2nd DULIA-bio Workshop

4-5 November 2019
Laboratori Nazionali del Gran Sasso

The RENOIR Experiment at the LNGS

"Radiation ENvirOnment triggers biological Responses in flies: physical and biological mechanisms"

G. Esposito, P. Anello, M. Ampollini, E. Bortolin, C. De Angelis, V. Dini, C. Nuccetelli, C. Quattrini, **M.A. Tabocchini**, C. Tomei, P. Morciano, A. Ianni, M. Balata, G. Carinci, M. Chiti, O. Frasciello, G. Cenci, F. Cipressa, A. De Gregorio, A. Porrazzo, E. Alesse, A. Tessitore

Istituto Superiore di Sanità

INFN Sezione di Roma — INFN Roma 1

INFN LNGS — INFN-LNGS

INFN — INFN LNF

Sapienza Università di Roma

NM State — WIPP Waste Isolation Pilot Plant

Flinders University

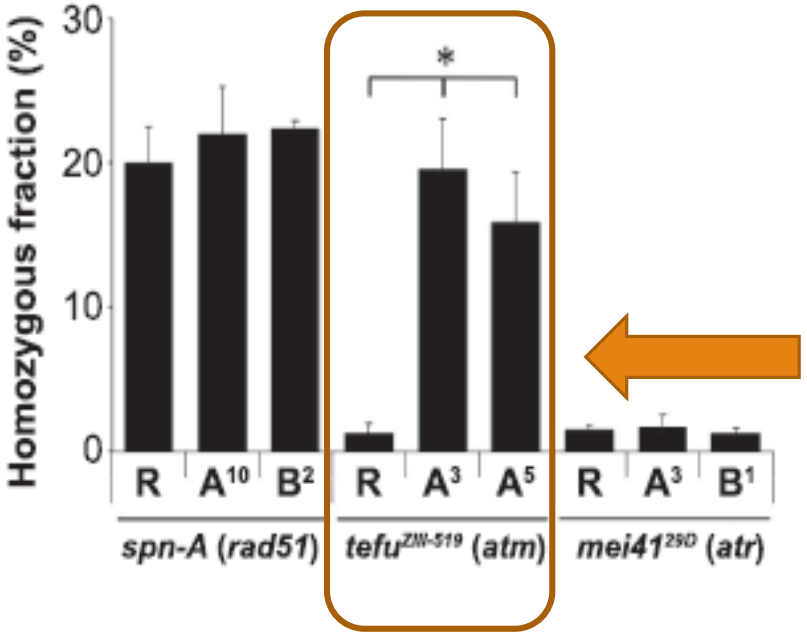
Centro Fermi — Museo Storico della Fisica e Centro Studi e Ricerche Enrico Fermi

ENEA

The LNGS results on Drosophila melanogaster

So far, we collected and published the first data at organism level using different *Drosophila* strains grown in parallel in low radiation environment at LNGS (LRE) and in the reference laboratory (RRE)

