Project Description

ZEMAD

Effects of uranium on ZEbrafish, *Danio rerio*: relationships between bioMArkers and life history traits using a Dynamic energy budget model.

1 – Research team:
This research team belongs to the Laboratory for Radioecology and Ecotoxicology (LRE) at IRSN. LRE conducts applied research for supporting the expertise of the institute on fate and effects of stressors (natural or artificial, stable or radioactive elements in isolation or in mixtures) within freshwater and terrestrial ecosystems on one hand and within the human food chain on the other hand. All the projects developed are either carried out as a part of IRSN's own research programme (ENVIRHOM), or on behalf various national (ANDRA, EDF, AREVA, etc.) or international contracts (EC-funded projects such as ERICA, PROTECT, FUTURAE). LRE acts therefore as an expert group for issues devoted to protection of the environment while conceiving and applying methods, tools, models and associated parameters in Environmental Risk Assessment (ERA). It is also present within international bodies in the field of radioprotection (e.g. International Atomic Energy Agency, United Nations Committee Energy Atomic Radiation).
The present research project was launched on the basis of preliminary results obtained within the framework of the ENVIRHOM research programme. The latter is developed since 2001 to enhance the methodology and associated knowledge to improve ecological and human risk assessment for situations of chronic low-level exposure to stressors (metals and radioactive substances) of ecosystems and human populations. This ENVIRHOM programme is widely developed at the LRE composed by a group of 23 permanent staff members, 9 PhDs, 5 Post-Docs and 5 to 10 master level students per year. Until now, 50 papers have been published under the umbrella of the ENVIRHOM programme. The LRE skills cover all science needed for pluridisciplinary environmental research science. The scientific architecture is based on three main sub-projects developed for freshwater and terrestrial ecosystems as follows:

(1) **Mobility and bioavailability of metals/radionuclides:** Understanding and modeling of the metals/radionuclides mobility with a focus on (bio)reactive transport processes and on their biogeochemical cycles within soils and sediments acting as ecosystems pollutants reservoirs. The link between speciation and bioavailability for the various sources of exposure of living organisms is also investigated (e.g. BLM-type approach).

(2) **Bioaccumulation and biological effects:** Once bioaccumulated, the biologically active fraction of the internalized metal/radionuclide may give raise to a variety of biological responses at all organisational levels. Our strategy is to focus at first on life-cycle traits at the individual level to establish dose(rate) – effects relationships. In parallel, the development of population dynamics modelling gives information on the way a response observed at the individual or subindividual level (e.g., energy budget) would propagate to population-level endpoint such as intrinsic growth rate or biomass.

(3) **Mode of actions, toxicity and detoxication mechanisms :** The main results and lessons learnt from effects observed on individual life-traits drive the investigation of elementary involved mechanisms with a focus on genotoxicity and oxidative stress known to be the primary interactions between ionizing radiation and/or metals and biomolecules. Other modes of actions are investigated if they are relevant

The research is mainly using *in vivo* models (both plants and animals) under laboratory controlled conditions. It is performed in collaboration with major French universities or public research laboratories and various international collaborations exist.

The present project leaders Christelle Adam and Béatrice Gagnaire are strongly implicated in the last research axis.

I will also be collaborating with Frederic Alonzo at the Laboratory of Ecological Modelling (IRSN/DEI/SECRE/LME), J-C Poggiale at the Laboratory of Microbiology, Geochemistry and Marine Ecology LMGEM, and Alexandre Péry at the INERIS.

2 – Project description and expected results

Abstract :

The aim of this project is to understand how the long term dynamics of *Danio rerio* are affected by exposure to environmentally relevant concentrations of waterborne uranium. Three previous theses within the LRE have worked on how uranium interacts with *Danio rerio*. Using the data collected as well as experimental protocols put in place this fourth project seeks to understand how early responses of the immune and the oxidative stress systems (measured at molecular and cellular levels) are coupled to responses measured at the level of the individual (mortality, reproduction) or even the population. The main focus of this work will be on linking these different levels of biological organisation through the DEB theory and understanding the mechanisms at stake.

