FREEBIRD Project - Fukushima Radiation Exposure & Effects in BIRD populations

Audrey STERNALSKI (Post-doctoral fellow)
Jean-Marc BONZOM,
Christelle ADAM-GUILLERMIN
Jacqueline GARNIER-LAPLACE

(IRSN/PRP-ENV/SERIS/LECO)

Workshop on uncertainties in field studies on chronic low level effects due to radiation - CEH Lancaster
Feb. 4-6th 2013
Why the FREEBIRD project?

From Chernobyl (26 April 1986)...

Many studies performed under controlled condition (lab.) to measure the effects of ionizing radiations on non-human organisms... but the majority have been realized under acute exposure condition.

Therefore, from these studies, it is difficult to identify and predict the eco-physiological and evolutionary consequences of ionizing radiations under chronic exposure on individuals in the wild.

Some studies have thus been conducted under natural conditions, mainly from bird populations.

However, two limitations exist in these studies:
- have been performed 20 years after the nuclear accident...
- ...from background radiation level measurements highly disputed;
Background: Effects of ionizing radiation exposure on wild organisms.

- **SURVIVAL**
- **RADIATION EXPOSURE**
  - **PHYSIOLOGY**
  - **PHENOTYPIC EXPRESSION**
  - **REPRODUCTION**
  - **INDIVIDUAL FITNESS**
  - **POPULATION DYNAMICS**

**CHERNOBYL**

- Lack of immediate and/or short-term data
- Natural selection, adaptation

20 years after the accident: February 4th, 2013
Background: Effects of ionizing radiation exposure on wild organisms

**Radionics Exposure**
- Internal Contamination
- External Irradiation

**Physiology**

**Phenotypic Expression**

**Survival**

**Reproduction**

**Individual Fitness**

**Population Dynamics**

**Chernobyl**

February 4th, 2013
Background

Why the FREEBIRD project?

From Chernobyl (26 April 1986)...to Fukushima (11 March 2011)

- To obtain short- & medium-term data set → to assess short-term effects of ionizing radiations on wild individuals

- To acquire accurate dosimetric measurements → from the IRSN experience

- To collect data on individual internal contamination → to assess robust dose-response effects
FREEBIRD project originality

- Project originality → **integrative approach** based on **behavioural ecology** & **eco-physiology theories**, associated to accurate dosimetric measurements

- In behavioural ecology, animal “choice” process → based on **signals**
  - **Signal**: **phenotypic trait** that informs about the **bearer quality**

- In the wild, it exists ≠ **signal types**, among which the **most used** are **pigmentary visual signals** (i.e. coloured traits)

- **Two main pigment types**, with particular properties:

  - **Melanin**
    - eu-melanin (grey to dark)
    - Pheo-melanin (ruffus to brown)

  - **Carotenoids**
    - (yellow to red)
Carotenoid pigments

Carotenoids characteristics & properties:
- not synthetizable by vertebrates
- used in different physiological functions

FOOD (i.e. environment)

Limiting resource

CAROTENOIDS

Self-maintenance function

IMMUNO-ENHANCERS

Communication function

COLORED TRAITS
(indicative of individual quality, used during mate choice and/or parent-offspring communication)

Carotenoid-based traits might thus reveal individual condition and quality
Melanin pigments & Glutathione GSH

Melanin characteristics & properties:
- melanogenesis controlled by available amount of GSH
- limiting resource in the environment
- used in different physiological functions

FOOD (i.e. environment) → Limiting resource → Tyrosine → Melanin (gluthatione) → Anti-oxidant

Melanin-based traits might thus reveal individual quality & oxidative status

Self-maintenance function

Trade-offs

Communication function

EU-MELANIN

PHEO-MELANIN

COLOURED TRAITS (indicative of social ladder)
Aims of the FREEBIRD project

1) To define key biological parameters that can inform, in real time, about organism response to their environment
→ to assess whether coloured traits can be used as such

2) To determine external irradiation and internal contamination levels that can affect individual physiology and health
→ to obtain accurate dose-response curves at individual level

3) To identify proximate physiological mechanisms (physiological and/or genetic) leading to a possible co-variation between individual coloured traits, external irradiation levels and internal contamination
→ to predict potential long-term effects on population dynamics
**General methodology**

- Study species
- Trapping methods
- Study sites

Characterisation of external irradiation level of study sites

Characterisation of individual contamination

Assessment of key biological parameters

Individual dose-response curves

---

**Methods & preliminary results**

**RADIATION EXPOSURE**

*INTERNAL CONTAMINATION*

*EXTERNAL IRRADIATION*

**PHYSIOLOGY**

**PHENOTYPIC EXPRESSION**

**IONIZING RADIATION**

**EFFECTS ON INDIVIDUAL FITNESS**
General methodology

Methods & preliminary results

- Study species
- Trapping methods
- Study sites

Characterisation of external irradiation level of study sites
Characterisation of individual contamination
Assessment of key biological parameters