R = reference flies (at RRE)
A, B = different generations at LRE

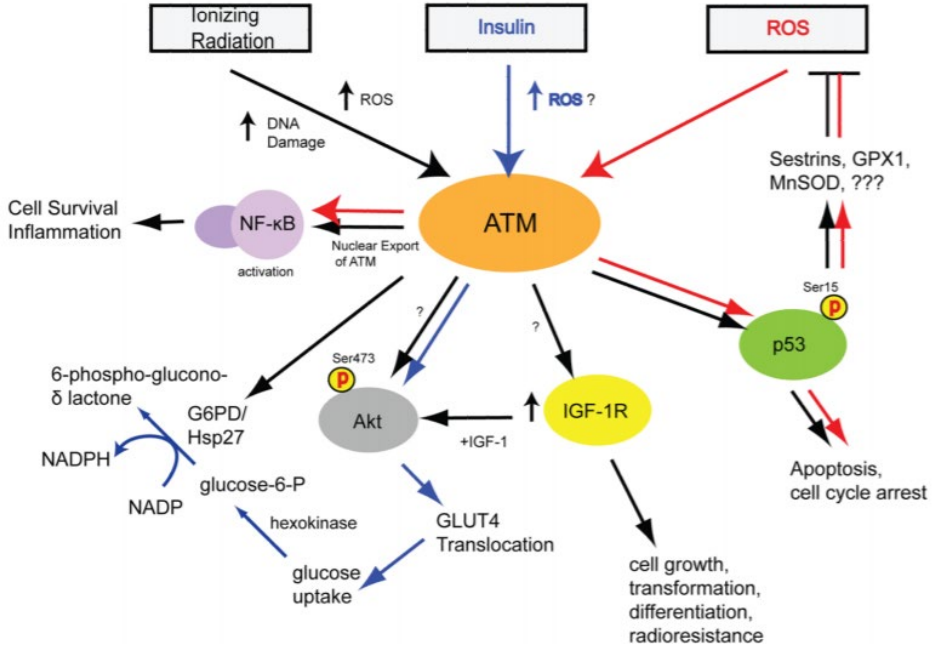


End point	Result
Life span	Increased life span (up to 15%) in flies growing at LRE: effect observed after 1 generation and maintained constant for several generations
Fertility	Reduced fertility (up to 40%) of both male and female adults growing at LRE: effect observed after 1 generation and maintained constant for several generations
DNA repair	Positive selection on the survival of mutant atm/tefu homozygous flies (with little ATM protein) at LRE: surprising effect observed even when mutant flies are moved back to RRE

(Morciano et al. J Cell Physiol 2018; Morciano et al. Radiat Res 2018)

ATM** is crucial protein of the DNA Damage Response (DDR), involved in different pathways, among them in the **early sensing** of DNA **double strand breaks

- *It is resonable to expect that the fitness of organisms lacking in ATM is strongly reduced in the **above ground environment**, where radiation represent a constant stimulus in the day life*



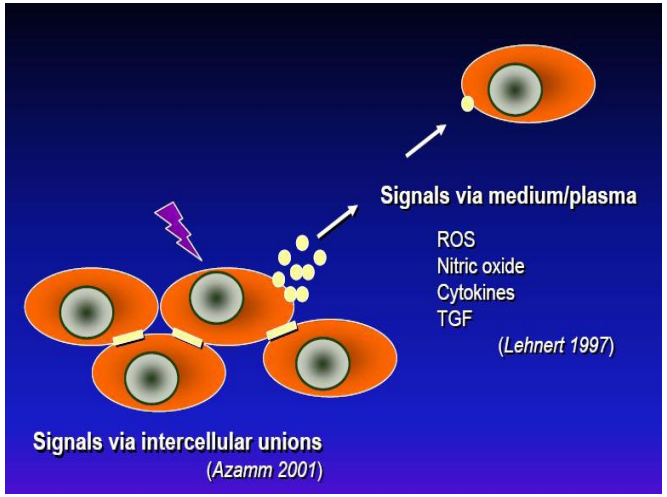
- *In a **«cosmic silence» environment** no activation of DDR is needed so individuals lacking in ATM are not disadvantaged*

It is interesting to note that *Drosophila melanogaster*, well known to be a radioresistant organism, responds so promptly to changes in the environmental radiation

- ***The doses/fluences of concern are so low that we should speculate about the triggering of bystander mechanisms, typical of the so-called «non-targeted effects», that involve cell-cell communication phenomena for amplifying such small signal(s)***

Bystander mechanisms

The target for radiation damage is greater than the initial irradiated volume



RENOIR experimental plans for the next 3 years

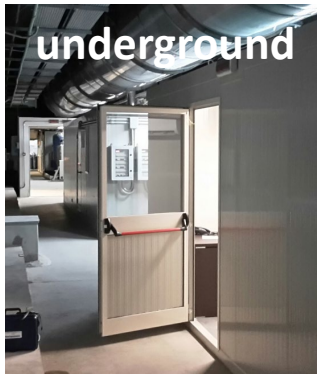
Two main aims:

1. To improve the knowledge of the radiation field inside the external (reference) and underground laboratories, with dosimetric and spectroscopic measurements and with simulations

2. To obtain information about the involvement of the different components of the radiation field, starting from gamma rays, on the biological responses of the fruit fly Drosophila *melanogaster*

