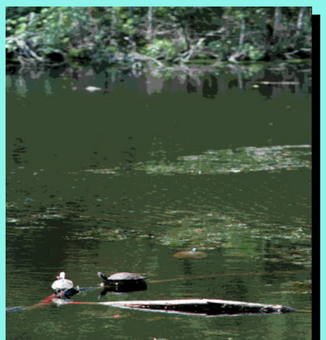
Determining Significant Endpoints for Ecological Risk Analysis



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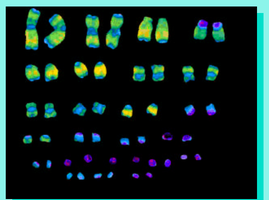


We would like to acknowledge our research associates: Maria Muhlmann-Diaz (CSU), Brant Ulsh (CSU), and Bill Hopkins (SREL)

Introduction

- Human and Ecological Risk analyses are necessary for determining:
  - which DOE sites should be remediated, and
  - DOE compliance with performance standards

- In sharp contrast to human risk analyses, the methods, endpoints and interpretation of ecological risk analyses are still being developed and lack standardization

Fundamental Differences In Human and Ecological Risk Analyses

Type	Unit of Observation	Endpoint	Dose-Response
Human	individual	lifetime cancer risk	relationships established
Ecological	varies	varies	not established

individual, population, community, ecosystem

molecular or cellular abnormality, < fecundity, > mortality ???

for chronic, low level exposure to radiation, alone, or mixed with other contaminants

Problem

- DOE faces complex decisions regarding clean-up of contaminated sites
- DOE needs a scientifically defensible protocol for measuring ecological risks
- A sound protocol is only possible when the relationship between sublethal, cellular damage is made with the performance of individuals and populations exposed to:
  - chronic, low-level radiation, and
  - radiation in combination with chemical contaminants

Potential Solution

- Assess ecological risks by coupling molecular damage to effects observed at the individual and population levels of biological organization

Research Objectives

Test the hypothesis that:

Sublethal Molecular Damage

leads to:

Changes in Metabolic Rate and Energy Allocation

leads to:

Changes in: Age-specific Survivorship, Reproductive Output, Age at Maturity, Longevity

Determination of Risk requires knowledge of:

- Site Characterization
- Environmental Transport
- Dose - Response Relationships

Our Emphasis To Date Has Been:

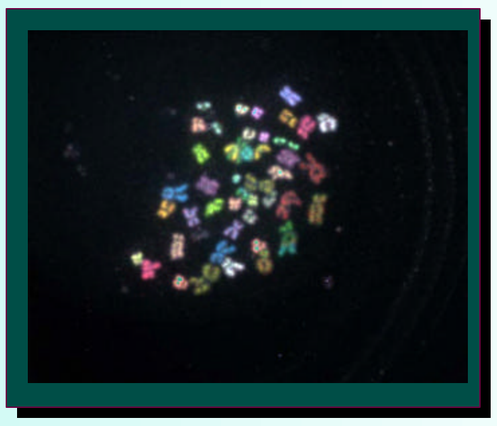
1) developing a biological dosimeter that reflects cumulative damage (panel #2)
2) quantifying responses of organisms exposed to contaminants (panel #3)
3) constructing an irradiation - mesocosm facility (panel #4)

Biological Dosimeter

Reciprocal translocation in turtle chromosome #1

- We have developed, what is to our knowledge, the first whole-chromosome specific probe for ecological risk analyses, using microdissection and polymerase chain reaction amplification techniques
- Using fluorescent *in situ* hybridization (FISH), the probe reflects cumulative damage in exposed individuals by detecting a stable form of chromosome damage called reciprocal translocations
- The probe detects chromosome exchanges that are biologically relevant (i.e. implicated in turmorigenesis in mammals and a reduction in reproductive success)

Our molecular probe provides a sensitive, biologically relevant endpoint that serves as a record of the organism's lifetime cumulative exposure.

