

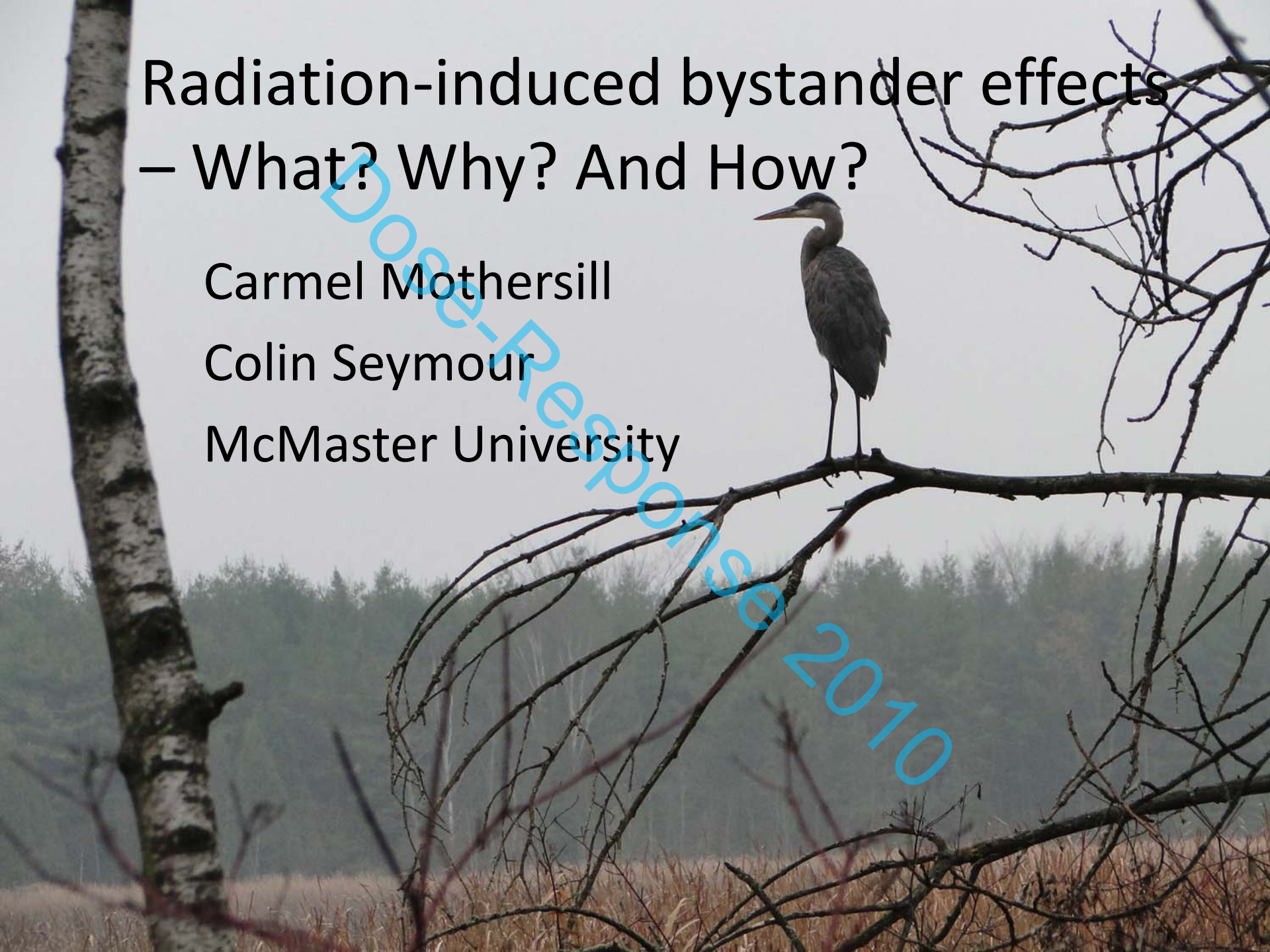
Radiation-induced bystander effects

– What? Why? And How?

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Outline

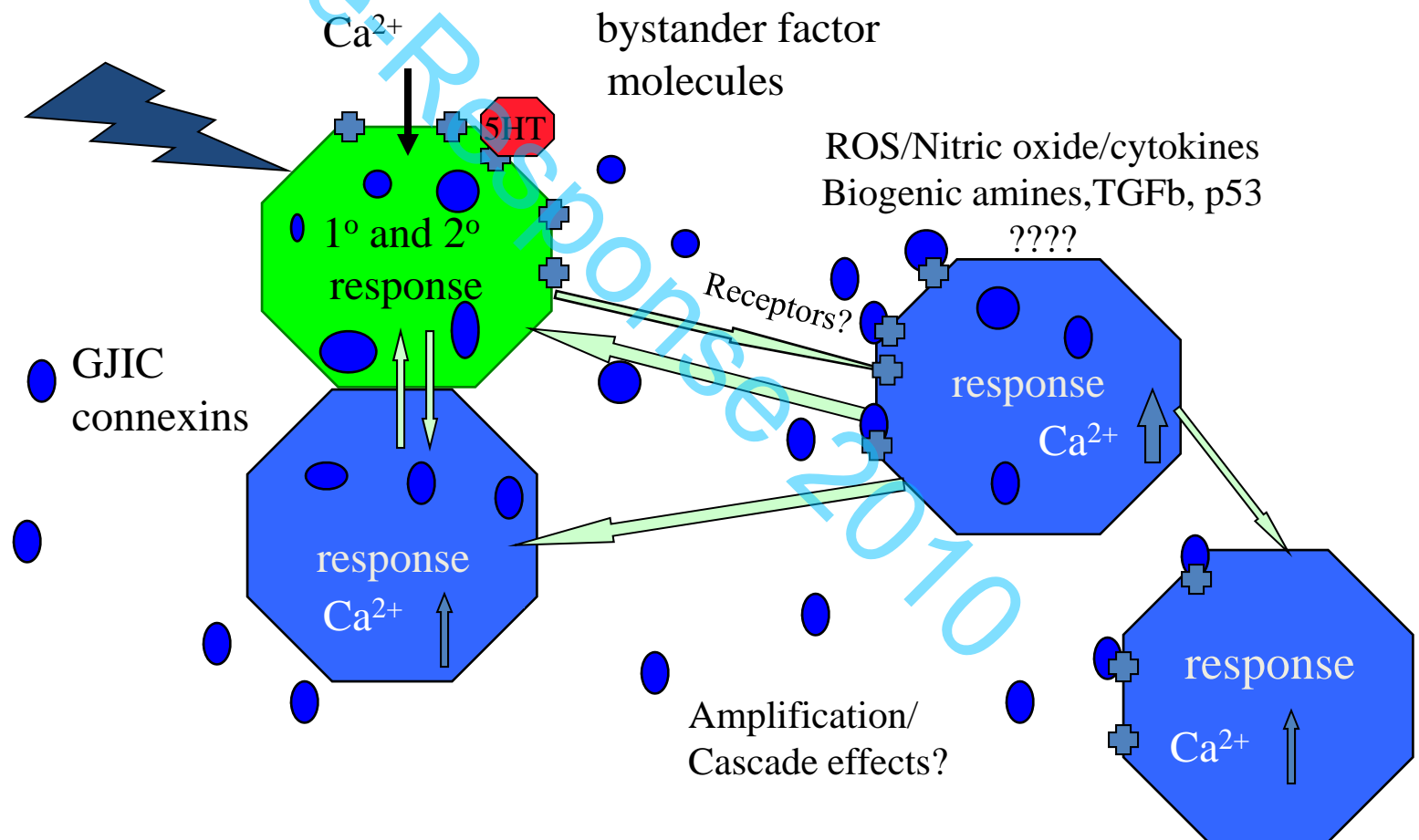
- Bystander effects and genomic instability
- In vivo relevance and evidence
- The fish model
- Mechanisms
- Multiple stressor issues
- Why do these effects happen?

Take home messages

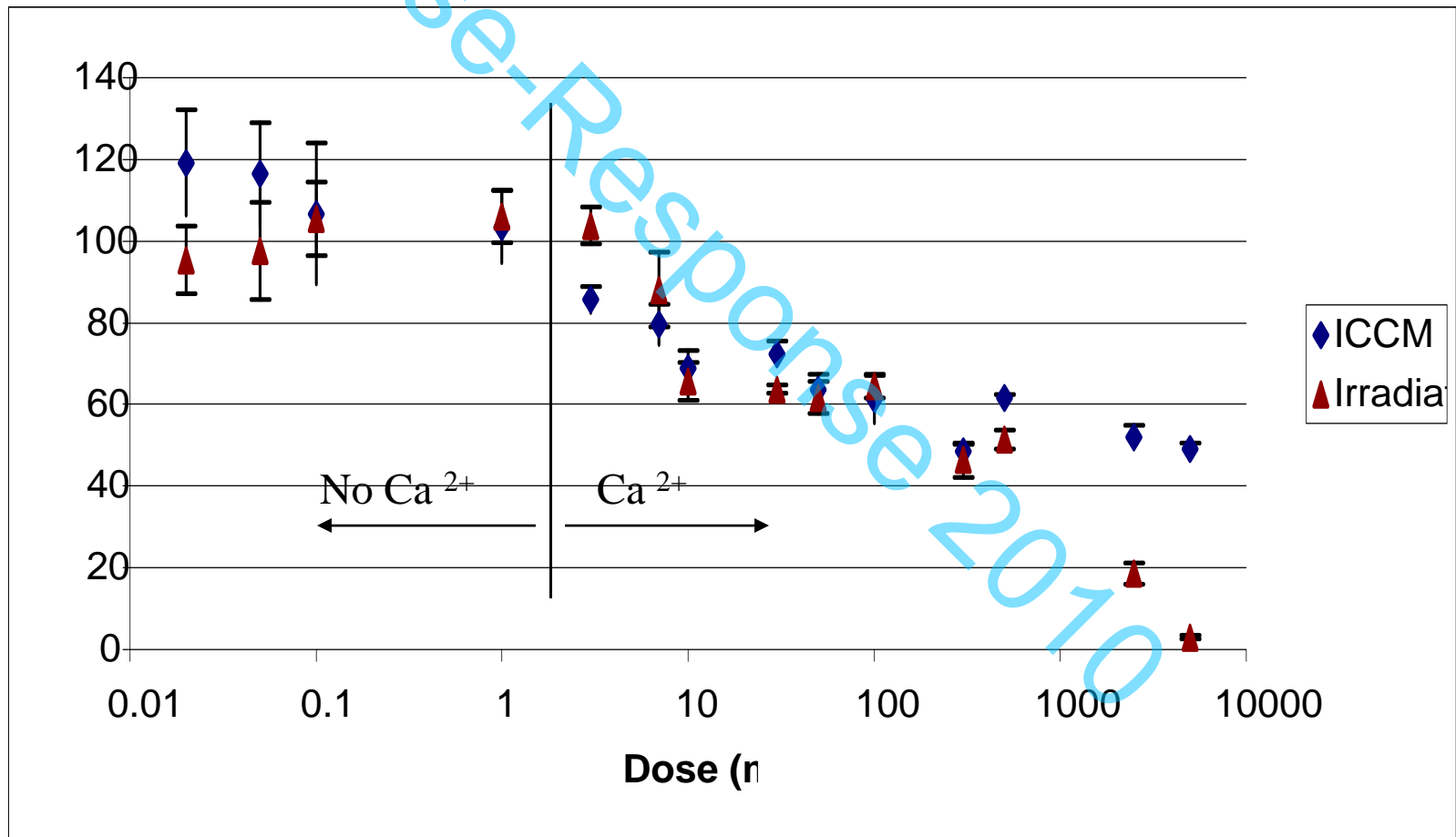
- Target theory, LNT and DNA centric ideas of low dose effects are dead
- Chronic (background) exposure to radiation is in no way related, even by elaborate “fudge factors” to acute exposure
- Environmental effects are not simple and multiple stressor exposure HAS to be part of the discussion
- Evolution is all about adaptation (or not?) to changed conditions