The radiation environment characterization of the different experimental sites where the biological experiments are performed **is crucial for the interpretation of results**

We have scheduled a ***new campaign of measurements underground*** *inside the* ***COSMIC SILENCE facility*** ***and in the reference external LNGS laboratory (located at the Servizio di Chimica e Impianti Chimici)***



Measurements will include the **characterization of the gamma field** through:

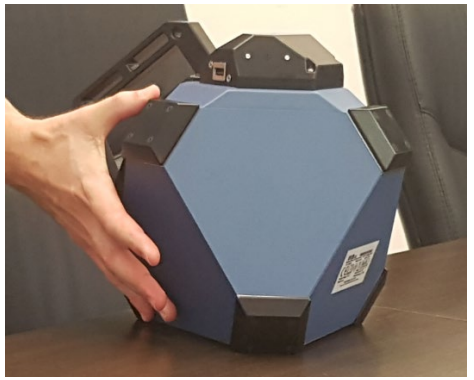
a) gamma **dose rate** measurements with Reuter Stokes, Automess and TLD 700H

b) in field **gamma spectrometry** with portable HpGe spectrometer

As for the **neutron component**:

so far, ***measurements have been carried out using BF_3 detectors*** *(in horizontal and vertical position)* ***in the bypass of the Pulex-Cosmic Silence area, showing a "geometric effect"*** *(observed range: 0.12 nSv/h - 0.55 nSv/h)*

We are presently in touch with Politecnico di Milano colleagues for testing the sensitivity of their ***neutron spettrometer*** *in our labs*



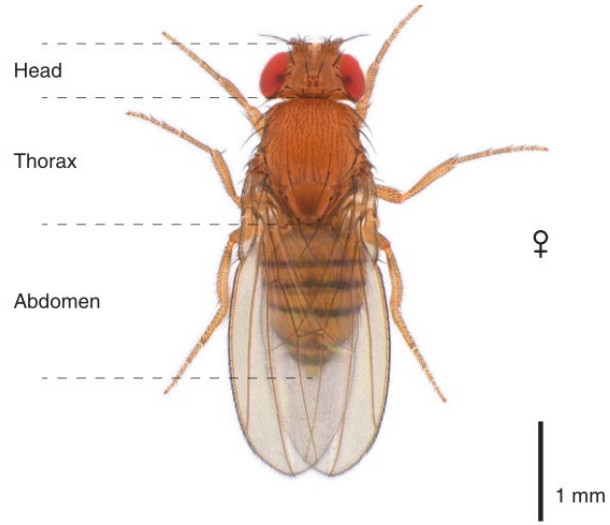
We will try to get information on neutron dose rate by comparing ***TLD 600H and 700H***

Other detectors/approaches ???

Simulations by means of FLUKA, GEANT4 and MCNP6 Monte Carlo codes will give information on the **fluxes/doses to the biological target** induced by **gamma rays and neutrons** both outside and inside LNGS laboratories

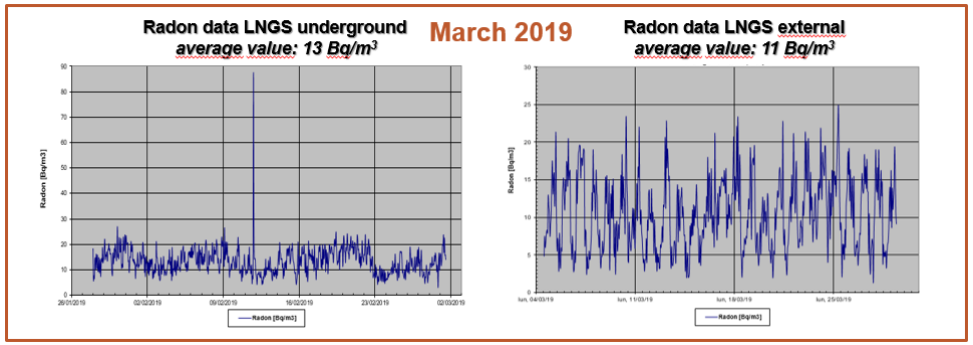
A ***Drosophila* geometric model** will also be implemented in the simulations

