Expression Based Technology

Adam Biales
NERL Postdoctoral Fellow

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

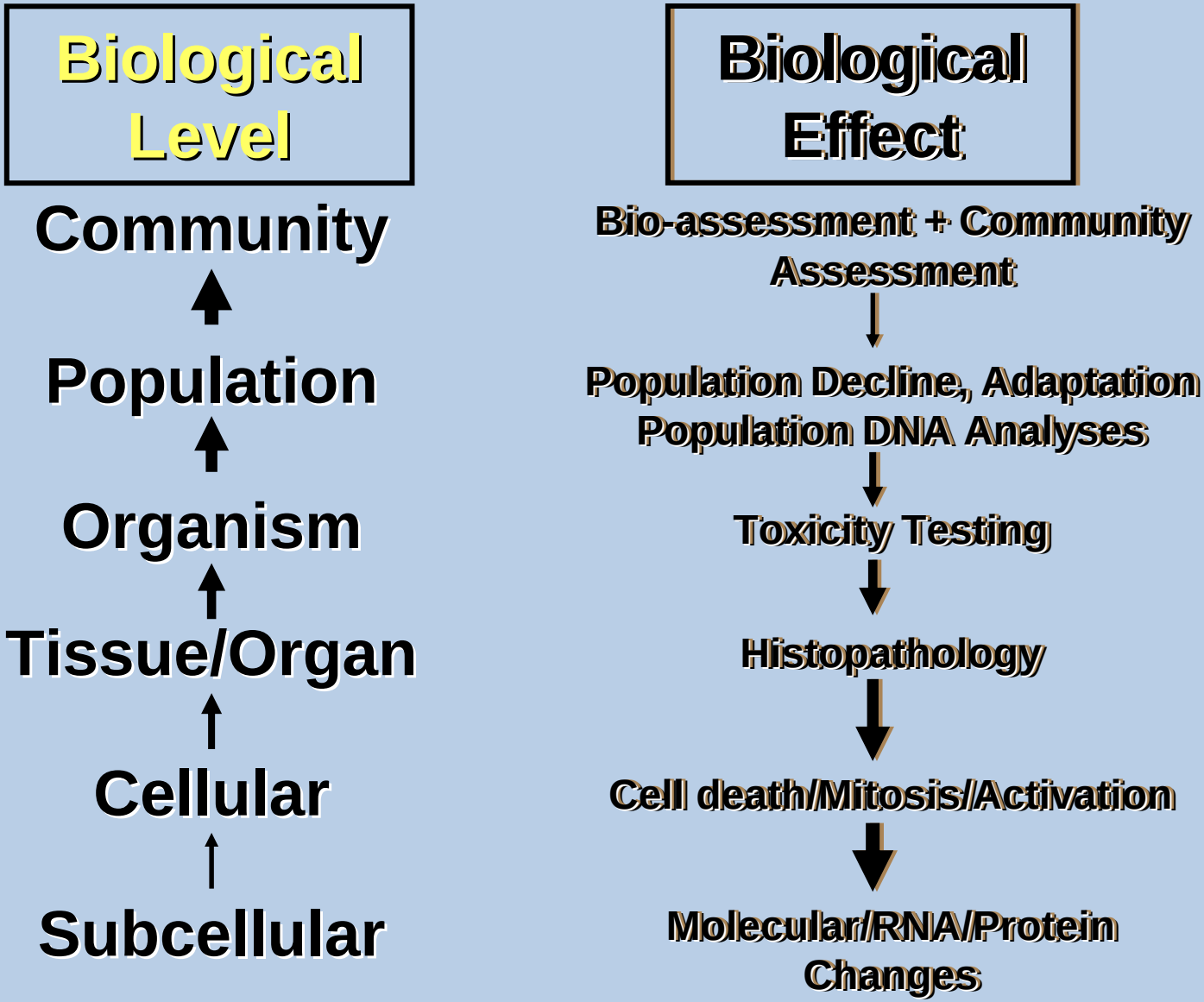
Expression Based Technology

- Immediate responses on cellular level
- Biological information
- Can be specific for a given stressor
- Rapid

