Risk from radionuclides: a frog’s perspective

Accumulation of $^{137}\text{Cs}$ in a riparian wetland, radiation doses, and effects on frogs and toads after low-dose rate exposure

Karolina Stark

Stockholm 2006

Department of Systems Ecology
Stockholm University
106 91 Stockholm
Sweden
There will come soft rains and the smell of the ground,  
And swallows circling with their shimmering sound;  

...  
Not one would mind, neither bird nor tree,  
If mankind perished utterly.  

Teasdale (1937)

We must begin thinking like a river  
if we are to leave a legacy of beauty and life  
for future generations.  

David Brower
Abstract
Threats from man-made radionuclides include waste issues, increasing number of power plants, underground bomb testing, nuclear weapons, and “dirty bombs”. Until recently the ionizing radiation protection system focused on protecting humans with an implied protection of biota. However, goals of sustainable development and precautionary principles for human activity are leading to an inclusion of plant and animal populations in the protection system.

From this perspective, the present thesis examines wetlands that function as sinks for the radionuclide $^{137}$Cs, and describes calculated and measured radiation doses to residing biota. Also, multi-level effects from exposure to low-dose rate ionizing radiation were studied. Accumulation of $^{137}$Cs after the Chernobyl accident fallout was studied in a riparian wetland with a mean activity concentration of 1 200 kBq m$^{-2}$ in Sweden (paper I). A mass balance budget of $^{137}$Cs showed that the sedimentation of new material was balanced by the decay process of $^{137}$Cs in parts of the wetland (paper I).

Frogs were identified as organisms of concern in this wetland. Internal radiation doses, based on whole body measurements of frogs, were estimated to be lower than external doses based on soil samples (paper II). Current dose models for biota resulted in a wide range of doses depending on different levels of conservatism in the models. Therefore, in situ measurements with frog-phantoms were found to provide valuable dose information (paper III). Measured doses using frog-phantoms were lower than calculated doses using several dose models. Although a dose conversion factor by FASSET was found to be useful for comparison with measurements in the field. A higher dose was measured to the phantom surface in comparison to inner parts, i.e. the sensitive skin of frogs receives the highest dose. Estimated and measured radiation doses to frogs were below suggested dose rate limits.

Low-dose rate $^{137}$Cs exposure of eggs and tadpoles from three amphibian species, Scaphiopus holbrookii, Bufo terrestris, and Rana catesbeiana, showed no increased levels of strand breaks in red blood cells, and no effects on development, survival or growth up to metamorphosis (paper IV). The ecological factor larval density had a stronger effect on metamorphic traits than low-dose rate radiation. Higher levels of strand breaks were detected after an acute dose in R. catesbeiana than after a chronic dose supporting a dose rate limit for protection of amphibians rather than a dose limit (paper IV).

Based on current knowledge, frogs in the contaminated wetland are probably not exposed to radiation doses from $^{137}$Cs that are harmful for the population. However, variations in sensitivity between populations and species, and adaptive responses have been shown for amphibians exposed to other stressors. This supports further research on effects of chronic low-dose rates of ionizing radiation on amphibians.
Svensk sammanfattning

Hot från antropogena radionuklider inkluderar olösta avfallsfrågor, ökande antal kärnkraftsverk, underjordiska bombtester, kärnvapen och ”smutsiga bomber”. Tills nyligen fokuserade strålskyddet mot joniserande strålning på skydd av människor med ett underförstått skydd av biota. Men en efterfrågan av en hållbar utveckling och att försiktighetsprincipen ska guida människors aktiviteter ledde till de nuvarande förändringarna att även inkludera växt- och djurpopulationer i skydds-systemet.

I detta perspektiv undersöker denna doktorsavhandling våtmarker som fungerar som sänkor för radionukliden $^{137}$Cs och beskriver beräknade och uppmätta stråldoser till biota. Avhandlingen studerar även multi-nivå effekter av låg-dosrat joniserande strålning. Omfördelningsprocesser och ackumulering av $^{137}$Cs efter nedfallet från Tjernobylolyckan studerades i en alsumpskog i mellan-öst delen av Sverige (paper I). En massbalans budget för $^{137}$Cs visade att ackumulationen var pågående och resulterade i höga aktivitetskoncentrationer av $^{137}$Cs (1 200 kBq m$^{-2}$). I delar av våtmarken balanserades sedimentationen av nytt material av sönderfallsprocesser för $^{137}$Cs (paper I).

Grodor identifierades som organismer som lever i denna typ av våtmark där $^{137}$Cs brukar ackumuleras. Interna stråldoserna från $^{137}$Cs, baserade på helkroppsmätningar av grodor, var lägre än externa doserna som baserades på jordprover (paper II). I detta hög kontaminerade område resulterar nuvarande dosmodeller för biota i en vid variation av doser beroende på olika grad av konservativt i modellerna. Därför bidrog in situ mätningar med grodfantom med värdefull dosinformation (paper III). Uppmätta doser med hjälp av grodfantomer i studieområdet resulterade i lägre doser än vad som kunde beräknas med hjälp av flera dosmodeller. Dock visade sig FASSETs dosomvandlingsfaktorer vara användbara för jämförelser med uppmätta doser i fält. En högre dos uppmättes till fantomytan i jämförelse med de inre delarna av fantomet, vilket betyder att den känsliga huden hos grodor får den högsta dosen. De uppskattade och uppmätta stråldoserna var lägre än de föreslagna dosratgränsvärdena för biota.


Baserat på kunskapen i dagensläget, utsätts grodor i det kontaminerade området troligen inte för stråldoser från $^{137}$Cs som skulle kunna vara skadliga för populationen. Variation i känslighet mellan populationer och arter samt adaptiva responser har dock påvisats hos amfibier som exponerats för andra stressfaktorer. Detta stöder behovet av ytterligare forskning om effekter av kronisk låg-dosrat joniserande strålning på amfibier.
TABLE OF CONTENTS

List of papers.................................................................................9
Introduction..................................................................................11
Radiation ecology........................................................................12
Wetland ecosystems.................................................................13
Frogs and toads...........................................................................15
Accumulation of cesium-137 by organisms.............................17
Effects of ionizing radiation.......................................................17
Models.........................................................................................18
Study sites....................................................................................19
Short summary of papers..........................................................20
Discussion....................................................................................22
    Ecological risk assessment....................................................24
    Synthesis of radiation doses in this thesis..............................24
    Comparison with suggested dose rate limits.........................26
    Are frogs less sensitive to radiation than fish?.....................26
Conclusions.................................................................................27
Acknowledgements....................................................................29
References..................................................................................30
Appendices..................................................................................Paper I - IV
List of papers

This doctoral thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III. Stark, K. and Pettersson, H.B.L. External radiation doses from $^{137}\text{Cs}$ to frog-phantoms in a wetland area: *in situ* measurements and dose model calculations. (*Manuscript*)

