24 months eating radium!

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Outline

Phenomenology

- Dosimetry
- Growth data
- Biochemical indices

Mechanisms

- Proteomic changes
- Bystander signaling
- Calcium transport

The future?

Goals of the study

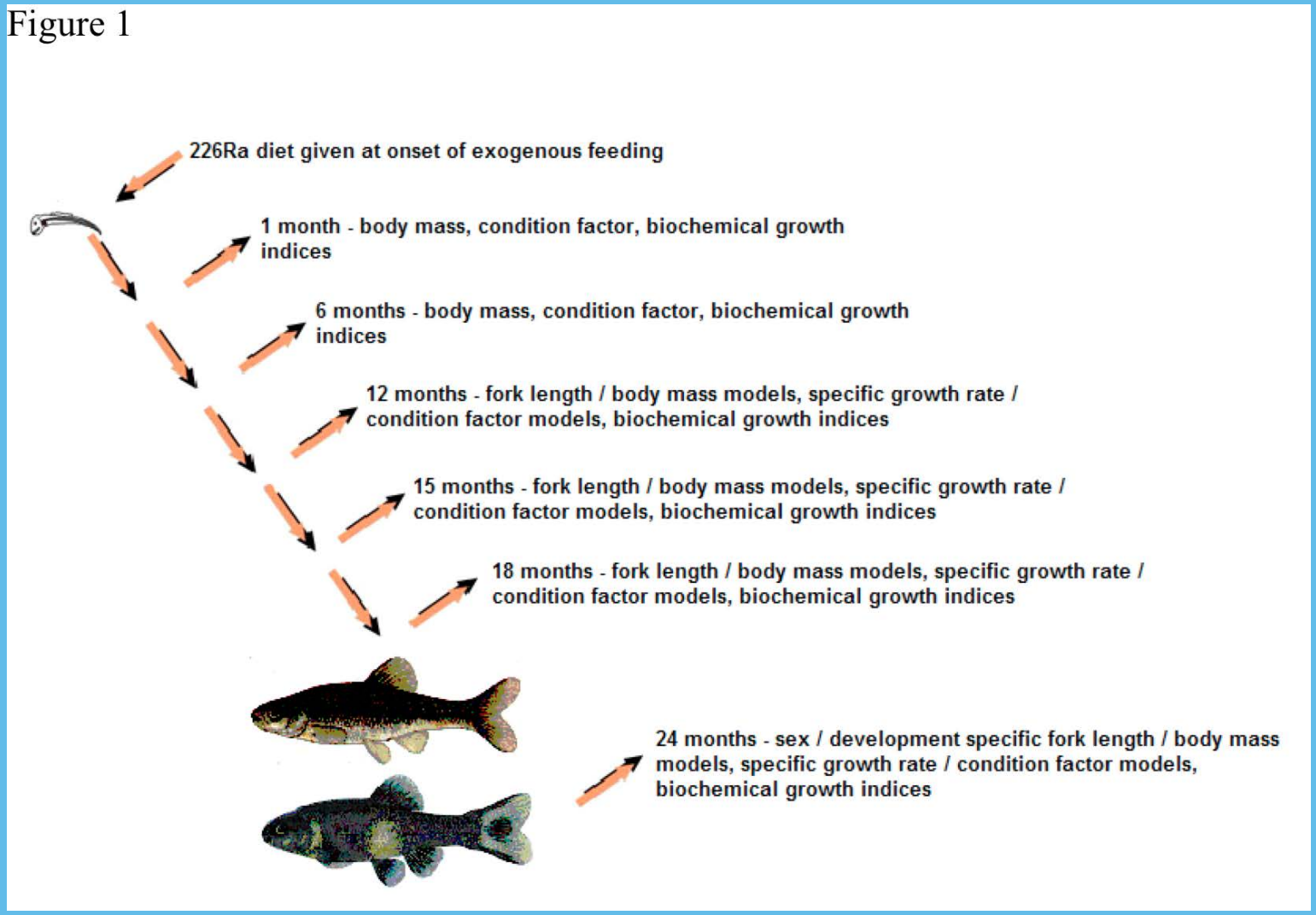
- To access impacts of environmentally relevant levels of ^{226}Ra in a fish species
- To determine mechanisms associated with chronic exposures
- To determine ultimately whether ^{226}Ra in the environment is a radiological risk to biota

Highlights of the work

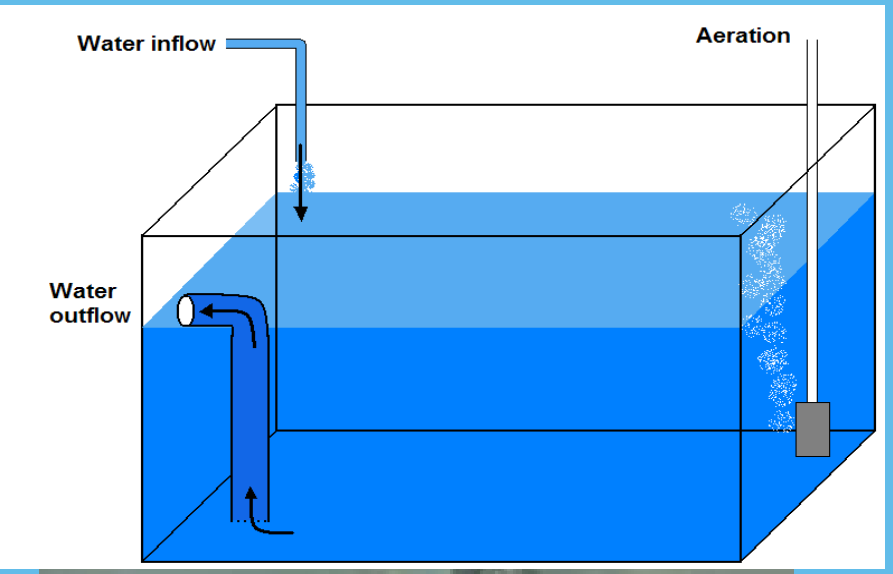
- Two year (lifetime) study in fish (FHM) concluded using environmentally relevant doses
- No increased or accelerated mortality, no gross pathology
- Minor and transient effects on growth at environmentally relevant doses and temperatures, virtually no effects at higher doses (up to 1000 times greater than seen in lakes)
- Proteomic changes observed
- Adaptive mechanism to rid Ra-226 from body induced
- Stress signaling remains throughout life and may be a homeostatic mechanism

Schematic of Experiment

Figure 1



Fathead minnow husbandry / ^{226}Ra feeding



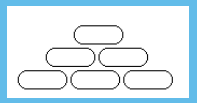
Water temperature: ambient, currently 8°C