The bystander effect

Ionizing radiation, UVA, UVB, ELF-EMF and heavy metals induce affected cell to signal to others. Responses to the signals include apoptosis, micronucleus formation, transformation, mutation, induction of stress and adaptive pathways. Serotonin (5HT) and Calcium ions known to be involved in signal production.

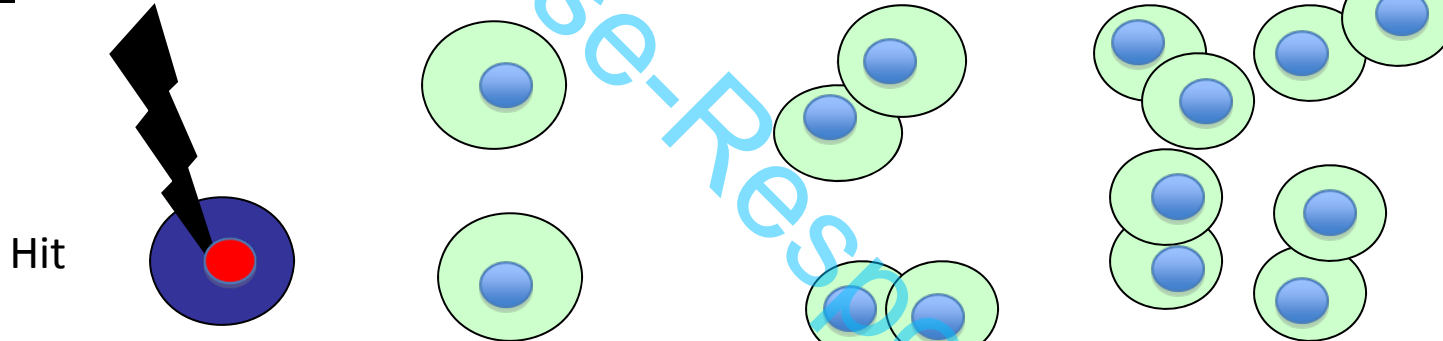


Bystander and direct dose survival curves
over six orders of magnitude ^{60}Co with calcium data



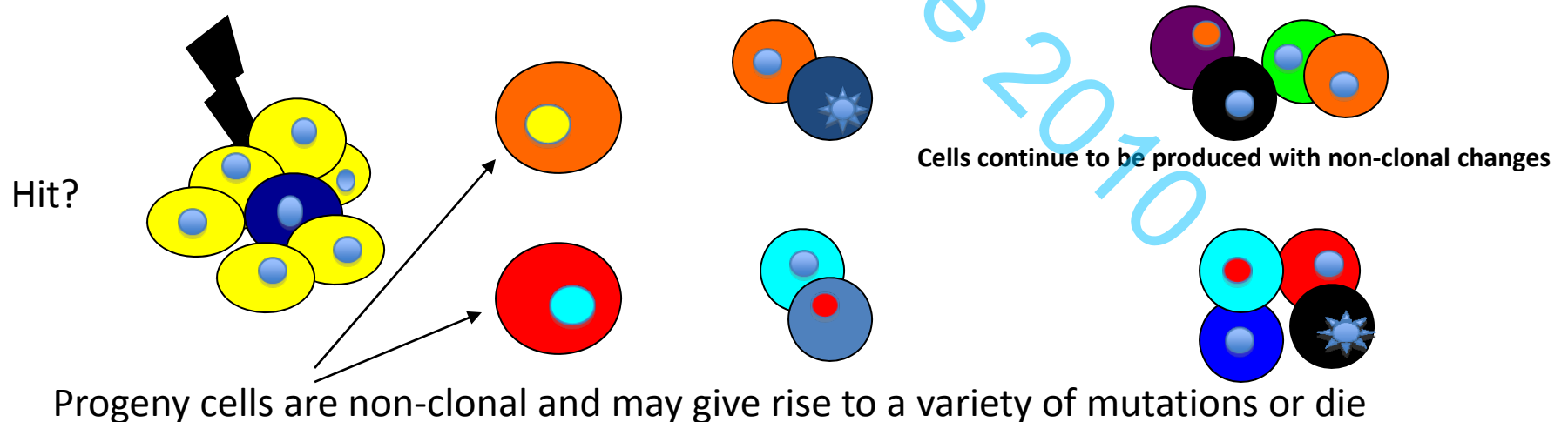
The link between bystander effects and genomic instability – twin pillars of the new paradigm

Old view- clonal outcome



Progeny are all clonal i.e. identical and mutation is passed to all progeny

New view-non-clonal, population-determined outcome



In vivo relevance

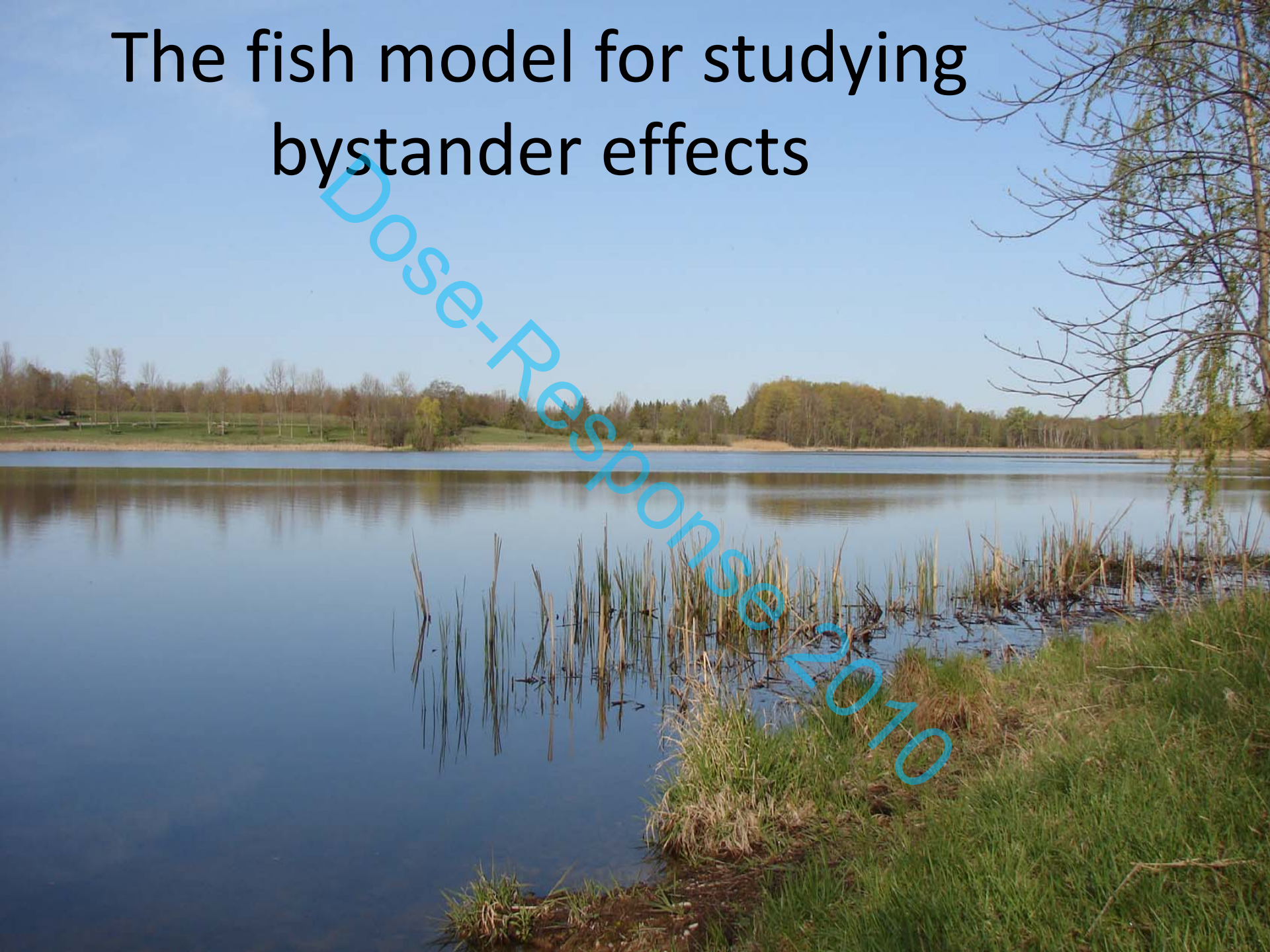
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In vivo evidence