IV. Stark, K., Scott, D.E., Tsyusko, O., Coughlin, D.P., Hinton, T.G. Multi-level effects of low-dose rates of ionizing radiation on amphibians after exposure to $^{137}\text{Cs}$. (*Manuscript*)

As first author, I was the main person responsible for planning the studies, carrying out the field or experimental work, as well as analysis of data, and writing of the papers. In paper III I did not take part in the reading of doses to thermoluminescence chips. The published papers are reprinted with the kind permission of the publisher Elsevier.
Introduction
Regardless of our increased awareness and complex regulations, environmental problems continue to emerge. The knowledge about effects of global and local environmental pollution has resulted in a demand from stakeholders for a system that assures protection of ecosystems and biota from impacts of contaminants (UNCED, 1992). This thesis consists of four papers that are part of the research field of ecotoxicology, which can be defined as the science of contaminants in the biosphere and their effects on constituents of the biosphere, including humans (Newman & Unger, 2003). One group of such contaminants is radionuclides. Anthropogenic radionuclides continue to require our attention due to unresolved waste issues, an increasing number of power plants, underground bomb testing, and threats of nuclear weapons and “dirty bombs”.

Although research on effects of radionuclides is, by the definition given above, a branch of ecotoxicology, it has to a large extent been kept in isolation from research on other environmental contaminants. In turn, two separate fields have evolved: radiation ecology, mainly studying radionuclides in the environment; and radiation biology, studying effects of radionuclides on humans.

Until recently the radiation protection system has focused on protecting humans with an implied protection of biota. The reasoning behind this viewpoint was that the International Commission on Radiological Protection (ICRP) stated that if humans are protected then it is also very likely that animals or plants would not receive a radiation dose that could affect their populations (ICRP, 1991). Additionally, the International Atomic Energy Agency (IAEA) calculated in a set of cases with radioactive contaminated areas that no biota populations would receive harmful doses exceeding the ones humans received (IAEA, 1992). However, radiation doses to individuals -of particular concern are endangered species- in environments where humans are not present, or radiation exposure to organisms lower in the food chain were not considered. Also, this approach was not compatible with the ecological risk assessment methodology used for other contaminants. In 1992 the UNCED meeting in Rio de Janeiro stated that a sustainable development was desired and that a precautionary principle should guide human activity (UNCED, 1992). This statement in addition to the concern for protecting individuals of endangered species as well as populations in ecosystems where humans are not present led to the present strategy to also include plants and animals. Since no such system was established, several initiatives were taken from different countries and organizations. In the USA, a graded approach for radiation protection of biota was developed by the US Department of Energy (USDOE, 2002), including an approach to risk assessment and dose models for plants and animals. An EU-project, FASSET, was conducted between 2000 and 2004 with the aim to construct a framework for the protection of the environment from ionizing radiation (FASSET, 2004). Within the project an extensive review of radioecological research on effects on biota was carried out and large knowledge gaps were identified. FASSET was followed by the EU-project ERICA, which focuses on a management system for radiation protection of non-human biota (ERICA, 2006). Also, in 2003 ICRP assigned a task group to construct recommendations for such a system (ICRP, 2003).

In this thesis, the four papers presented are all conducted from the perspective of protecting the environment (plants and animals) from impacts of ionizing radiation.
The origin of this project was when airborne gamma spectrometry measurements of the ground deposition of $^{137}$Cs over Sweden following the Chernobyl fallout (SSI, 1998) revealed that many “hot spots” with elevated levels occurred in wetland areas. The first study aimed to clarify the redistribution processes of the accumulation of $^{137}$Cs in a wetland ecosystem by calculating a mass balance budget for $^{137}$Cs (Paper I). In wetlands, frogs were identified as an organism that could be exposed to high radiation doses due to their physiology and ecology. Therefore, external and internal radiation doses from $^{137}$Cs to frogs living in a wetland were estimated (Paper II) and external radiation doses were measured using frog-phantoms (Paper III). Furthermore, as there was a lack of literature data regarding effects on amphibians from ionizing radiation, especially chronic low-dose rate exposures to $^{137}$Cs, this was experimentally studied in three species of frogs and toads (Paper IV).

Radiation Ecology

This thesis is part of the research field of radiation ecology. The research field progressed as a result of society’s request for understanding the impacts of military and civilian activities with nuclear material on the environment, and possible ecological effects of radiation that could eventually lead to human harm or exposure. Radiation ecology, or radioecology as it is also called, is a relatively old field of research that can be traced back to Russia and the year of 1896 (Whicker & Schultz, 1982). However, the USA can be considered to be the place of origin for modern radioecology (Shaw, 2005).

Radioecology has been defined as a science that: 1) attempts to understand and predict the transport of natural and man-made radionuclides through natural and agricultural ecosystems to various receptors such as plants, animals and humans; 2) studies the effects of environmental radioactivity on plants and animals, particularly at the population and community levels of biological organization; and 3) uses radioisotopes as tracers of ecological processes with the goal of understanding those processes (Whicker, 1991, cited in van der Stricht & Kirchmann, 2001). Just like ecotoxicology, it involves many sciences primarily physics, chemistry, biology, and geology.

Even though the foundation in radioecology might be strong in this relatively mature research field, there are bricks missing in the house of radioecology. Radioecology has evolved and been kept as a separate field even though it has much in common with ecotoxicology. Instead of taking a holistic perspective on radionuclide transport and effect on the environment, a series of specific nuclear accidents at nuclear power plants, or nuclear releases has been the focus of radioecology’s progress. For example: the atmospheric nuclear bomb tests in 1952-1963; the 1957 Kyshtym accident in the former USSR; the Windscale accident in UK in 1957; and the Chernobyl accident in Ukraine in 1986. (e.g. Warner & Harrison, 1993). As a result specific transport and accumulation models, and remediation approaches have been developed instead of general tools. Consequently, important knowledge gaps are still present in e.g. the understanding of effects on most non-human species and whole ecosystems, and on radionuclide behavior in certain ecosystems such as in forests, wetlands, and tropic and Polar Regions (Bourdeau, 2001).

The field of radioecology is now faced with new challenges of assuring protection and creating protection systems for non-human species. It has been suggested that
radiation ecologists and radiation biologists need to collaborate to a higher extent if we are to succeed in the work of assuring protection of the environment from ionizing radiation (Hinton et al., 2004a). Accordingly, in this thesis multi-level effects, i.e. both DNA damage and changes in resource allocation, were examined in exposure experiments with frogs and toads (paper IV).

**Wetland ecosystems**

Wetland ecosystems are ecotones, i.e. transition areas between land and water, but also have their own typical characteristics. Historically, wetlands were often seen as trouble areas, containing too much water to serve as good agricultural or forestry land, and breeding mosquitoes and disease. Therefore, they have often been ditched and drained for conversion to agricultural or forestry land, causing large areas of wetland loss around the globe. In 1971 the convention on wetlands was created for the protection of wetlands and their resources (Ramsar, 1971). In Sweden, government subsidies for ditching were stopped and an environmental objective to maintain “Flourishing wetlands” was created in 1999 (Svanberg & Vilborg, 2001). However, wetland areas are still being lost due to new infrastructure, houses, and forestry activities. Also, the protection of smaller sized wetland areas (< 1.2 ha) is limited (Semlitsch & Bodie, 1998), which means that large areas of edge effect with a rich biodiversity are lost.

