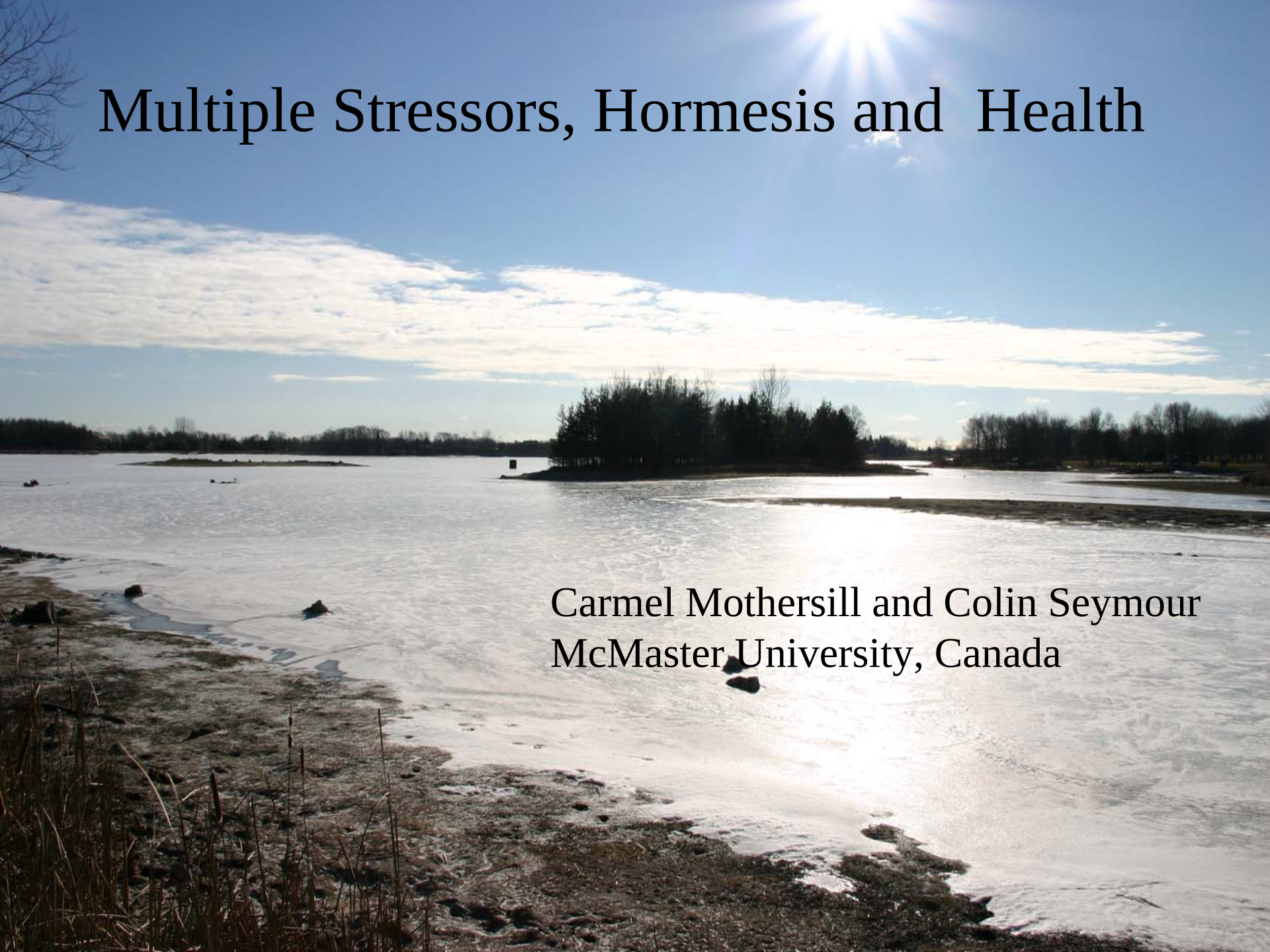
Multiple Stressors, Hormesis and Health



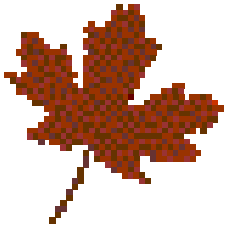
Carmel Mothersill and Colin Seymour
McMaster University, Canada

What are “multiple stressors”?

- Buzz word to indicate exposure to combined pollutants
- Used in stress biology to indicate that several agents combine to produce the biological perturbation being studied
- In environmental science the term means “mixed contaminants”
- In hormesis research the term probably means “multifactorial effect causation”

Hormetic effects and multiple stressor response

- Induction of an adaptive response by one agent making the system more resistant to a second agent
- Saturation of receptors for agents such as bystander signals by one agent so a second agent cannot increase the response
- Interference between agents at the mechanistic level eg pro and anti apoptotic inducing agents present together
- Threshold effects- e.g. in the transition from HRS to IRR, a modulating protective or sensitising chemical could push the threshold for the transition



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What multiple stressor problem does to environmental protection

- Multiple inducers of stress effects therefore dose and effect are not simply linked
- Response based approach needed
- How to link biological effect with adverse outcome at the organism level
- Mechanistic uncertainty at low doses
- Non-targeted effect predominate at low doses

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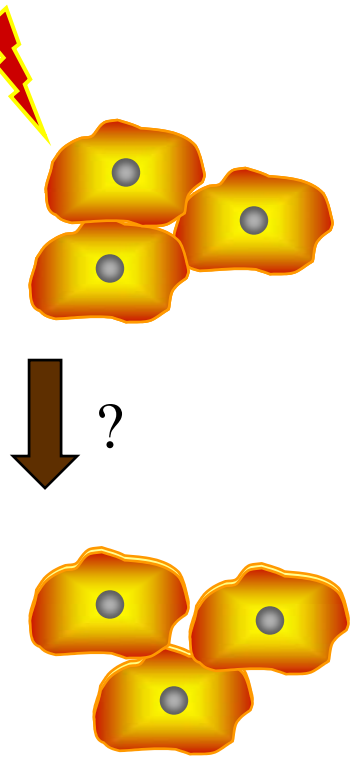
The challenge

- How to extrapolate
  - From effect to harm
  - From harm to risk
  - From individual risk to population risk
  - From population risk to ecosystem risk

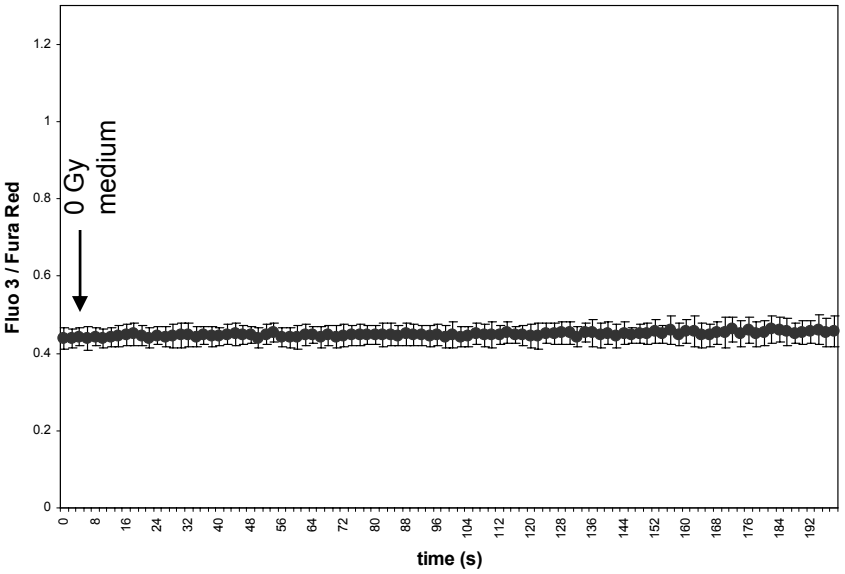
Bystander Effect-A unique signature?

