

The incidence of electromagnetic pollution on the amphibian decline: Is this an important piece of the puzzle?

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(Received 26 October 2005)

Abstract

A bibliographical review on the possible effects of radiofrequency radiation (RFR) from wireless telecommunications on living organisms and its impact on amphibians is presented. The technical characteristics of this new technology and the scientific discoveries that are of interest in the study of their effects on wild fauna and amphibians are described. Electromagnetic pollution (in the microwave and in the radiofrequency range) is a possible cause for deformations and decline of some amphibian populations. Keeping in mind that amphibians are reliable bio-indicators, it is of great importance to carry out studies on the effects of this new type of contamination. Finally, some methodologies that could be useful to determine the adverse health effects are proposed.

Keywords: *Athermal effects, electromagnetic pollution, effects on amphibians, microwaves, phone masts*

Introduction

Amphibians are important components of the ecosystem and reliable bio-indicators; their moist skin, free of flakes, hair or feathers, is highly permeable to water chemicals (particularly larvae) and air pollutants (especially adults). Amphibian eggs are also directly exposed to chemicals and radiation. These characteristics make amphibians especially sensitive to environmental conditions, changes of temperature, precipitation or ultraviolet (UV) radiation and reliable monitors of local conditions [1].

A recent report from the International Union for Conservation of Nature (IUCN), prepared by 500 scientists from 60 countries, analyzed populations of 5743 amphibian

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species in the world and concluded that 1856 (32%) of them were considered threatened of extinction. Nine species have become extinct since 1980 and another 113 have not been observed in the recent years, and probably are also extinct [2]. The results demonstrate that amphibians are far more threatened than either birds or mammals, and the factors causing ‘enigmatic’ declines are driving the species toward extinction particularly rapidly. Unless these declines are quickly understood and reversed, hundreds of amphibian species can be expected to become extinct over the next few decades [3]. The disappearance of amphibians together with other organisms is a part of the global biodiversity crisis [4,5].

An associated phenomenon is the appearance of large numbers of deformed amphibians, with absent or extra limbs [5]. From 1995, at least 60 different species were affected with a high incidence of deformities, with several species affected in one place, in 46 states of United States and in regions of Japan, Canada, and several European countries [5,6]. The problem seems to have become more prevalent, with deformity rates of up to 25% in some populations, which is significantly higher than in previous decades [6].

The problem of deformities is complex because it is related to water quality, physiology, development, anatomy, and ecology [5]. The reduction in populations and the increase in deformities are a warning of serious environmental degradation [5].

Evidence exists that several populational declines are probably the result of complex interactions among several biotic and abiotic factors [1,4,7,8]. The proposed explanations are an increase of ultraviolet radiation (UV-B)[1,5,9–14]; chemical pollutants (pesticides, herbicides, fungicides, fertilizers, etc.) [5,15]; pathogen and parasites [1,6,16], destruction and alteration of habitat, changes in meteorological patterns (climatic change) [4,17], and introduced species [1,5].

The amphibian population declines are also occurring in relatively pristine places such as National Parks, or rural areas far from urban centers [3,14]. Humans and other animals can also be affected by the same environmental factors that damage amphibians [6].

A type of contamination whose effects on amphibians have not been studied up to now, is the electromagnetic pollution, especially microwaves and radiofrequencies from mobile telecommunications and radio station transmitters that will be discussed in this review. Before the 1990s, radiofrequencies were mainly from a few radio and television transmitters, located in remote areas and/or very high places. Since the introduction of wireless telecommunication in the 1990s, the rollout of phone networks has seen a massive increase in the electromagnetic contamination in cities and in the wilderness [18,19]. At the moment, new types of antennas are being investigated to reduce the power needed to establish communication [20,21]. Recently, there has also been an increase of other wireless transmitters (radio or television stations).

The objective of this review is to detail advances in the knowledge of biological mechanisms and effects from radiofrequencies and microwaves on animals, and some considerations are made on its possible relationship with deformations and the population decline of amphibians.

Main causes of populational decline and appearance of deformations in amphibian populations

Ultraviolet radiation

UV-B radiation (1) induces mutations and cellular death, (2) weakens the immune system, (3) reduces growth, and (4) induces several types of damage, like malformations

of the limbs, body, and eyes [1,5,12,14]. Not all the species respond in the same way [14]. Embryos with higher photolyase levels (DNA photorepair enzyme) are more resistant to UV-B radiation [11,12].