There are many types of wetlands and many different names are used to describe them and their features. Thus, the literature is rather confusing. In many English speaking societies wetland ecosystems include the structural groups: marshes, swamps, bogs, and fens (Table 1; Tiner, 1999). *Marshes* are defined as regularly or constantly inundated wetlands characterized by emergent herbaceous vegetation adapted to saturated soil conditions. In European terminology, a marsh does not accumulate peat and has a mineral soil substrate (Mitsch & Gosselink, 2000). *Swamps* are dominated by trees or shrubs. In Europe, wetlands dominated by reed grass (*Phragmites*) and forested fens are included in swamps. *Bogs* are peat-accumulating wetlands that have no significant inflows or outflows and support acidophilic mosses, particularly *Sphagnum* sp. *Fens* are also peat-accumulating wetlands but they receive some drainage from the surrounding mineral soil and usually support marshlike vegetation. This results in a more alkaline pH than found in bogs. In comparison with the English system, the wetlands in Sweden are classified differently (Landin, 2002). Four groups are used: *mires*, including bogs, fens and mixed mires; *freshwater shorelines*, including wet meadows, marshes, reed belts and areas with emerged and submerged vegetation; *marine shorelines*; and *wetland forests*, including alder forest swamps, noble deciduous forest swamps, birch forest swamps; *salix* forest swamps and coniferous forest swamps (Table 1). In order to consider the biological diversity, the wetland forest swamps is classified as one group. Wetlands can also be divided into functional groups of permanent and temporary wetlands that have a dry period.
Table 1. Two systems for classifying wetland ecosystems in English speaking societies according to Tiner (1999) and Mitsch & Gosselink (2000), and in Sweden according to Landin (2002).

<table>
<thead>
<tr>
<th>English system</th>
<th>Swedish system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marshes</td>
<td>Freshwater shorelines (wet meadows, marshes, reed belts)</td>
</tr>
<tr>
<td>Swamps</td>
<td>Marine shorelines</td>
</tr>
<tr>
<td>Bogs</td>
<td>Wetlands forests (alder-, noble deciduous-, birch-, Salix-, and coniferous forest swamps)</td>
</tr>
<tr>
<td>Fens</td>
<td>Mires (bogs, fens, and mixed mires)</td>
</tr>
</tbody>
</table>

Wetlands provide many ecological services such as flood prevention, and have been called the kidneys of the landscape because of their ability to filter nutrients and contaminants (Mitsch & Gosselink, 2000). Floodplains, marshes and riparian swamps are often inundated by rivers, streams or other watersheds and affected by overbank sedimentation. This is why they function as sinks for radionuclides such as $^{137}$Cs (Walling & Bradley, 1988; Burrough et al., 1999; Paper I).

The main factors affecting the accumulation of $^{137}$Cs in wetlands are summarized in Table 2. $^{137}$Cs mainly occurs as a positive charged ion, Cs$^+$, and has a high affinity to colloidal and particulate material. If $^{137}$Cs is released in to a watershed a large part becomes quickly adsorbed to particles in the water and is deposited on the bottom (Avery, 1996). As a result $^{137}$Cs follows, to a large extent, the pathways of sediment particle transport in a watershed. Other particle-bound radionuclides could be assumed to follow the same transport routes as $^{137}$Cs. In an outlet stream of a lake, sediment particles with associated $^{137}$Cs will be transported from the lake and during spring floods end up on floodplains. This redistribution process and accumulation in a downstream riparian wetland was studied in Paper I. The adsorption of $^{137}$Cs in soils is promoted by high clay content. However, in highly organic soils $^{137}$Cs remains mobile (Kudelsky et al., 1996, Rigol et al., 1999). Hence, $^{137}$Cs is more mobile in peat-accumulating bogs and fens that have a high organic content and they may thereby leak out their content of $^{137}$Cs. In wetlands with constant saturation the content of $^{137}$Cs in soil will decrease due to a higher mobility of $^{137}$Cs. Further, if the vegetation functions as a filter for flooding water, it can help retain $^{137}$Cs associated with colloids and particles in the wetland. Low concentrations of potassium (K) result in high uptake of $^{137}$Cs and, thus, cause retention of $^{137}$Cs by the vegetation. In addition, ponding water in hollows and depressions usually results in enhanced sedimentation and thus, higher deposition of $^{137}$Cs in these areas.

A mass balance budget showed that 29 % of the $^{137}$Cs discharged between 1986 and 2002 from the upstream lake was retained in the downstream wetland (Paper I). Also, in some parts of the wetland, the sedimentation of new material in 2003 was balanced by the decay process of $^{137}$Cs. Wetlands have so far not been well studied in radioecology because of no easily identifiable direct pathway to humans. However, from the perspective of protecting plants and animals, many species could be exposed to radiation in wetlands, among them amphibians.
Table 2. The main factors influencing an accumulation of $^{137}\text{Cs}$ in a flooded wetland.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Example</th>
<th>Mechanism of retention</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of soil</td>
<td>peat or mineral soil</td>
<td>$^{137}\text{Cs}$ high affinity to clay minerals</td>
<td>Sawhney (1972), Broberg &amp; Andersson (1991), Avery (1996)</td>
</tr>
<tr>
<td>Duration of saturation</td>
<td>permanent or temporary</td>
<td>dry periods, in constantly saturated area $^{137}\text{Cs}$ is more mobile</td>
<td>Nylén &amp; Grip (1991), Hilton et al. (1993), Saxén (1994)</td>
</tr>
<tr>
<td>Type of vegetation</td>
<td>tall or short, thick or sparse</td>
<td>tall and thick vegetation filter colloids &amp; particles</td>
<td>Horrill (1984), Mungur et al. (1995)</td>
</tr>
<tr>
<td>Degree of salinity</td>
<td>marine water or freshwater</td>
<td>freshwater, in saline water $\text{Cs}^+$ is more mobile</td>
<td>Horrill (1984)</td>
</tr>
<tr>
<td>Level of pH</td>
<td>high, neutral, low</td>
<td>high or neutral pH, high level of $\text{H}^-$-ions (low pH) releases $\text{Cs}^+$ from soil particles</td>
<td>Staunton &amp; Roubaud (1997), Munthe et al. (2001)</td>
</tr>
<tr>
<td>Level of nutrients in soil</td>
<td>high or low</td>
<td>high uptake of $^{137}\text{Cs}$ by plants if low $\text{K}^+$ and high $\text{NH}_4^+$ concentration</td>
<td>Camps et al. (2003)</td>
</tr>
<tr>
<td>Topography</td>
<td>elevated or low areas</td>
<td>hollows and depressions can enhance sediment deposition</td>
<td>Jeffries et al. (2002), van der Perk et al. (2002)</td>
</tr>
</tbody>
</table>