Context :

This PhD project takes place within the context of pollution to the environment by radionuclides in environmentally relevant conditions (programme ENVIRHOM). Aquatic ecosystems are submitted to chronic rejections of radionuclide's coming from different anthropic sources (industry, agriculture, etc). These radionuclides can then directly interact with aquatic organisms. Fish are particularly sensitive to such pollution. The alteration of one or several biological functions can modify the fish's homeostasis and potentially affect their reproduction, their growth, and their survival thus endangering the whole population (Van der Oost et al. 2003).

Uranium is a naturally occurring trace metal, a member of the actinides group, that is widely distributed throughout the environment. Its concentration in terrestrial and aquatic ecosystems may be increased in connection with various anthropogenic contributions, originating from uses throughout the different stages of the nuclear fuel cycle (mines and waste storages sites in particular), agricultural use (phosphate-based fertilizers), research laboratories, and military and industrial use of depleted uranium.
Typical concentrations in surface waters range from below 0.01 µg/L to in excess of 1500 µg/L (WHO, 2001). Current legal drinking water limits for uranium ranges from 10 µg/L in Canada to 20 µg/L in the USA, while the World Health Organisation (WHO) recommends that uranium should not exceed 15 µg/L in drinking waters. There are no European recommended levels to protect the aquatic life from adverse effects of uranium.
Natural uranium has an average natural composition of 0.0057 % ^{234}U, 0.719 % ^{235}U and 99.275 % ^{238}U. ^{235}U and ^{238}U are the parents of series of radioactive daughters (comprising ^{234}U in the case of ^{238}U) ending with stables isotopes of lead, ^{207}Pb and ^{206}Pb respectively.

The chosen biological model is a small cyprinidae, the zebrafish *Danio rerio*, that reproduces easily and rapidly (sexually mature at 3 months) in the laboratory. This species is widely used for ecotoxicity tests, as well as for medical research in the domains of genetics, cancer or for the study of the nervous system. Furthermore its genome has been entirely sequenced.

Danio rerio exposed to waterborne uranium show signs of oxidative stress associated with membrane lipid peroxidation and the inhibition of several enzymes involved in anti-oxidative

defence (Cooley et al. 2000; Labrot et al. 1999). Several studies conducted within the Laboratory of Radioecology and Ecotoxicolgy (LRE) on uranium effects have comforted these results on *Danio rerio* using biochemical (catalase, superoxide dismutase, glutathione peroxidase activities) (Barillet et al. 2007) and molecular markers (expression of genes involved in anti-oxidative stress) (Lerebours et al., accepted). Studies are currently in progress in our laboratory to develop immunological biomarkers as well (phagocytosis, production of reactive oxygen species involved in oxidative stress).
Studies conducted within the LRE at the individual level showed that uranium could modify *Danio rerio* life traits in both early stages (delays in hatching and development/growth in larvae, larvae mortality at high concentrations) (Bourrachot *et al.* 2008) and in adults (decrease of fecundity). In that context the overall aim of the project is to link biological effects observed at different biological organization levels. One way to extrapolate effects induced by pollutant toxicity on physiological and life history traits to population dynamics is to measure how such a stress impacts the overall energy budget of an organism (Kooijman and Bedaux 1996; Kooijman 2000). Dynamic Energy Budget (DEB) models describe the acquisition of energy by food uptake and its distribution to the major physiological functions of an organism (maintenance, growth and reproduction) considered at different stages of its development. DEB models have not yet been applied to *D. rerio*. The novelty of this work consists in linking subcellular responses (biomarkers) to individual effects for an aquatic vertebrate exposed to waterborne uranium.

Research Project Aims:

The research project proposed here aims at quantifying the impact of exposure to waterborne uranium on the overall energy budget of *Danio rerio*. An important part of this work will be elucidating how much energy is allocated to the immune and anti-oxidative stress systems of the cell when uranium is present in concentrations ranging from 0 to 100µg/L. The analysis of the output of a DEB model I will build will give strength to this discussion and help interpret results.

This project revolves around two main axes:

(1)An experimental approach where we measure the activity of the immune system and the anti-oxidative system with carefully chosen biomarkers and try and link that activity to changes in the global and relative composition of the energy reservoirs of the cell (quantity of proteins, lipids and glucose) as well as metabolic parameters of the individual (such as assimilation rate and respiration).