Standing water volume: 15 l

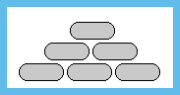
Water flow through: 200 ml min^{-1}

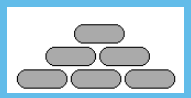
Feeding: once daily, to satiation

^{226}Ra-labelled pellet diets – derived from commercial fish food



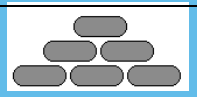
Untreated food





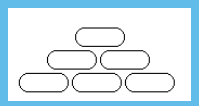
10 mBq/g diet
100 mBq/g diet

Based on field data



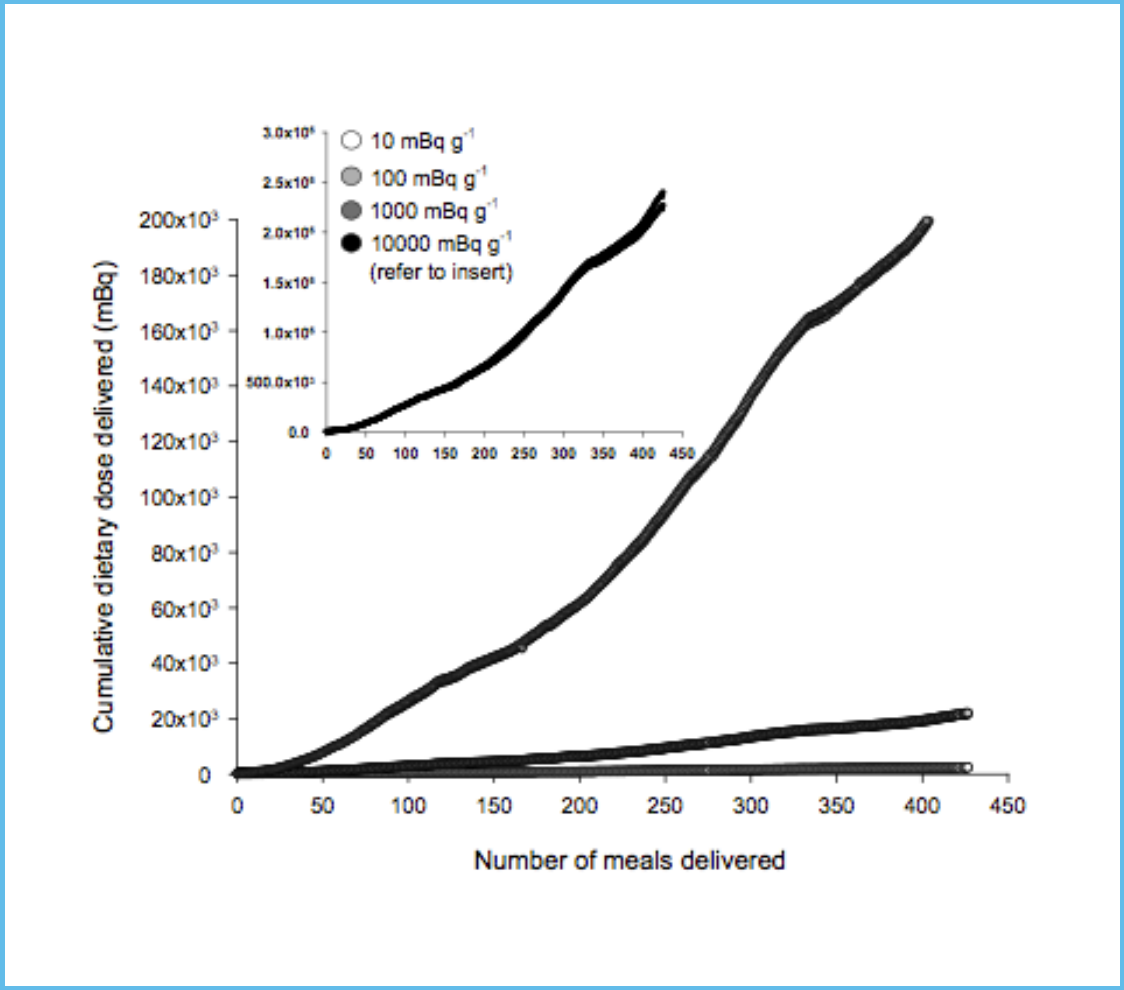
1000 mBq/g diet

10000 mBq/g diet



Acid control food (0.98 mM HNO_3)

Activity (mBq/g) delivered since start of feeding



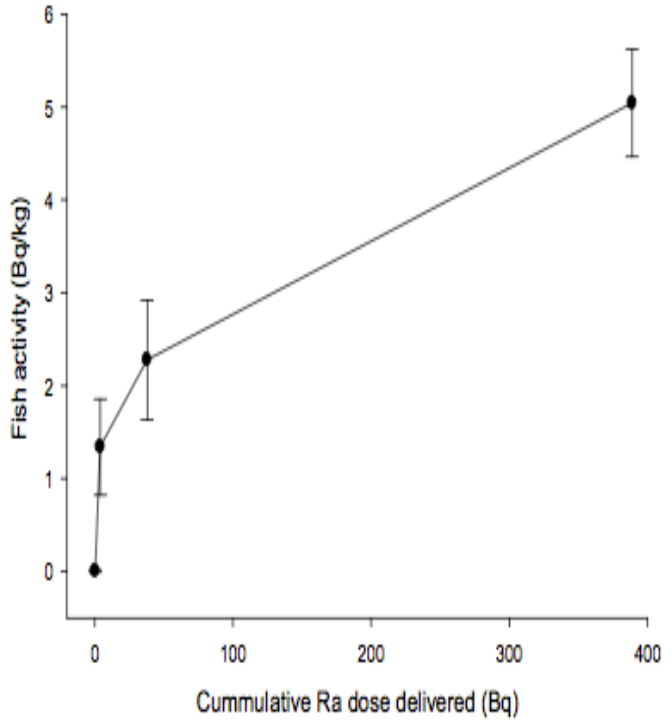
Chronic high LET dosimentry data

Fed ^{226}Ra for 1 month

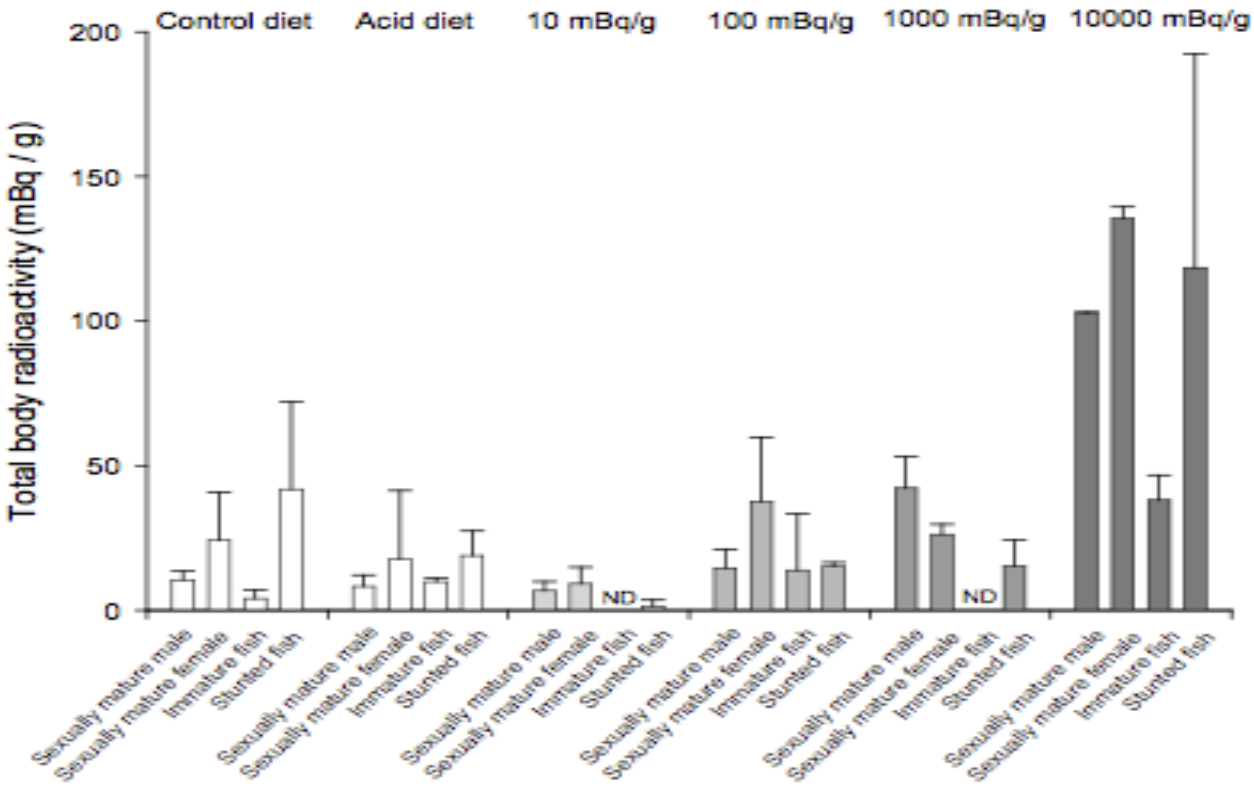
ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Control Fish	36 ± 22	0,9 ± 0,5
Control Fish	28 ± 28	0,7 ± 0,7
Fed 10 mBq g^{-1}	39 ± 15	1,0 ± 0,7
Fed 10 mBq g^{-1}	23 ± 8	0,6 ± 0,2
Fed 100 mBq g^{-1}	11 ± 12	0,2 ± 0,2
Fed 100 mBq g^{-1}	9 ± 12	0,2 ± 0,3
Fed 1 Bq g^{-1}	26 ± 11	0,7 ± 0,3
Fed 1 Bq g^{-1}	33 ± 13	0,8 ± 0,3
Fed 10 Bq g^{-1}	100 ± 18	2,5 ± 0,4
Fed 10 Bq g^{-1}	124 ± 16	3,0 ± 0,4