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

The Exposure-Effect Hierarchy



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

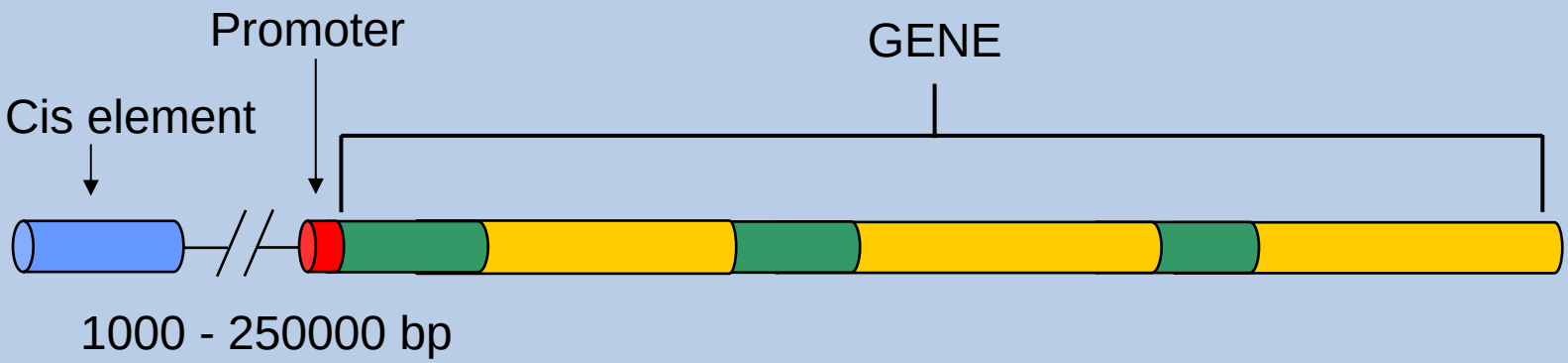
Regulation

- Needs to be heavily regulated
- Appropriate cell activation
- If not regulated leads to pathology
  - Cancers
    - Cell-cycle
    - Signaling
- Occurs on each level:
  - DNA, RNA, Protein