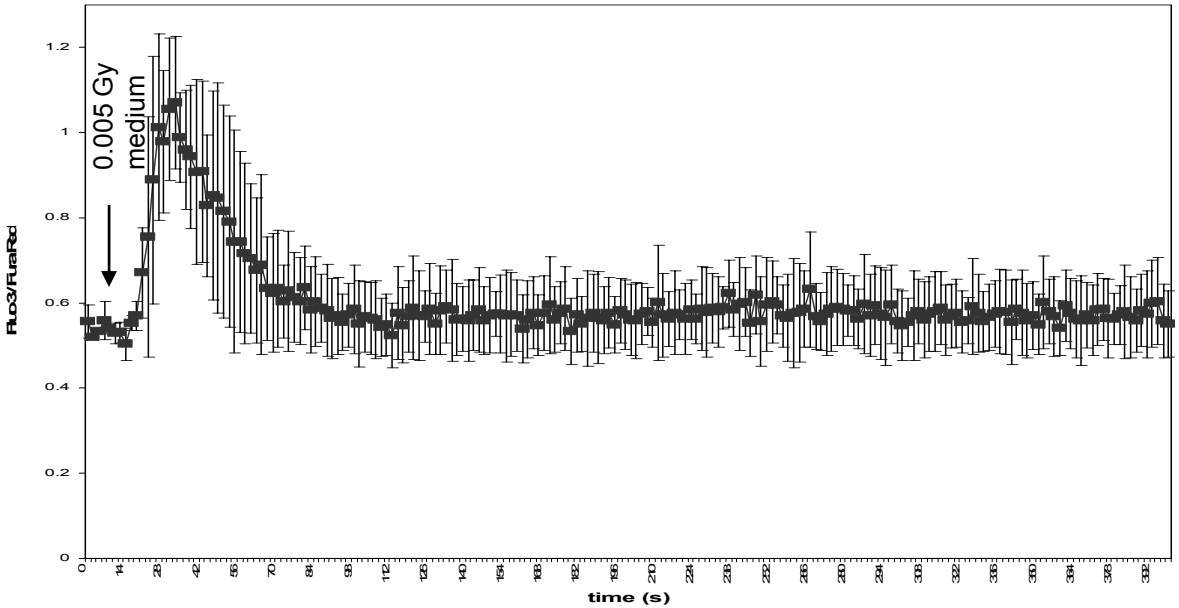
- Communicated damage

- Non-linear dose response
- History - Clastogenic factors
- Search for the “effector”