**Frogs and toads**

Amphibians, including frogs, toads, salamanders, and caecilians, are an interesting group to study exposure to and effects from ionizing radiation due to their physiology and ecology. The frog and toad species that were studied in this thesis have a complex life cycle (Figure 1) and an external embryonic development, i.e. they lay their eggs directly in the water without protection of a shell or amnion. This could make them sensitive to ionizing radiation. Also, they are poikilothermic or exothermic animals, which prolong the biological half-life for $^{137}\text{Cs}$ at low temperatures (Reichle et al., 1970, Prosser, 1973). Frogs have a thin skin consisting of a living cell layer that could make them radiosensitive. They have a small home range (200 m$^2$ for *Rana* sp.; Loman, 1994) and they return every year to the same watershed or pond to breed (Duellman & Trueb, 1986). In Sweden, frogs hibernate for 6 months by digging into the soil or mud at the edge of a stream or lakeshore where $^{137}\text{Cs}$ tends to accumulate. All these factors could make them susceptible to exposure of ionizing radiation. Furthermore, amphibians are threatened and declining world-wide and are therefore of global biodiversity concern (Storfer, 2003, Stuart et al., 2004). Radiation doses from $^{137}\text{Cs}$ for frogs living in a contaminated riparian wetland were estimated and measured in this thesis (**Paper II and III**). The studies showed that the external doses from $^{137}\text{Cs}$ in the soil were found to be higher than the internal doses from $^{137}\text{Cs}$ accumulated by the frogs (**Paper II**). Also, the radiation dose to the skin of a frog was found to be higher than to the rest of the body (**Paper III**).
Figure 1. The life cycle of an anuran, from fertilized egg, through larval stages and metamorphosis, to sexually mature adult. From Storer et al., 1979.

In a recent review of the ecotoxicology of amphibians it was reported that only 2.7% of the literature over the last 25 years (up to 1998) concerned amphibians (Sparling et al., 2000). A lack of knowledge on effects on amphibians after exposure to ionizing radiation, especially chronic low-dose rate exposures, was identified by FASSET (2004).

Frog and toad embryos and tadpoles are relatively large in size and sensitive to a great number of environmental pollutants dispersed in the aquatic environment (Sparling et al., 2000). Moreover, frog and toad larvae are available in large numbers (>2300 eggs per female for the species in paper IV) and a detailed staging series of normal embryonic development has been established for anurans (Gosner, 1960). In general, amphibians constitute a suitable model for monitoring early embryo-larval development and advanced development, including metamorphosis (Reviewed by Fort et al., 2004). Accordingly, effects of low-dose rate ionizing radiation from $^{137}$Cs on frogs and toads were experimentally studied in Paper IV. The experiments focused on examining the effects on stages of egg development, larvae, and metamorphosis, as well as DNA damage in red blood cells. Larval density was found
to have a larger effect than ionizing radiation on the development and body size of the three species studied (Paper IV).

**Accumulation of cesium-137 in organisms**

Fission processes of uranium and plutonium form $^{137}\text{Cs}$ in nuclear reactors and atomic bombs. $^{137}\text{Cs}$ emits beta and gamma radiation when it disintegrates. The physical half-life of $^{137}\text{Cs}$ is 30.2 years which makes it a radionuclide of long-term concern if released into the environment. $^{137}\text{Cs}$ characteristics make it function as an analogue to potassium, K, and micro-organisms can accumulate Cs$^+$ via active K$^+$ transport systems (e.g. Bossemeyer et al., 1989). Accumulation of $^{137}\text{Cs}$ by herbivorous and carnivorous animals mostly occurs through consumption of contaminated foods. When ingested it distributes fairly homogeneously in the soft tissues of the body with slightly higher levels in muscle tissues and lower levels in bone and fat (Avery, 1996).

The body content of $^{137}\text{Cs}$ in frogs was measured in Paper II. The type of soil in a contaminated area influences the bioavailability of $^{137}\text{Cs}$ because it has a high affinity to clay minerals that make it less available and partly irreversibly bound (e.g. Sawhney, 1972; Avery, 1996). It can also bind to organic material, but here $^{137}\text{Cs}$ stays more reversibly bound (Kudelsky et al., 1996; Rigol et al., 1999). Low levels of nutrients can increase uptake of $^{137}\text{Cs}$ by plants increasing the amount of $^{137}\text{Cs}$ that is recirculated in biological systems.

**Effects of ionizing radiation**

Exposing living tissue to ionizing radiation can result in direct damage to the DNA in the cell nucleus in the form of single and double strand breaks on the DNA spiral. If breaks are not repaired correctly they can lead to cell death or loss of genetic information in the form of mutations or chromosome aberrations. Indirect damage to the DNA can arise when radiation interacts with water in the cell and free oxygen radicals are formed. The free radicals can in turn cause strand breaks on the DNA. In radiation research a linear relationship is assumed between the amount of strand breaks that are created and the received radiation dose (UNSCEAR, 2000). For example, 1 Gy creates about 1000 single strand breaks and 40 double strand breaks in a mammalian cell. Also, the number of mutations is proportional to the absorbed radiation dose. All of these types of damages are in turn dependent on the cell nucleus size and chromosome volume in a cell (e.g. Sparrow et al., 1970, Conger & Clinton, 1973), in combination with the function of the cell and the efficiency of the cell’s repair mechanisms (UNSCEAR, 2000). It is known that a high dose of ionizing radiation, given at a high dose-rate, is harmful to living organisms. However, some studies reported that a high dose-rate but low dose radiation can activate several biological processes including triggering the immune system and repair mechanisms, and augmentation of growth rate (reviewed by Ina & Sakai, 2005). In addition, Ina & Sakai (2005) found that chronic low-dose rate radiation could activate the immune system of the whole body in mice (1.2 mGy h$^{-1}$ up to 17 weeks).

Although a linear relationship is assumed between radiation dose and harmful effects in humans using cancer as a major endpoint, there is still uncertainty at lower dose ranges and regarding harmful effects from chronic low-dose rate radiation. Furthermore, FASSET (2004) identified a large knowledge gap in effect data for biota, especially concerning chronic low-dose rate radiation. There is also an ongoing debate on which endpoints to use when ensuring protection of biota. In this case, generally, populations of biota are to be protected and cancer is not chosen as an
endpoint; instead mortality, reproduction, fecundity, and morbidity have been suggested (FASSET, 2004). Also, a molecular endpoint, such as chromosome aberration, has been suggested (ICRP, 2003). However, there is a lack of knowledge concerning what level of molecular damage is harmful for populations of biota (Hinton & Bréchignac, 2005). Accordingly, in **Paper IV** effects on frogs and toads from exposure to low-dose rate radiation from \(^{137}\)Cs was examined by studying DNA damage in red blood cells in combination with effects on development, survival, and growth.