Fed 226Ra for 6 months

Approx 50μBq per Bq assimilated in 6 months



Radium levels after 18 months of feeding



24 month dosimetry data

Control diet

ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Background (all fish)	2.7 ± 9.3	0.1 ± 0.3

10 mBq g^{-1} diet

ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Sexually mature males	4.7 ± 5.6	0.12 ± 0.14
Sexually mature females	5.9 ± 7.1	0.15 ± 0.17
Immature fish	0.6 ± 1.6	0.02 ± 0.09

100 mBq g^{-1} diet

ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Sexually mature males	3.1 ± 3.8	0.1 ± 0.1
Sexually mature females	0.0 ± 0.0	0.0 ± 0.0
Immature fish	4.5 ± 10.0	0.1 ± 0.2

1000 mBq g^{-1} diet

ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Sexually mature males	11.8 ± 10.3	0.3 ± 0.3
Sexually mature females	10.1 ± 4.2	0.2 ± 0.1
Immature fish	23.1 ± 13.3	0.6 ± 0.3

10000 mBq g^{-1} diet

ID	Activity (Bq kg^{-1} wet)	Annual dose (mGy y^{-1})
Sexually mature males	39.2 ± 9.4	1.0 ± 0.2
Sexually mature females	14.9 ± 17.8	0.4 ± 0.4
Immature fish	55.0 ± 21.0	1.4 ± 0.5

Calculated CF from Dr Lariviere

Averaged concentration factor (CF) calculated for various fish age

	Fish age (months)				
Food activity ($Bq\ kg^{-1}$)	1 (n=2)	6 (n=16)	18 (n=8)	24 (n=16)	Average
10	3.1	0.375[a]	0.692	0.92	1.27
100	0.1	10.06	0.2321	0.099	2.62
1 000	0.0295	1.70825	0.02788	0.0174	1.78
10 000	0.0112	0.378143	9.865×10^{-3}	4.55×10^{-3}	0.10

a. Only two fishes test had activities above DL.

Radium purging

- Confirms data seen based on a small pilot study
- Suggests a modification of calcium transport mechanisms
- Supports the pattern of adaptive effects during chronic exposures (Hinton, Stuart, Mitchel and others)

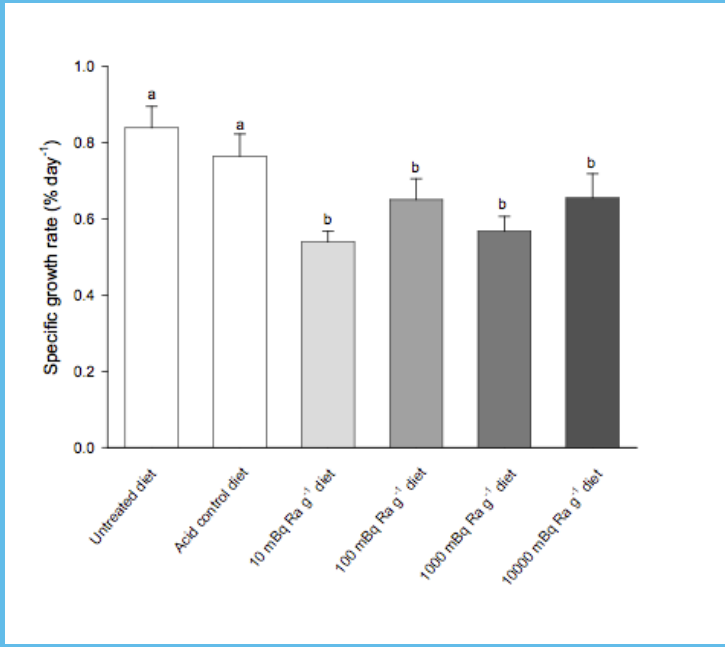
Despite very low retention biological *effects* ARE seen

After 6 months all ^{226}Ra diets yield smaller fish

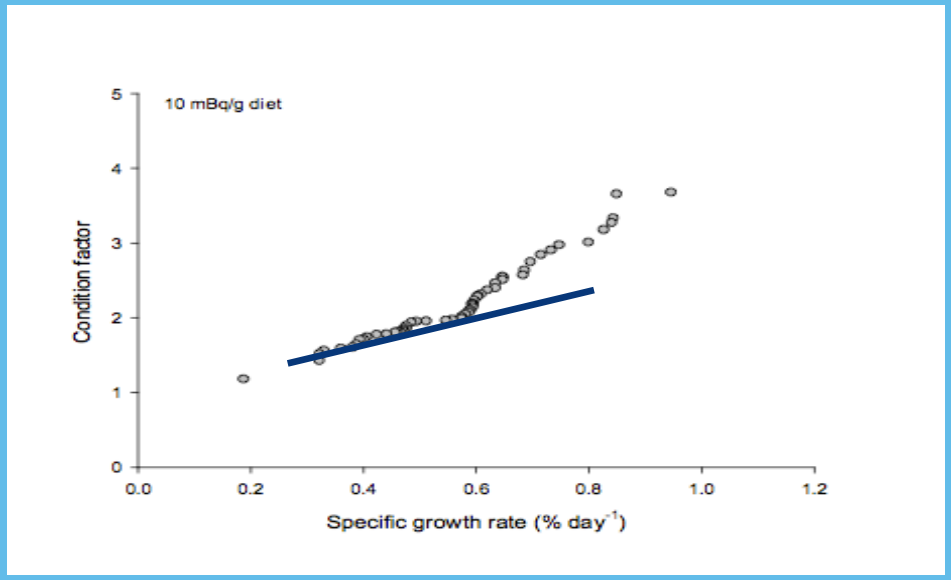
Relationship between K and SGR deviates in Ra fed fish. Points above the line show small (slow growing) fish with greater than expected K factor

SMALL FAT FISH!