(2)A modelling approach which consists of building a pertinent DEB model to experiment with hypothesis on impacts of waterborne uranium on the energy budget. This work will also cover defining the parameters and their biological significance, and performing an exhaustive sensitivity analysis.

The ultimate goal of this project will be in integrating the DEB model, which helps explain how the individual will be affected when exposed over long periods of time to small concentrations of uranium, to a model which will help study the possible behaviours of fish populations when exposed over long periods of time to the contaminant. Thus I will work on extrapolating effects observed at the scale of the individual to effects potentially observed at the level of the population. This will concretely be carried out by integrating the DEB model into a population dynamic model and performing bifurcation analysis to discuss different behaviours of the mathematical system in different regions of parameter space in ecological context.

A close dual collaboration with M. Kooijman from the TB department in Amsterdam and with B. Gagnaire and C. Adam from the LRE will give strong foundations for carrying out aims (1) and (2). My collaboration with the Free University of Amsterdam will be reinforced by yearly work visits.

Research strategy

It will first be necessary to synthesize all the data already available in the literature pertaining to the measure of metabolic parameters for teleostei and DEB models as well as the data obtained from the previous three thesis in the LRE on how uranium interacts with *Danio rerio* (S. Barillet, A. Lerebours in progress, S. Bourrachot, in progress) (task1). The DEB model must then be developed. It will be possible to obtain supplementary data in laboratory-controlled experiments in order to parameterize the model. These experiments should enable us to evaluate how toxic stress (measured indirectly by the perturbations of immune and anti-oxidative systems) impacts the energy budget of *Danio rerio* (tasks 2 and 3). The final step will involve extrapolations towards population levels of organisation (task 4).

Task 1: Elaboration of experimental and modelling strategy:
I will begin the project by synthesizing the literature relative to toxic pathways of heavy metals as well as the effects of these toxicants on the metabolism of Teleostei. Then I will proceed to synthesize the literature pertaining to measurements of cellular and individual metabolic parameters for Teleostei (and the protocols for these measures). Finally I will synthesize the work on Dynamic Energy Budget (DEB) models applied to Teleostei as well as DEB models combined with population dynamic models (such as matrix population models) in order to compare the uses of these different models and analyse their strengths and weakness's. My other objective will be to gather and organize all the data obtained by the previous three theses at the LRE on how uranium interacts with *Danio* rerio.
This bibliography will enable me to build the structure of this project. I will then elaborate a precise experimental strategy and know what gaps to fill in the data sets available here at the LRE.

Deliverable (publication): Critical Review on Parameters and Models used for assessing the energetic metabolism of Teleostei at different scales of biological organisation.

Task 2 : Acquiring experimental data/modelling
We will measure immunotoxicity and oxidative stress to *Danio rerio* exposed to different concentrations of waterborne uranium in order to attempt to link the activity of the defence system and anti-oxidative stress system to changes in the energy reservoir of the cell (measured as the quantity of proteins, lipids and glucose) (Smolders *et al.* 2002, Smolders *et al.* 2003).
We will also measure individual effect parameters such as respiration, feeding rate, maintenance costs, and assimilation.
During this time I will finish the elaboration of the model and perform a sensitivity analysis to understand which parameters have to most critical effect on model output. Laboratory experiments will enable me to parameterize the model.

Deliverable (publication): Biological effects of uranium on Danio rerio's cellular energy budget. I aim to submit the article to Aquatic Toxicology.

Task 3: Construction of DEB model and integrating the data obtained
The first step is the construction of the DEB model as well as defining the parameters. I will follow the DEB tele-course where I will interact with scientists working on DEB applied to different organisms. I will also make a yearly work visit to Amsterdam to work directly with the department of Theoretical Biology.
Once I have parameterized the model and integrated the necessary experimental data I will analyse the output of the DEB model taking into account the assumptions used to build the equations. I will attempt to discuss the shift in energy allocated to the immune and anti-oxidative stress systems when *Danio rerio* is exposed to uranium and link biochemical

effects of the pollutant on the cell to individual effects (which are often typical endpoints in risk assessment).