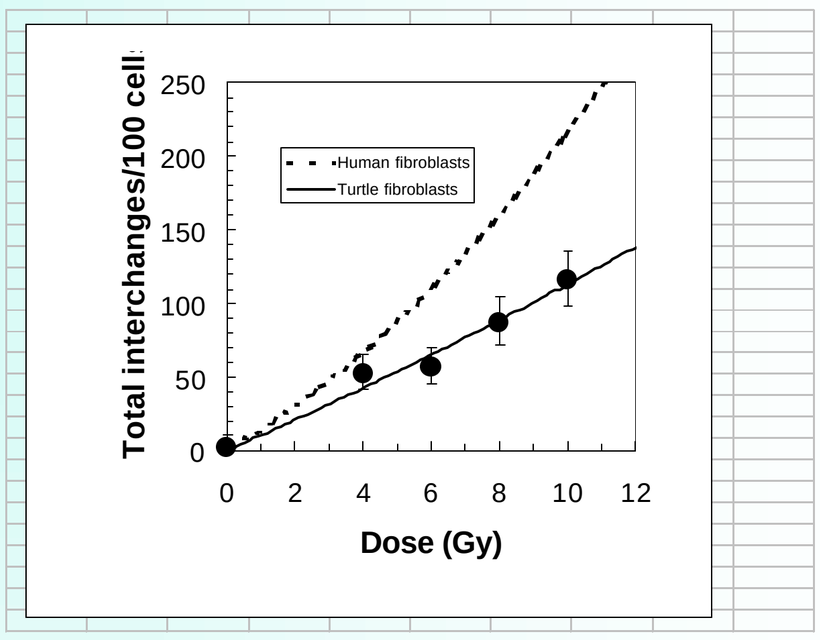


Multiple probes (mFISH) for human cell; currently we have developed two such probes for turtles

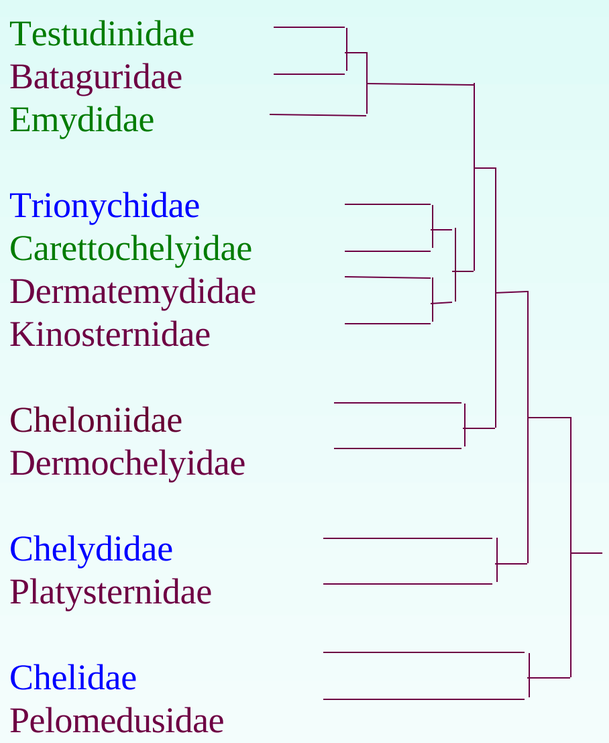
The probe was developed for a common turtle species, the yellow-bellied slider.

- long-lived (30 y)
- found in numerous contaminated locations on DOE sites
- can accumulate an appreciable dose
- has a wide geographical range

Frequencies of detected chromosomal exchanges are highly correlated with dose ($R^2 > 0.95$)



Turtle fibroblasts appear to be about twice as radioresistant for aberration induction as human fibroblasts.



Because the same species do not occur at all DOE sites, we are encouraged that:

- **the probe has wide applicability across numerous species**
  (it has worked in five genera of turtles within three different families, coded in green, and is being tested on three others, coded in blue)
- **it may also be a useful tool for comparative genomics**