**Models**

Models are often used in science to simplify something complicated and complex. Models can generally not be constructed to exactly mirror the reality but are very useful in order to get an overview of a system, highlight the most important processes or features, and to point out knowledge gaps. However, models are dependent on good data inputs and assumptions. Three types of models are used in this thesis, a conceptual model for mass balance budget calculations (**Paper I**), dose models (**Paper II and III**) as well as phantoms of frogs to measure doses (**Paper III**).

In a conceptual model the identified significant abiotic components are illustrated as compartments or boxes and processes or interactions in-between are represented by arrows. In **paper I** a conceptual model was used as the basis for calculating a mass balance budget for \(^{137}\)Cs in a contaminated riparian wetland.

Dose models facilitate dose calculations to an organism and instead of calculating the radiation dose to all limbs and organs, a frog can be represented by an ellipsoid of a certain size. This makes it possible to apply a single calculated dose conversion factor (DCF) in calculations of the dose in the risk assessment. Currently, there is no internationally established dose model or DCFs for non-human biota but several models and DCFs have been suggested (e.g. USDOE, 2002; ISCORS, 2004; FASSET, 2003b). In general, a tiered approach is recommended for dose estimations starting with a screening and if the screening value is exceeded, continuing with a more site-specific analysis. Usually, when there are large knowledge gaps, a conservative approach is preferred to cover the uncertainties in a risk assessment and ensure sufficient protection. However, too conservative dose estimations can result in unnecessary and destructive remediation (Whicker et al., 2004). Therefore, in highly contaminated areas where a site-specific analysis is required, realistic dose models and DCFs are needed (**Paper II and III**).

The phantoms developed for this purpose in **Paper III** are made of polymethyl methacrylate (PMMA) with a size of 6 x 3 x 3 cm, based on the average size of frogs captured in **Paper II**. Thermoluminescence chips were placed in constructed holes in the phantom to measure the radiation dose to a frog in the soil of a contaminated wetland. The advantage with the use of phantoms is that it can integrate the radiation dose in a contaminated area to a whole body, and in **Paper III** to the skin, over a longer time period. Thereby, variations due to e.g. humidity in the soil can be included in the measurement. Phantoms can be used where it can be difficult to place an ordinary detector; in soil, in sediments, in water or high up in trees. Also, phantoms can provide valuable site-specific dose information that can be used in improvement and verification of existing dose models (**Paper III**).
Study sites
In paper I-III a riparian alder forest swamp in Utnora, 10 km north of the town Gävle, in the central-eastern part of Sweden was used as study site (Figure 2). This wetland is situated downstream Hille lake, which has previously been studied within radioecological research (in e.g. Sundblad, 1991; Björnstad et al., 1994; Brittain et al., 1997). The outlet stream Verkmyra from Hille Lake passes through the wetland and flows into the Baltic Sea. Every spring the Verkmyra Stream inundates the wetland causing overbank sedimentation. After the Chernobyl fallout, the wetland adjacent Verkmyra Stream was found to contain activity concentrations of $^{137}$Cs up to 2.5 MBq m$^{-2}$ (Tjärnhage et al., 2000).

![Figure 2. The study site in Utnora, in the central-eastern part of Sweden. The study site consists of a wetland area adjacent to Verkmyra stream. Verkmyra stream eventually flows into the Baltic Sea. A = alder forest swamp, B = stream bank, C = reed belt, D = alder carr, E = inner basin of the Baltic Sea, F = outer basin of the Baltic Sea, and G = spruce forest site used as reference site.](image)

In paper IV an outdoor Low Dose Rate Irradiation Facility (LoDIF) at the University of Georgia’s Savannah River Ecology Laboratory (SREL) in South Carolina, USA, was used in the exposure experiments (Figure 3). The facility consists of 40 tanks that receive water from a nearby lake in a flow-through system. Thirty tanks have a $^{137}$Cs radiation source mounted above them; the remaining tanks are used as controls. Experiments in this outdoor facility represent something between controlled but unrealistic laboratory studies and uncontrolled but realistic field experiments.
The main objectives of this thesis were:

• to clarify the redistribution processes behind the accumulation of $^{137}\text{Cs}$ in a riparian wetland adjacent to Verkmyra stream, in the central-eastern part of Sweden (Paper I).

• to estimate and measure external and internal radiation doses received by frogs living in this wetland contaminated by $^{137}\text{Cs}$ (Paper II and Paper III).

• to test if the suggested dose rate limit for aquatic populations (10 mGy d$^{-1}$) sufficiently protects amphibians by exposing frogs and toads to low-dose rate ionizing radiation from $^{137}\text{Cs}$ during critical life stages (Paper IV).

Short summary of papers

Paper I
To clarify the redistribution processes behind an accumulation of $^{137}\text{Cs}$ in a downstream wetland, a mass balance budget of $^{137}\text{Cs}$ was calculated based on soil and sediment samples and reports in the literature. Results showed that accumulation occurred over several years. Of all the $^{137}\text{Cs}$ activity discharged between 1986 and 2002 from the upstream lake, 29% was estimated to be retained in the wetland. The geometric mean activity concentration of $^{137}\text{Cs}$ in the wetland was 1200 kBq m$^{-2}$, seven times higher than in the surrounding spruce forest. In 2003, measurements showed that 17 kBq m$^{-2}$ sedimented on the stream banks of the wetland. The sedimentation of new material is balanced by the decay process of $^{137}\text{Cs}$ in some parts of the wetland. Continuing overbank sedimentation by spring flooding prolongs the time that the wetland will contain high activity concentrations of $^{137}\text{Cs}$ and may expose organisms living there over long time periods to high activity concentrations.
Paper II
Various dose models were used to estimate radiation doses to moor frogs (*Rana arvalis*) in a wetland contaminated with $^{137}$Cs. External dose estimations were based on activity concentrations of $^{137}$Cs in soil and water, considering changes in habitat over a life-cycle. Internal doses were calculated from the activity concentrations of $^{137}$Cs in moor frogs measured with a whole body counter. Depending on the dose model used, the results varied substantially. External dose rates ranged from 21 to 160 mGy y$^{-1}$, and internal dose rates varied between 1 and 14 mGy y$^{-1}$. The results show that realistic assumptions in dose models are particularly important at high levels of contamination.

Paper III
External radiation doses from $^{137}$Cs were measured using frog-phantoms of PMMA with thermoluminescence chips in a contaminated wetland area. Measured doses were compared to calculated doses based on soil samples using two dose models. The average external dose rate measured was 0.026 mGy d$^{-1}$ and calculated dose rates ranged from 0.018 to 0.128 mGy d$^{-1}$. A higher dose was measured to the phantom surfaces in comparison to the inner parts of the phantom, i.e. the sensitive skin of a frog receives the highest organ dose. The results suggest that the use of phantom measurements *in situ* could be provide valuable dose information useful for improvement and verification of dose models. They could also be a good complement to dose model calculations for external doses in a site-specific risk assessment for assuring protection of non-human biota from ionizing radiation.