INPUT
***gamma ray flux spectrum** measured both outside and inside LNGS laboratories*
***neutron flux spectrum** both outside and inside LNGS laboratories obtained by literature data and possibly measurements*



Simulation results will also help to **optimize the experimental set up**

The first planned experiments will be focused **to evaluate whether the restoring of underground radon level to values similar to the external one have any influence on our previous findings**



- *We will carry out the **fertility analysis** and the maintenance of **positive selection of specific DNA repair mutants** already identified in previous experiments **as a quick and reliable "sentinel" tests***
- *Furthermore, we will study the **molecular mechanisms** underlying the influence of environmental radiation in Drosophila **using different mutants** and undertaking **genome wide approaches to understand which changes at both protein and DNA/RNA levels undergo flies kept at LRE with respect to RRE***

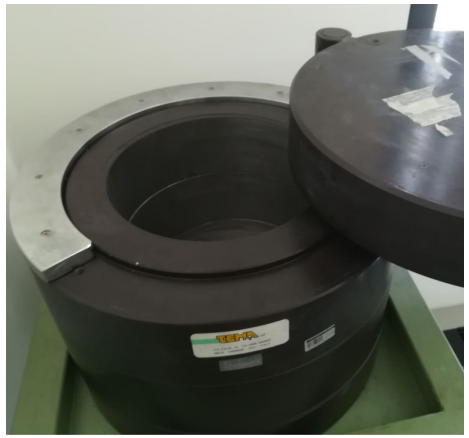
We will also focus on the possibility to modify the external environment (RRE now at the LNGS) using shielding or natural sources (tuff/pozzolana) to reduce or increase the gamma component

***We already collected in vitro evidence**, changing the location of the reference laboratory or removing the 5 cm thick iron shield* around *the PULEX cell culture incubator, **that a limited reduction of the gamma dose rate does not significantly change the biological response.** We want further investigate this aspect in vivo, possibly making negligible this component*

To this aim we will adapt to our purposes a standard gamma spectrometry shield. It is 10 cm thick Pb hollow cylinder, that allows the reduction of the gamma component by some orderd of magnitude

TLD will be used to monitor the dose rate inside and outside the shield

*Biological sample will be put inside **the hollow cylinder that will be further shielded in the bottom. Small holes** through the bottom shield will allow the **passage of humidified warmed air and light** (to provide a day/night cycle)*

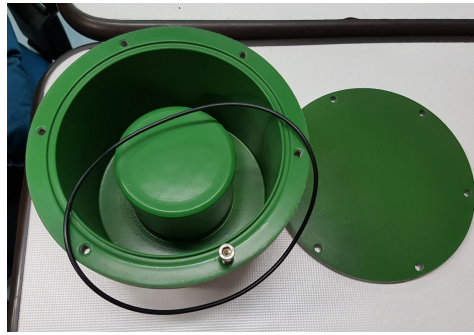


Shielding the gamma component

We want also to increase the gamma exposure obtaining a spectrum similar to the external radiation background

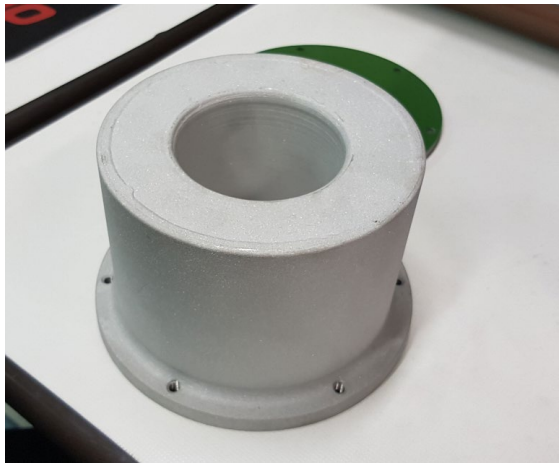
To this purpose, we will realize and use large "Marinelli beakers", able to fit all the available volume inside the shield, filled with natural building material *(tuff and/or pozzolana)* **and sealed to avoid any radon exposure**