The eggs of some of the amphibian species experienced high mortality that may contribute to the populational declines [9]. UV acts in conjunction with other agents like pesticides to induce defects in the development [10]. UV also decreases defense mechanisms against illnesses making individuals more susceptible to pathogen and parasites, affecting normal development and increasing mortality that consequently impacts on the decline of some populations [10]. The egg mass protected from UV-B radiation have significantly more hatching, less deformities, and develop more quickly [10].

Synergy between a pathogenic fungus and UV-B radiation increased mortality among amphibian embryos [12]. The synergy may occur when developing amphibians have reduced ability to respond to a stressor in the presence of another stressor. For example, contamination exerts more deleterious effects with UV-B [1]. Animals use molecular and physiologic mechanisms and certain behaviors [22] to limit their exposure to UV-B and repair from UV-B damage [14].

Although cellular repair mechanisms of several species are not effective in the presence of persistent increase in UV-B radiation levels [14], amphibians are relatively resistant to this radiation if they can repair the damage effectively [14]. In some species, photoreactivation is the most important repair mechanism of UV-damaged DNA [9]. Heat shock proteins may also play a role in protecting cells from UV-B damage, since they prevent the denaturation of proteins during exposure to environmental stress [14].

Chemical pollutants

Chemical pollutants appear in areas where pesticides and fertilizers are applied extensively and produce mortality and deformities in amphibians. Although on a broad scale, no correlation between pesticide contamination and amphibian deformities was found, pesticides cannot be completely ruled out as causal agents [5].

Pathogens and parasites

Three pathogens received attention recently for having produced an amphibian populational decline in some areas: *Batrachochytrium dendrobatidis*, *Saprolegnia ferax*, and an iridovirus (*Ambystoma tigrinum virus*) [1]. The parasite *Ribeiroia ondatrae* is an important source of malformations of amphibian extremities in western USA [16]. Larvae with malformations experience higher mortality before and during metamorphosis than the normal ones. The relevance of infection by *Ribeiroia* and the influence of habitat alteration on the pathology and biological cycle of this trematode, requires further investigation [16]. In relative pristine environments, the incidence of snails infected with *Ribeiroia* is low, but the habitat alteration can increase the rate of infestation [16]. Infection of amphibian larvae by the trematode *R. ondatrae* may represent a threat to amphibians or species in decline. Although deformities can be the cause of declines in some places, numerous populations of amphibians have greatly declined in the absence of any deformity, for which there must be other factors [6].

Climatic change

Climatic change influences breeding patterns of certain organisms which affect their populational structure and may be reflected in the populational declines of very sensitive

species such as amphibians. The pattern found up to now in the published studies is that some anurans of temperate areas show an early reproduction tendency [17]. Climate-induced reductions in water depth at egg-laying sites produced high embryo mortality by increasing their exposure to UV-B radiation which is more worrying than the reduction in ozone layer. Climate also increases their vulnerability to *S. ferax* [4].

Physical and technological characteristics of mobile telephone

Electromagnetic radiation (EMR) transmits small packages of energy denominated photons [23]. The radiofrequencies occupy the range from 10 MHz to 300 GHz. Cellsite antennae emit a frequency of 900 or 1800 MHz, pulsed at low frequencies, generally known as microwaves (300 MHz–300 GHz). Microwaves carry sound information by blasts or pulses of short duration, with small modulations of their frequency, that are transferred between wireless phones and base stations over dozens of kilometres.

The main variable that measures these radiations is ‘power density’ (measured in W m^{-2} , or $\mu\text{W cm}^{-2}$) expressing radiant power that impacts perpendicularly to a surface, divided by the surface area; and ‘electric field intensity’ (measured in V m^{-1}), a vectorial magnitude to the force exercised on a electric loaded particle, independent of their position in space.

For a concrete address with relationship to an antenna, the power density at a point varies inversely proportional to the square of the distance to the source. Though EMR have many and varied outputs, at a distance of 50 m the power density is about $10 \mu\text{W cm}^{-2}$ [24], while at distances of 100 m at ground level it measures above $1 \mu\text{W cm}^{-2}$ (pers. obs.). Between 150 and 200 m, the power density of the main lobe near the ground is typically some tenth of $1 \mu\text{W cm}^{-2}$ [25].