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

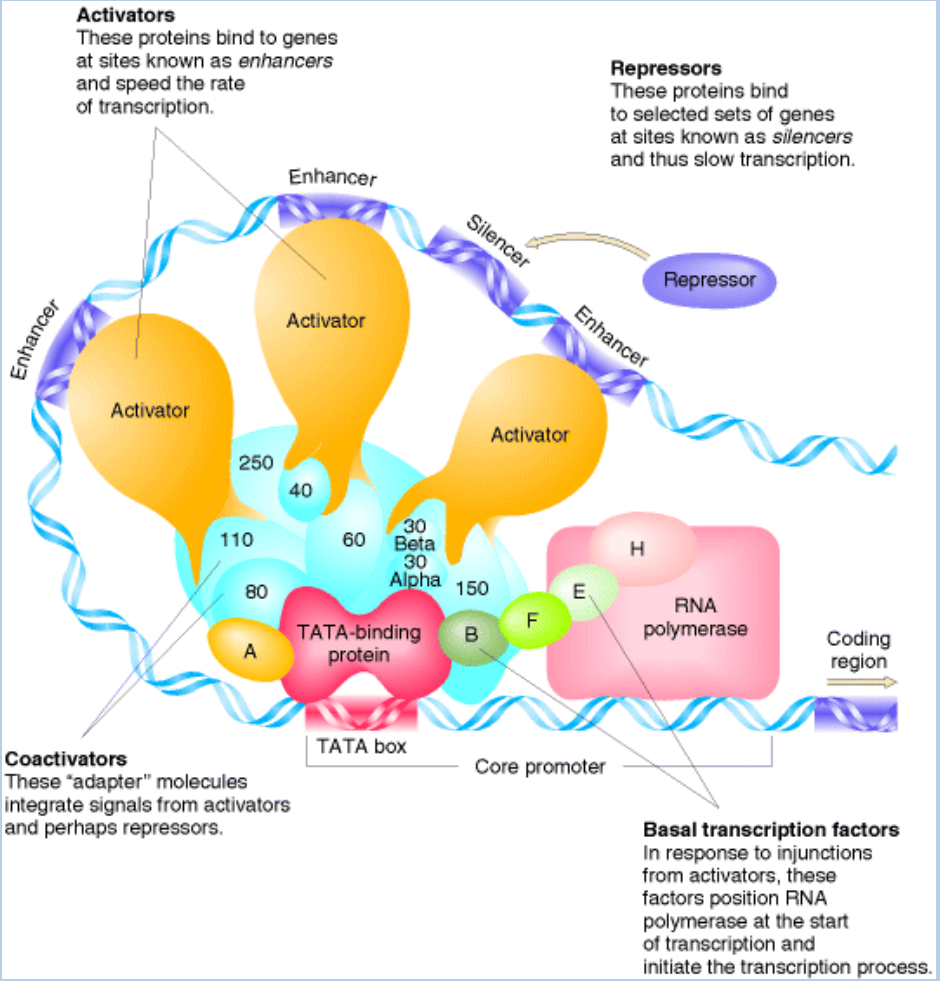
Gene Structure



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

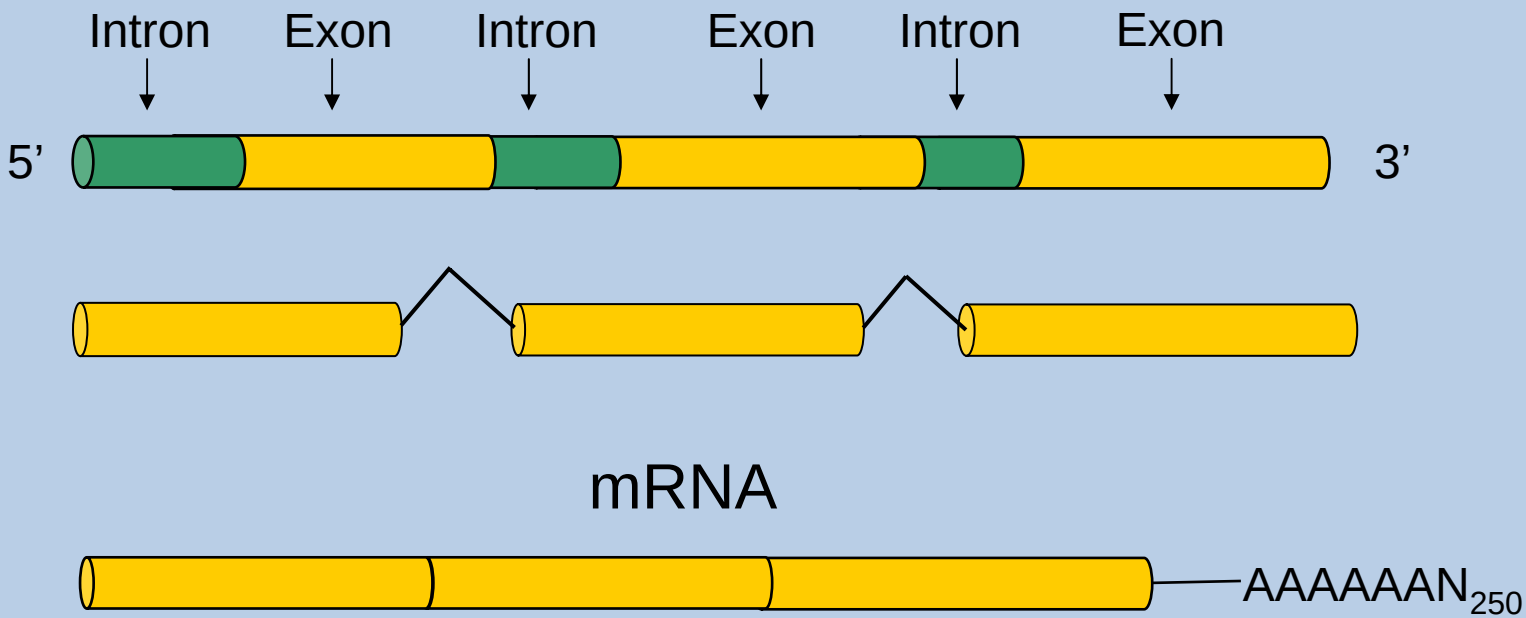
Regulation



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

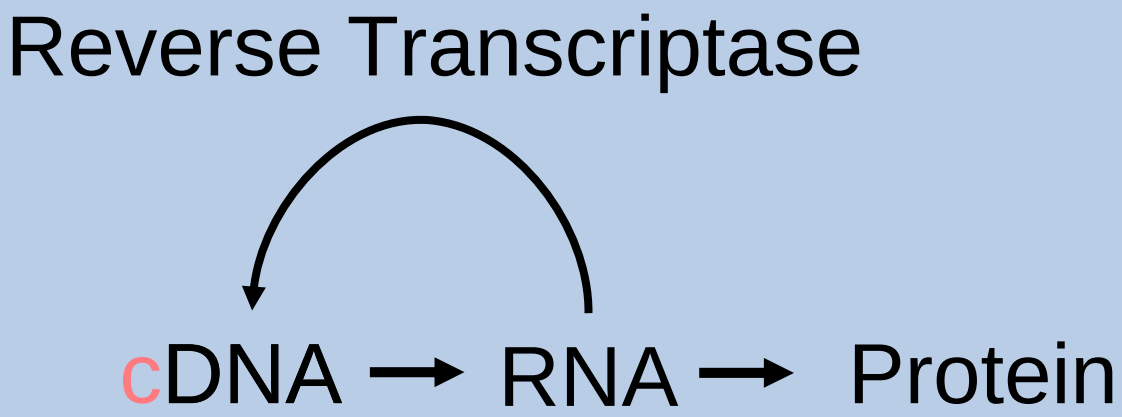
Transcription/RNA processing



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

The Central Dogma



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

QPCR

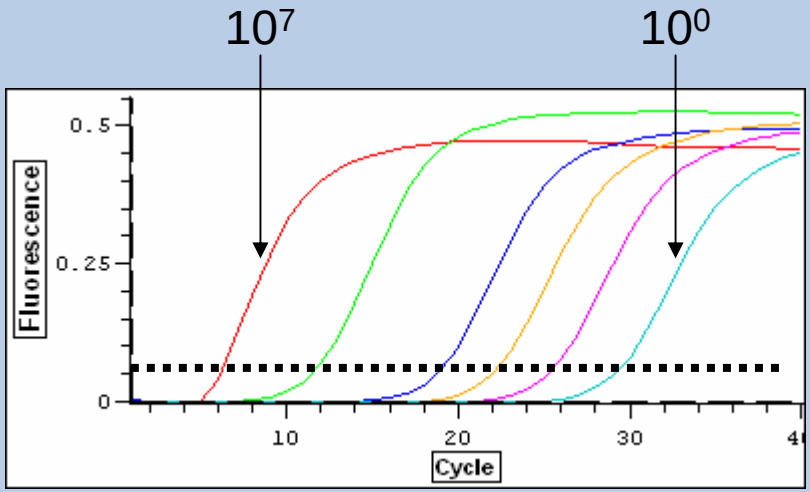
- Realtime quantitative PCR
  - Advantages
    - Quantitative
    - Sensitive
    - Reproducible
    - Minimal tissue requirements
    - Rapid results