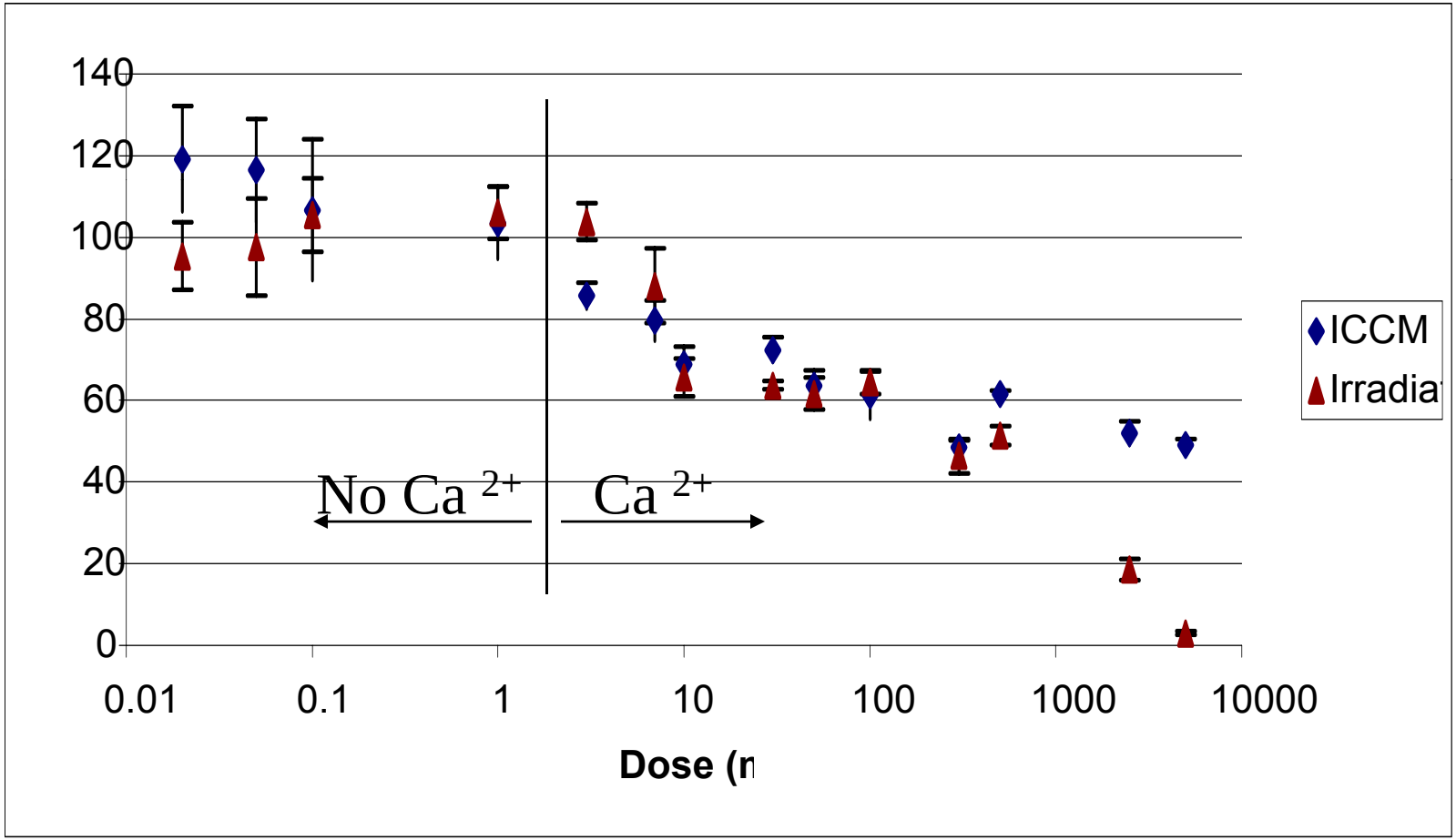


Signal after exposure to ICCM from 5mGy irradiated cells



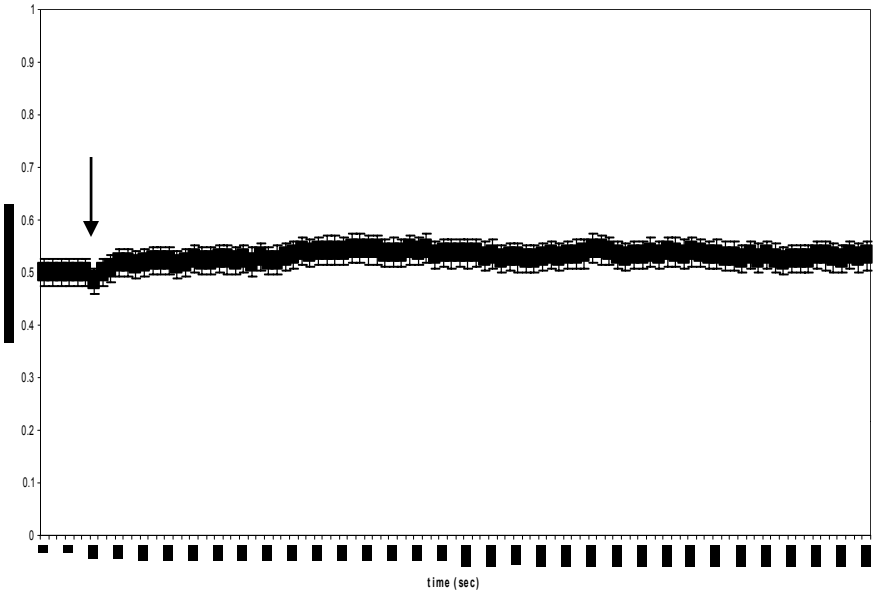


Bystander and direct dose survival curves over six orders of magnitude ^{60} Co with calcium data

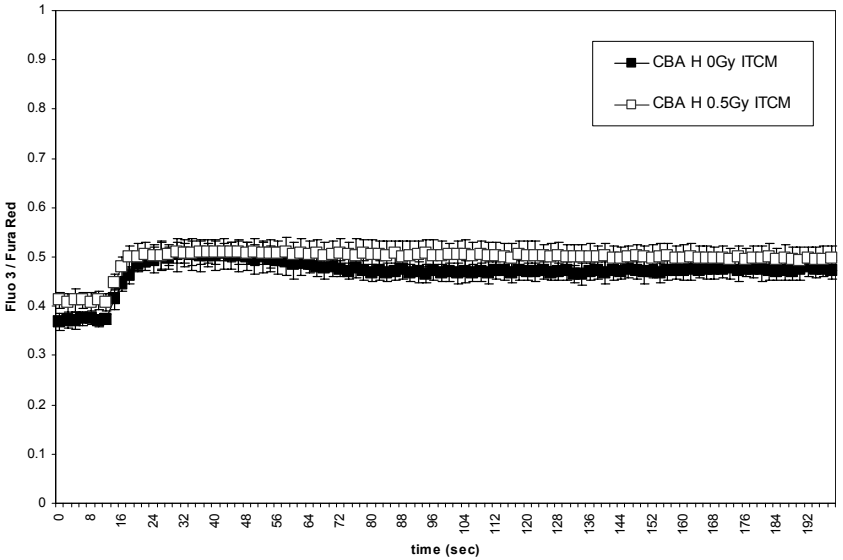


Calcium ratios in control and 0.5Gy TBI CBA/Ca and C57BL/6 mice

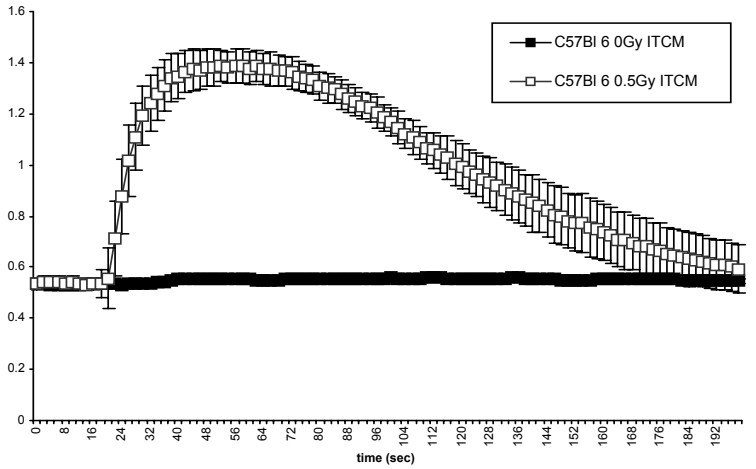
Medium from unirradiated tissues from both strains