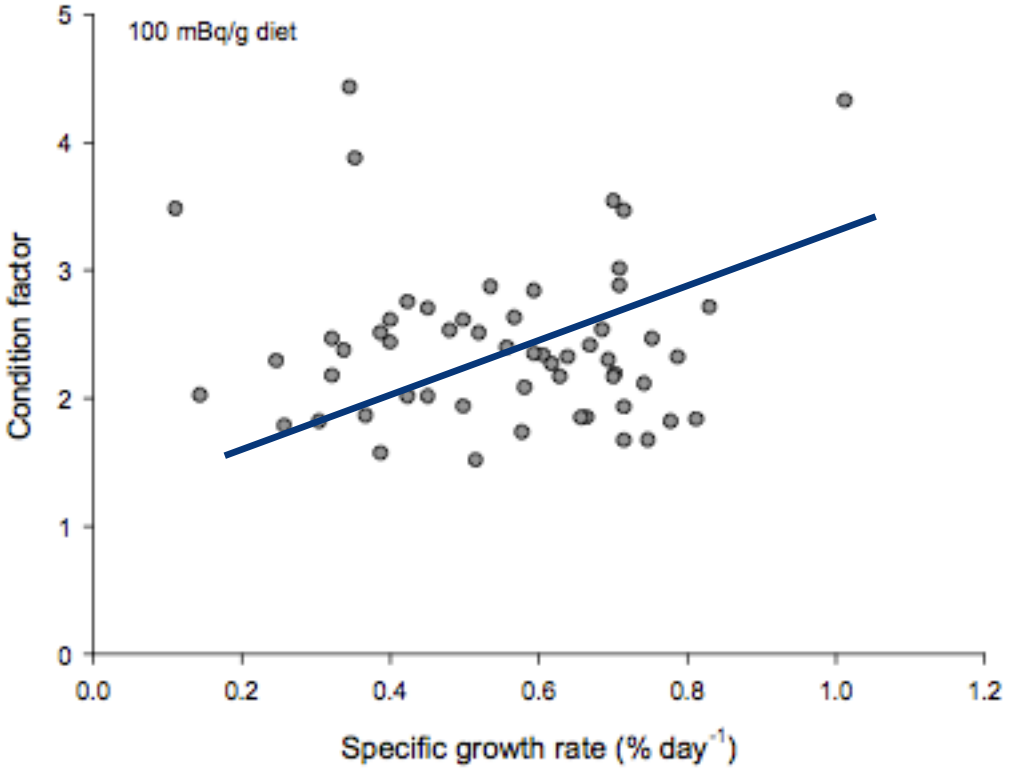
6 months on ^{226}Ra diet



1 year on diet (10mBq/g)

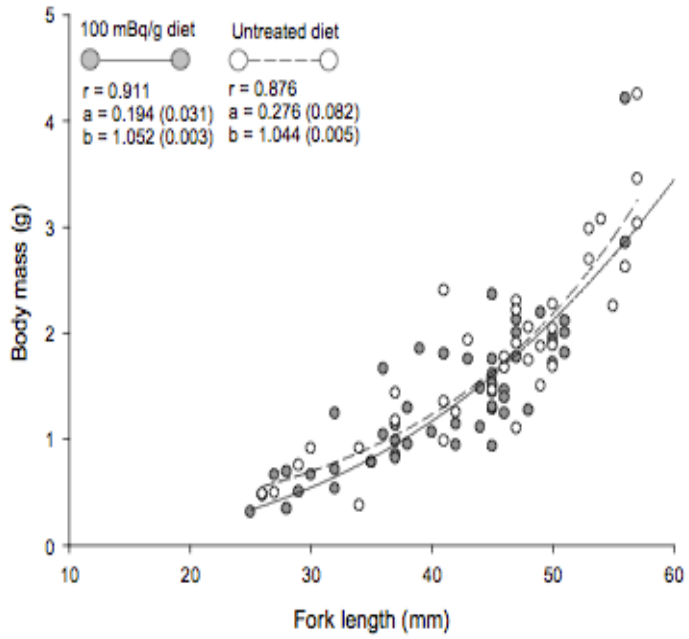


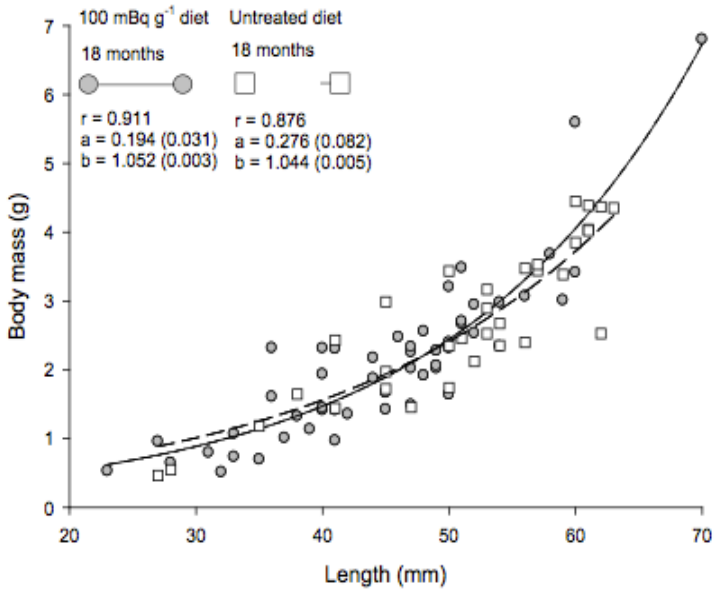
I YEAR DATA: 100mBq/g diet data with control line of best fit superimposed (blue). points above the line show small (slow growing) fish with greater than expected K factors. Points below the line have lower than expected K factors for their growth rate.



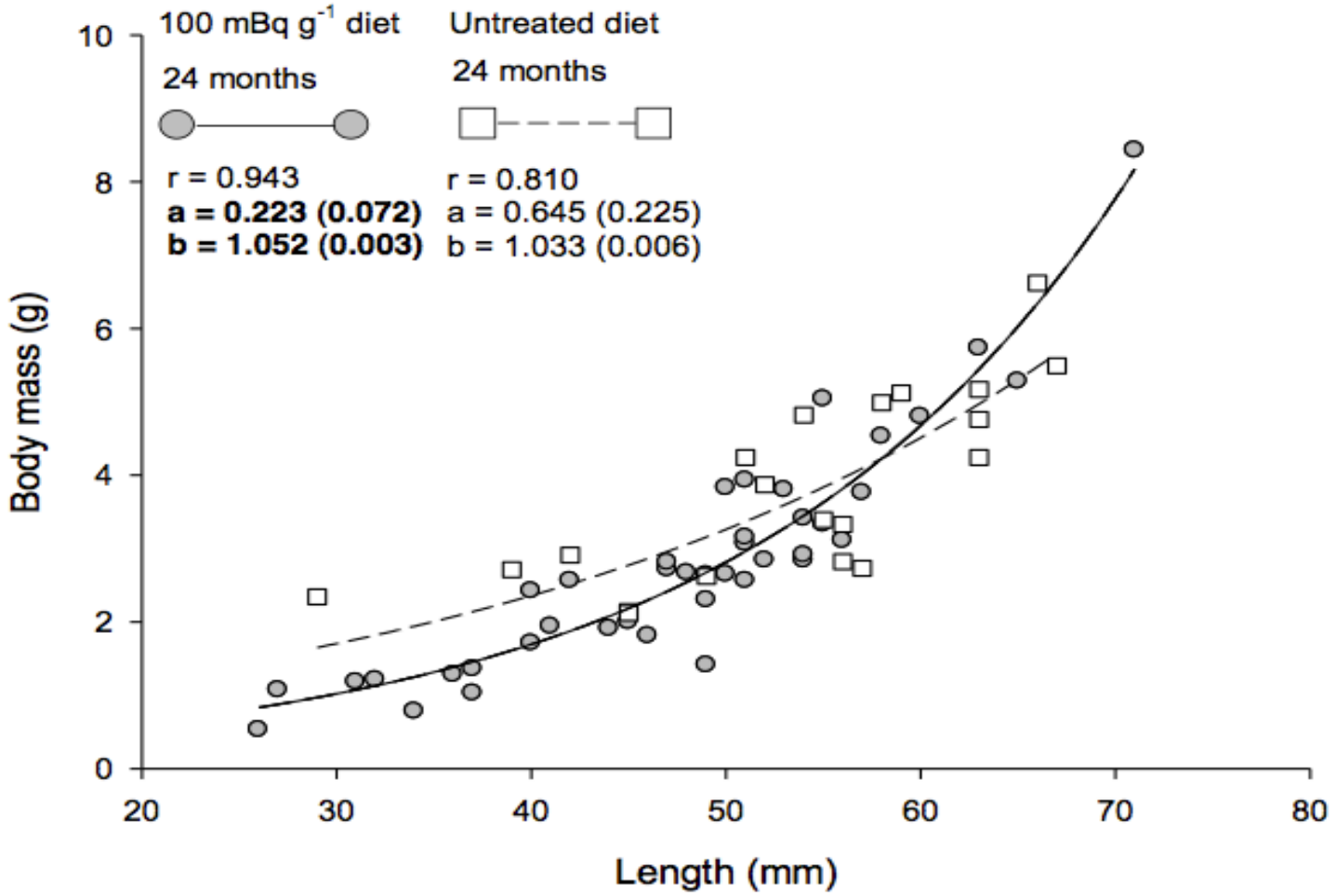
Relationship between K and SGR disrupted

However…….. Effect gone at 15 and 18 months





Back at 24 months as a reverse effect ?