Experimental difficulties

Experiments that study the effects of EMR on living organisms are complex, since a high number of variables exist that need to be controlled. Microwave radiation produces different effects depending on certain methodological positions such as frequency, power, modulation, pulses, time of exposure, etc. [26–28]. Some studies demonstrated different microwave effects depending on the wavelength in the range of mm, cm or m [28,29]. The dose–response relationships (of non-thermal effects), are not simple to establish since they present a non-linear relationship [30–32].

Pulsed waves (in blasts), as well as certain low frequency modulations exert greater biological activity [26,28,31,33]. These radiations also have accumulative effects that depend on the duration of exposure [19,34,35]. It is possible that each species and each individual, show different susceptibility to radiations, since the vulnerability depends on the genetic tendency, and the physiologic and the neurological state of the irradiated organism [31,36–41].

Effects and action mechanisms on biological systems

One of the well known effects of microwaves is their capacity to excite water molecules and other components in food, elevating their temperature. The resulting heating level depends on the radiation intensity and the exposure time. At a power density above $500 \mu\text{W cm}^{-2}$

(microwave ovens) heating effects take place, below that level the effects are 'athermal non-heating'.

Animals are sensitive complex electrochemical systems that communicate with their environment through electrical impulses. In cellular membranes and body fluids, ionic currents and electrical potential exist [42]. Electromagnetic fields (EMFs) generated in biological structures, are characterized by certain specific frequencies. It is possible a frequency-specific, non-thermal electromagnetic influence, of an informational nature exists [25,31,43]. Some organs or systems like the brain, heart, and nervous system are especially vulnerable.

The wave systems have properties such as the frequency, which affect resonance capacity of living organisms to absorb the energy of an electromagnetic field [25]. Electromagnetic fields induce biological effects at "windows of frequency" (window effect) [44]. Living organisms are exposed to variable levels of radiofrequency electromagnetic fields, according to (1) distance to phone masts, (2) presence of metallic structures which are able to reflect or obstruct the waves (buildings or other obstacles), (3) number of phone masts, and (4) orientation and position [24].

Microwaves emitted by phone antennae affect organisms living in their vicinities, like vertebrate [45–47], insects [48–55], vegetables [56–58], and humans [25,31,59–63]. Small organisms are especially vulnerable: size approach to resonance frequency and thinner skull, facilitates an elevated penetration of radiation into the brain [24,31,64]. In a recent study carried out with bees in Germany, only few irradiated bees returned to the beehive and required more time to reach the hive. The weight of honeycombs is also smaller in the bees that were irradiated [54].

The microwave effects were investigated in a variety of living organisms, but the results found in vertebrates have special interest to amphibians. For more than 30 years, there is growing evidence on the existence of athermal effects on birds [65,66]. The exposed animals suffer a deterioration of health in the vicinity of phone masts [67,68]. Rats spent more time in the halves of shuttle boxes that were shielded from illumination by 1.2 GHz microwaves. The average power density was about 0.6 mW cm^{-2} . Data revealed that rats avoided the pulsed energy, but not the continuous energy, and less than 0.4 mW cm^{-2} average power density was needed to produce aversion [69]. Navakatikian and Tomashevskaya [70] described a complex series of experiments in which they observed disruption of a rat behavior (active avoidance) by radiofrequency radiation (RFR). Behavioral disruption was observed at 0.1 mW cm^{-2} (0.027 W kg^{-1}) power density.

It has been documented that the radiofrequencies induce biological effects on biomolecules [27,51,71] that include changes in intracellular ionic concentration [72,73], cellular proliferation [74], interferences with immune system [19,75,76], effects on animals reproductive capacity [77,78], effects on stress hormones [79], in intrauterine development [80], genotoxic effects [81–87], effects on the nervous system [32,88–92], the circulatory system [93,94], and a decline in the number of births [47,95]. Firstenberg [18] proposed a connection between EMR, deformations, and the worldwide decline and extinction of amphibians.

Evidence that electromagnetic contamination may be responsible for the appearance of deformities and decline of amphibians

Some athermal effects of EMR on amphibians have been well known for more than 35 years [96,97]. The radiation of frogs with $30\text{--}60 \mu\text{W cm}^{-2}$ produced a change in the heart

rhythm, probably due to the nervous system activation (Levitina, 1966 cited in [96]). When toad hearts were irradiated with pulses of 1425 MHz at a power density of $0.6 \mu\text{W cm}^{-2}$, an increase in the heart rate and arrhythmia were observed [96]. Radiofrequency burst-type dilated arterioles were observed on the web of the anaesthetized frog (*Xenopus laevis*) by a athermal non-heating mechanism [93].