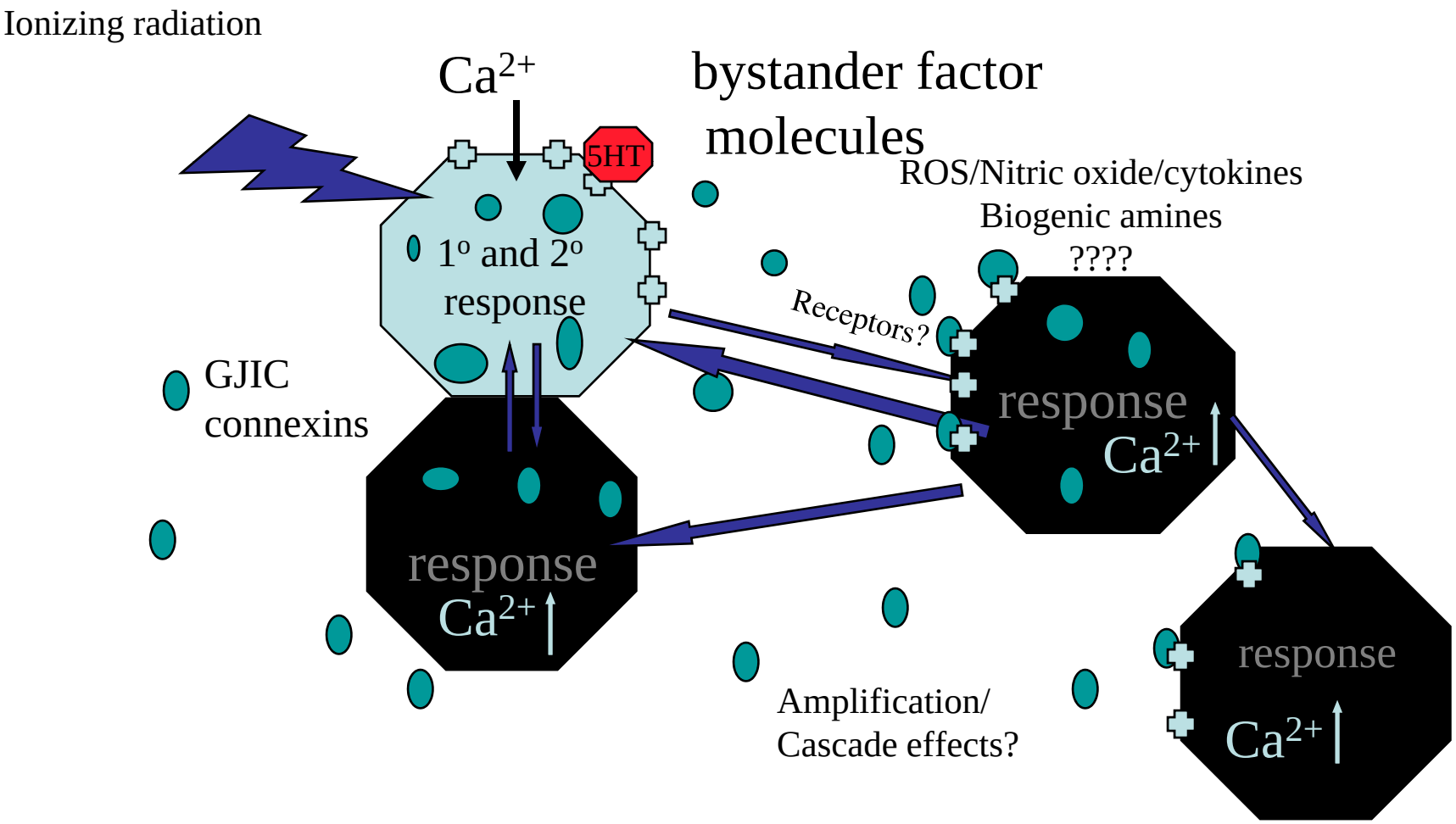
CBA/Ca



C57BL/6



The bystander effect



Advantages of Fish as Sentinel Species

A growing consensus from fish carcinogenesis studies indicates that fish offer unique opportunities to examine universal mechanisms of carcinogenesis, and to identify and predict human health effects from exposure to environmentally relevant compounds

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Rainbow trout in vivo exposure to multiple stressors

- Set up in Agricultural University of Norway - team of 8!
- Live rainbow trout exposed to 0.5Gy ± sub lethal levels of Cd, Al and Cd+Al
- Four tissues cultured, (liver, fin, pronephros and gill), medium harvested, data for primary culture, bystander effect, physiological stress.

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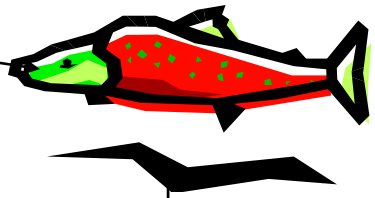
Design of multiple stressor in vivo experiments



mGy Cs137

Al+Cd

5hr exposure

Harvest tissues and set up explants

Gill

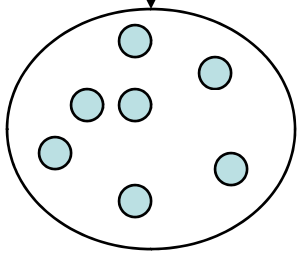
Fin

Kidney

2 days

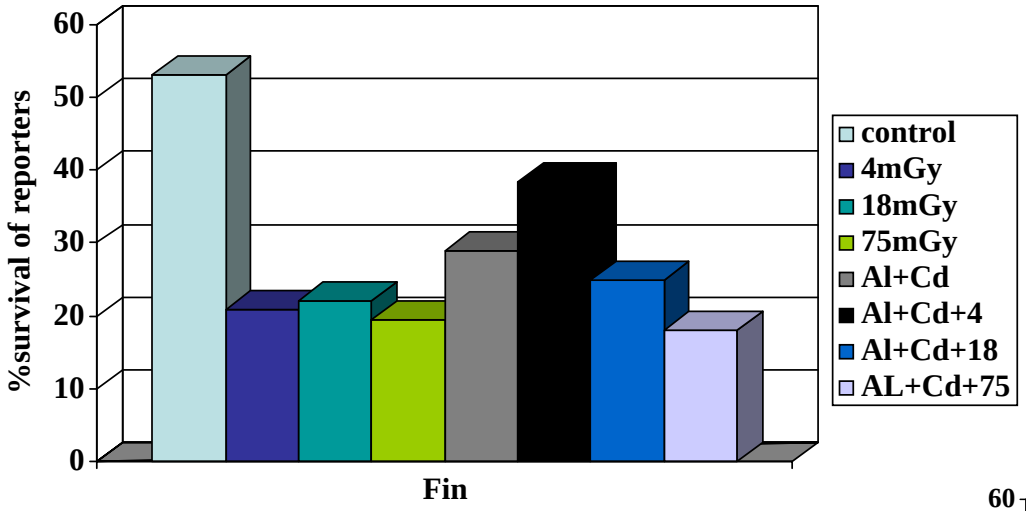
Medium harvest, filtration, addition to reporter cells