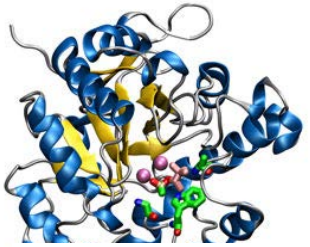


Details of the Study

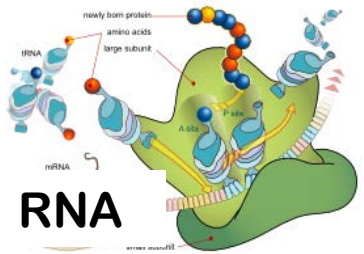
- Exposure to low dose alpha radiation from Ra-226 through the drinking water.
- 40 mice per treatment (20 females and 20 males).
- One control group and four treatment groups (0.01, 0.1, 1.0 and 10.0 Bq/L Ra-226).
- 40 individuals per group (kept until the fourth generation is obtained).
- Breeding between 8 weeks and 16 weeks of age.

White muscle biochemical growth indices

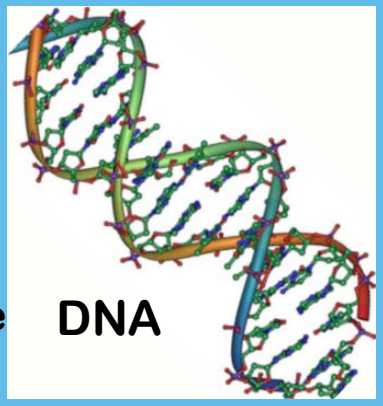
Perchloric acid + NaOH



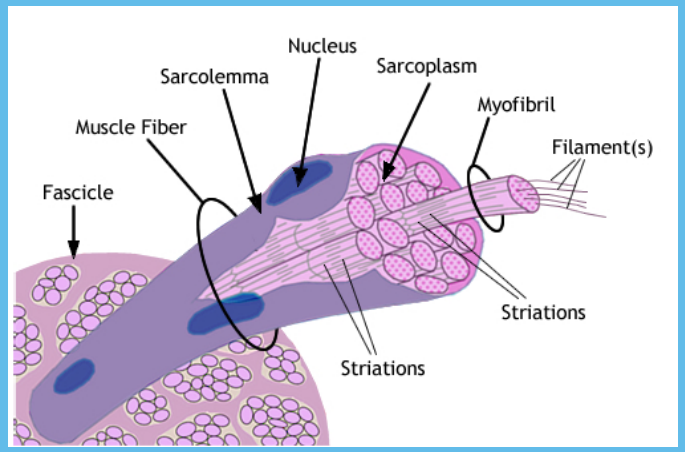
Protein



RNA



DNA

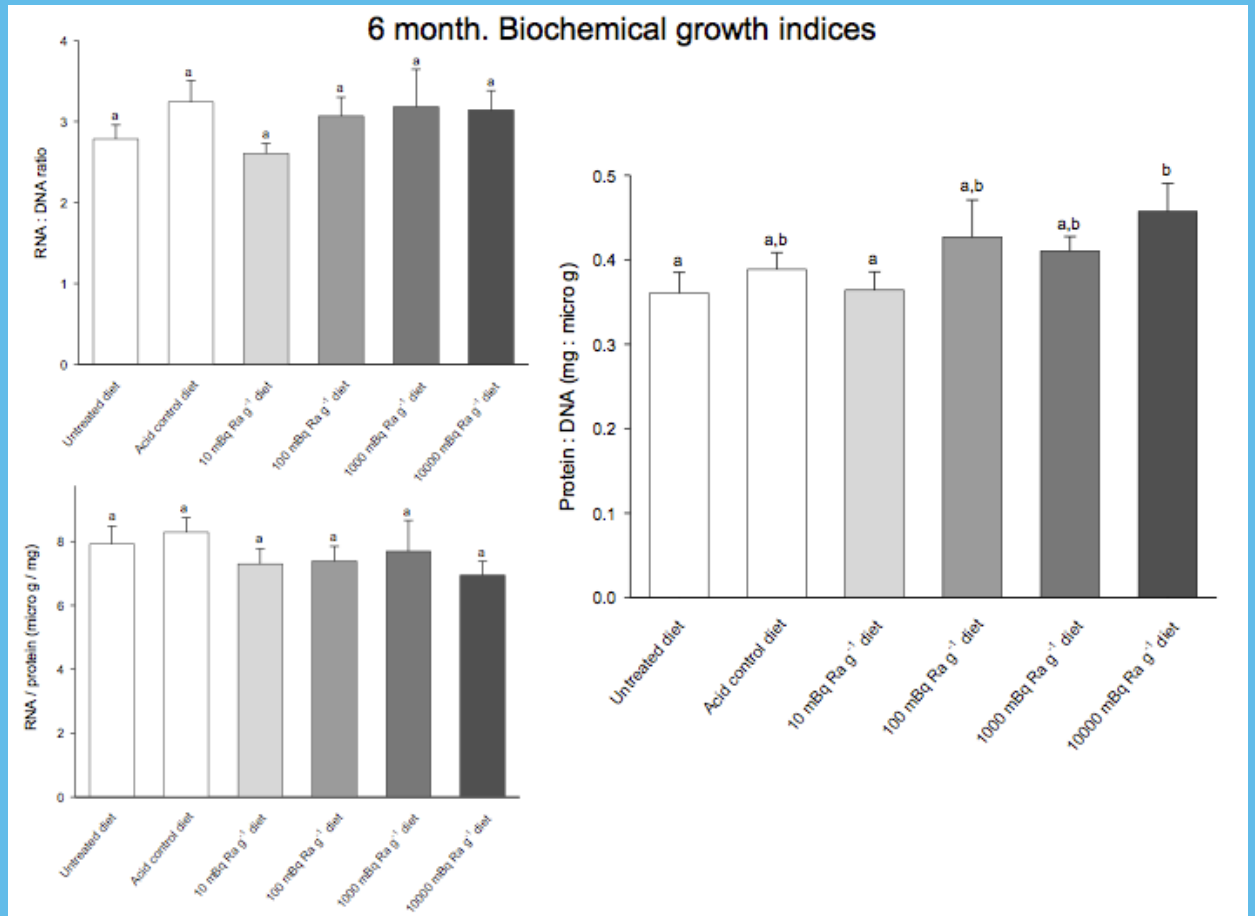


RNA : protein = index of ribosome number

RNA : DNA = index of cell size

Protein : DNA = index of extracellular protein per cell

No biologically significant effects at six months (or 18 or 24 months)



Summary of growth/biochemical data

- Clear transient growth perturbations, resulting: in
  - smaller fish at 6/12 months,
  - No effect at 15/18 months
  - Bigger healthy fish after 24 months
- Some statistically significant effects on biochemical growth indices but very small.