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

"Real-Time" visualization of amplified products

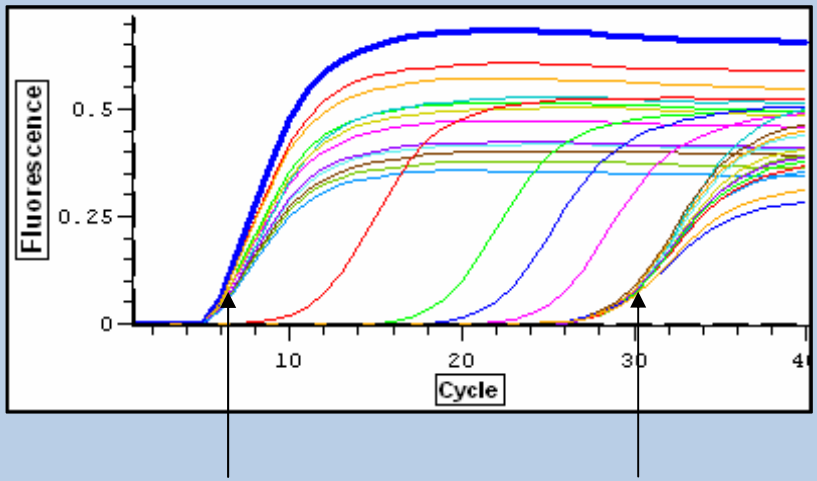
Dynamic range



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

"Real-Time" visualization of amplified products

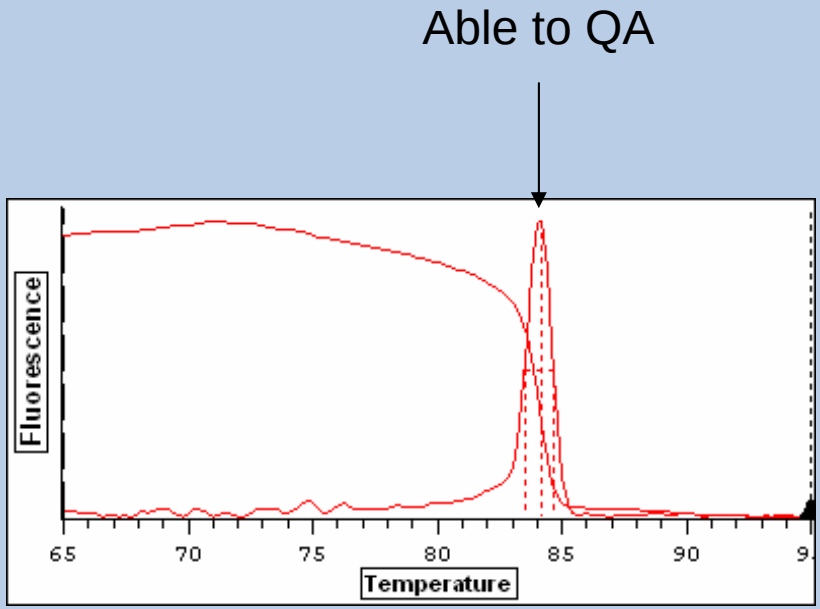


Highly reproducible across entire range
12 replicates each ΔCt < 0.50 cycles

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

"Real-Time" visualization of amplified products



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Microarrays

- Monitor changes across 1000s of genes in 1 experiment
  - Available for rainbow trout
  - Zebrafish
  - FHM – 2006
  - Daphnia
- Signatures – 1 set of genes = 1 chemical
- Systems biology
- Information about mechanism
  - Cluster analysis

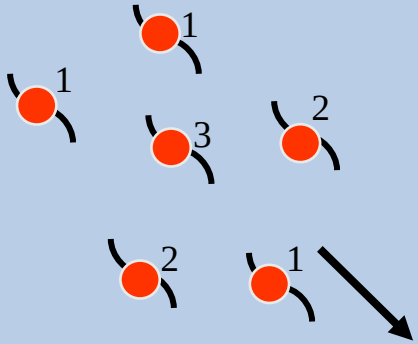
RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Treatment (ie. effluent)

Isolate Tissue → RNA

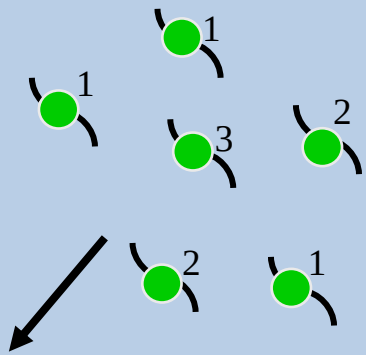
RT & label



Control (reference sample)