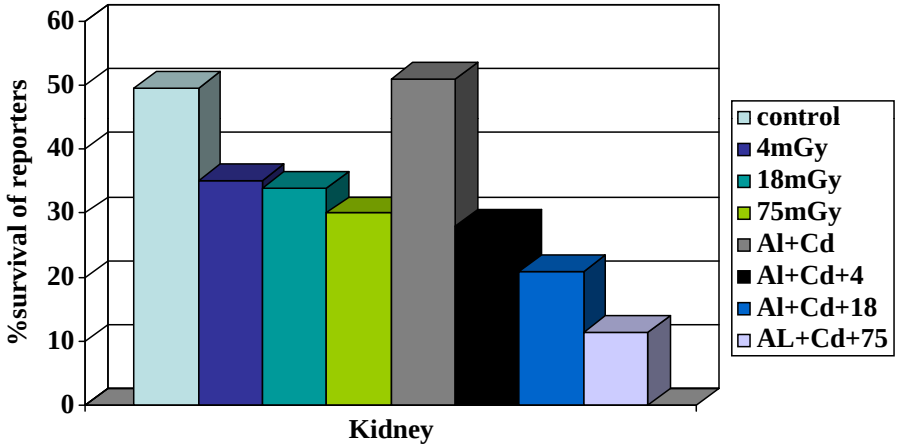
9 days

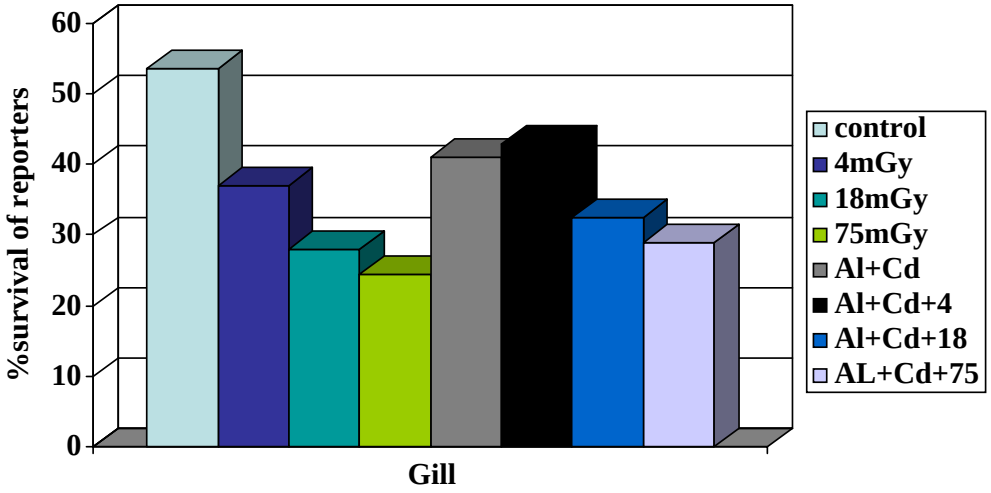


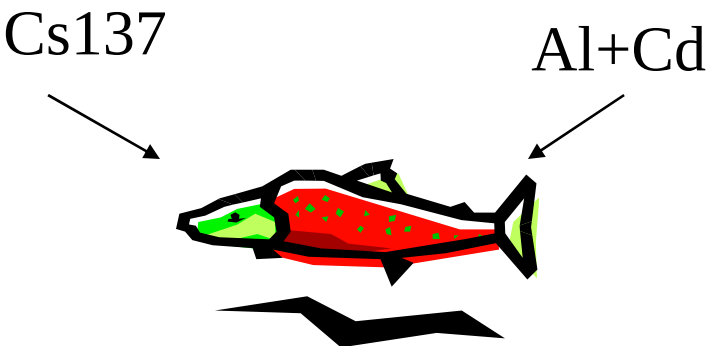
Stain colonies and calculate Toxicity of bystander signal

Comparison of in vivo mGy radiation exposure±metals on production of bystander signals

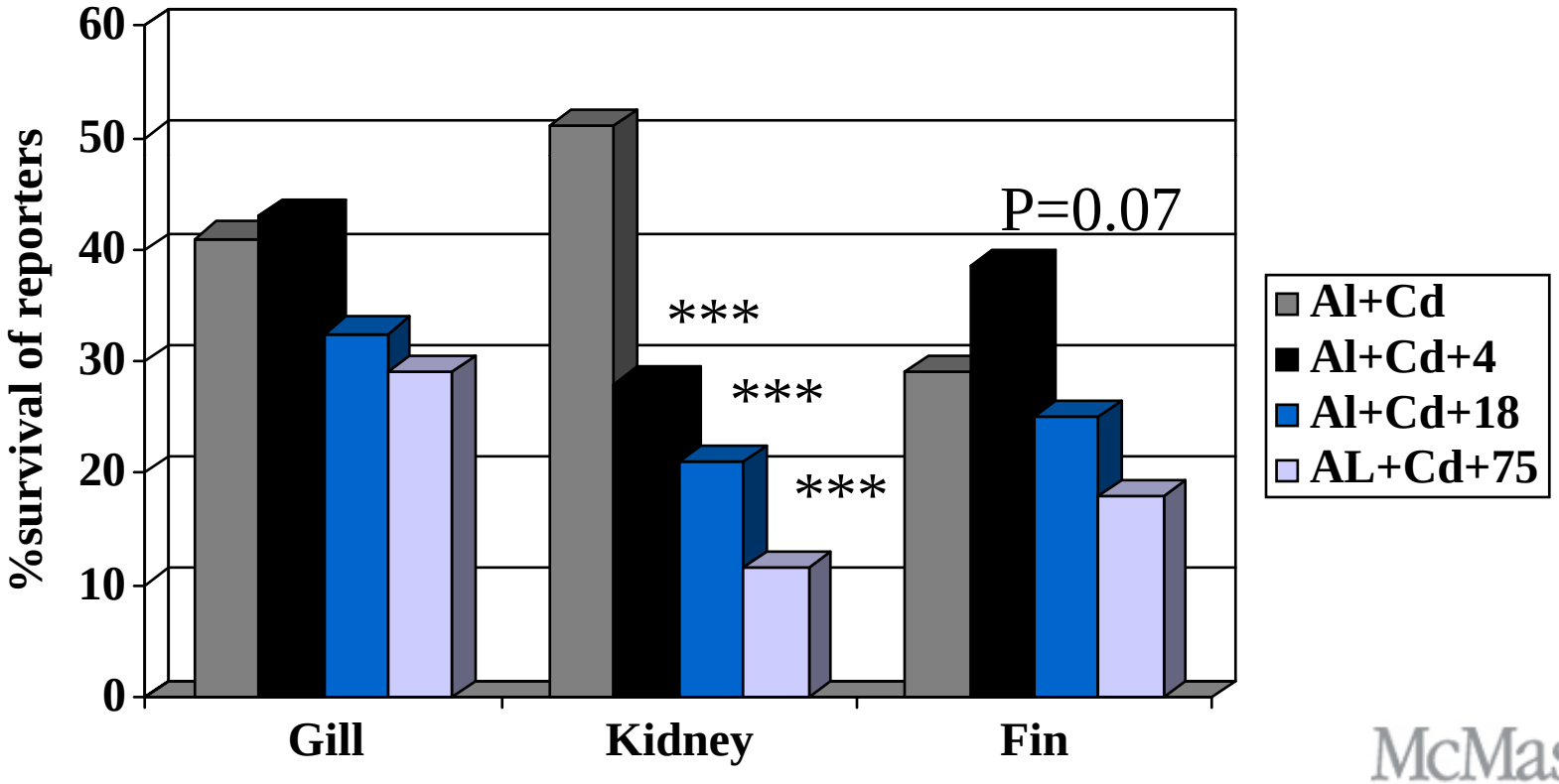








Effect of in vivo radiation with metals



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Multiple stressor data for rainbow trout gill