Mechanisms are very important at low doses

- Search for bio-markers or bio-indicators
- Search for population level markers of system perturbation
- Search for adaptive mechanisms

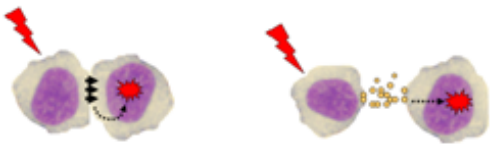
Low dose effects which might act as bio-indicators

- Bystander effects
- Genomic instability
- Low dose hypersensitivity
- Adaptive responses

All related? All driven by bystander signals?

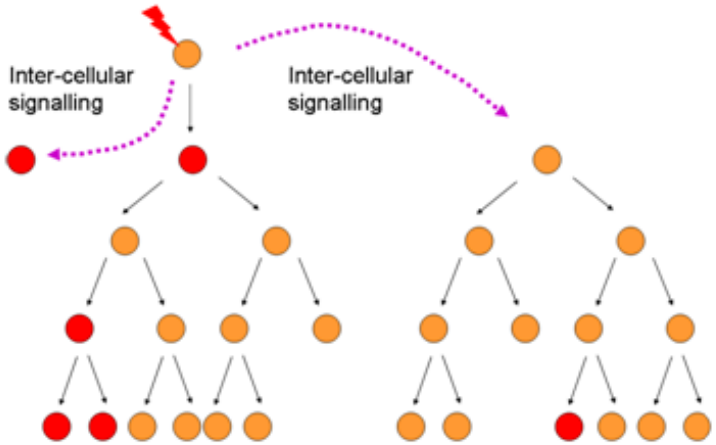
Bystander effects

Effects in neighbouring cells



Genomic Instability

Effects in unirradiated descendant cells

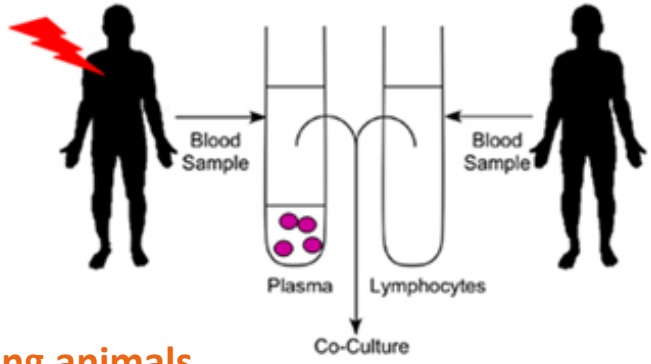


Abscopal effects

Effects in neighbouring tissues

Clastogenic factors

Ex vivo effects in cultured cells



Inflammatory Processes may provide mechanistic link

Inter-animal signaling

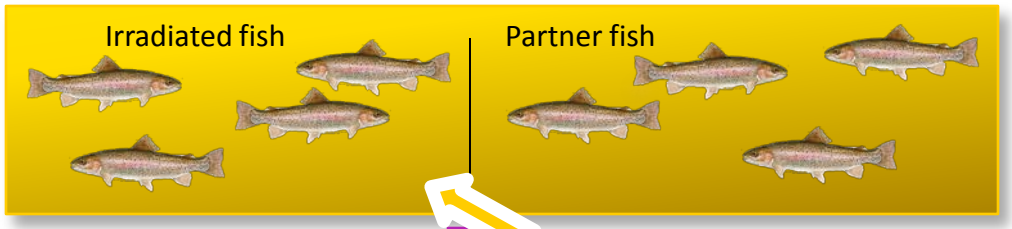
Effects in neighbouring animals

Long-term effects on innate immune response function may occur

Bystander signaling mechanisms in our system

- Nature of signaling within and between fish and cell cultures which leads to bystander response
  - Physical component?
  - Neurochemicals
  - Bioenergy
- Pathways involved in low dose response
  - p53
  - TGFb
- Dose rate and radiation quality effects

Measuring bystander response to radiation *in vivo* adapted from Mothersill et al 2006



Radium exposed fish allowed to swim with unexposed partner for 2hrs

Unexposed fish introduced into water from irradiated or sham fish
After 2hrs. Dissect tissues
Do proteomics

Explant pieces taken from skin, fin, gill, spleen and kidney
Do tissue culture

Culture of explants for 2 days

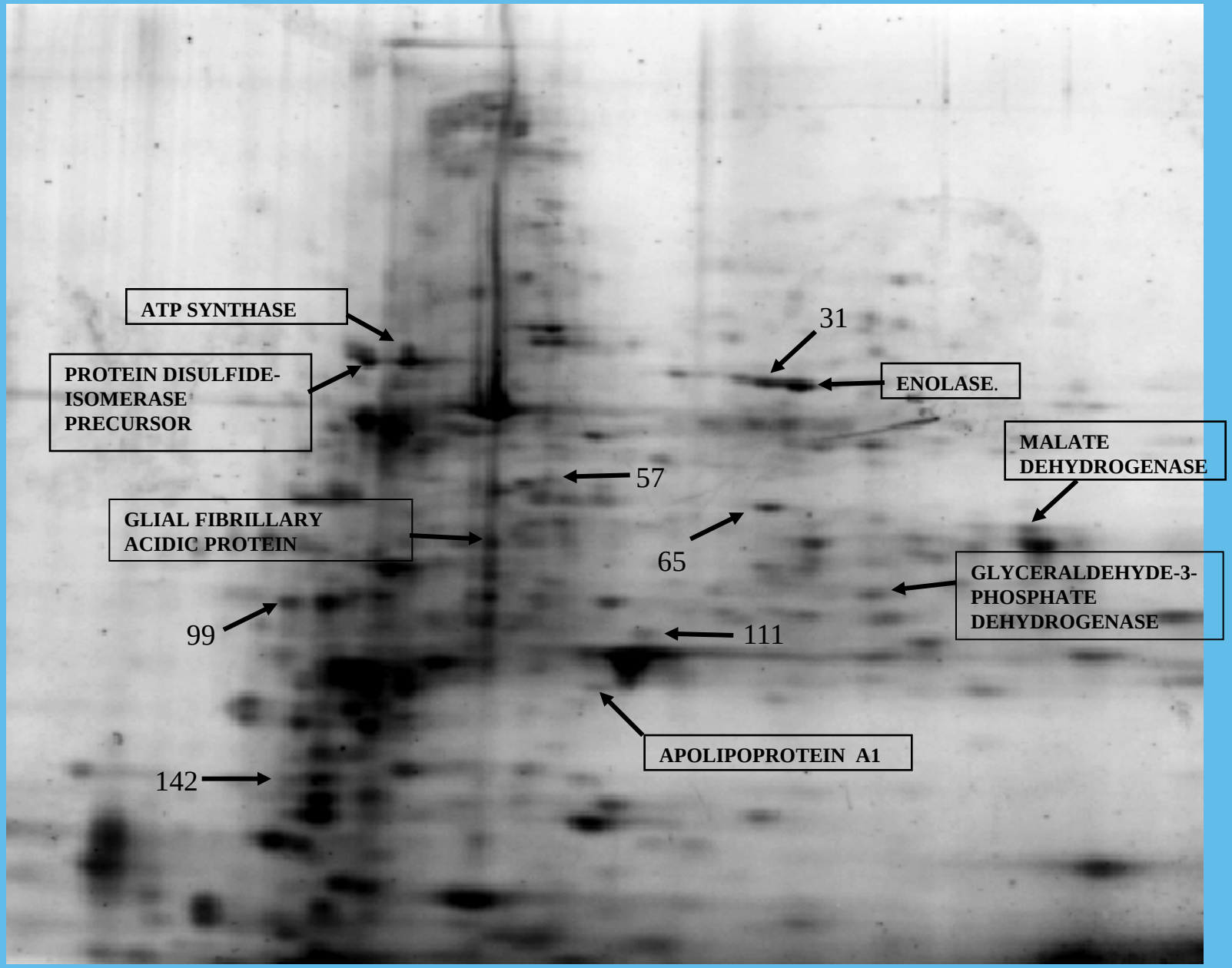
Harvest culture medium for calcium flux, ELISA and clonogenic assays

Grow up culture

examine explant outgrowth do immunocytochemistry

Add medium to unirradiated clonogenic cell line
determine surviving fraction by counting colonies after 10 days

Protein spots excised for identification



Proteomics Conclusions

Low dose dietary ^{226}Ra appears to exert a greater influence than higher doses.