Isolate Tissue → RNA

RT & label



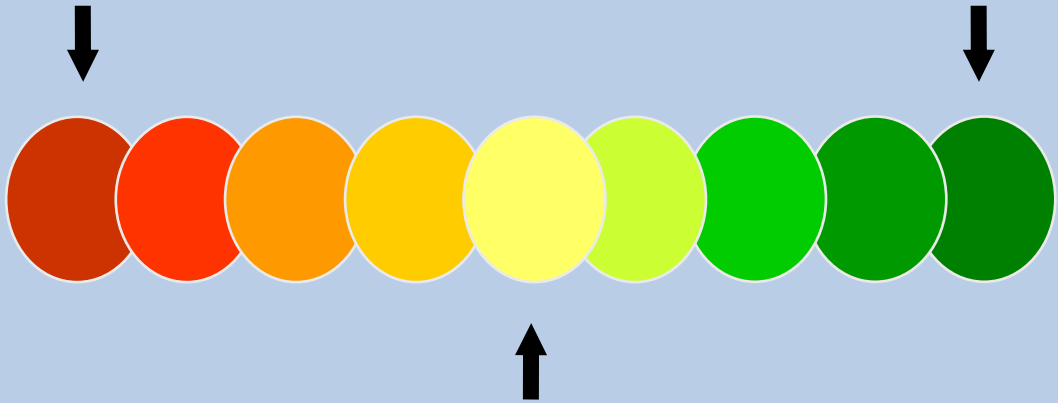
Microarray

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Message present only in treatment

Message present only in control



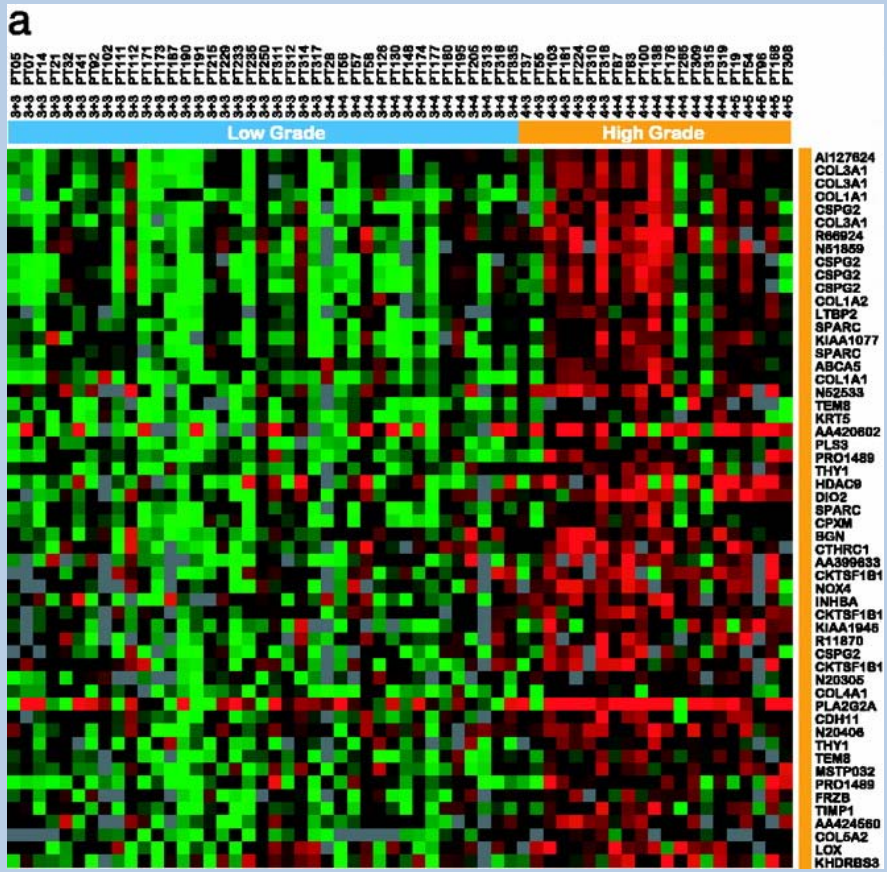
Message present at equal levels in both treatment and control

Adapted from H. Hamadeh and C. Afshari, American Scientist 88:508-515

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Genes Expression Comparison between High and Low Grade Cancer



Lapointe, Jacques et al. (2004) Proc. Natl. Acad. Sci. USA 101, 811-816

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Proteomics

Study of protein populations of one cell versus another

- **Similar to microarrays – global changes**
- **Protein data more biologically relevant**
  - **Higher biological level**
  - **Less variable**
- **2-D gel electrophoresis**
  - **Non-directed, no assumptions**
  - **Can see modifications**