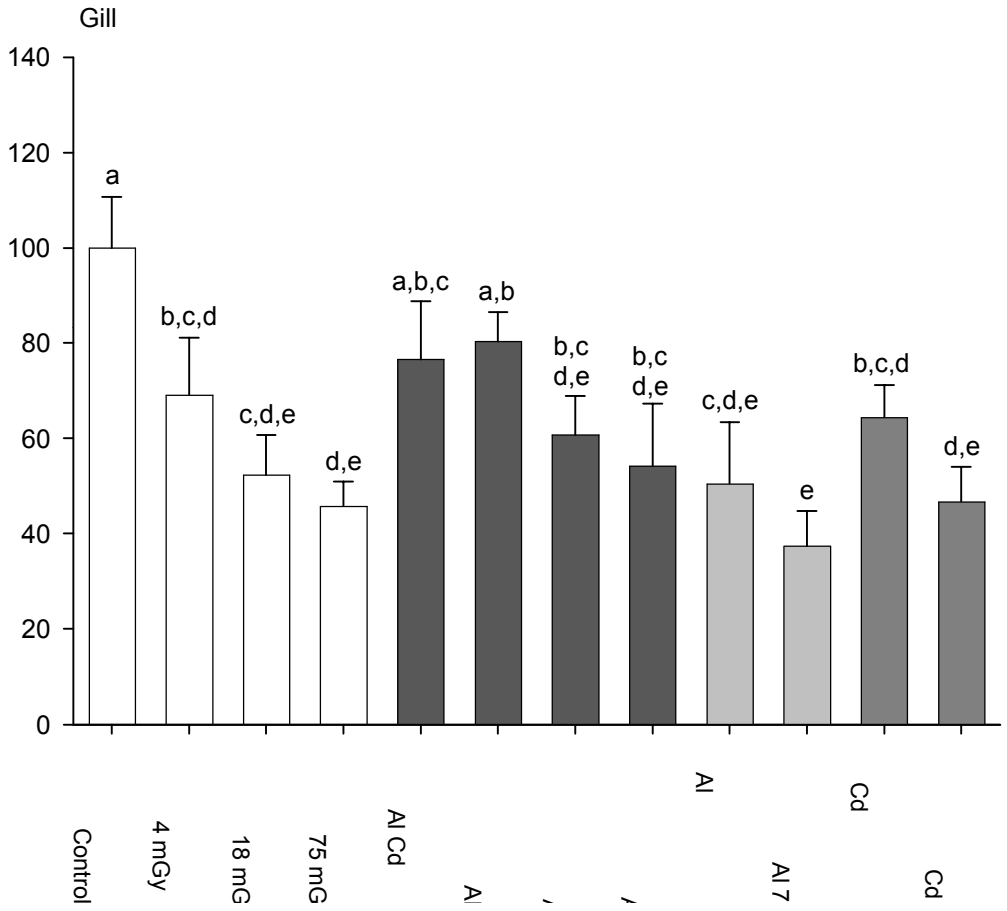

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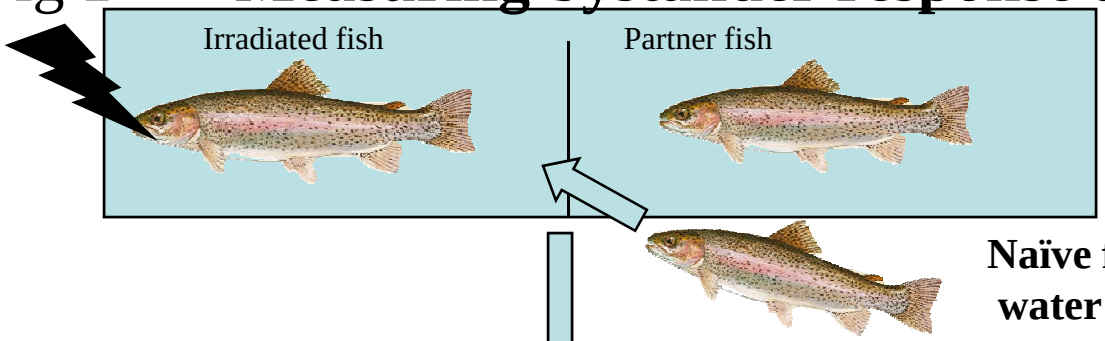
Fig 1 **Measuring bystander response to radiation *in vivo***



Irradiate or sham irradiate one fish, allow to swim with unexposed partner for 2hrs

Naïve fish introduced into water from irradiated or sham fish After 2hrs

Explant pieces taken from skin, fin, gill, spleen and kidney for culture or proteomics