The exposure to magnetic fields on two species of amphibians induced deformities [48].

Frog tadpoles (*Rana temporaria*) developed under electromagnetic field (50 Hz, 260 A m^{-1}) have increased mortality. Experimental tadpoles developed more slowly and less synchronously than control tadpoles, remain at the early stages for a longer time. Tadpoles developed allergies and EMF causes changes in the blood counts [98].

Amphibians can be specially sensitive: thresholds of an overt avoidance response to weak electrical field stimuli down to 0.01 V m^{-1} were found in *Proteus anguinus* and 0.2 V m^{-1} in *Euproctus asper* at 20–30 Hz, but sensitivity covered a total frequency range of below 0.1 Hz to 1–2 kHz [99].

Deformities in nature

Ultraviolet radiation, UV-B. UV-B radiations produce deformities in amphibian embryos that go from lateral flexure of the tail to abnormal skin, eye damage, and lower survival rate [6,10]. However, numerous experiments carried out did not provide evidence that this exposure induces all types of deformities observed in nature, nor the appearance of extra limbs, one of the most frequent deformities noted [5,6]. On the other hand, most of the deformations for UV-B radiation occur in the legs or in reduction of the number of bilateral fingers. However, in the wild, amphibians exhibit a wide diversity of aberrations that are limited to only one side of the body, including problems in the skin, loss of legs, and twisted internal organs, reasons for which it was considered that this radiation is not the only source [5]. Similar abnormalities found in the wild and not induced by UV-B radiation have been obtained in laboratory studies, by exposing amphibian larvae to magnetic fields [48]. A similarity exists in the deformations of amphibians observed by Levengood [48] and Blaustein and Johnson [5]. Several studies addressed behavior and teratology in young birds exposed to electromagnetic fields [39,41]. Typical abnormalities include malformation of the neural tube and abnormal twisting of the chicken embryo. The electric currents are believed to have a significant role in the control of development and it is also possible that external EMR could influence these control systems [100]. The appearance of morphological abnormalities influenced by pulsed electromagnetic fields during embryogenesis in chickens [33,101] are similar to those produced by ultraviolet radiation [36]. The pulses are in fact a characteristic of mobile telephone radiations that have increased from 1995, when a marked rise in deformations started. Several experimental studies point out that the exposure to UV-B produced deferred effects (early exposure causes delayed effects in later stages) [1]. The exposure to electromagnetic fields also induces delayed effects and the tadpoles are the same as the control until the beginning of metamorphosis. The extra limbs and blistering were induced during the gastrula stage of the development which appeared to be the most sensitive stage [48]. The early *Rana pipiens* embryonic development was also inhibited by magnetic fields [97]. In rats, brief intermittent exposure to low-frequency EMFs during the critical prenatal period for neurobehavioral sex differentiation can demasculinize male scent marking behavior and increase accessory sex organ weights in adulthood [102]. Biological effects resulting from EMR field exposures might depend on the dose (e.g. duration of exposure). Short-term exposures up-regulate cell repair

mechanisms, whereas long-term exposures appear to down-regulate protective responses to UV radiation [103].

Parasites. The parasite *R. ondatrae* is an important and extensive cause of malformations in amphibian extremities in western USA [16]. Tadpoles with malformations experience higher mortality than the normal ones before and during metamorphosis. The *Ribeiroia* infection represents a threat for amphibian populations that are in decline. However, with a growing volume of data based on the experimental evidence, the infection from parasites does not seem to be the cause of all the malformations on limbs, since in some places with the presence of deformations, the parasite *R. ondatrae* was absent [5]. Further certain deformities like the absence of eyes, limbs, and twisted internal organs was not induced by the parasite [5].

In a laboratory study, eggs and embryos of *Rana sylvatica* and *Ambystoma maculatum* were exposed to magnetic fields at several development stages. A brief treatment of the early embryo produced several types of abnormalities: microcephalia, scoliosis, edema, and retarded growth [48]. Several of the treated tadpoles developed severe leg malformations and extra legs, as well as a pronounced alteration of histogenesis which took the form of subepidermal blistering and edema [48]. In chick embryos exposed to pulsed EMR a potent teratogenic effect was observed: microphthalmia, abnormal trunkal torsion, and malformations on the neural tube [33,36,101,104]. One of the possible reasons for these deformities appearing more often [5], may be due to wireless telecommunications and exponential increase of electromagnetic contamination.