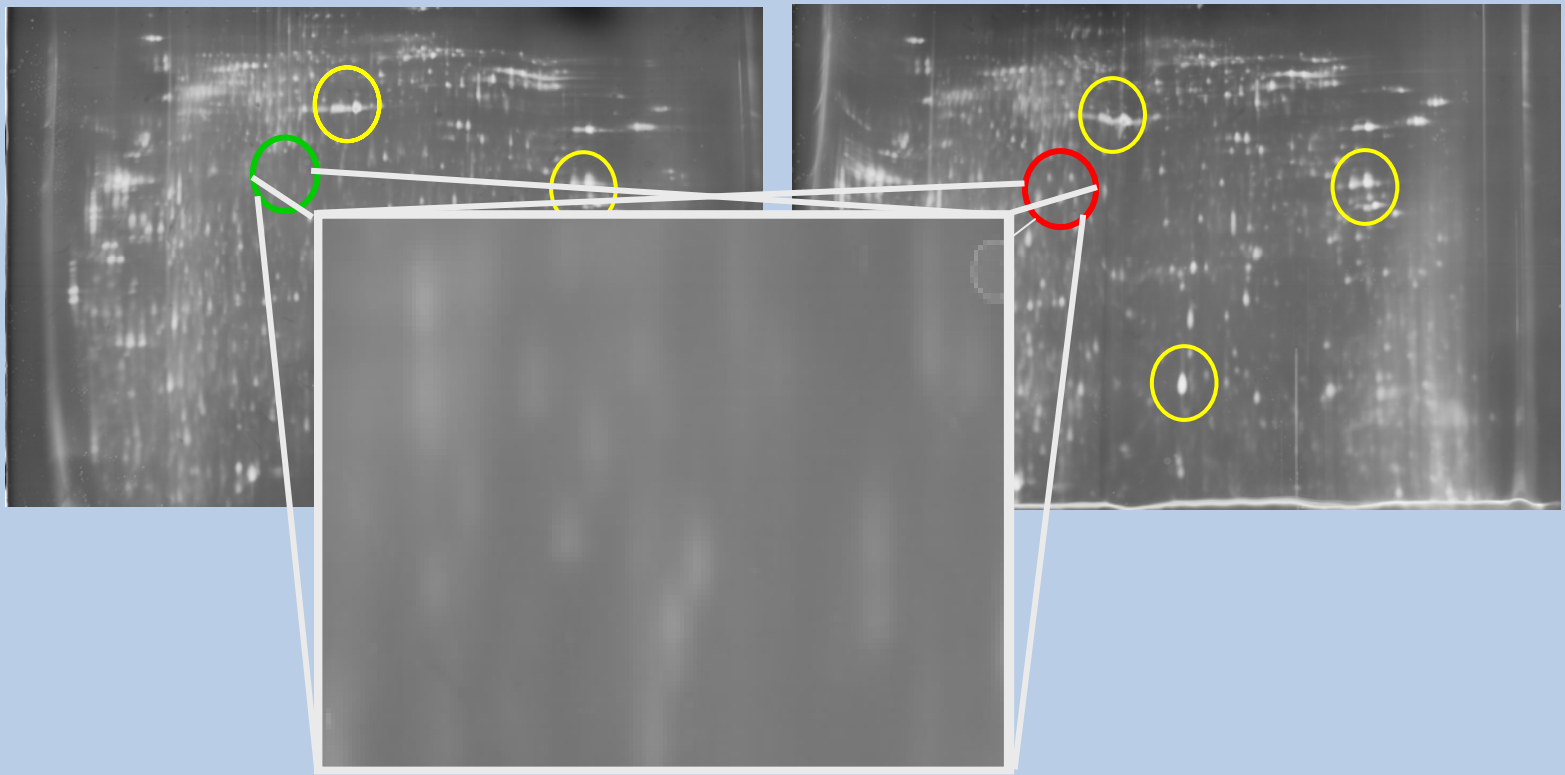
RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

2-D Gel Electrophoresis

Control

Treatment



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Current Molecular Assays

- Vitellogenin
  - Egg protein – only females in breeding season
  - Males turn it on when exposed to estrogen
  - All kinds of fish
    - Fathead, trout, bass, gudgeon, shiners, roach, flounder
  - Can be used as an indicator to estrogen exposure
  - LOEC Vg protein 0.1 ng/L

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Estrogens

- Estradiol, estrone, EE_2
- Sources
  - Farm runoff
  - Contraceptives
- Environmental levels - EE_2
  - Surface water **0.05 – 30.8 ng/L EE_2**
  - Sewage effluents **0.05 – 62.0 ng/L EE_2**
- Can have additive effects
- Potent responses at low levels
  - (4 ng/L EE_2)

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Estrogenic Effects

- Male fish with ovatestes
  - LOEC sex interchange 0.6 ng/L
- Decreased male fitness
- Decreased size/physical abnormalities
- Decreased reproductive success (.32 ng/L)
  - NO EGGS PRODUCED at 3.5 ng/L
- Skewed sex ratios
  - 0 males with secondary sex characters
  - 3.5 ng/L
- May have long-term effect on reproductive success
  - 50% reduction in reproductive success 29 days after exposure
  - 5 months following treatment – decreased fertility

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Issues

- Fish swim
  - Not sitting by effluent for whole life
  - Level of real world exposure
- Linkage between biological levels not completely established
- Why are there any fish at all?
- Needs field testing
- Mixtures
- Can we use gene expression as a metric in bioassessment