Paper IV
In this low-dose rate exposure study no effects were found on any of the three species (*Scaphiopus holbrookii*, *Bufo terrestris*, and *Rana catesbeiana*) on body size and age at metamorphosis, and no effect on hatching success of eggs and larval survival for *B. terrestris* when exposed to dose rates up to 222 mGy d$^{-1}$ (and 343 mGy d$^{-1}$ for the eggs) and total doses up to 32 Gy. Also, no increase in strand breaks in DNA in red blood cells could be detected after low-dose rate exposure in the three species. The results suggest that the ecological factor of larval density has a much stronger effect on amphibian life history traits than low-dose rate ionizing radiation. Higher level of strand breaks was detected after an acute dose of 3.0 Gy than a chronic dose of 3.0 Gy over 144 days in *R. catesbeiana* tadpoles, which suggests that a dose rate limit for protection of amphibians is appropriate.
Discussion

In order to also include plant and animal populations in the radiation protection system, ongoing changes entail that several aspects has to be considered. One important aspect is to identify likely exposure scenarios for non-human biota. Wetlands are one example of ecosystems where data and scenarios are lacking. Riparian wetlands that are affected by overbank sedimentation can act as sinks for particle-bound radionuclides such as $^{137}$Cs. In paper I it was also shown that wetlands relatively small in size (0.024 km$^2$) can accumulate high activity concentrations over time. Many species, such as amphibians, crustaceans, and aquatic insects, depend on small sized wetlands (e.g. Semlitsch & Bodie, 1998) and will thereby risk coming in contact with or being exposed to the accumulated $^{137}$Cs. Another aspect in the changing radiation protection system is to identify biota that is representative of different ecosystems. ICRP and FASSET/ERICA have already started a process of choosing reference organisms for risk assessments (in the same way as there is a reference human). The criteria for a reference organism should be based on exposure probability, extent of exposure, bioaccumulation abilities, and radiosensitivity of the organism. Frogs fulfill these criteria since $^{137}$Cs tends to accumulate where frogs reside, they have a small home range, and a relatively high radiosensitivity (paper II-IV). The knowledge about the radiosensitivity of amphibians is limited, although has been reported to be within the same range as for mammals if the observation period after exposure in the experiments is prolonged due to them being poikilothermic animals (FASSET, 2003a).

The system of radiation protection in humans is far more developed and explored in comparison with other human ecotoxicological protection systems. After the nuclear bombs over Hiroshima and Nagasaki, the radiation protection of humans has made impressive progress. For example, relative biological effectiveness (RBE) values for different radiation types have been established, weighting factors for different organs with different sensitivity to radiation have been developed, and even special weighting factors for a fetus exposed to radiation has been investigated. Every new innovation has often been followed by a new unit to use in risk estimations, such as Sievert (Sv), including a weighting factor, for effective dose. For instance, a risk factor for developing cancer after radiation exposure for the public has been examined mounting to 0.05 Sv$^{-1}$ (ICRP, 1991). In contrast, no weighting factors, special units, or risk factors have been developed for non-human biota. Also, there is no internationally established dose model for biota. In paper II and III we used several different dose models that are currently being used or recently suggested for use with biota. By using several models, a picture of the variation of possible doses is gained and can be compared. Because of the high activity concentration of $^{137}$Cs in the study area and the variation in doses gained from calculations, measurements with frog-phantoms in the area were a good complement. However, the phantoms cannot create a conservative worst-case-scenario and should only be used in site-specific analyses. Due to the current large knowledge gap in the effect data, some conservatism is motivated to assure protection of biota. In addition, phantoms could be useful and provide valuable dose measurements in environmental effect studies that often lack dose information (e.g. Lamb et al., 1991; Sugg et al., 1996).

Dose models in paper II and III are based on dose conversion factors (DCFs). The most realistic DCFs are calculated for certain geometries such as an ellipsoid representing an organism, and include a limited radiation source taking in to account...
the heterogeneous distribution of $^{137}$Cs in the soil. To cover the uncertainties with heterogeneous distribution and limitations in soil sampling (Bunzl et al., 2001), a probabilistic method (Avila & Larsson, 2001) can be applied in the calculations (Paper II). However, it is important to include the residence time and home range of the organism of interest in a risk assessment because these factors will affect the extent of the exposure.

Only small effects can be expected in chronic low-dose rate ionizing radiation exposure, leading to a need for large sample sizes in effect studies. The major target for ionizing radiation is the DNA where subsequent damages can be studied as strand breaks (e.g. Jarvis & Knowles, 2003; Paper IV), micronuclei (Krauter et al., 1987; Fernandez et al., 1993), mutations (Somers, 2006), and chromosome aberrations (Ulsh et al., 2003). Since strand breaks are repaired quickly, other types of damages such as micronuclei (in large sample sizes) may be better suited for low-dose rate effect studies. Aberrations in the form of reciprocal translocations could have an impact on a population because an organism with the translocations in the germine stem cells will produce offspring that is semi-sterile (Ulsh et al., 2003). However, techniques generating large sample sizes in short time such as flow cytometry in the analysis would be preferable. Jarvis & Knowles (2003) detected a higher level of strand breaks in whole fish larvae after exposure to 1.2 mGy h$^{-1}$ for 1 and 24 h. However, given the small amount of strand breaks that low dose rates are expected to create, it is unlikely that the damage was a direct effect of radiation. Furthermore, there is still a lack of connection between increased DNA damage and subsequent effects on populations. Therefore, resource allocation changes in organisms and effects on life history traits (Congdon et al., 2001) could be suitable for population effect studies after low-dose rate exposure. For example, changes in energy assimilation and demands affecting reproduction, fecundity, mortality, and morbidity could be studied.

The dose rate limit of 10 mGy d$^{-1}$ for aquatic populations based on a review by IAEA (1992) was in this thesis found to protect the development of three amphibian species (paper IV). One debated question at present is if there should be a dose rate limit or a dose limit for protection of biota, or perhaps both. In paper IV a higher level of strand breaks was detected after an acute dose of 3.0 Gy than after a chronic dose of 3.0 Gy over 144 days. This suggests that the repair mechanisms are efficient enough for the new damage caused by low-dose rate exposure. The result might suggest that a dose rate limit for amphibians is more appropriate than a dose limit. However, misrepair leading to chromosome aberrations and mutations that can accumulate over time were not studied in paper IV. Furthermore, a dose rate of 10 mGy d$^{-1}$ would be a very high level of contamination in an area ($\geq 300$ MBq m$^{-2}$) that probably would not be acceptable to nearby living humans. Based on the predicted no-effect-concentration (PNEC) method used in risk assessments for other contaminants, ERICA (2006) suggested a screening dose rate value of 10 μGy h$^{-1}$ (0.240 mGy d$^{-1}$) for tier 2 (out of 3 tiers) in a dose assessment. This dose rate is lower than the previously suggested but foremost based on a scientific method for dealing with knowledge gaps on effects from contaminants in the same way as for other contaminants in the field of ecotoxicology, such as chemicals, and it includes a safety factor for precaution.
Ecological Risk Assessment

In ecological risk assessments (ERAs) the National Academy of Science (NAS) paradigm, which consists of four steps, is often used (Newman & Unger, 2003). The steps are: hazard identification, exposure assessment, dose-response assessment, and risk characterization (Figure 4). Hazard identification is when relevant data on the situation of interest is gathered, with contaminants of potential concern highlighted. Exposure assessment includes identifying possible pathways of exposure and estimating potential exposure. In a dose-response assessment exposure data are related to relevant effects. Finally, the risk characterization integrates all the information to assess a potential risk of adverse effects. The four papers included in this thesis address the ERA steps as presented in Figure 4.