- Clastogenic factors in blood of irradiated people and experimental animals
- Abscopal effects in distant organs
- Bone marrow ablated mice receiving opposite sex marrow transplant show instability in the regenerating marrow
- Soluble factors from explanted tissues after in vivo exposure

The fish model for studying bystander effects



Measuring radiation induced bystander response *in vivo*

Irradiate fish

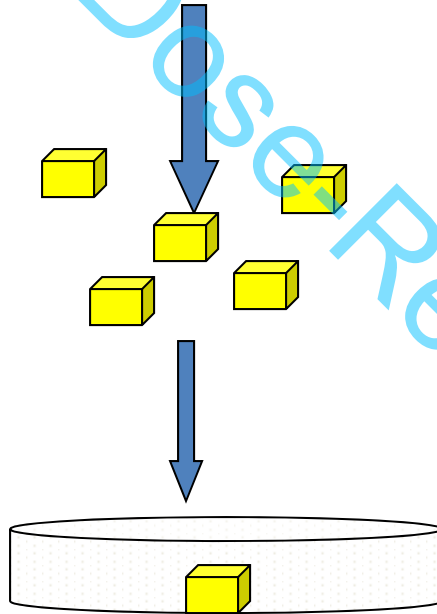
± Metals in water



Dissect fresh tissue from animal

Do *proteomics*

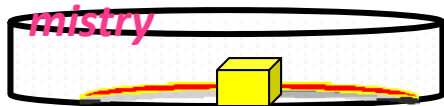
Chop tissue to provide explant pieces



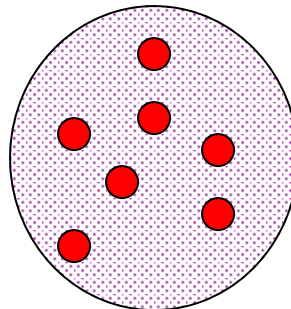
Culture of explants

Harvest culture medium containing
stress signal molecules/metabolomics

Examine *explant*
outgrowth/immunocytochemistry

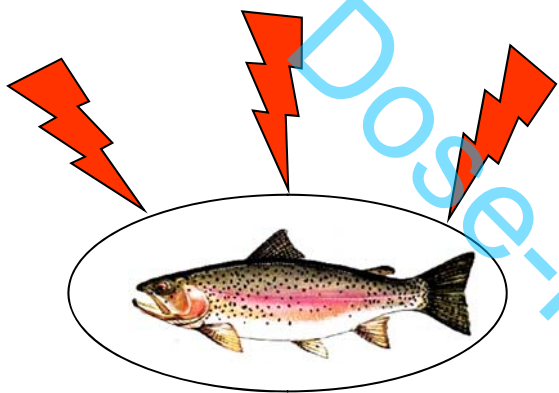


Add to unirradiated
clonogenic cell line and
determine *survival*
or other stress endpoints

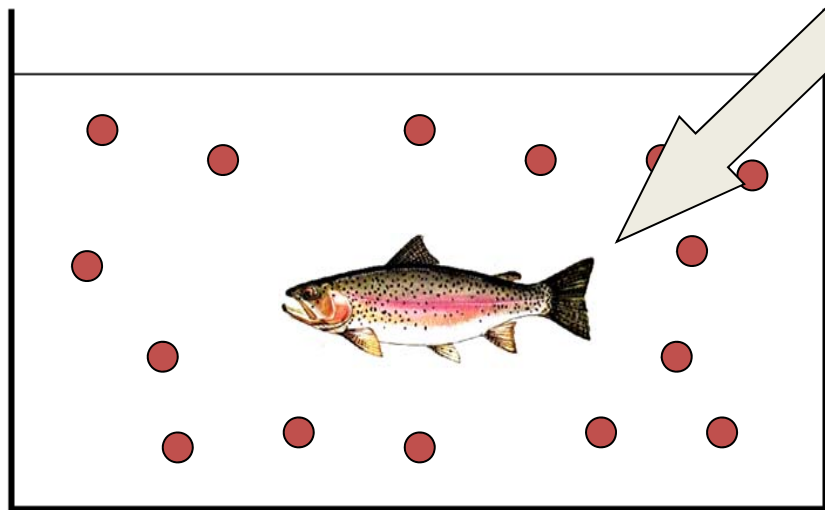




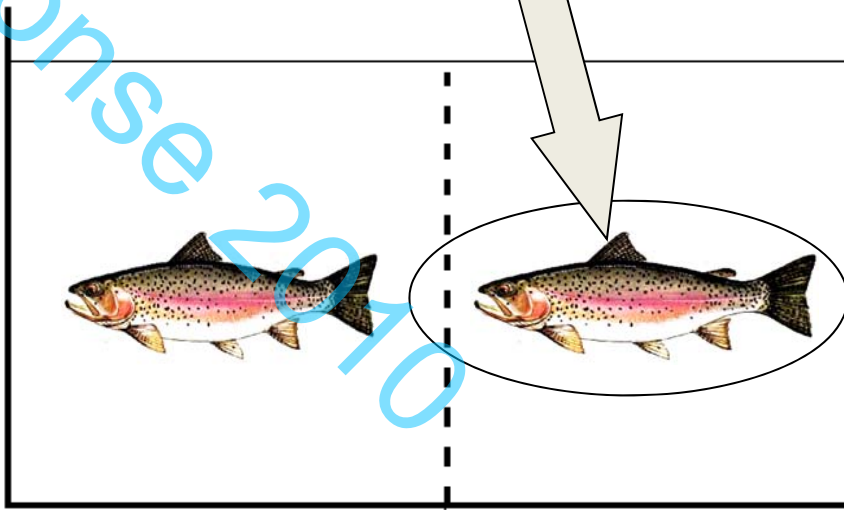
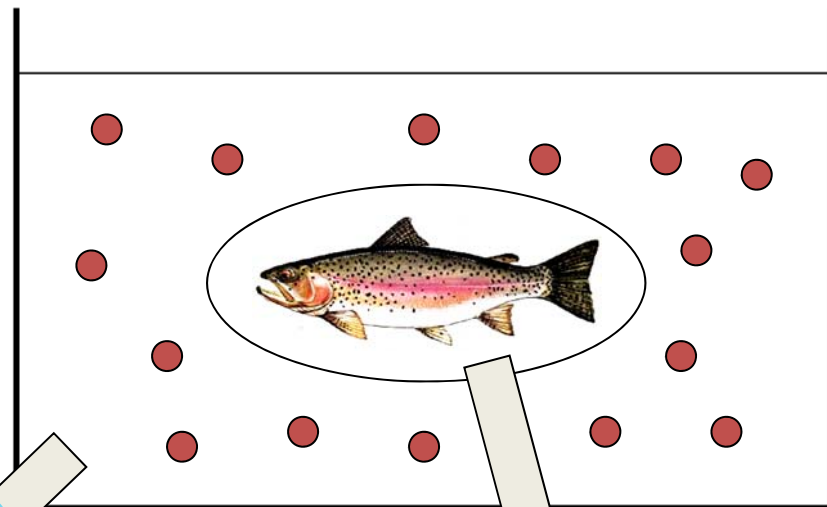
INDUCTION OF THE BYSTANDER EFFECT IN A DIFFERENT FISH



X-rayed fish



Waterborne bystander effect



Partner bystander effect

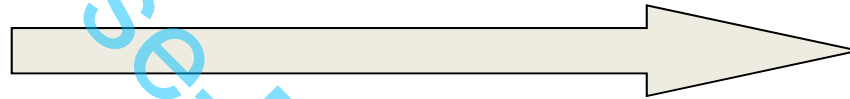


BYSTANDER EFFECT INDUCED *IN VIVO* IN RAINBOW TROUT, MEDAKA & ZEBRAFISH

0.5Gy X-ray dose



Water soluble bystander signals



Rainbow trout (Mothersill et al 2006)

Non X-rayed fish



0.5Gy X-ray dose

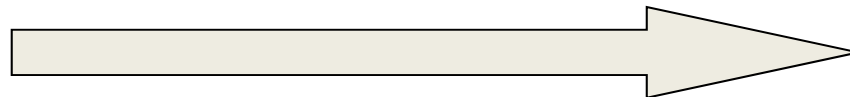


Zebrafish (Mothersill et al 2007)