Of the proteins identified so far; no absolute contradictions between early (juvenile) and long term (adult) dietary exposure but some dose-dependent specific changes.

However protein spot 57 shows an increase in early exposure but a decrease in long term exposure

Broadly speaking the response of the gill proteome centres on energetic and structural proteins. All of those identified so far have a relevance to radiation exposure.

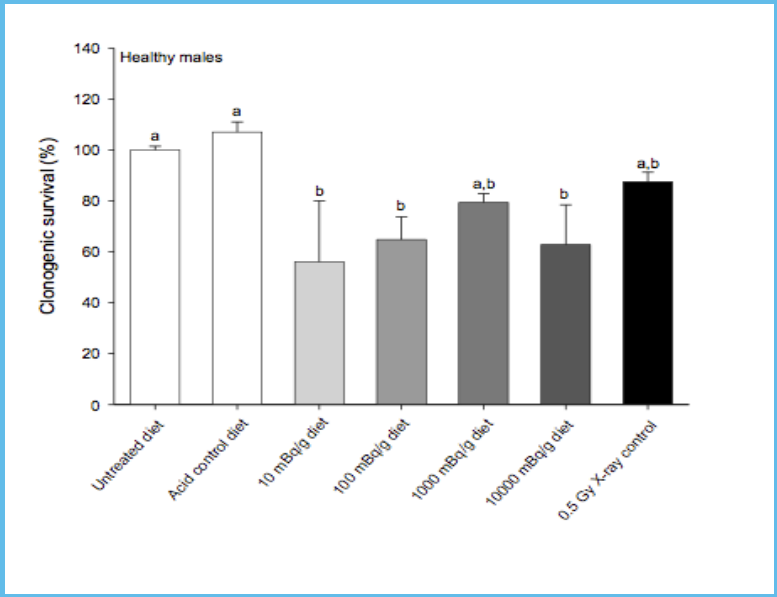
Evidence for both adverse and adaptive responses in the gill proteome

Stress signaling

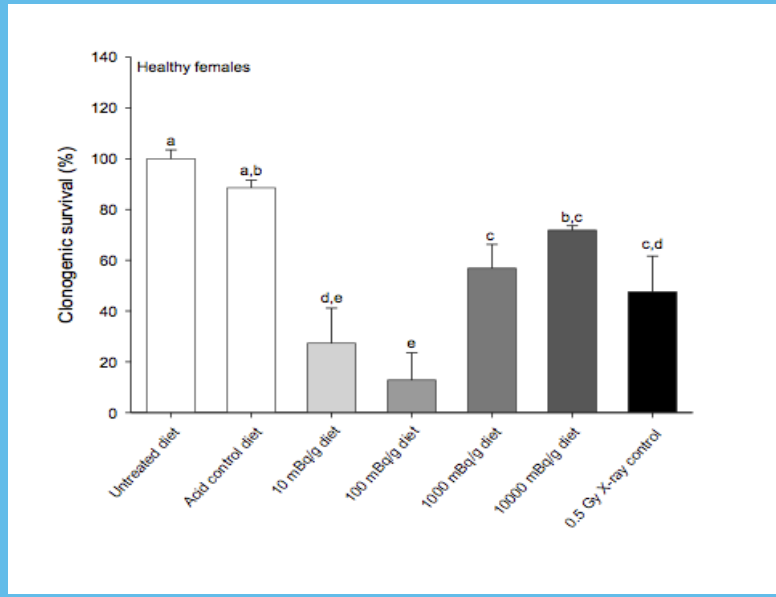
- Measured using bystander signal clonogenic assay
- Measured using Calcium flux or mitochondrial membrane leakiness assay
- Based on recent data showing link between radiation stress and inflammatory/immune response
- Can be done as a non-invasive test using fin clip