Deliverable (publication): Linking subcellular and cellular effects of exposure to uranium to individual effects with a DEB model. I aim to submit the article to Ecological Modelling.

Task 4 : From the individual to population dynamics
I will couple the DEB model to population dynamic model in order to discuss consequences of continuous exposure to environmentally relevant concentrations of uranium at the population level. I will work with methodologies put in place by Klanjscek *et al.* 2006 and Billoir *et al.* 2007.

Deliverable (publication): Linking biological effects of uranium toxicity from molecules to populations in a model vertebrate Danio rerio.

Milestones:
Milestone 1: Optimise experimental and modelling protocols (9 months)
Milestone 2: Construction, definition of parameters for model, sensitivity analysis (4 months)
Milestone 3: Acquisition of all data, analysis of model output (12 months)
Milestone 4: Extrapolating individual effects to population dynamics (8 months)
Milestone 5: Compile thesis from published papers (3 months)

Expected Output:
The results obtained from this project will contribute towards understanding flows of energy reserves on a subcellular level. This project will also contribute towards understanding the sublethal modes of action of uranium on the organism of aquatic vertebrates and give some insight on the construction of tools to evaluate effects of pollutants in the environment.
Relationships between different levels of biological organisation will be developed to gain an understanding on how the long term trajectories of ecosystems could be influenced by continuous exposure to small quantities of contaminants that often don't have immediate recognisable effects on the environment.
Finally I hope to contribute towards the development of tools for translating the biological significance of ecotoxicological data (such as cellular and molecular biomarkers).

Key scientific results of the project will also be presented at international conferences (oral contributions and posters).

References:

Barillet S, Adam C, Palluel O, Devaux A. 2007. Bioaccumulation, oxidative stress, and neurotoxicity in *Danio rerio* exposed to different isotopic compositions of uranium. *Environ Toxicol Chem* 26(3):497-505.

Billoir E, Péry A, Charles S. 2007. Integrating lethal and sublethal effects of toxic compounds into the population dynamics of *Daphnia magna*: a combination of the DEBtox and matrix population models. *Ecol. Model.* 203:204-214.

Bourrachot S, Simon O, Gilpin R. 2008. The effects of waterborne uranium on the hatching success, development, and early life stages of zebrafish (*Danio rerio)*. *Aquatic toxicology*. 90:29-36.

Cooley HM, Evans RE, Klaverkamp JF. 2000. Toxicology of dietary uranium in lake whitefish (*Coregonus clupeaformis*). *Aquatic Toxicology* 48(4):495-515.

Klanjscek T, Calwell H, Neubert M, Nisbet R. 2006. Integrating dynamic energy budgets into matrix population models. *Ecol. Model.* 196:407-420.

Kooijman SALM. 2000. Dynamic energy and mass budgets in biological systems. Cambridge, Royaume-Uni: Cambridge University Press. 424 p.

Kooijman SALM, Bedaux JJM. 1996. The analysis of aquatic toxicity data. Amsterdam, Pays-Bas: VU University Press. 149 p.

Labrot F, Narbonne JF, Ville P, Saint Denis M, Ribera D. 1999. Acute toxicity, toxicokinetics, and tissue target of lead and uranium in the clam *Corbicula fluminea* and the worm *Eisenia fetida*: comparison with the fish *Brachydanio rerio*. *Arch Environ Contam Toxicol* 36(2):167-78.

Lerebours A, Gonzalez P, Adam C, Bourdineaud J-P, Camilleri V, Garnier-Laplace J Accepted. Gene response of the zebrafish (*Danio rerio*) exposed to environmentally relevant waterborne uranium concentrations.

Smolders R, Bervoets L, De Boeck G, Blust R. 2002. Integrated conditions indices as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environ Toxicol Chem* 21:87-93.

Smolders R, De Boeck G, Blust R. 2003. Changes in cellular energy budget as a measure of whole effluent toxicity in zebrafish (*Danio rerio*). *Environ Toxicol Chem* 22:890-899.

Van der Oost R, Beyer J, Vermeulen NPE. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13(2):57-149.

WHO (2001). Depleted uranium: source, exposure and health effects. Geneva: 209