Non X-rayed fish



0.5Gy X-ray dose



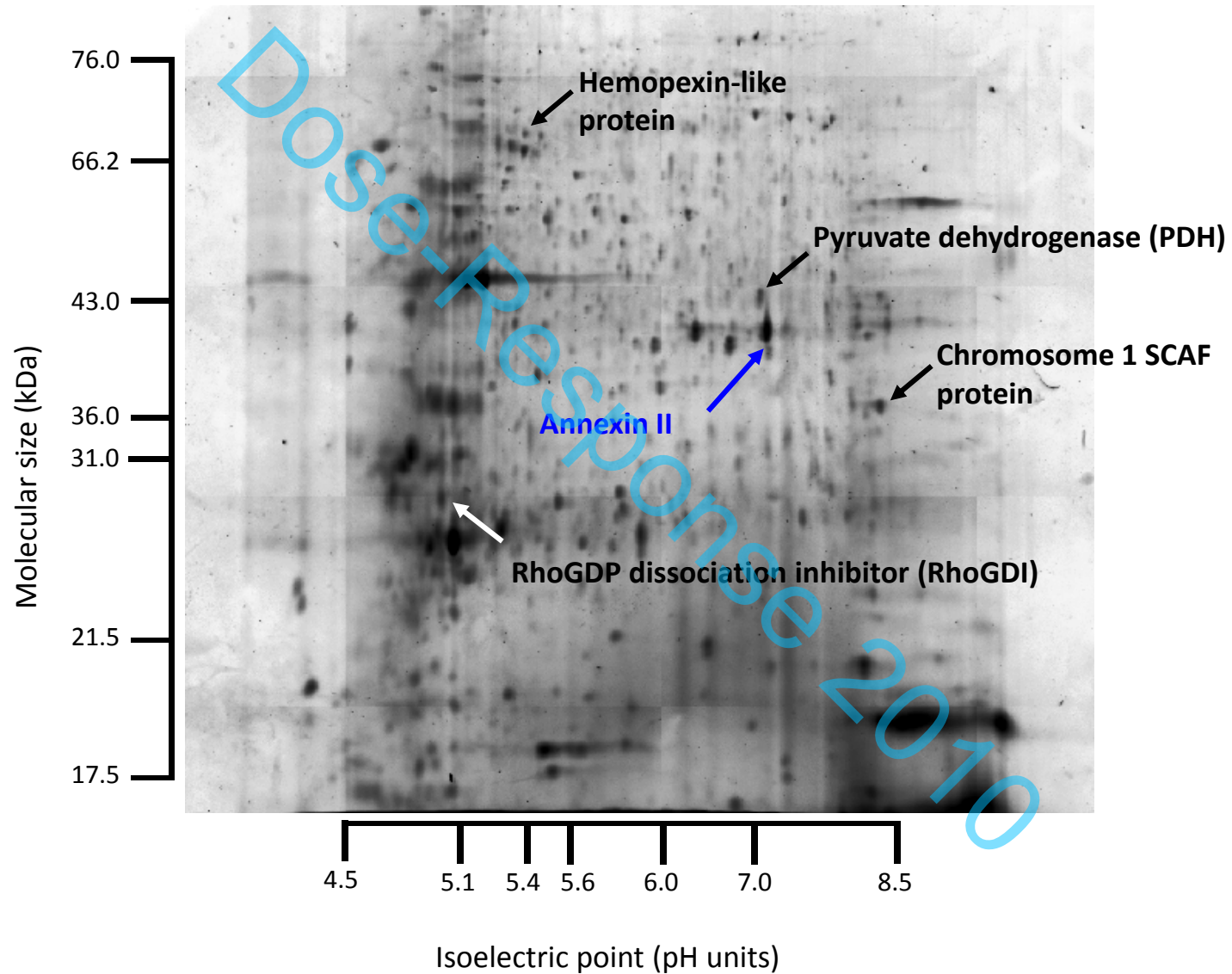
Medaka (Mothersill et al 2009)

Non X-rayed fish



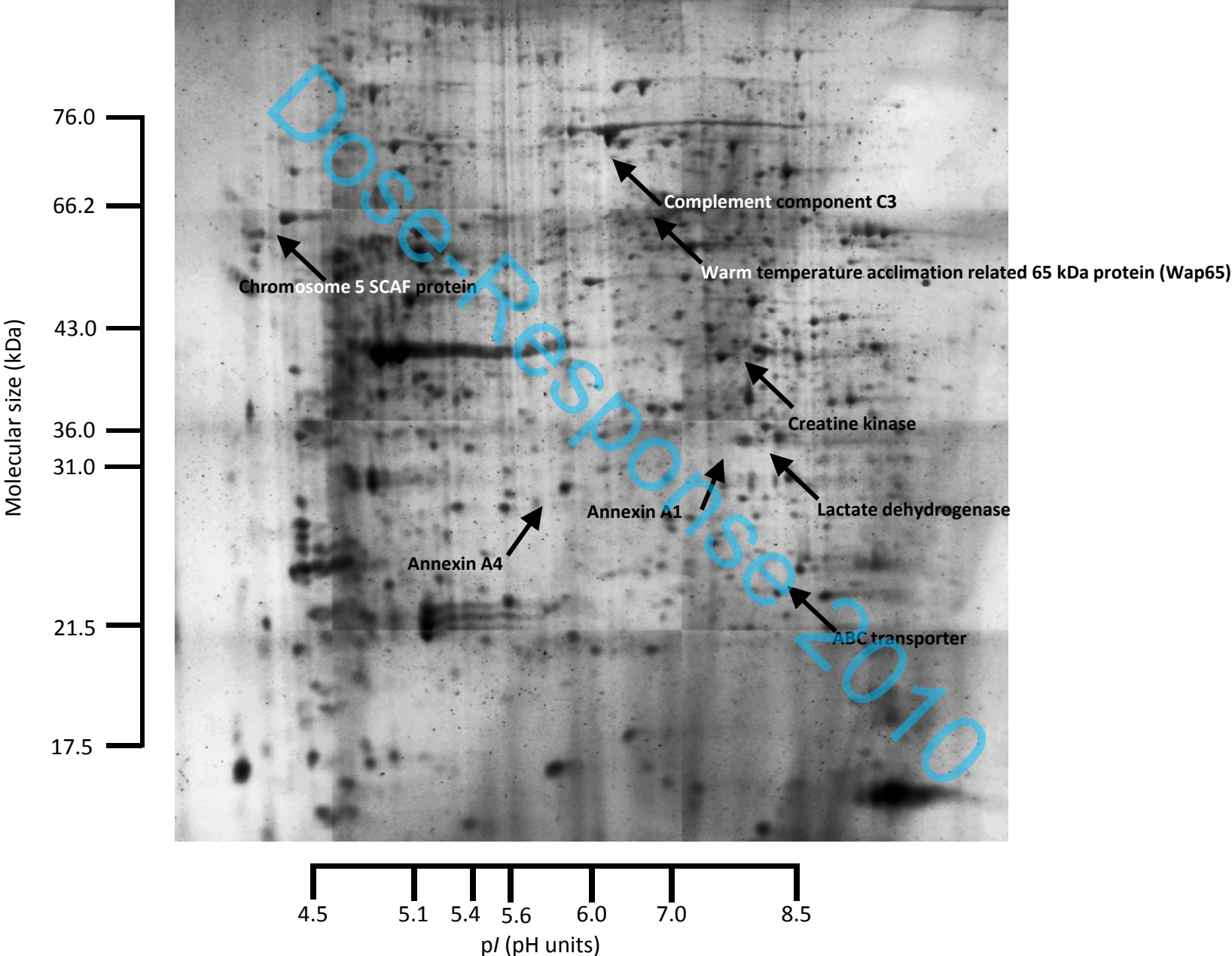


X-RAY AND BYSTANDER EFFECT INDUCED CHANGES TO THE TROUT GILL PROTEOME



SCAF = SR-like CTD-associated factor (SR = Serine – arginine Rich, CTD = C Terminal Domain)

Medaka gill proteins affected



Bystander effect proteomics

- Protective response against reactive oxygen species
- Bystander effect proteomic changes are transcriptionally regulated (SCAF proteins)
 - Virtually identical proteomic response in rainbow trout and medaka

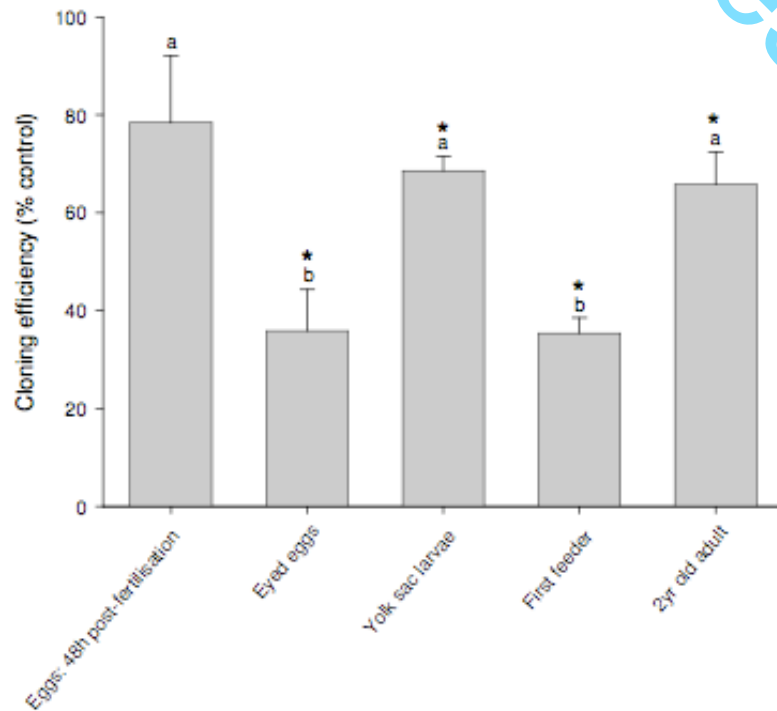
Is the bystander effect...

... an immediate protective response (Smith et al 2007) or an adaptation to possible future radiation damage (Kadhim et al 2004)?