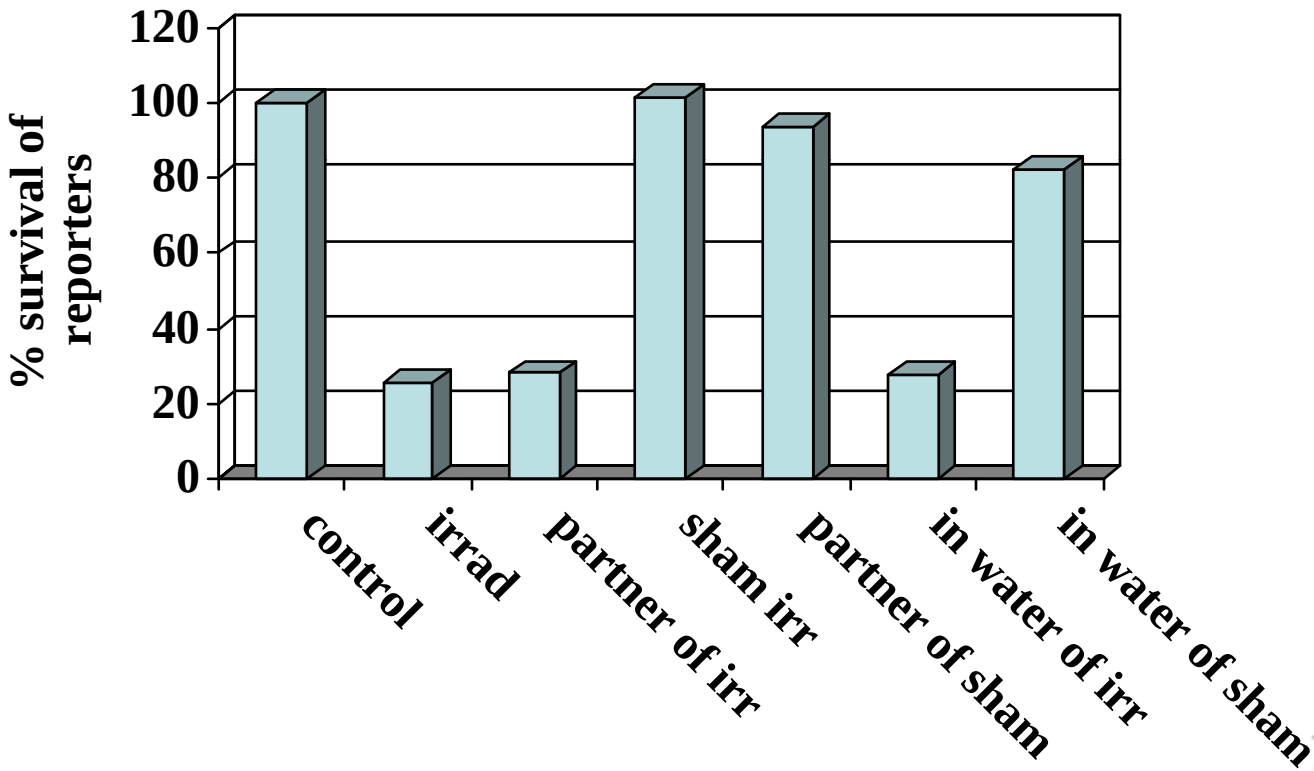
Culture of explants for 2 days

Grow up then examine explant outgrowth after direct or bystander exposure

Harvest culture medium

Add to unirradiated clonogenic cell line and determine surviving fraction

Gill



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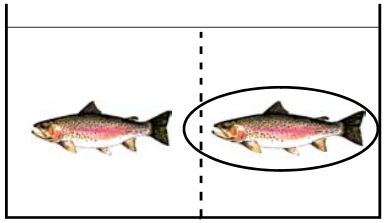
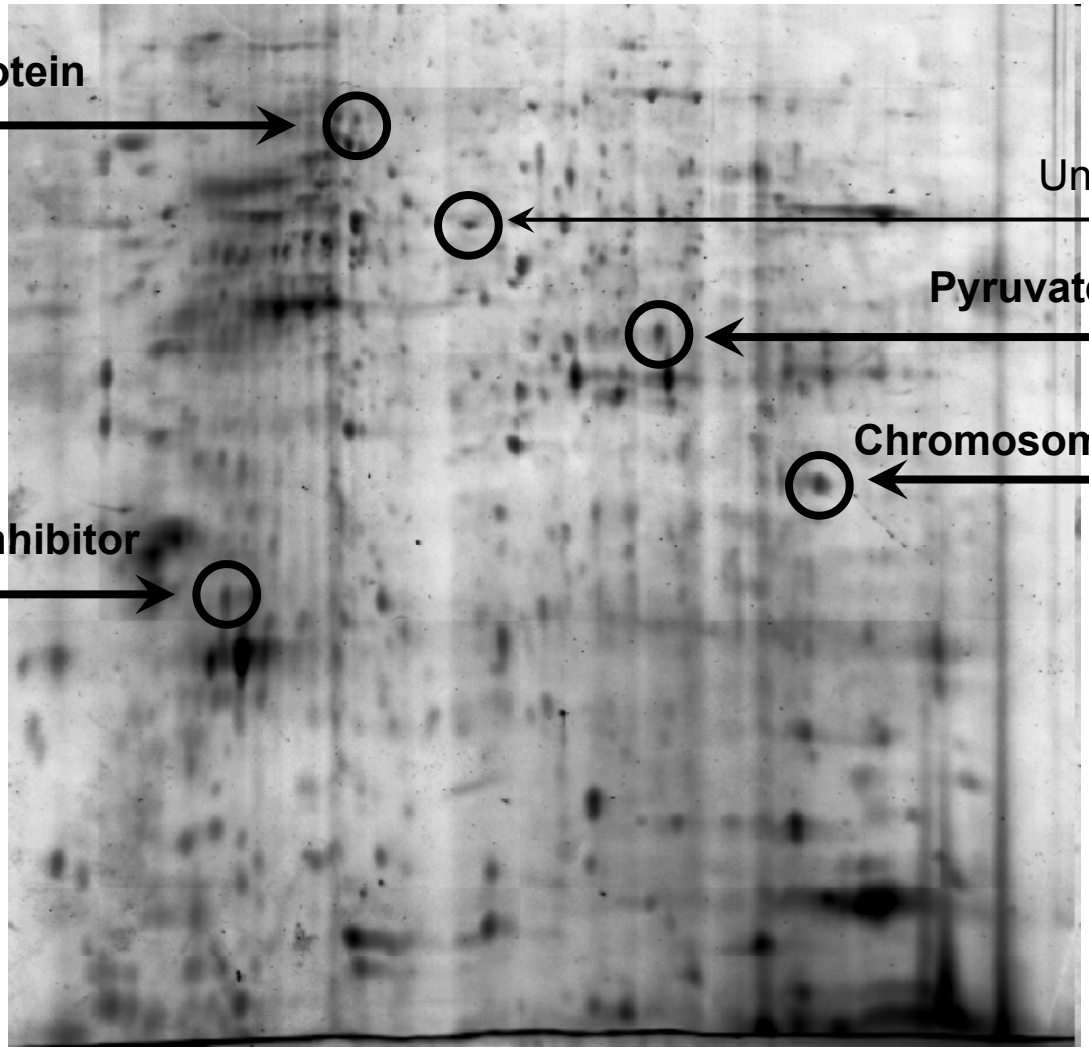
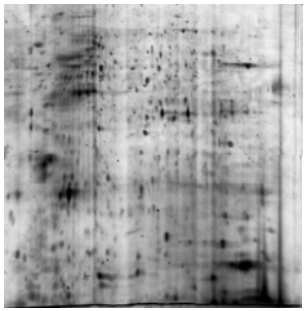


BYSTANDER PROTEIN IDENTITIES



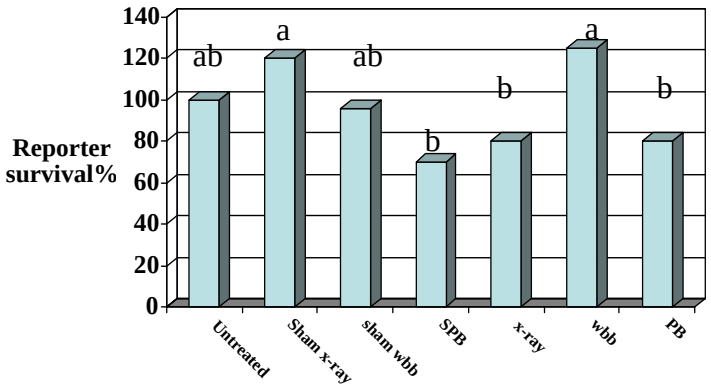


Proteomics conclusions

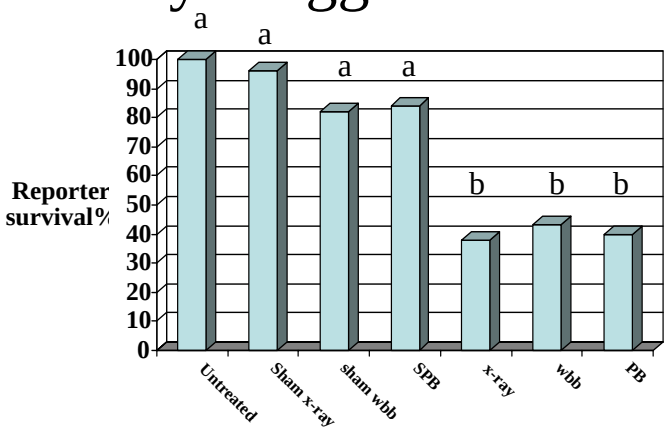
- **The bystander effect includes the up-regulation of specific gill proteins in rainbow trout swimming in water from irradiated fish.**
- **These bystander proteomic changes differ from those associated with direct X-radiation (or stress from sham X-ray handling).**
- **The known functions of these proteins suggest that up-regulation leads to specific protective, restorative or adaptive responses. These involve…**
  - **Metabolic regulation**
  - **Tissue repair**
  - **Maintaining specific aspects of gill function, including epithelial polarity and barrier properties, and ionic regulation.**