MANUSCRIPTS

- Chromosome Translocations in Turtles: A Biomarker in a Sentinel Animal for Ecological Dosimetry. B. Ulsh, M. Muhlmann-Diaz, W. Whicker, T. Hinton, J. Congdon, and J. Bedford. Submitted to *Radiation Research*
- Culture Methods for Turtle Lymphocytes. B. Ulsh, J. Congdon, T. Hinton, W. Whicker, and J. Bedford. Submitted to *Copeia*
- Conservation of Chromosome-1 in Turtles over 66 Million Years. M. Muhlmann-Diaz, B. Ulsh, W. Whicker, T Hinton, J. Congdon, J. Robinson, J. Bedford. Submitted to *Trends in Ecology and Evolution*
- Chromosome Translocations in *T. scripta*: The Dose-Rate Effect and *In Vivo* Lymphocyte Radiation Response. B. Ulsh, W. Whicker, T. Hinton, J. Congdon and J. Bedford. Submitted to *Radiation Research*

for more information on this aspect of our work
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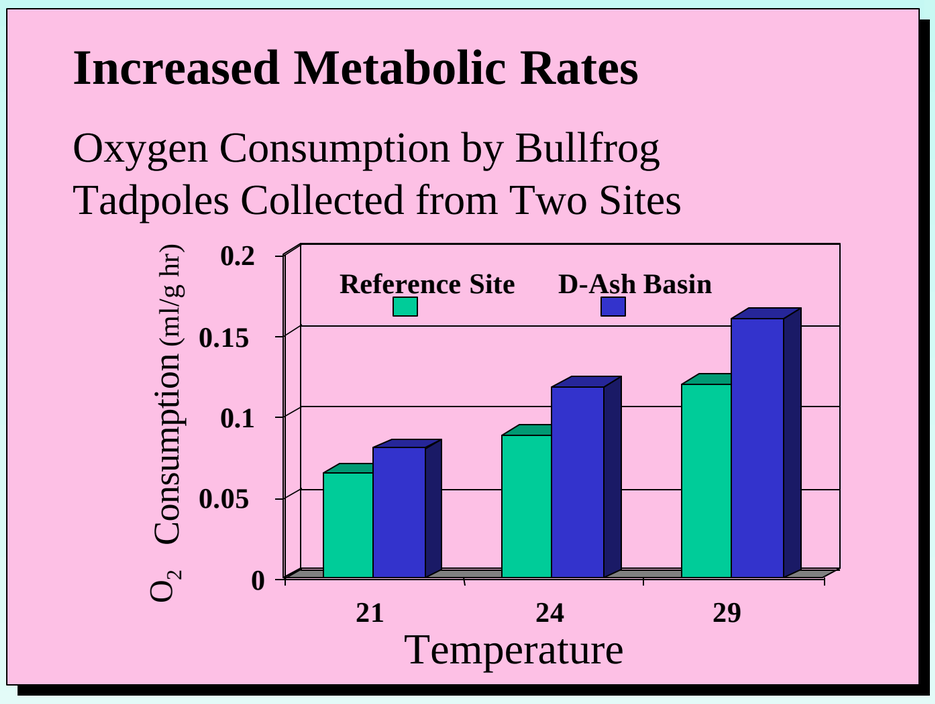
Metabolic Rate as an Indicator of Stress from Contaminant Exposure

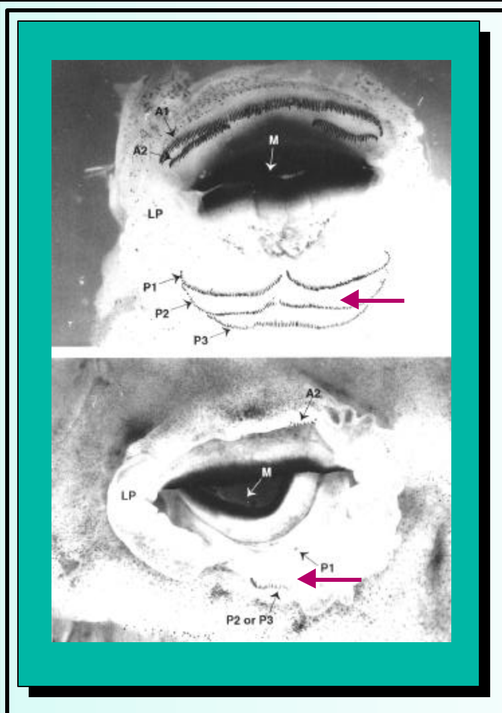
- Metabolic rate measures an animal's cost of maintenance or activity

- Changes in metabolic rates have implications for processes such as energy storage, growth and reproduction

- Metabolic rate of exposed organisms:
  - may be an excellent physiological measure of sublethal stress because it reflects multiple processes occurring within an organism
  - may be a criterion for determining whether cellular effects from contaminant exposure are relevant to higher levels of biological organization

- We have documented increased metabolic rates in numerous species of animals exposed to nonradioactive contaminants from a coal ash basin





Typical bullfrog tadpole mouth structure

Deformed bullfrog tadpole mouth from D-Area ash basin

Oral deformities are found in > 90 % of ash basin tadpoles.