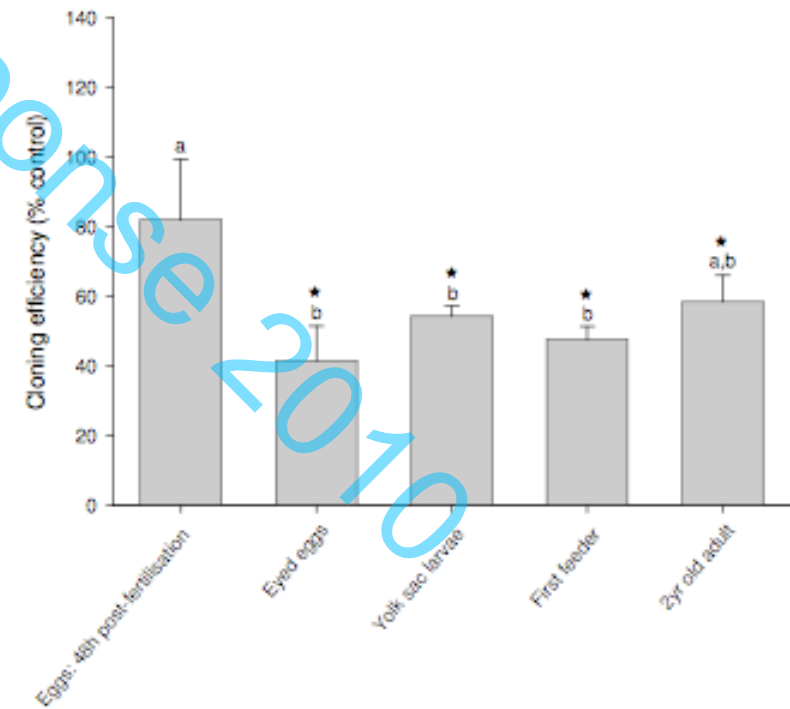
- Evidence from trout and medaka suggests the bystander effect is immediately protective
- Additionally the induction of an adaptive response may be species specific and apply particularly in radiosensitive biological systems

Legacy effect of single acute 0.5Gy X-ray exposure to eggs

Direct X-ray exposure

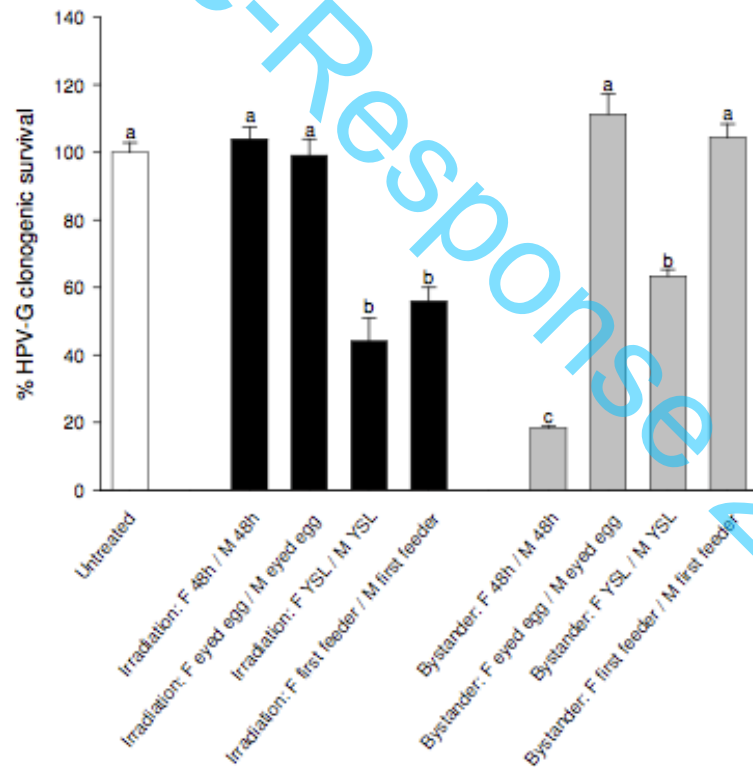


Partner bystander

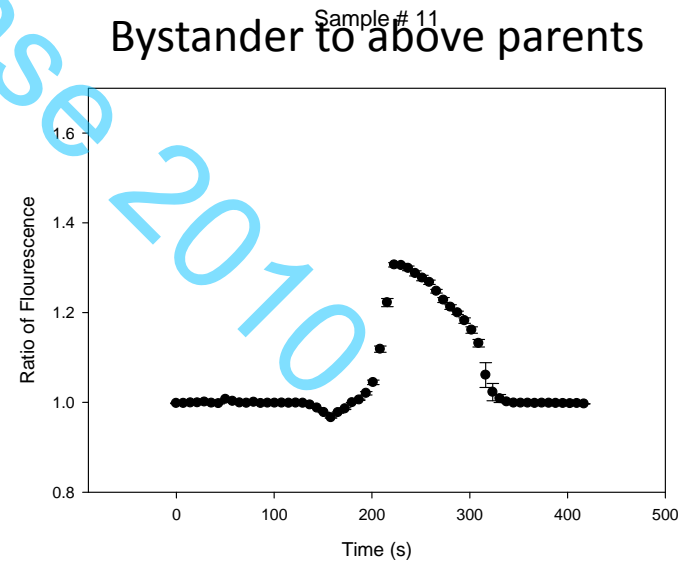
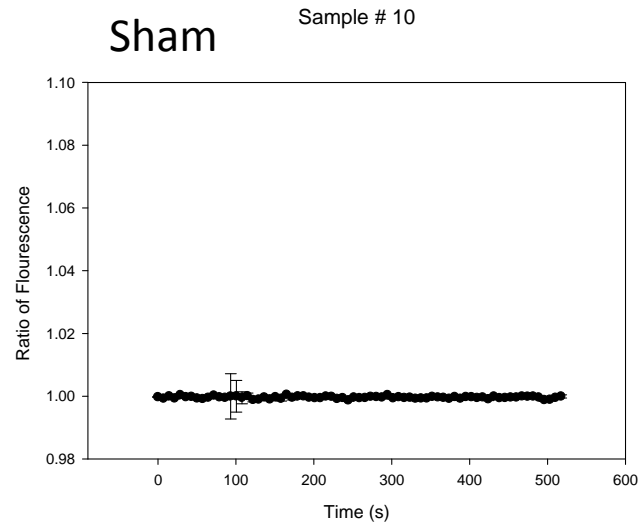
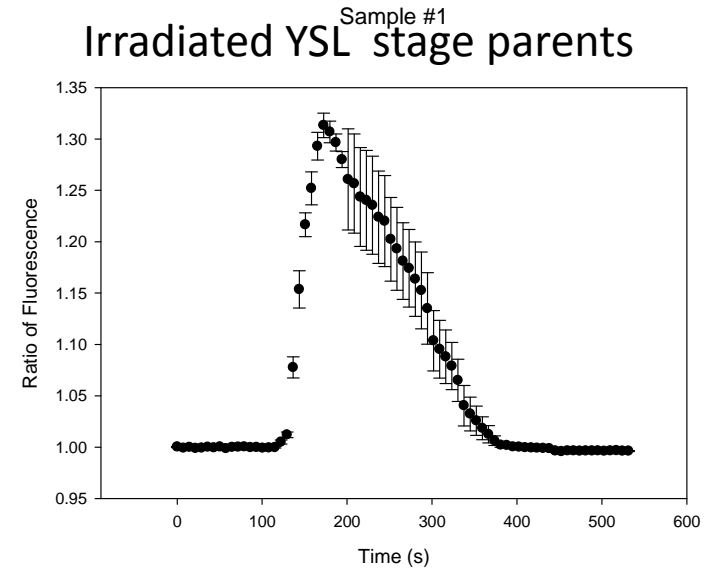
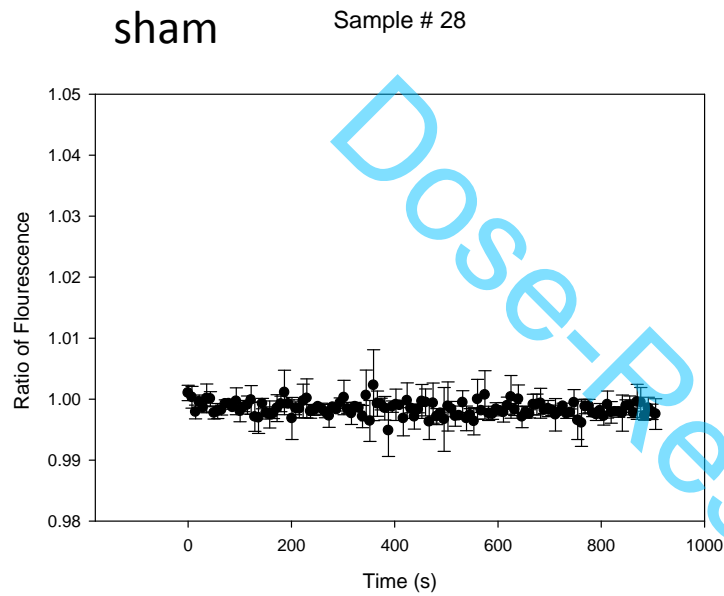


Transgenerational study (in progress)

F1 eyed egg data from same treatment crosses



Transgenerational memory of irradiation calcium signal

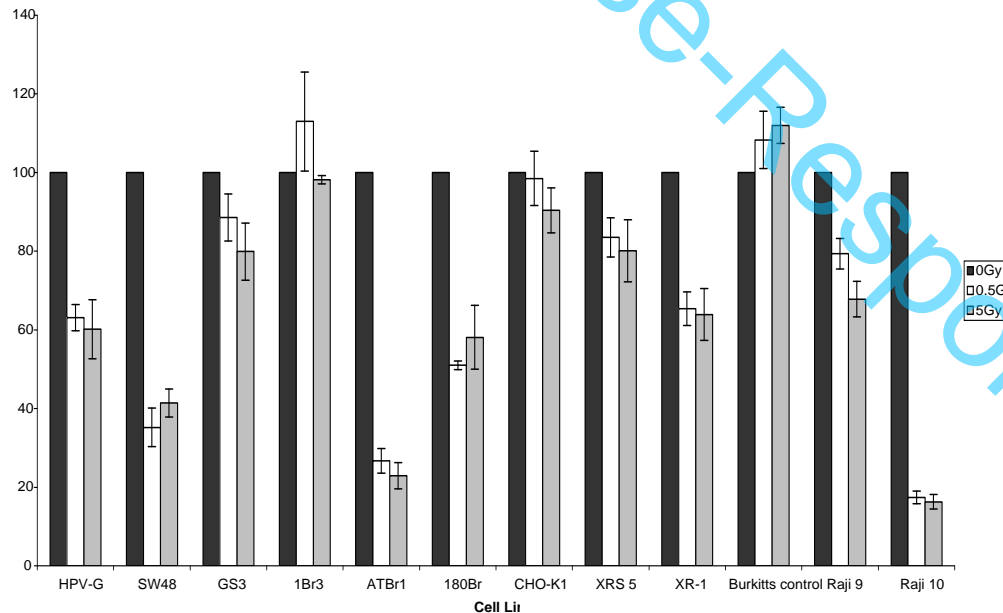


Some mechanistic questions

- Role of DNA repair
- Role of serotonin
- Role of p53

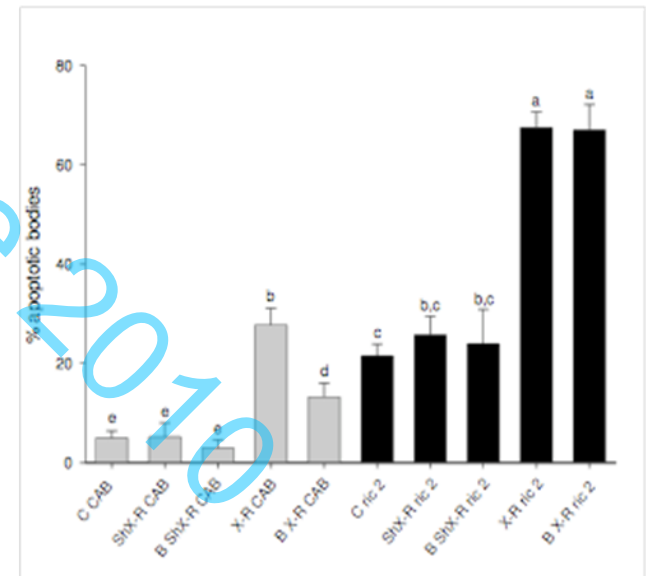
DNA repair is important in vivo and in vitro