Bystander signaling after 24 month on ^{226}Ra diet

Healthy Males

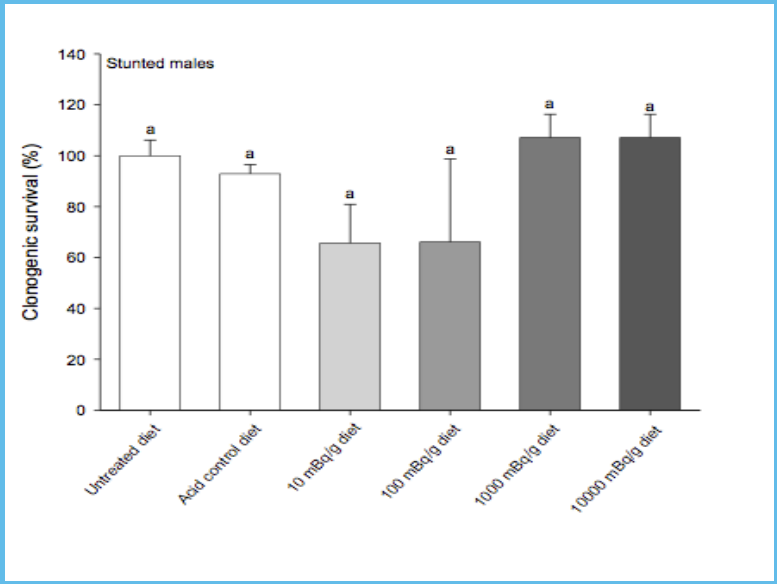


Healthy Females

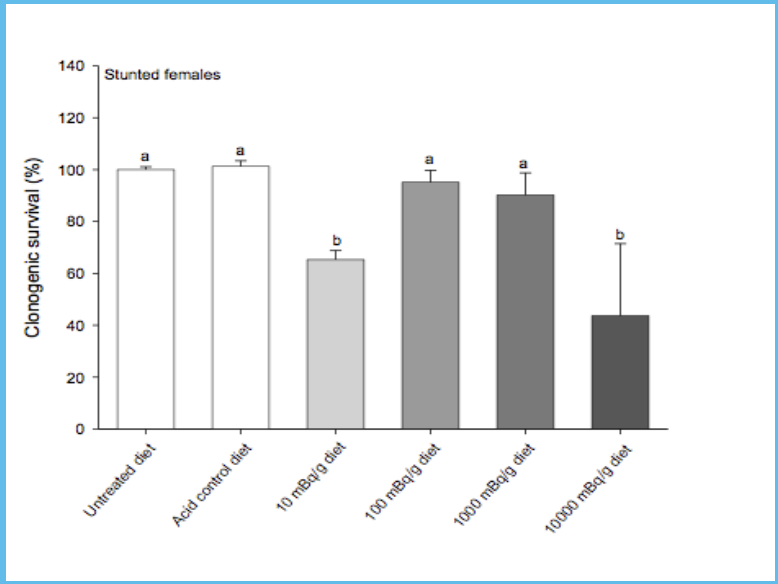


Bystander signaling after 24 month on ^{226}Ra diet

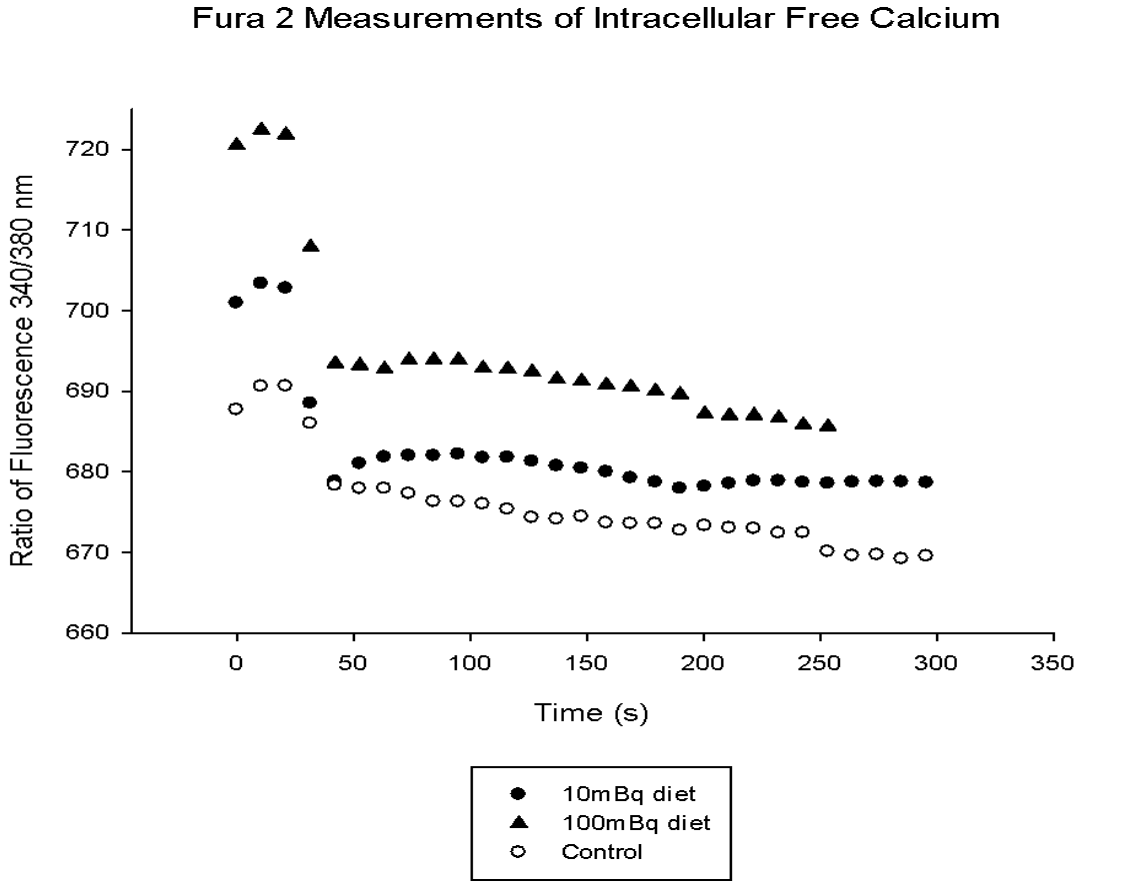
Stunted/deformed males



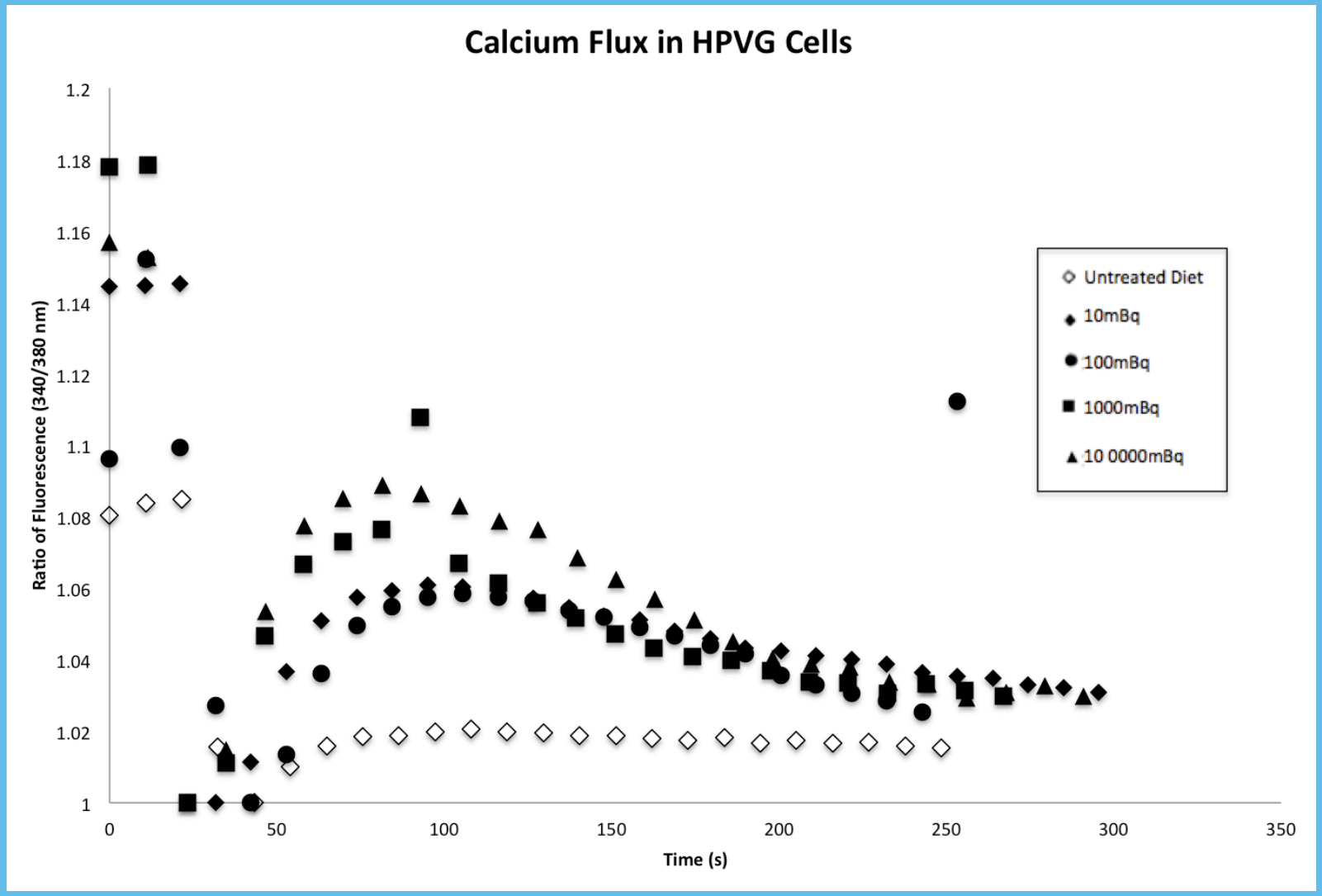
Stunted/deformed Females



Calcium traces for 18month ^{226}Ra fed fish (same as 6 mth)



Calcium traces for ^{226}Ra fed fish after 24 months



Summary of findings

- Small growth perturbations mainly at lower doses from 12 months onward
- Small changes in biochemical indices which are most apparent in females at 24 months
- Bioaccumulation is very low at 6 months and ^{226}Ra has gone in bodies of 18 and 24 month fish
- Clear proteomic changes relating to energy and structure
- Stress signaling persists throughout life and is not dose dependent – may be driving adaptive responses
- No real effects over the life span which would be likely to impact individual or population survival

Bottom Line

- Environmentally relevant levels of Ra-226 in Canadian lakes are unlikely to impact fathead minnow
- Extrapolation to other species seems reasonable
- Novel purging mechanism needs to be investigated mechanistically and in other species

Temporal Hormesis?

- Dose rate is important at low doses
- Need to consider time dependent hormesis not just dose dependent hormesis
- Implications for understanding chronic exposure responses, adaptive and stress induced evolutionary responses.

Acknowledgements

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