As an example, after filling the toroidal volume of a standard Marinelli beaker (3 l volume) with a mix of tuff and/or pozzolana we have carried out at ISS dose rate measurement inside the inner hole (8.5 cm dia x 8 cm h) using the Automess dose meter, obtaining a value above background of at least 150 nSv/h



With this approach we are able to increase the gamma-component underground by a factor of about 10

Starting from the value of about 20 nSv/h, that is the gamma-dose rate contribution at LNGS, we can gradually increase the gamma-dose rate value up to the external value and even beyond. We are planning to test 3 different dose-rates in the interval 20-150 nSv/h



	LNGS RRE w Pb-shield	LNGS External (RRE)	LNGS LRE w Tuff/Pozzolana	LNGS LRE w Pb-shield	LNGS Underground (LRE)	LNGS LRE w Tuff/Pozzolana
Photons and directly ionizing cosmic rays (low LET) *(nSv/h)*		**41.5**[a]			**negligible**	
Neutrons from cosmic rays (high LET) *(nSv/h)*		**21.2**[a]			**0.12-0.55***	
Total γ-rays (terrestrial, low LET) *(nSv/h)*	**<<<**	**22**[b]	***> 100 above bk***	**<<<**	**20**[c]	***> 100 above bk***
^{40}K (internal exposure, low LET) *(nSv/h)*		***19***[a]			***19***[a]	
^{222}Rn (high LET) *(Bq/m^3)*		***11***			***13***	

(a) Evaluation based on UNSCEAR 2008 (United Nations Scientific Committee on the Effects of Atomic Radiation) Vol I. Sources and Effects of Ionizing Radiation
(b) G. Di Carlo, personal communication
(c) Reuter Stokes, Automess and TLD measurements (just terrestrial, no cosmic rays)

* *neutrons measurements **underground** (dependent on the device axis of measurement)*

Previously: γ-rays at UNIVAQ (RRE) = 75 nSv/h ; radon at LNGS (LRE) = 72 Bq/m^3

Milestones	Year 1	Year 2	Year 3
M1	0-34		
M2	0-10m		
M3	0-10m		
M4		10-22m	
M5			22-34
M6		10-36	

M1. Characterization of the radiation environment in the scenarios of interest: measurements and simulations

M2. Biological measurements underground and in the reference lab to confirm the previous results in similar conditions of radon concentration

M3. Implementation of devices for biological measurements after modulation of the gamma dose rate

M4. Biological measurements at the external laboratory in conditions of shielded γ-rays and underground after increasing the gamma dose rates

M5. Biological measurements underground in conditions of shielded γ-rays and at the external laboratory after increasing the gamma dose rates

M6. Statistical analysis of the biological results

Experimental plan – GANTT CHART

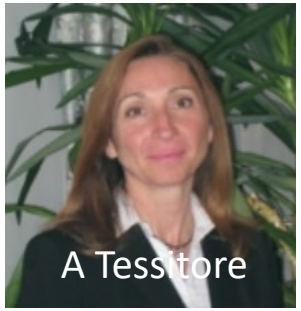
The COLLABORATION

Institutions involved:

1. **Istituto Superiore di Sanità** (Centro nazionale tecnologie innovative in sanità pubblica; Centro nazionale protezione dalle Radiazioni e fisica computazionale; Servizio grandi strumentazioni e core facility) **and INFN-Sezione di Roma 1**
2. **INFN - LNGS** (Divisione Ricerca; Servizio di Chimica e Impianti Chimici)
3. **INFN - LNF** (Servizi tecnici della Fisica Sanitaria)
4. Department of Clinical and Biotechnological Sciences, **L'Aquila University**
5. Department of Biology & Biotechnology "C. Darwin", Section of Genetics, **"La Sapienza" University, Rome**
6. ***Radon laboratory*** *of Istituto Nazionale di Metrologia delle Radiazioni Ionizzanti (**INMRI**) of Ente nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile (**ENEA**)*

Other collaborations:

- **New Mexico State University and WIPP** Facility, underground repository, **New Mexico, USA**
- **Flinders University, Adelaide, Australia**



On behalf of the PULEX-COSMIC SILENCE collaboration

Thank you for the attention !