Reduced reproductive survival in vitro

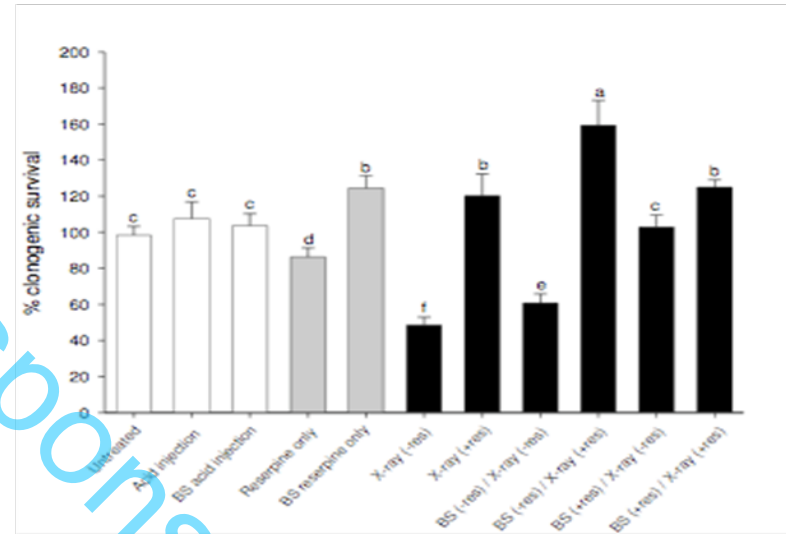
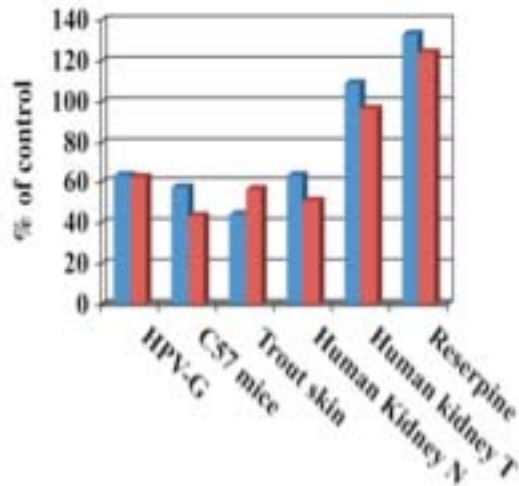


DNA repair deficient cell lines and transgenic medaka both produce highly toxic bystander signals after low dose irradiation

Increased apoptosis in vivo



Serotonin important in vivo and in vitro



| Sham treatment | % clonogenic survival |
|--|------------------------------|
| Sham X-ray (-reserpine injection) | 103.8 ± 8.1 ^{*,‡,†} |
| Sham X-ray (+reserpine injection) | 111.7 ± 8.6 [‡] |
| Bystander (-reserpine) / sham X-ray (-reserpine) | 102.0 ± 4.8 ^{*,†} |
| Bystander (-reserpine) / sham X-ray (+reserpine) | 137.0 ± 6.8 ^{*,‡} |
| Bystander (+reserpine) / sham X-ray (-reserpine) | 93.5 ± 3.6 [†] |
| Bystander (+reserpine) / sham X-ray (+reserpine) | 130.6 ± 4.3 [‡] |

* significantly different to equivalent X-ray treatment

‡ significantly different to untreated and reserpine injected fish.

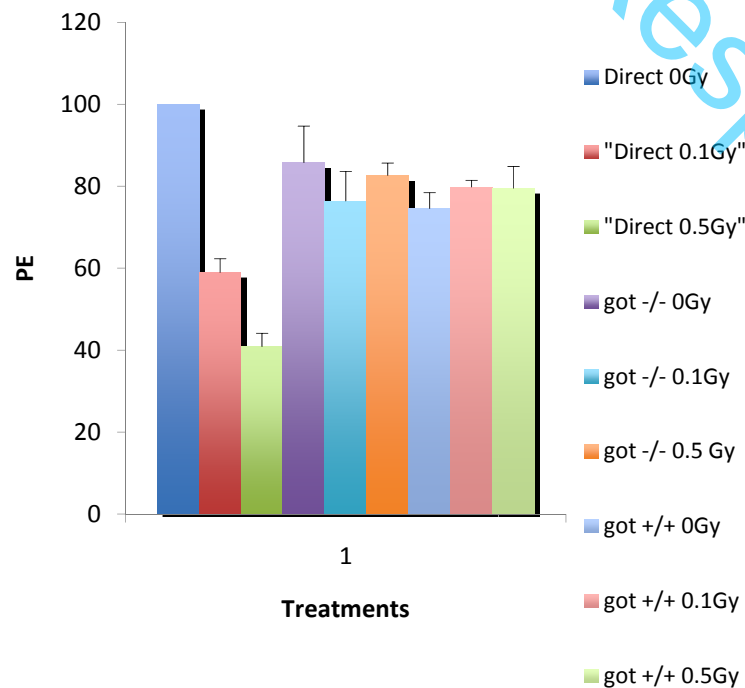
† significantly different to reserpine injection only induced bystander effect.

Serotonin bound by irradiated cells
In vitro, leading to Calcium pulse.

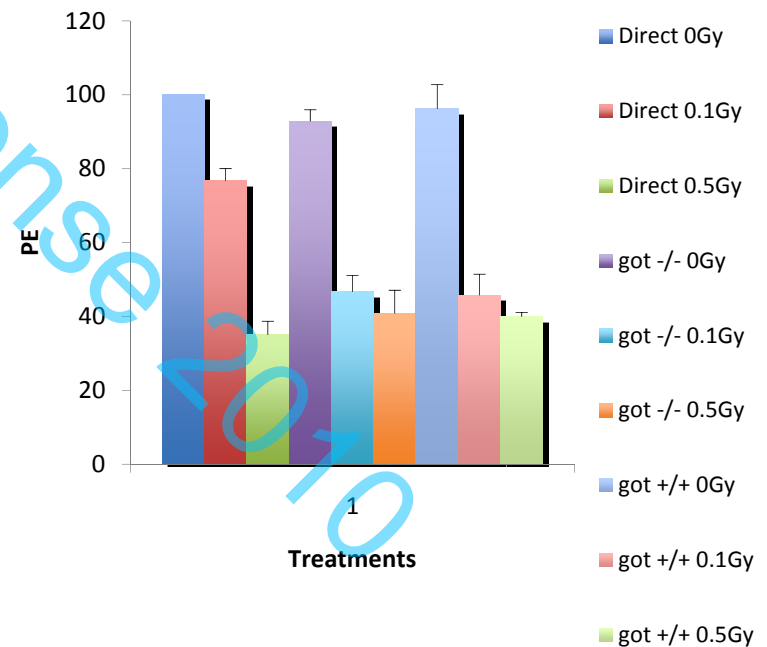
Reserpine inhibits serotonin binding
and prevents the bystander effect in
vitro and in vivo

Role of p53 in response to signal but not in generation of signal

HCT -/- Reporters



HCT +/+ Reporters



Multiple stressors

- Radiation seldom acts alone
- Isotopes are by definition a radioactive chemical
- Little information on synergistic effects
- Little low dose information
- Little in vivo information

What the multiple stressor problem does to radiation 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, population and ecosystem level
- Mechanistic uncertainty at low doses
- Non-targeted effect predominate at low doses

Examples of complex scenarios

- Radiation induces a cell to undergo apoptosis, removing it from the potentially carcinogenic pool. Substance 2 (eg Cd) interferes with the signaling cascade and the cell lives – survival assay suggests protective effect of interaction?
- Radiation induces an adaptive response in population A, a further stressor has little effect but pristine population B has no adaption and is devastated by the same stressor.