Stage of irradiation and bystander challenge

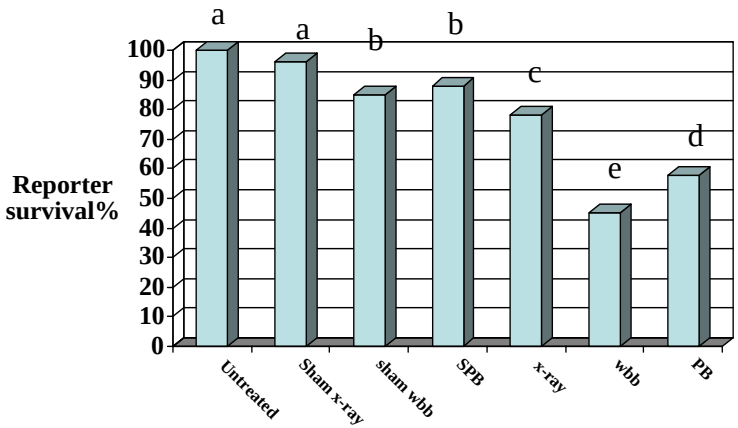
Eggs



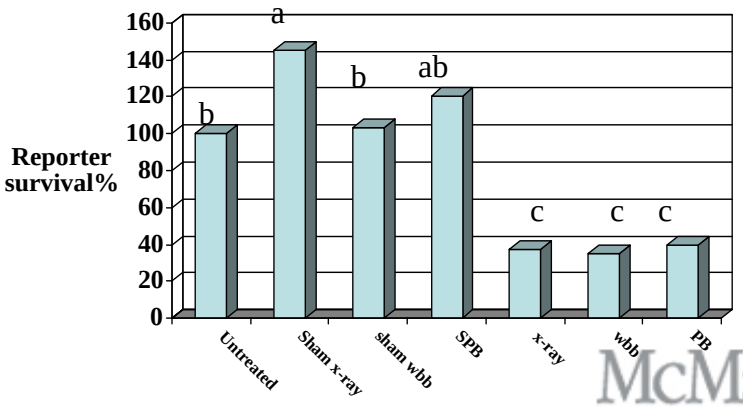
Eyed eggs



Yolk sac larva



Juvenille



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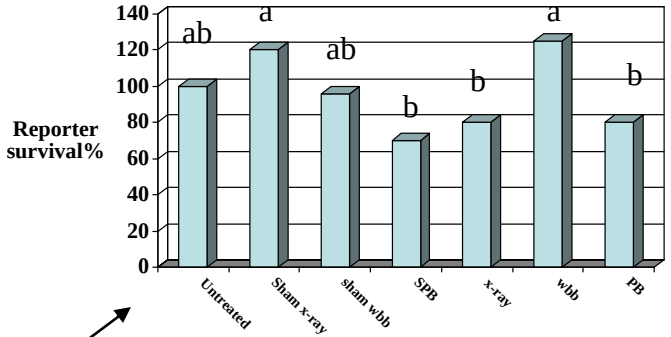
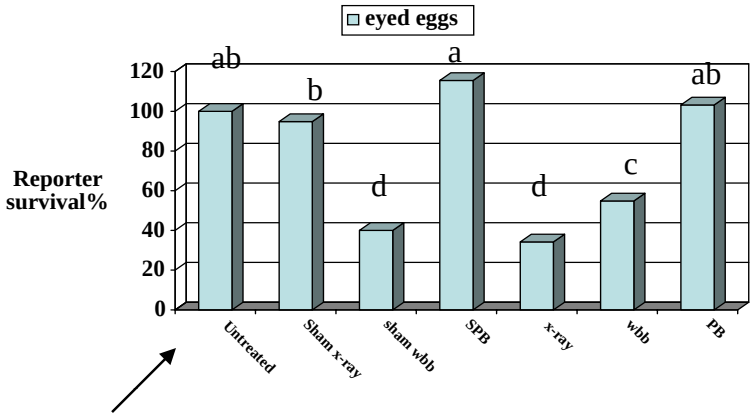
Legacy effect: Eggs irradiated with a single dose of 0.5Gy x-rays at 40hrs then at successive development stages put swimming with never exposed individuals

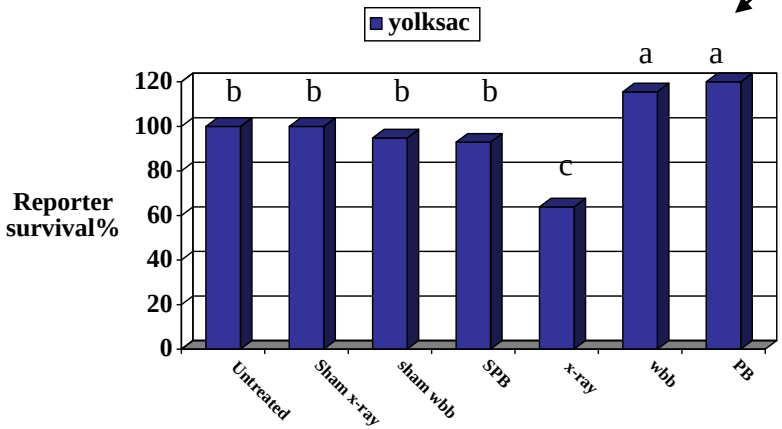
Eggs with eggs

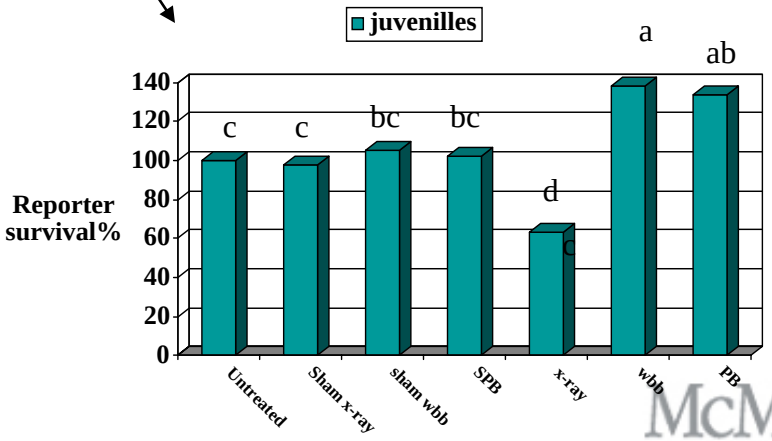




No effect

Life effect in legacy bystanders





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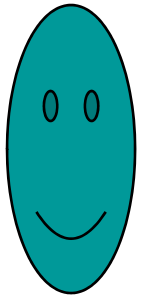
Evolutionary implications

- In a stable situation an organism must establish and defend it’s position
- In an unpredictable situation complexity theory requires the organism to be based on “the edge of chaos” providing just enough structure to allow it to capture the best opportunities

So what? Why this strange “anti-order” mechanism

- Deleterious mutations lead to malfunction and maybe death
- Favourable mutations aid survival and reproductive success
- The balance is critical and control is vital
- What is the cost of fidelity? How is it manipulated?
- Is there a role for instability in evolutionary progress?

The unifying theory!



Original phenotype

Environmental stress

Genetic background

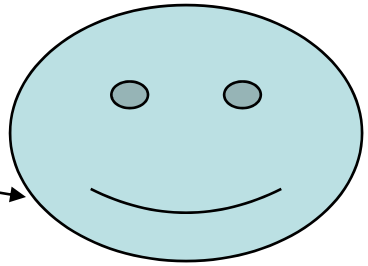
Unstable cell

Hypermutable

Selection pressure

Chance to change

Adaptation



New phenotype

Summary

Multiple stressors - science needs

- Stressors seldom act alone
- Little information on synergistic/sub-additive effects
- Little low dose information - mostly extrapolation assuming linearity
- Little in vivo mechanistic information- mostly in vitro

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The way forward

- Need to get away from the “mono-stress mentality”
- Need to get away from the “dose mentality” and start scoring “health of the population” i.e. response, not what the dose is
- Need to realise Ed is right!

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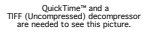
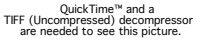
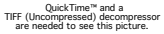
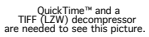
The team at work!









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