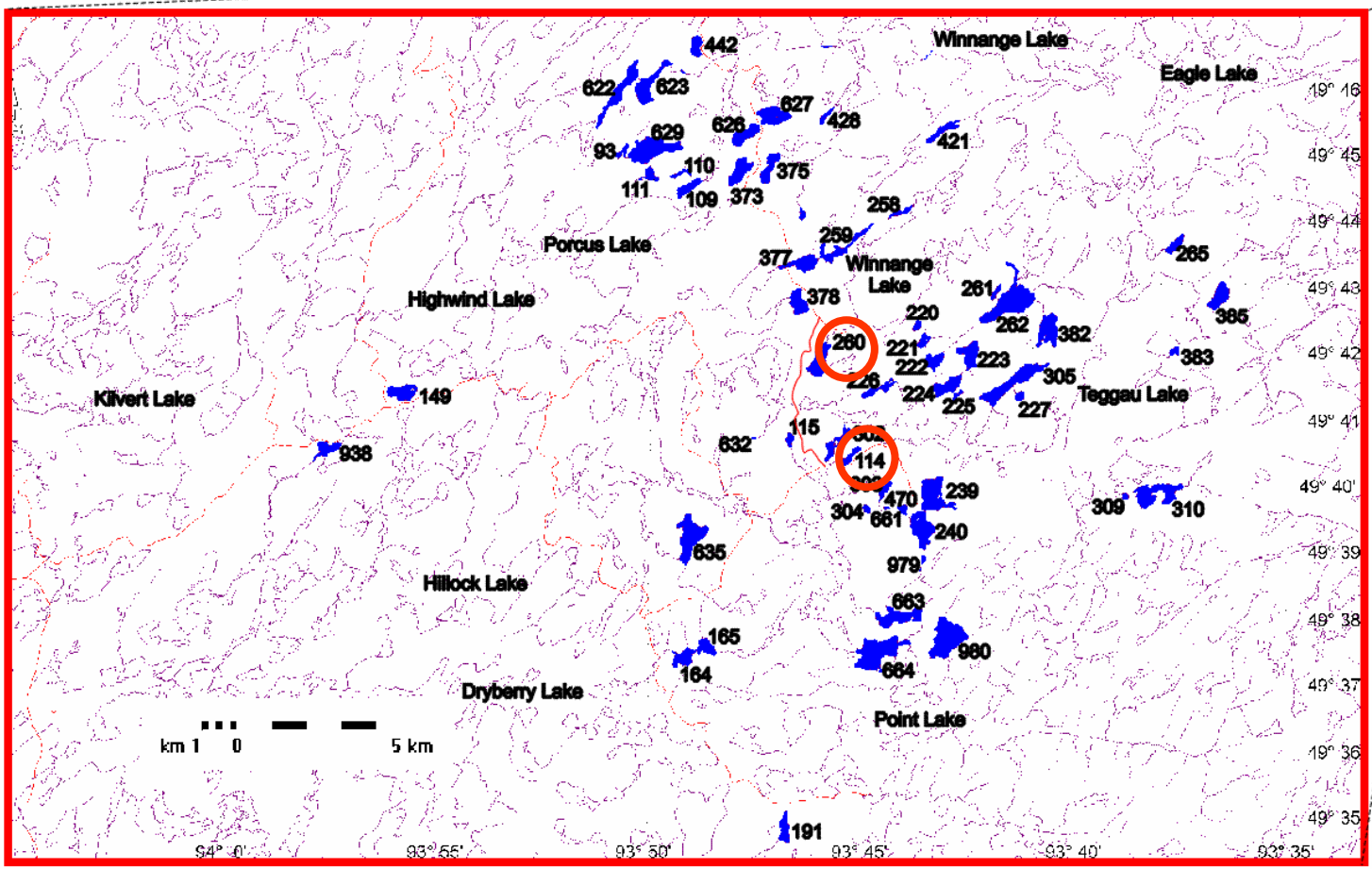
RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

ELA

58 Designated Research Lakes and their Watersheds Detailed Monitoring since 1969

Located in northwestern Ontario approximately 250 km east of Winnipeg and 50 km east-southeast of Kenora.



K. Kidd

Experimental Lakes Area

Designated Research Lakes shaded Blue

Boreal Shield of northwestern Ontario

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

ELA

Objective: To study the long term effects of xenoestrogens on wild fish population

- Dose lake with 4-6 ng/L EE2 or not for 3 continuous years (2001-03)
- Measure Vg expression in wild fish, deployed fish, laboratory, embryo/larval exposures
- Water chemistry
- Sediment elutriate exposure in fry
- Other biological measurements – aggression, mortality

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

ELA results

- Male FHM had elevated Vg expression from 24 hours until October (last sample of year)
- Females had elevated Vg levels past end of breeding season
- Male pearl dace also exhibited high Vg levels
- In fall of second year no age 0 fish found
- Histopathology
  - liver hypertrohpy
  - fibrotic sperm ducts