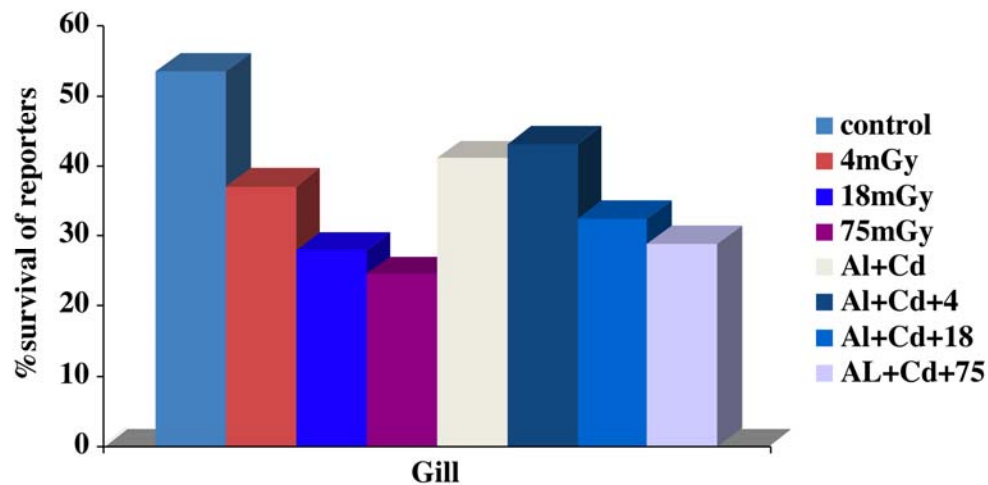
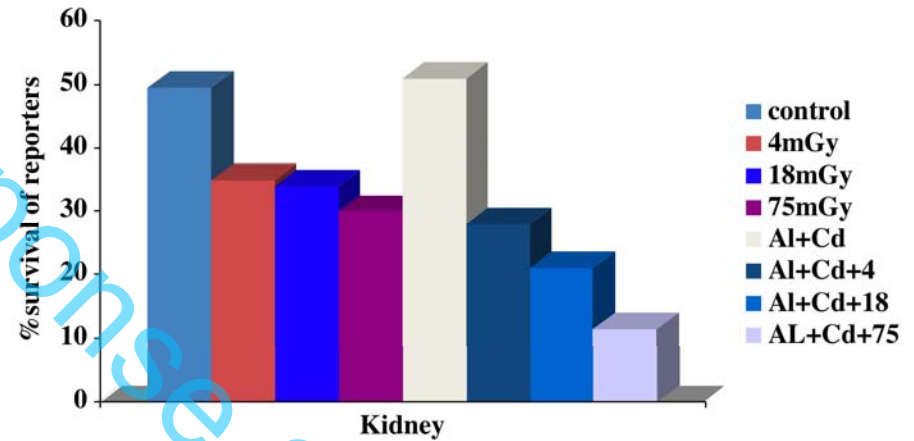
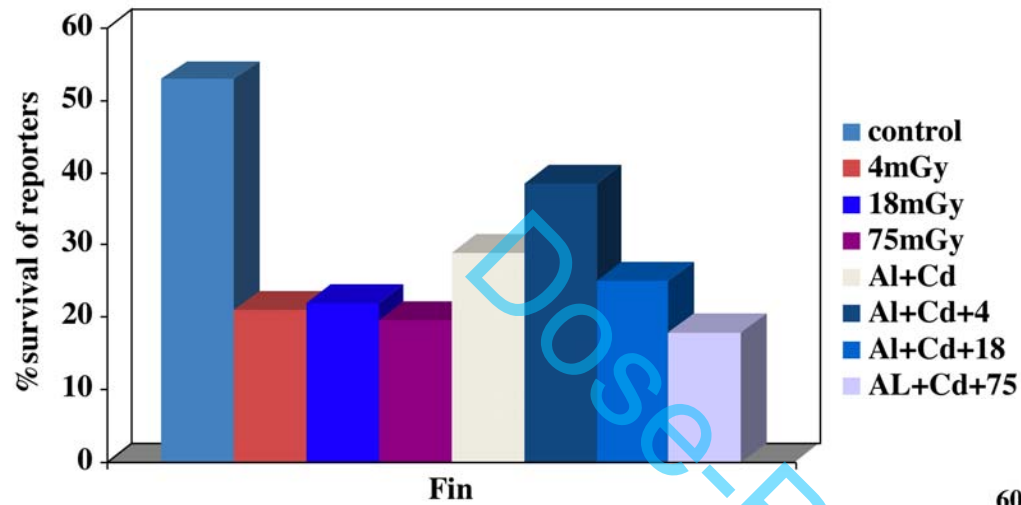
Fish irradiation in Norway: (lets get some low dose data!)

- Exposure of fish in aerated tanks to mGy doses over 5-48 hours



Metals in the water

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

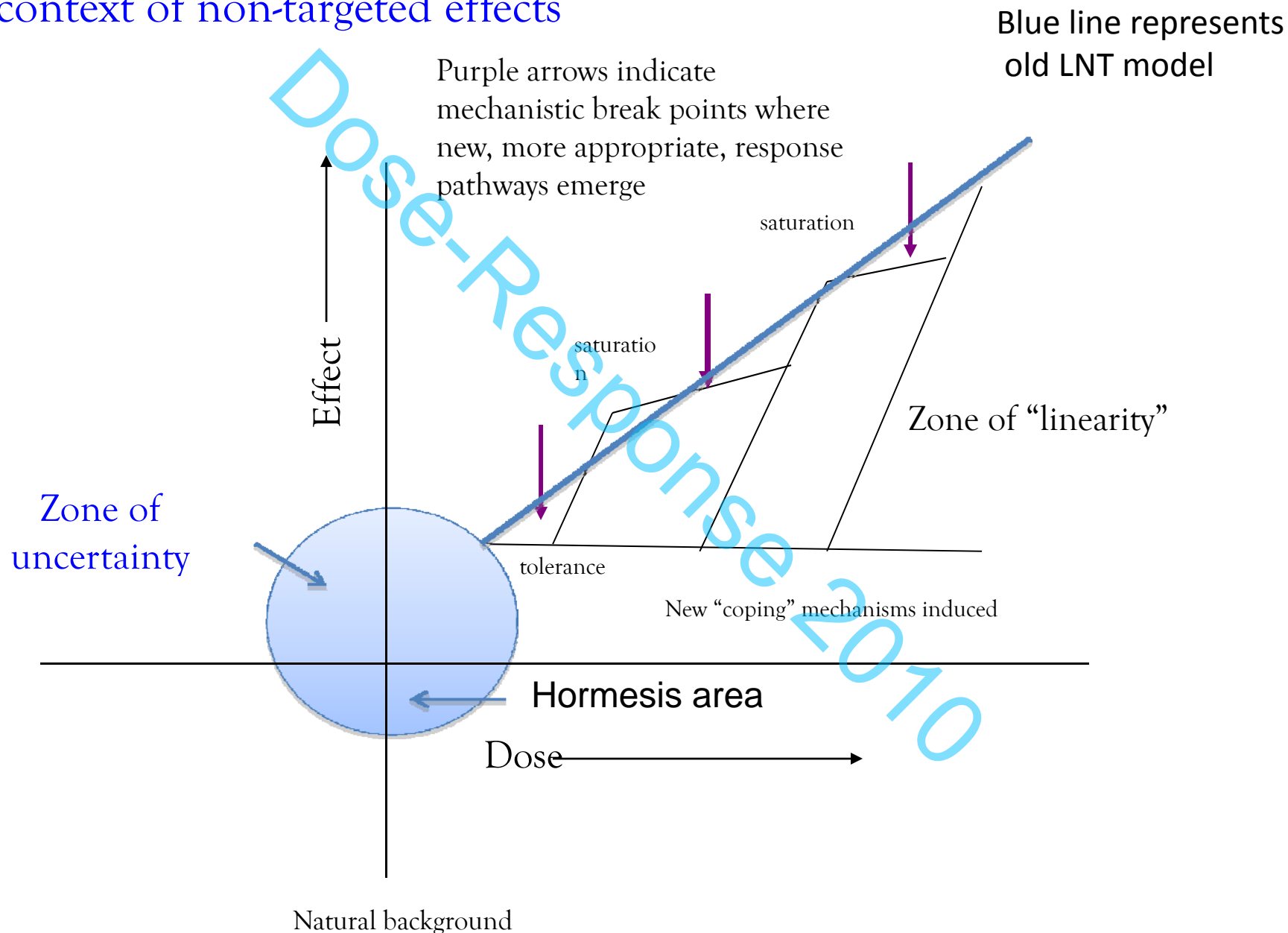


Co60

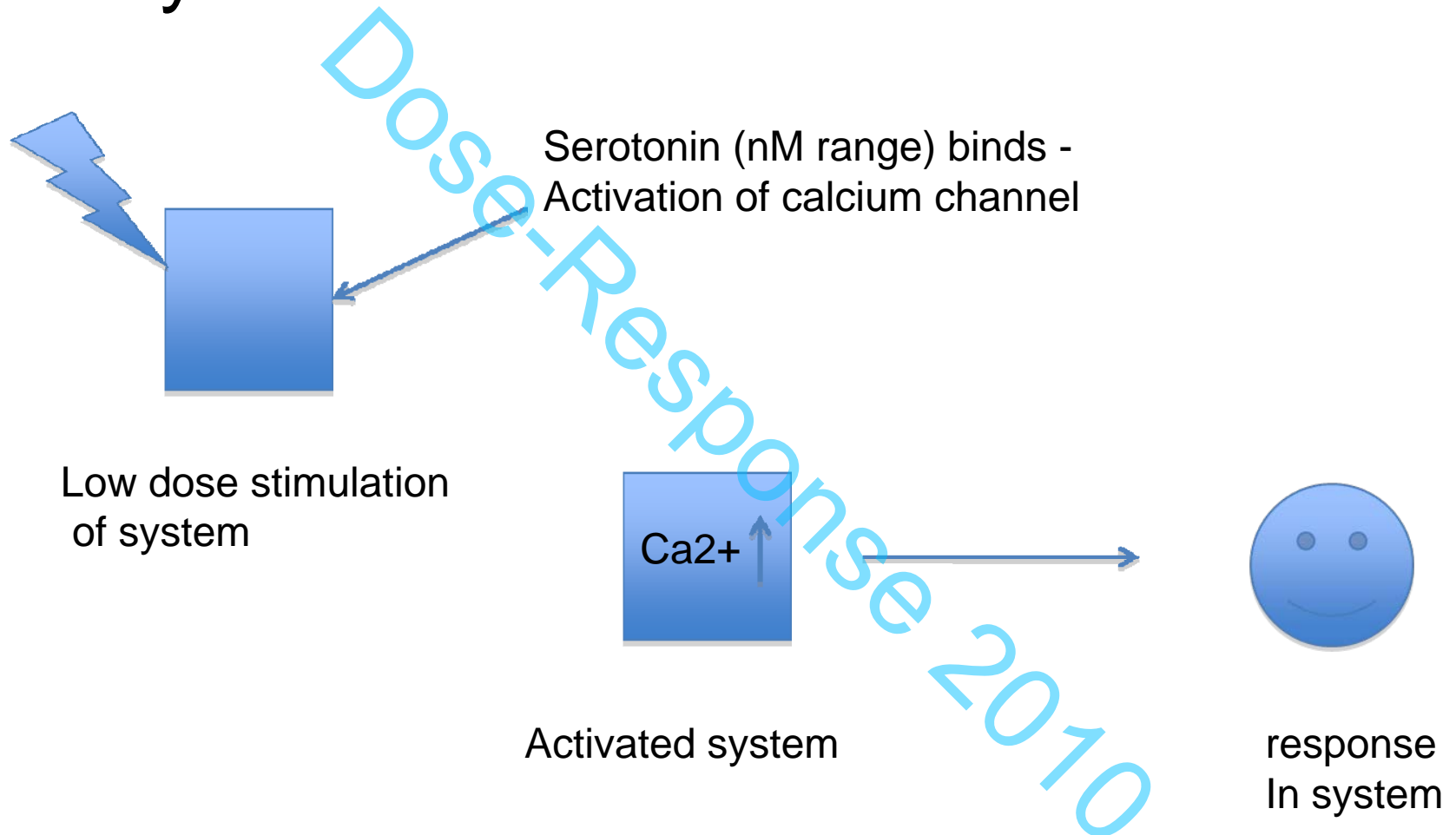
Al+Cd



Proposed dose response relationship for radiation in the context of non-targeted effects



