786.

Susanto, H. (1989): Budidaya Kodok Unggul. Jakarta, Penebar Swadaya.

Suzuki, S., Ohe, Y., Okubo, T., Kakegawa T., Tatemoto, K. (1995): Isolation and characterization of novel antimicrobial peptides, Rugosins A, B and C, from the skin of the frog *Rana rugosa.* Biochem. Biophys. Res. Commun. **212**: 249-254.

Tyler, M.J. (1997): The exploitation of frogs: past, present and future. Aust. Biol. **10**: 65-69.

Tyler, M.J., Smith, B.P. (2001): Pharmacological significance of frog skin gland secretions. The Fourth World Congress in Herpetology, December 2-9, Bentota (Colombo), Sri Lanka [Abstract].

Tyler, M.J., Stone, D.J.M., Bowie, J.H. (1992): A novel method for the release and collection of dermal, glandular secretions from the skin of frogs. J. Pharmacol. Toxicol. Meth. **28**: 199-200.

Tyler, M.J., Watson, G.F. (1998): Beneficial aspects of human changes to frog habitats in Australia. Aust. Biol. **10**: 65-69.

VanCompernolle, S.E., Taylor, R.J., Oswald-Richter, K., Jiang, J., Youree, B.E., Bowie, J.H., Tyler, M.J., Conlon, J.M., Wade, D., Kewalramani, V.N., Dermody, T.S., Ailcen, C., Rollins-Smith, L.A., Unutmaz, D. (2005): Antimicrobial peptides from amphibian skin potently inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. J. Virol. **79**: 11598-11606.

Visser, H.J. (2005): Array and Phased Array Antenna Basics. Southern Gate. Chichester, England, John Wiley & Sons.

Watanabe, M., Moroji, T., Tada, K, Aoki, N. (1984): A therapeutic trial of Caerulein to a long-term heavy marihuana user with amotivational syndrome. Prog. Neuro-Psychopharmocol. Biol. Psychiat. **8:** 419-421.

Whitehouse, R.H., Grove, A.J. (1930): The Dissection of the Frog. London, University Tutorial Press.

Williams, C.R., Smith, B.P.C., Best, S.M., Tyler, M.J. (2006): Mosquito repellents in frog skin. Biol. Letters **2**: 242-245.

Zasloff, M. (1987): Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms and partial DNA sequence of a precursor. P.N.A.S. USA **84**: 5449-5453.

Accepted: October 17, 2006.