Individual dose-response curves

RADIATION EXPOSURE
INTERNAL CONTAMINATION
EXTERNAL IRRADIATION

PHYSIOLOGY
PHENOTYPIC EXPRESSION

IONIZING RADIATION EFFECTS ON INDIVIDUAL FITNESS
Bird species... and a frog species

- usually display **coloured traits** (i.e. carotenoid- and/or melanin-based)
- **easy to trap and handle**
- common and distributed along a **contamination gradient**
- widely studied at **Chernobyl** → **never studied!!**

**Study species**

**Eurasian Tree sparrow**  
(*Passer montanus*)

**Varied tit**  
(*Parus varius*)

**Great tit**  
(*Parus major*)

**Japanese tree frog**  
(*Hyla japonica*)
Methods & preliminary results

Mist-net catching

Trapping methods

&

Nest-box use

Physiology

Phenotypic expression

Breeding performance
Catching within rice field

Physiology
Phenotypic expression
External irradiation level gradient

Study sites

Methods & preliminary results
External irradiation level gradient

- Tree sparrows
- 3 study sites (3 - 7 μGy/h)

Study sites

Debit de dose dans l'air (μSv/h) à 1 m du sol
- > 9.5
- 3.8 à 9.5
- 1.9 à 3.8
- 1.0 à 1.9
- < 1.0

Zone de prélèvement

Carte établie par J.M. Métivier d’après le relevé aérien du 28 août 2011 effectué par le MEXT
Methods & preliminary results

- External irradiation level gradient
- Tree sparrows
- 3 study sites (3 – 7 µGy/h)

- homogenous

- Additional feeding

Study sites

Carte établie par J.M. Métivier d’après le relevé aérien du 28 août 2011 effectué par le MEXT
External irradiation level gradient

- Tit species
- 15 nest-boxes (3 – 21 µGy/h)

Study sites

Homogenous

Carte établie par J.M. Métivier d'après le relevé aérien du 28 août 2011 effectué par le MEXT
**Methods & preliminary results**

- **External irradiation level gradient**
  - Japanese tree frog
  - 5 study sites (0.3 – 8 µGy/h)

- **Homogenous**

---

**Study sites**

- External irradiation level gradient
  - Japanese tree frog
  - 5 study sites (0.3 – 8 µGy/h)

- Homogenous
General methodology

Study species
Trapping method
Study sites

Characterisation of external irradiation level of study sites

Characterisation of individual contamination

Assessment of key biological parameters

Individual dose-response curves

RADIATION EXPOSURE
INTERNAL CONTAMINATION
EXTERNAL IRRADIATION

PHYSIOLOGY
PHENOTYPIC EXPRESSION

IONIZING RADIATION EFFECTS ON INDIVIDUAL FITNESS
Dosimetry: external irradiation level

Combination of **passive** & **active dose measurements**

**Dosimeters (RPL)**
- integration time = 6 months
- 3 ≠ heights (10 cm / 1m / 2m)

**Active Measurement**
- Radiameter
- integration time = real time output dose
- 3 ≠ heights (10 cm / 1m / 2m)
Dosimetry: external irradiation level

- Measurement type "Passive vs Active" effect: higher recorded radiation level with passive measurement;

- Location "10cm vs 1 & 2m" effect: higher recorded radiation level on the ground;

Ex: site S03

Dosimetry: external irradiation level
Characterisation of external irradiation level

Source
N. Dubourg

Ex: site S03

Dosimetry: external irradiation level
Characterisation of external irradiation level

Source
N. Dubourg
Dosimetry: external irradiation level

- **Tree sparrows**
  - 3-7 µGy/h

- **Varied & Great tits**
  - 3-21 µGy/h

- **Japanese tree frog**
  - 0.3-8 µGy/h

---

**Methods & preliminary results**

Characterisation of external irradiation level

**Dosimetry: external irradiation level**

**Tree sparrows**
- Study sites: S05, S03, S06
- Levels: 3, 5, 8 µGy/h

**Varied & Great tits**
- Study sites: N1, N2, N3, N5, N6, N7, N8, N9
- Levels: 1, 2, 6, 9, 15, 21 µGy/h

**Japanese tree frog**
- Study sites: T, F, E, G, C
- Levels: 0.3, 1, 3, 6, 8 µGy/h

---

Workshop
CEH Lancaster
February 4th, 2013
General methodology

Methods & preliminary results

- Study species
- Trapping method
- Study sites

Characterisation of external irradiation level of study sites

Characterisation of individual contamination

Assessment of key biological parameters

Individual dose-response curves

RADIATION EXPOSURE
- INTERNAL CONTAMINATION
- EXTERNAL IRRADIATION

PHYSIOLOGY
- PHENOTYPIC EXPRESSION

IONIZING RADIATION EFFECTS ON INDIVIDUAL FITNESS

February 4th, 2013
Internal individual contamination

Method: in the field

Integration time = 600 sec → trade-off between measure accuracy & bird stress

Background measurement and then individual measurement

Currently analysing

Methods & preliminary results

Characterisation of individual contamination

Workshop

CEH Lancaster

February 4th, 2013
Internal individual contamination

Method: in the field / in the lab

Individual capture in the field

Acid-wet digestion on dryied individuals → measurement of whole body contamination by spectrogamma technique (germanium detector)
Internal individual contamination