Tadpoles have:

1) elevated levels of As and Se

2) increased metabolic rates

Bullfrog juveniles

As: 15.6 ± 4.4 ppm

Se: 26.9 ± 0.3 ppm

Bullfrog tadpoles

As: 48.9 ± 2.6 ppm

Se: 25.7 ± 3.6 ppm

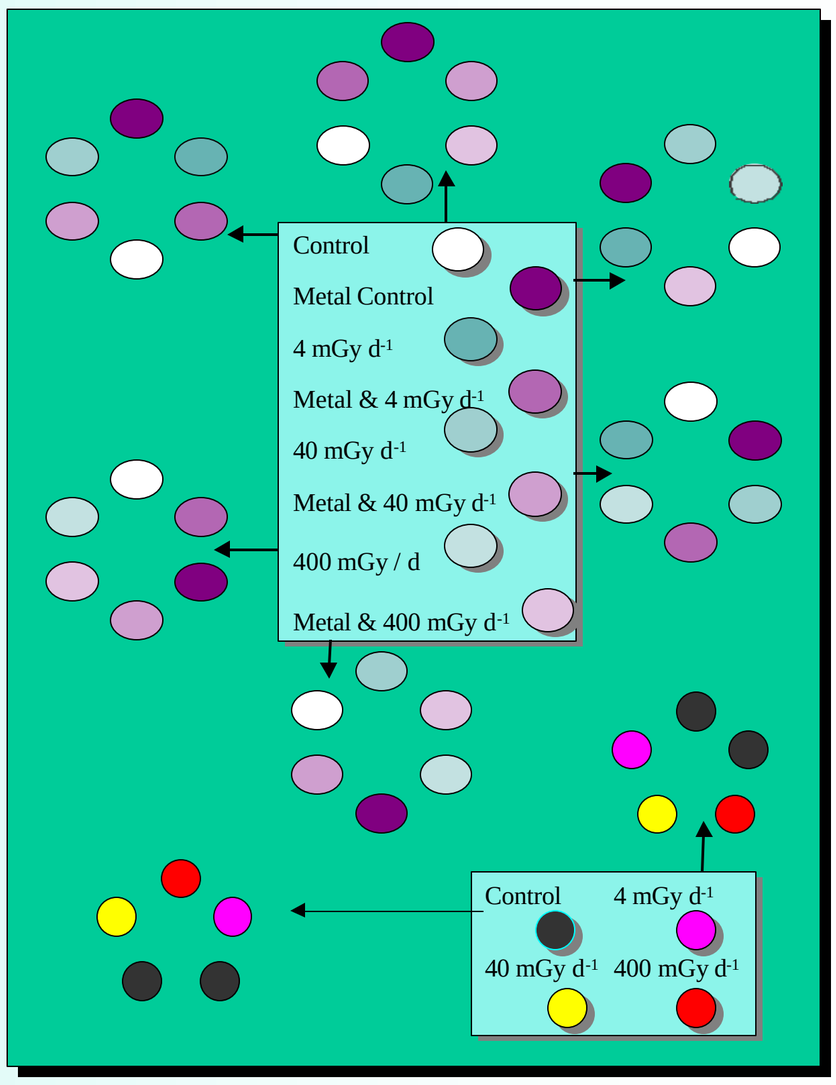
Currently we are testing:

- whether metabolic rates increase in animals exposed to chronic, low level radiation
- if there are synergistic effects when organisms are exposed to both radioactive and non-radioactive contaminants
- how previous exposures to contaminants alter the metabolic response from subsequent exposures

for more information on this aspect of our work contact:
Dr. Justin Congdon; 803 725-2472; congdon@srel.edu