Bioelectric fields have long been suspected to play a causal role in embryonic development. The electrical field may directly affect the differentiation of some tail structures, in particular those derived from the tail bud. Alteration of the electrical field may disrupt the chemical gradient and signals received by embryo cells. It appears that in some manner, cells sense their position in an electrical field and respond appropriately. The disruption of this field alters their response. Endogenous current patterns are often correlated with a specific morphogenetic events such a limb bud formation. The most common defect in chick embryos experimental group was in tail development. Internally, tail structures (neural tube, notochord, and somites) were frequently absent or malformed. Defects in limb bud and head development were also found in experimentally treated chick embryos, but less often than the tail defects [105]. Amphibians can be especially sensitive because their skin is always moist, and they live close to, or in water, which conducts electricity easily.

Populations' decline

Deformities found in nature can directly affect embryonic mortality and survival after hatching [10]. It seems interactions that exist among UV-B radiation and additional factors contribute to embryo mortality [9]. Water pollution and excessive ultraviolet radiation act jointly, producing specific problems and alter the immune system, making amphibians more vulnerable to parasitic invasions and pathogen infections [6,8,12,14]. It is proposed that there exists a possible relationship between the decline of amphibians and exponential increase of electromagnetic pollution. Several experiments with bird eggs showed a high mortality of embryos exposed to EMR from mobile phones [36,106,107]. EMFs increases mortality of tadpoles [98]. The EMR alters the immune, nervous, and endocrine systems, and operates independent or together with other factors like UV-B radiation or chemical pollutants. Death of embryos in nature is not due to UV radiation

as the capacity of DNA repair mechanisms like photolyase (photoreactivating enzyme) is effective [9]. EMR produces stress on the immune system [76,98] that obstructs DNA repair [42,108,109]. Heat shock proteins may play a role in protecting amphibians from UV-B damage [14] and animals exposed to EMR [27,51,71,110,111]. Different susceptibility to UV among species and even among populations exists [112], as seen with EMR [31,40].

Hallberg and Johansson [108,109] proposed that radiofrequencies increase the effects of UV radiation. A study on the causes of melanoma in humans conclude that the incidence increases and the mortality associated with this skin tumor cannot only be explained by the elevation in UV sun radiation, but rather by the continuous alterations on mechanisms of cellular repair, produced by EMR (radiofrequencies) resonant with the body, that amplify the carcinogenic effects of the cellular damage induced by the UV-B radiation. The cases of melanoma experienced a significant increase from the 1960–70s [108] that continues today, and also asthma and several types of cancer associated with deterioration of immune system. Data suggest there is an increase of electromagnetic pollution [108,113]. The public health situation in Sweden has become worse since the autumn of 1997. There is a correlation between the massive roll-out of GSM mobile phone antennae and adverse health effects [109].

Enigmatic decline of amphibian species are positively associated with streams at high elevations in the tropics and negatively associated with still water and low elevations [3]. In high places, the electromagnetic contamination is usually higher [47]. Microwave measurements of power density as low as $0.0006 \mu\text{W cm}^{-2}$ show strong correlation with symptoms like depressive tendency, fatigue, and insomnia in humans [63].

Proposed research

To demonstrate the conclusive effect of microwave radiation on amphibians it is necessary to approach research with a control (non-exposed) and an experimental group. This methodological position is complicated at present due to the ubiquity of these radiations [98]. Studies that try to correlate populational evolution, appearance of deformities, or the presence or absence of amphibians with measurements of electromagnetic fields from radiofrequencies will be of great interest. Field investigations of urban park populations and phone masts surrounding territories need to be high-priority. A radius of 1 km^2 laid out in concentric circumferences at intermediate distances may be useful to investigate the differential results among areas, depending on their vicinity and corresponding levels of EMR. Laboratory studies on amphibians exposed to pulsed and modulated microwaves would also be of great interest.

Acknowledgments

The author is grateful to Denise Ward who revised the English version of this article and thanks Sam Kacew and Miguel Lizana for the valuable suggestions to a first manuscript of this article. The author would like to thank “Centro de Información y Documentación Ambiental” in Castilla y León (Spain) and Roberto Carbonell for providing some articles.

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