Figure 4. Schematic figure of the four steps in an ecological risk assessment (ERA) and how the papers (I-IV) in the thesis address the different steps.

Synthesis of radiation doses estimated and measured in this thesis

In paper II and III different dose models and methods were used in an exposure assessment for the study area. The different models resulted in a range of external dose rates as presented in table 3. The exposure scenarios for a frog in the dose models are shown in figure 5. For all dose models summarized together the dose rates range from 4 to 160 mGy y\(^{-1}\), which is a large range. The lowest ranges were calculated using Microshield based on soil in dry weight indicating that the programme might underestimate the dose rate slightly, and the highest range using a dose conversion factor (DCF) from Amiro (1997) and a probabilistic approach. The Environment Agency (EA) and RESRAD-BIOTA model uses the same DCFs for an ellipsoid and assume an infinite body of soil as radiation source. The dose rates from EA’s model are higher in table 3 than from RESRAD-BIOTA because they were based on soil in dry weight and used a probabilistic approach. The assumptions in EA’s and RESRAD-BIOTA’s models result in slightly higher dose rates than using FASSET’s DCF. A probabilistic method that results in a wider range of possible dose
rates is suitable for the screening steps (tier 1 and 2) since it includes some conservatism that accounts for and covers knowledge gaps. For tier 3 and a more site-specific analysis, phantoms are suitable because they can be used to measure the actual dose (from soil in fresh weight) to an organism in an area. In this case the measured dose rates (5 – 16 mGy y\(^{-1}\)) show that the true dose rates probably are in the lower range of that estimated with a probabilistic method and FASSET’s DCF (13 - 69 mGy y\(^{-1}\)). Thus, the result in this thesis suggests that FASSET’s DCF without a probabilistic approach could be used for comparison to measurements in the field if based on soil samples in fresh weight. Since there usually is a variation in the fresh weight of soil during the year in different seasons, frog-phantoms that can integrate the dose rate over a long period could provide valuable dose information.

**Table 3.** Ranges of external radiation dose rates to frogs from \(^{137}\)Cs in a contaminated wetland in Sweden using several dose models.

<table>
<thead>
<tr>
<th>Dose model</th>
<th>Exposure scenario</th>
<th>Activity conc. based on soil in:</th>
<th>Dose rate (mGy y(^{-1}))</th>
<th>Reference</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiro, DCF</td>
<td>10 cm in soil</td>
<td>dry weight</td>
<td>49 – 160</td>
<td>1</td>
<td>II</td>
</tr>
<tr>
<td>Probabilistic method, EA, DCF</td>
<td>50 % on surface, 50 % in soil, infinite source</td>
<td>dry weight</td>
<td>21 – 96</td>
<td>2</td>
<td>II</td>
</tr>
<tr>
<td>RESRAD-BIOTA</td>
<td>on soil surface, infinite source</td>
<td>fresh weight</td>
<td>18 – 47</td>
<td>3</td>
<td>III</td>
</tr>
<tr>
<td>Probabilistic method, FASSET, DCF</td>
<td>on soil surface, 10 cm, 25 cm in soil of 50 cm</td>
<td>dry weight</td>
<td>13 – 69</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>FASSET, DCF</td>
<td>on soil surface, 10 cm, 25 cm in soil of 50 cm</td>
<td>fresh weight</td>
<td>7 – 18</td>
<td>4</td>
<td>III</td>
</tr>
<tr>
<td>Microshield</td>
<td>on soil surface</td>
<td>dry weight</td>
<td>4 – 19</td>
<td>5</td>
<td>II</td>
</tr>
<tr>
<td>Microshield</td>
<td>3 cm in soil</td>
<td>dry weight</td>
<td>5 – 22</td>
<td>5</td>
<td>II</td>
</tr>
<tr>
<td>Frog-phantom</td>
<td>5 cm in soil</td>
<td>fresh weight</td>
<td>5 – 16</td>
<td>6</td>
<td>III</td>
</tr>
</tbody>
</table>

1 = Amiro (1997)
2 = Copplestone et al. (2001)
3 = ISCORS (2004)
4 = FASSET (2003b)
5 = Microshield Version 5
6 = Paper III
Figure 5. Exposure scenarios for different dose models. 1 = Amiro (1997), 2 = EA (Copplestone et al., 2001), 3 = RESRAD-BIOTA (ISCORS, 2004), 4 = FASSET (2003b), 5 = Microshield Version 5, and 6 = Frog-phantom, in situ measurement. Arrows illustrate an infinite character of the radiation source. In models with an ellipsoid (2, 3, 4, and 6) the dose is calculated as an average to the ellipsoid. In the other two models (1 and 5) the dose is calculated to a point.

Comparison with suggested dose rate limits
None of the dose models (Table 3) produced dose rates that exceeded the suggested dose rate limits of 1 mGy d\(^{-1}\) (365 mGy y\(^{-1}\)) and 10 mGy d\(^{-1}\) (3650 mGy y\(^{-1}\)) for terrestrial and aquatic populations, respectively. Also, the dose limit or realistic environmental no effect value (ENEV) for amphibians recommended by the Canadian Nuclear Safety Commission (CNSC) of 0.2 mGy d\(^{-1}\) (80 mGy y\(^{-1}\)) is only exceeded by the Amiro (1997) and EA models (CNSC, 2001, cited in Bréchignac, 2003). Furthermore, the predicted no effect dose rate limit calculated by ERICA (2006) of 10 µGy h\(^{-1}\) (88 mGy y\(^{-1}\)) is also only exceeded by the Amiro (1997) and EA models. However, since two dose models exceeded current recommended dose limits and results from one more model is close to the dose limit values (FASSET’s DCF, 13 – 69 mGy y\(^{-1}\)) further measurements were motivated. In situ measurements with frog-phantoms resulted in dose rates well below the suggested dose limits by CNSC and ERICA. Hence, based on current knowledge, frogs in the contaminated wetland are probably not exposed to radiation doses from \(^{137}\)Cs that are harmful for the population.