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Ohio River

Collaboration with Ohio River Valley Water Sanitation Commission
ORSANCO

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

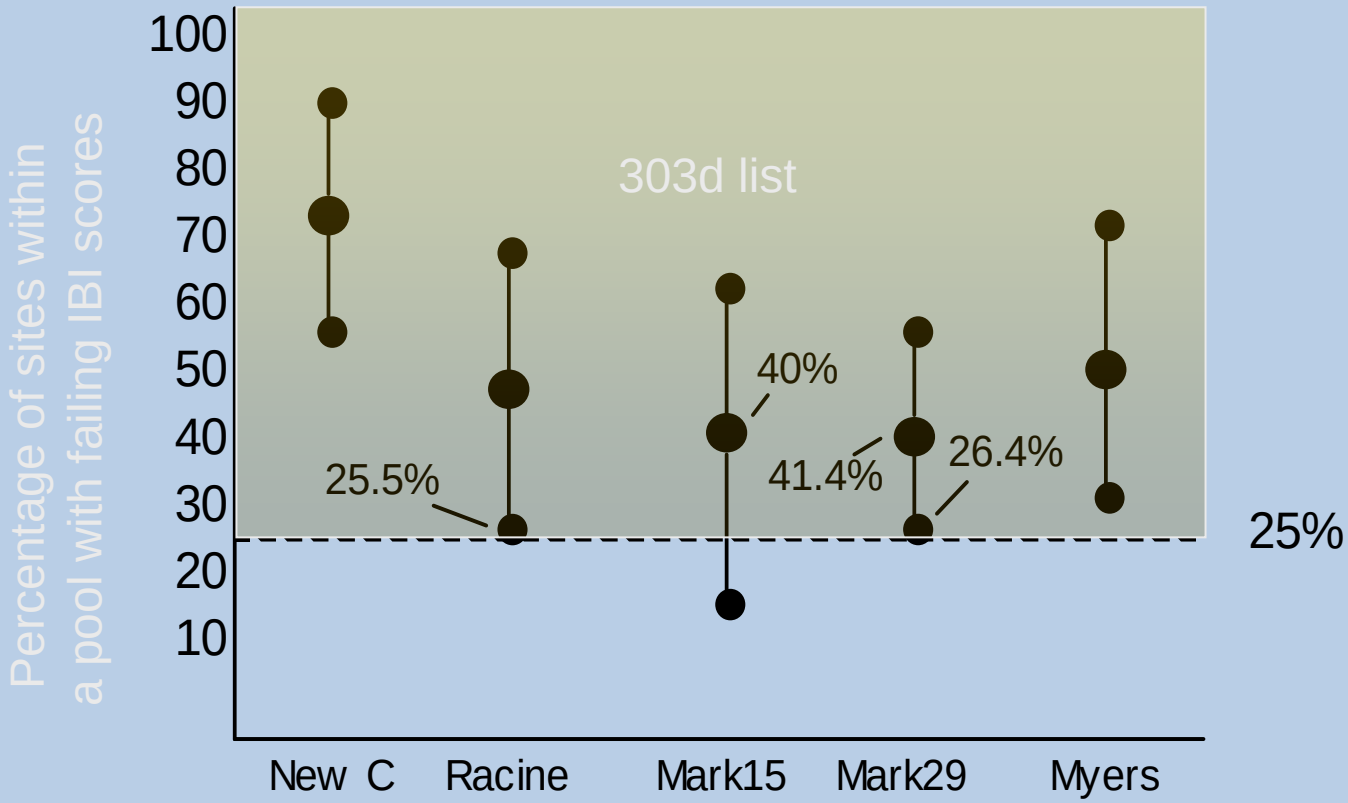
Vg – Sewage Treatment Plants

- Objective: Create Vg assay and identify the number of species and geographic extent effected by estrogenic compounds found in effluents
- 3 sampling sites
  - Region 3 – Wheeling, ALCOSAN, Parkersburg
    - Downstream – proximal
    - Downstream – distal
    - Upstream – reference
- Multiple species
- Multiple exposures – lab, deployment – high & low flow
- Chemical analysis
- Histopath analysis
- Sex Ratios
- Questions
  - Are male fish in reference sites producing Vg?
  - If fish aren't stuck at effluent is there an effect?

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Assessment of Ohio River Pools, 2004



RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Ohio River - EDC

- Using probabilistic sampling to determine extent of exposure in a pool of the Ohio River
  - Fish localized to a given pool - Dams
  - 15 probabilistic sites
  - Exposure differences – ecology
    - Multiple species
      - Bottom feeders
      - Water column
  - Sex ratios
  - Vg expression
  - Result: does expression aid in identifying causes

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Forthcoming Projects

- Several other projects targeting non-estrogenic compounds
- Atrazine
  - 2-D gels ⟶ Gene expression markers
    - 5 different tissues
- Androgen indicators
- Invertebrate sources
- Mixtures
- Pulsed exposures

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Expression Technology Summary

- Informative
- Available - microarrays are online or under developed for a number of aquatic species
- Sensitive
- Targets changes early in exposure
- High through-put
- Making linkages to higher biological levels
- Assays are being developed for a number of different species representing an array of different ecological catagories (habitat, feeding groups, etc.