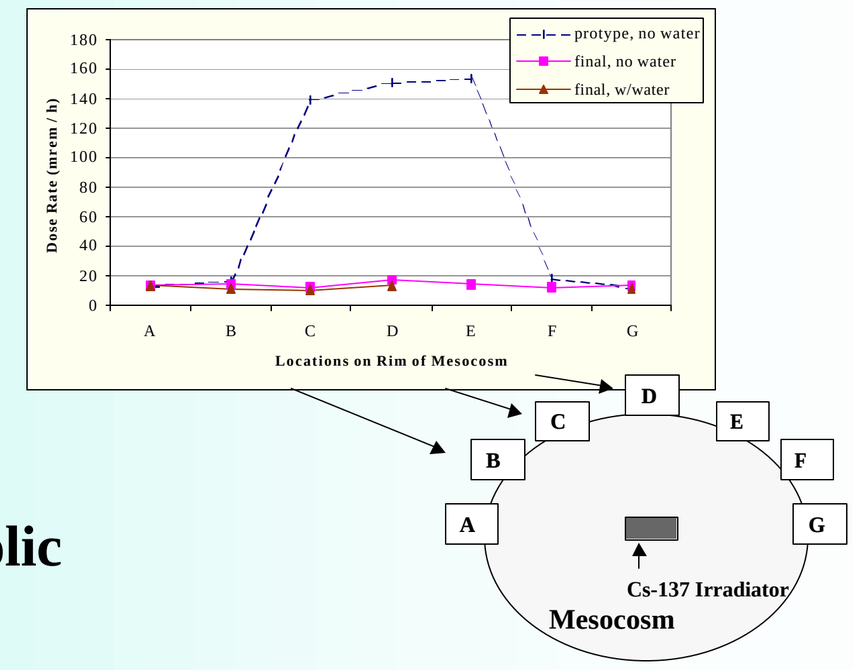
Irradiation - Mesocosm Facility

- Animals can be reared in 50 outdoor mesocosms
- Replicate treatments and powerful statistical methods are possible
- Each mesocosm has a sealed ^{137}Cs source located above it
- Experiments can be conducted using radiation, heavy metals, or a combination of the two



Array of mesocosms at the irradiation facility showing various treatments

- Radiation exposures to animals can be quantified using thermoluminescent dosimeters



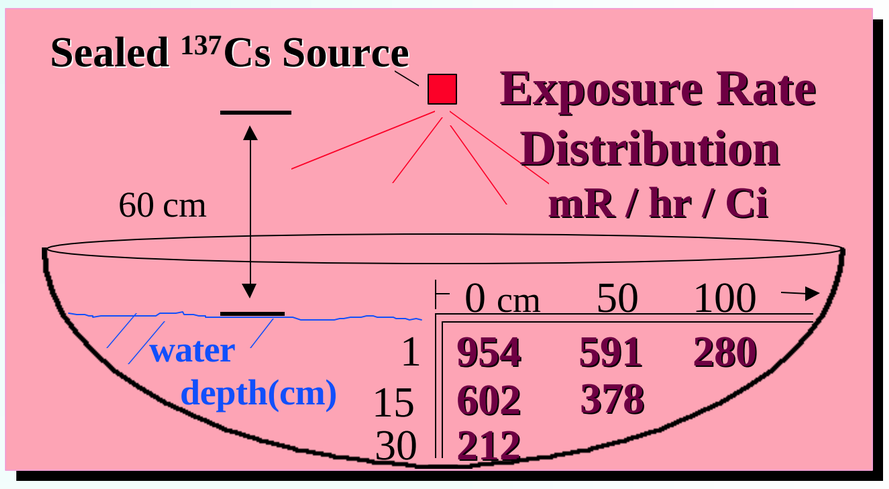
- Using turtles and transgenic fish in the irradiation-mesocosm facility we can determine:

1) the frequency of chromosome damage at various radiation exposures and metal contaminant concentrations

2) the relationship between cellular damage and metabolic rate

3) treatment effects on an individual's energy allocation pattern, growth and survival

4) endpoints that are significant and therefore of use to DOE in ecological risk analyses



Benefits to the DOE

- reduce uncertainties associated with ecological risk analyses
- provide defensible scientific evidence on which cleanup decisions can be based
- broad application across DOE sites
- reduce DOE's need to take an ultra-conservative approach to cleanup, resulting in substantial cost savings

Funded by an EMSP grant from the DOE;
for more information contact Dr. Tom Hinton,
803 557 7454; thinton@srel.edu (thanks to Juanita Blocker for help preparing the poster).