Are frogs less sensitive to radiation than fish?
In low-dose rate exposures of three amphibian species (paper IV) no effects on the development, survival or growth were found due to ionizing radiation in comparison with controls. Also, no increased levels of strand breaks in red blood cells of the three species could be detected after continuous exposure to a low-dose rate level up to 9.2
mGy h\(^{-1}\). Could the fact that amphibians are in terrestrial environments, which results in a higher exposure to ionizing radiation on average, result in that frogs have evolved a better developed and efficient repair system for radiation damage than fish? Effect data reviewed by FASSET (2003a) indicate that it is not so. Chronic dose rate exposures of developing fish eggs up to 4.0 mGy h\(^{-1}\) did not have any significant effects on subsequent growth (length and weight). In contrast, Hinton et al. (2004b) found that the Japanese fish Medaka exposed to low-dose rate ionizing radiation (350 ±150 mGy d\(^{-1}\)) produced fewer viable eggs. Also, in a review Sazykina & Kryshev (2003) found that dose rates of 2 – 5 mGy d\(^{-1}\) affected the reproduction in fish.

Amphibians have not been studied to a large extent in radiation research, which results in large knowledge gaps. For example, the effects of chronic low-dose rate radiation on reproduction success of exposed juveniles have not been examined. Different species even within taxonomic groups can vary greatly in their sensitivity to contaminants. Populations of *Rana temporaria* have been found to vary in sensitivity to synergistic effects of low pH and UV-B radiation (Räsänen, 2002). Also, in a review of the global decline of amphibian populations, Blaustein & Kiesecker (2002) found that both different species and different populations of the same species could react in diverse ways to the same environmental stressor. From an evolutionary point of view, Räsänen (2002) also found that female frogs originating from an area with low pH invested in larger egg sizes than females from areas with neutral pH. This indicates a rapid evolution response to human induced changes in the environment. Perhaps the same kind of variation of sensitivity between populations and adaptive responses could be shown for exposure to chronic low-dose rate ionizing radiation. Perhaps future research will tell.

**Conclusions**

In this thesis accumulation processes of \(^{137}\)Cs after the Chernobyl fallout were studied in a riparian wetland in the central-eastern part of Sweden. A mass balance budget showed that the accumulation was continuous and resulting in high activity concentrations of \(^{137}\)Cs. Still 17 years after the fallout the sedimentation of new material was balanced by decay processes of \(^{137}\)Cs in parts of the wetland. Frogs were identified as organisms that reside where \(^{137}\)Cs tends to accumulate. Their small home ranges, external embryonic development, and thin skin also make them susceptible to radiation exposure. The estimated and measured radiation dose rates to frogs in the wetland were found to be below suggested dose rate limits. However, in this highly contaminated area, current dose models for biota resulted in a wide range of doses due to different levels of conservatism in the models. Therefore, *in situ* measurements with frog-phantoms were found to be motivated and provided valuable dose information in the assessment. The external doses were found to be higher than internal doses to frogs, and the sensitive skin of frogs received the highest dose. Furthermore, there are large knowledge gaps of effects on amphibians exposed to chronic low-dose rate ionizing radiation. Low-dose rate exposure experiments in this thesis found no increased levels of strand breaks, and no effects on development, survival or growth up to metamorphosis in three amphibian species exposed from the egg stages. The ecological factor of larval density had a stronger effect on metamorphic traits than low-dose rate ionizing radiation.
Based on the findings of this thesis several areas requiring further research can be defined. This could involve performing a survey of highly contaminated riparian wetlands in Sweden and calculate and measure doses to biota in these ecosystems. Other areas affected by similar accumulation processes such as leaching from waste dumps, ash piles etc. could also be surveyed with the perspective of protecting the environment from radiation. A further development of the frog-phantoms could be performed including construction of new phantom shapes representing other biota to provide valuable dose information in environmental studies. Also, effects of chronic low-dose rate radiation from $^{137}$Cs or other radionuclides could be examined, separately or in combination with other stressors. Endpoints could be reproduction, survival, and dispersal in the field for amphibians exposed from egg stages to reproduction, or as adults to reproduction.
Acknowledgements
I would like to take the opportunity to thank the persons who have helped me during the journey of creating this doctoral thesis. I could not have done it without you. Thank you all!

Special thanks are due to:
My supervisor Nils Kautsky, for your positive support and for your last minute help when I was finishing this thesis.
My co-advisors: Petra Wallberg, for your never ending good advice, encouragement, reading of drafts, and last minute editing of text; Torbjörn Nylén, for interesting field work trips to the swamps of Gävle; Michael Gilek, for your statistical advise and reading of drafts.
All my co-authors on the papers in this thesis, for good cooperation. I have learnt a lot.
Co-author Håkan Pettersson, thanks for believing in the idea of constructing frog-phantoms and helping me make it possible.
Co-author and friend Olga Tsyusko, for teaching me all about the Comet Assay and blood sampling. Thanks for putting in so much of your time when we did the analyses and for fun dinners in Aiken!
Co-author and foremost advisor Tom Hinton, for making it possible for me to perform the frog experiments in your lab at SREL, I appreciate it very much. Thanks also for letting me stay in your beautiful house by the Savannah River and take care of Cooter, I enjoyed that very much!
Everybody at PAR Pond lab (at SREL), Yi Yi, Dan, Koichi, Cathy, Laura, I liked working with you among your Medakas and enjoyed our lunch breaks.
My room-mates at Systems Ecology through the years, Lisa, Ninni, Kerstin, and Susanne. Thanks for putting up with me and for brightening up the day from time to time.
Everybody at Systems Ecology, and especially the Ecotox-group, for inspiration and interesting discussions.
Colleagues at SSI during my first year as a graduate student. It was fun to be able to work and help with writing the evaluation report for the environmental objective a Safe Radiation Environment in 2003. Thanks also to Lena and Inger for your expertise in the SSI lab.
My parents, mother Astrid and father Arne, for always believing in me, and your love and support.
My sister Stina with husband Dan and newborn Lukas, for your support and understanding. Lukas, I’ll teach you all about frogs when you grow up, I promise!
My grandfather Bertil, for your positive attitude to new ideas and fun stories.
My Stefan, for your love, support, and understanding in my struggles with this thesis. I am happy that I belong with you and you belong with me.

I am also grateful for doctoral research funding and travel awards from:
The Swedish Radiation Protection Authority (SSI)
The Education Program at the University of Georgia’s Savannah River Ecology Laboratory (SREL)
Faculty funding from Stockholm University’s Department of Systems Ecology
L. Namowitsky’s fundation
The Royal Academy of Science (KVA)
The Swedish Society of Radiation Physics
References


Microshield Version 5. Shielding analysis and exposure estimation from gamma radiation. Grove Engineering, USA.


SSI, 1998. Airborne gamma spectrometry measurement (137Cs) maps. SSI project 998.97 & 1075.98. SGU report FRAP2001402. Swedish Radiation Protection Authority, SSI, Sweden.