Site effect on individual whole body contamination:

**GENMOD**: $F_{6,118} = 178.19, p < 0.0001$

External irradiation level effect on individual whole body contamination:

**GLIMMIX** (random Study site):

$F_{1,5.56} = 40.63, p = 0.0009$

Individual whole body contamination differs between study sites and increase with external irradiation level
General methodology

Study species
Trapping method
Study sites

Characterisation of external irradiation level of study sites

Characterisation of individual contamination

Assessment of key biological parameters

Individual dose-response curves
Key biological parameters

- **Physiological parameters**

- **Methods**
  - from blood sample
  - feathers
  - and tissues

- **Results**
  - centrifugation
  - plasma
  - pellet
### Physiological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colouration</td>
<td>Carotenoids</td>
</tr>
<tr>
<td></td>
<td>Vit. A/E</td>
</tr>
<tr>
<td>Immune system</td>
<td>Blood smear test</td>
</tr>
<tr>
<td></td>
<td>Plasmatic lysis</td>
</tr>
<tr>
<td></td>
<td>capacity</td>
</tr>
<tr>
<td>Oxidative status</td>
<td>TBARS</td>
</tr>
<tr>
<td></td>
<td>Oxide nitric</td>
</tr>
<tr>
<td></td>
<td>Glutathione</td>
</tr>
<tr>
<td>Hormones</td>
<td>Testosterone</td>
</tr>
<tr>
<td></td>
<td>Corticosterone</td>
</tr>
</tbody>
</table>
### Physiological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colouration</td>
<td>Carotenoids</td>
</tr>
<tr>
<td></td>
<td>Vit. A/E</td>
</tr>
<tr>
<td>Immune system</td>
<td>Plasmatic lysis capacity</td>
</tr>
<tr>
<td>Oxidative status</td>
<td>TBARS</td>
</tr>
<tr>
<td></td>
<td>Oxide nitric</td>
</tr>
<tr>
<td></td>
<td>Glutathione</td>
</tr>
<tr>
<td>Hormones</td>
<td>Testosterone</td>
</tr>
<tr>
<td></td>
<td>Corticosterone</td>
</tr>
</tbody>
</table>

#### Key biological parameters

**External irradiation (µGy/h) effects on number of micronuclei (per 1000 counted RBC cells):**

**GENMOD (Distr. Poisson):** $F_{2,61} = 2.69, p = 0.0757$
### Genetic parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA damage</td>
<td>Single strand DNA break</td>
</tr>
<tr>
<td>Epigenetic</td>
<td>DNA methylation</td>
</tr>
<tr>
<td>Gene expression</td>
<td>4 focus genes</td>
</tr>
</tbody>
</table>

**Key biological parameters**

Methods & preliminary results

Workshop

CEH Lancaster

February 4th, 2013
**Key biological parameters**

- **Phenotypic expression**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body condition</td>
<td>Biometric measures</td>
</tr>
</tbody>
</table>

**Methods & preliminary results**

GLMM (Random ‘nest-box’):

\[ F_{1,29} = 2.06, p = 0.1623 \]

External irradiation (\(\mu\)Gy/h)

- (Nest-boxes): 10.90 (N11), 12.37 (N2), 12.90 (N4), 14.00 (N3), 14.20 (N6), 21.40 (N5)

- Varied tit (\(Parus\) \textit{varius})
- Great tit (\(Parus\) \textit{major})
### Methods & preliminary results

#### Key biological parameters

- **Phenotypic expression**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body condition</td>
<td>Biometric measures</td>
</tr>
<tr>
<td>Coloured traits</td>
<td>Picture analyse</td>
</tr>
<tr>
<td>(carotenoids / melanin)</td>
<td>Carotenoids in feathers &amp; tissues</td>
</tr>
</tbody>
</table>
Acknowledgement

C. Xerri – Ambassade de France au Japon, E. Simon & K. Mimata for logistic

N. Dubourg & JF Guerre-Chaley – IRSN, for dosimetric measurements & analysis

Every lucky meeting during fieldwork... as K. Inoue

Japanese colleagues, Prof. K. Ueda-sensei, S. Kasahara & S. Matsui, for their great help in Japan... and their good moon!