Unifying Theory: transduction of bystander effect



NOTES

: “good” response at one level in system may be “bad” at another level
Links stress even mental stress, physiological response and outcome

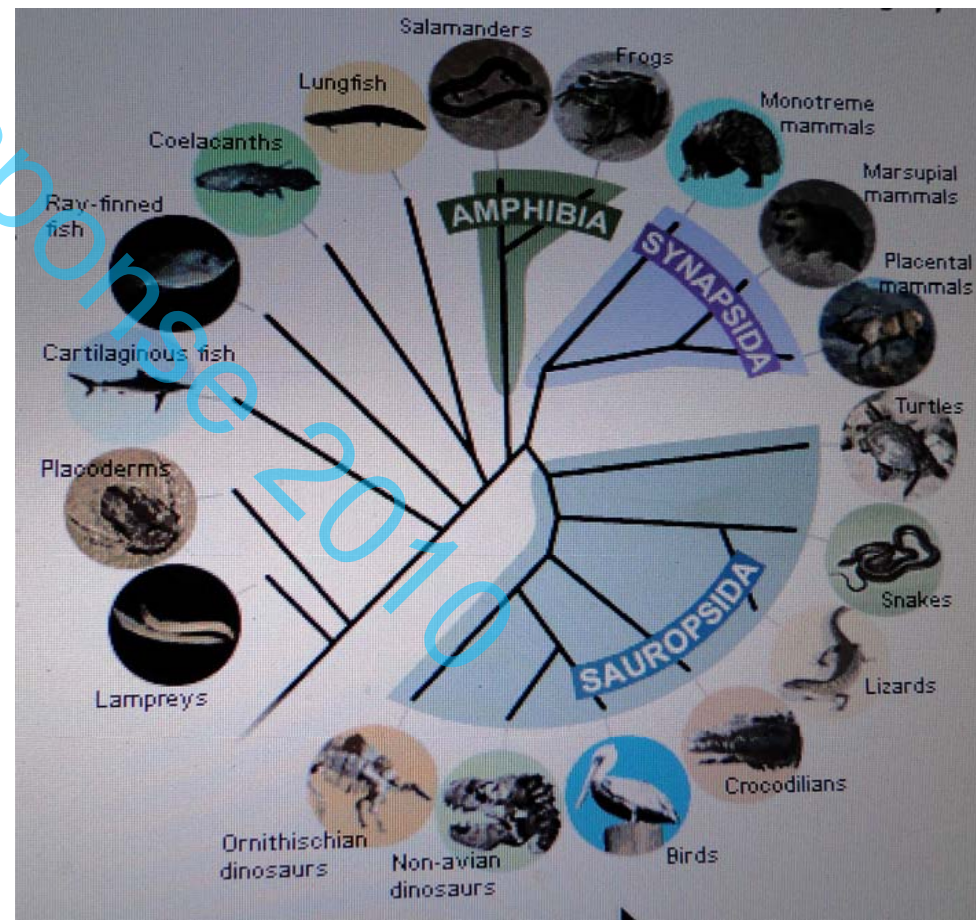
So why do these effects happen?

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WHY ARE THESE MECHANISMS SO WIDESPREAD AND PERSISTENT?

- In terms of evolution there is conservation of the mechanism and bystander pathway across species and this suggests a very primitive origin in the vertebrates since teleost fish split from the main vertebrate line early in vertebrate evolution.

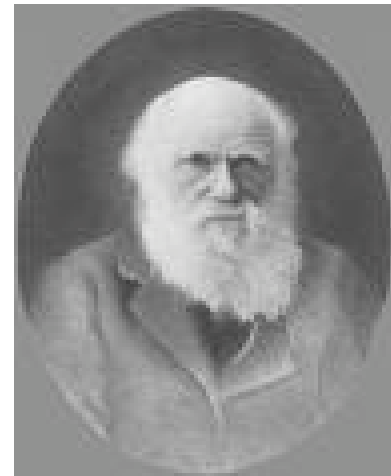
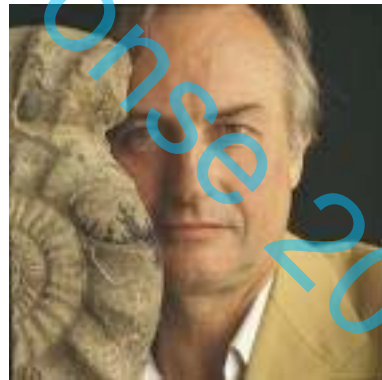


Population based response?

- Are non-targeted effects a reflection of population level regulation to optimise population fitness (tissue or individual level)?
- Is the function of radiation-induced bystander signaling to co-ordinate behaviour at higher hierarchical levels of organisation?
- Quorum sensing in bacteria is an example of this at the population level as are hormones at the organism level

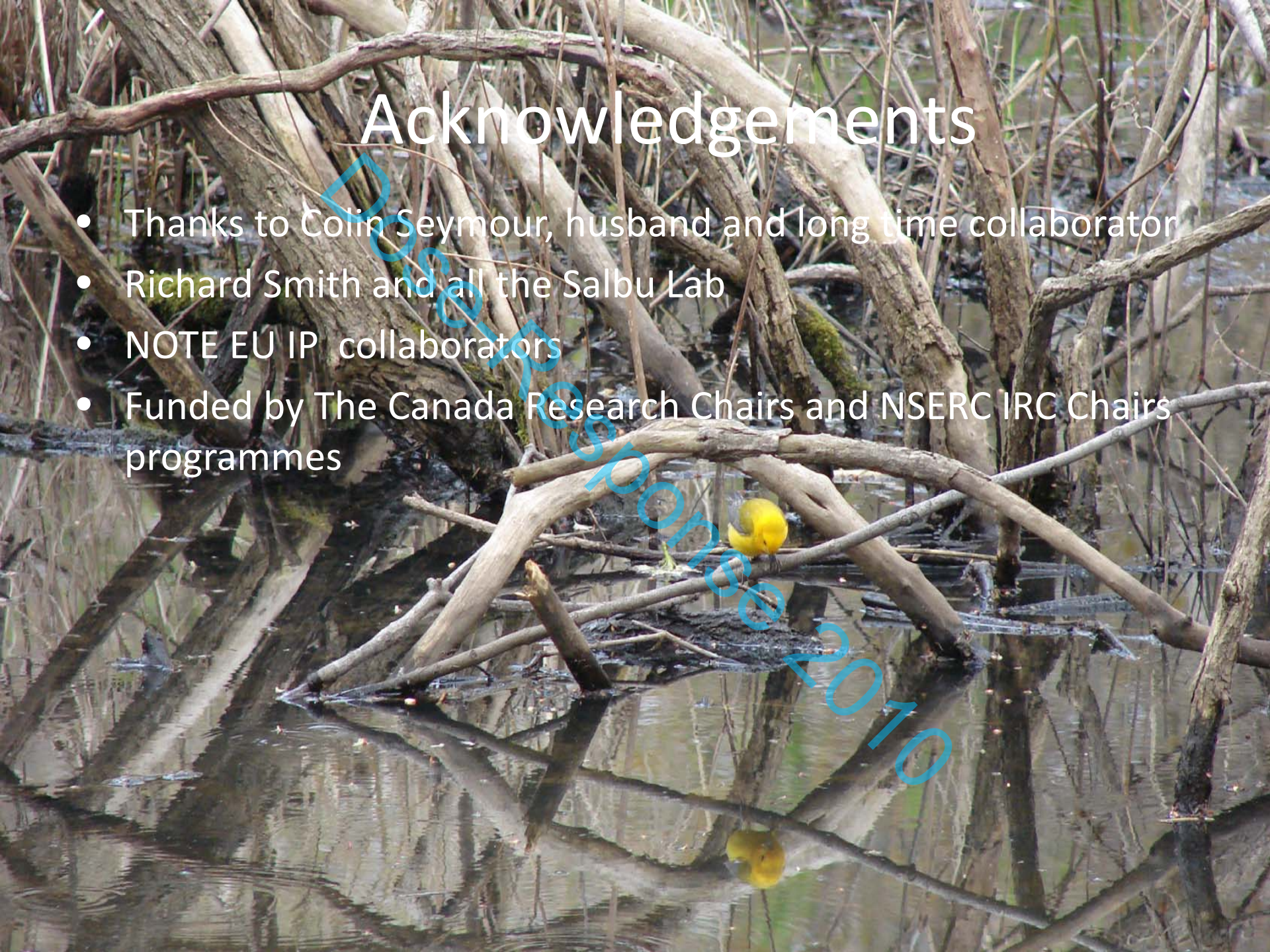
SUMMARY

Non targeted effects exist
They manifest at high frequency in many ways
They cause “stress-like” symptoms
We know a lot about the mechanisms but little about the reasons why they are tolerated
The underlying debate about purpose or chance is as old as Plato and Aristotle



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