

Protecting Canada's Drinking Water: Developing Real-time Early-Warning Biomonitoring Technology

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Problem

- Drinking water supply can be contaminated with heavy metals, pesticides, PAHs, halogenated organic compounds, pharmaceuticals, pathogens
 - can pose health risk
- How to detect them before going into drinking-water facilities?
 - chemical analysis – too slow
 - biological stress reactions – real-time, fast

Miner's canary

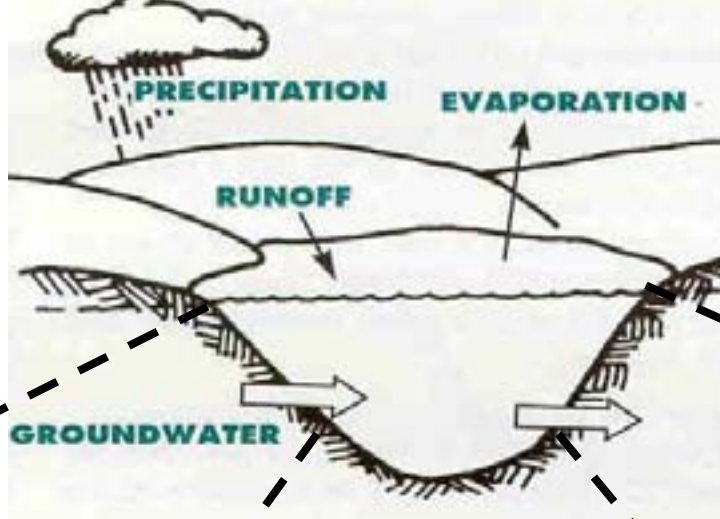
- Early-warning system
- Holistic, multi-organism-based strategy
- Develop ecotoxicity models based on responses of individual organisms to specific class of stressors
- Provide water-utilities managers with information about nature of stressor

To achieve this, our project will:

1. Develop suite of measurable responses in aquatic plants, invertebrates, vertebrates to chemical stressors, at environmentally-relevant concentrations in laboratory
2. Develop quantitative methods to detect presence and abundance of pathogens

3. Build/test flow-through system in drinking- water facility for stress-response determination in real-time
4. Develop models for suite of stereotyped responses to chemical contaminants, pathogens

Ultimately, models will predict nature of contamination when biological stress responses are observed



Water Column



Larval Amphibians



Daphnia magna



Euglena gracilis



Pseudokirchneriella subcapitata



Lemna minor



Anodonta grandis



Elodea densa



Hyalella arteca



Hydra viridissima

Sediment



Lumbriculus variegatus

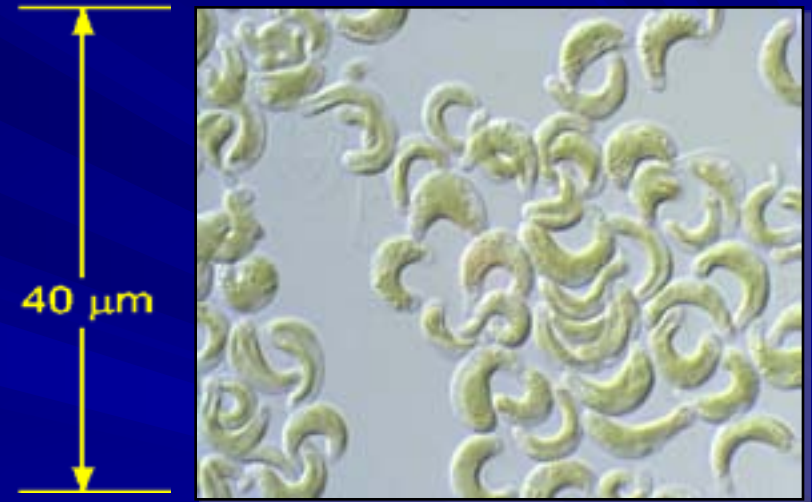


Chironomus tentans

Pseudokirchneriella subcapitata

Photosynthesis, Respiration, Delayed Fluorescence

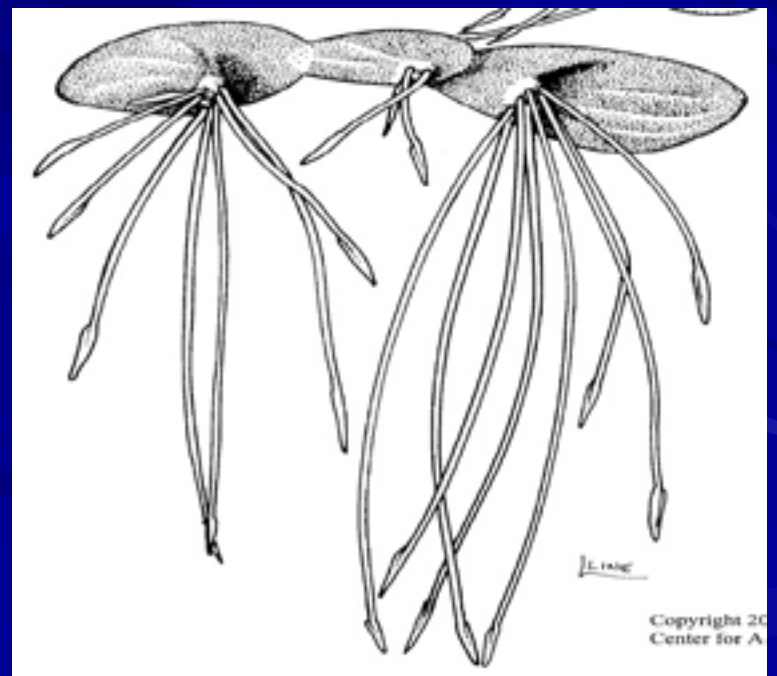
- C: Chlorophyceae
- planktonic
- doubling time 18-20 hrs
- acute, chronic, reproductive toxicity
- Env. Canada 1982
- US-EPA 1978



Lemna gibba (duckweed)

Growth

- C: Monocotyledoneae
- emergent macrophyte in still or slow-moving fresh water.
- acute (4 d)
- frond doubling time – 0.7 days
- Environment Canada, 1999



Elodea densa

Photosynthesis, Respiration, Growth

- submerged macrophyte
- littoral zone
- uptakes nutrients through roots, shoots, leaves
 - assess water column and sediment toxicity
- rapid growth



Euglena gracilis

Photosynthesis, Respiration, Motility, Shape

- single-celled protist
- chloroplasts, light-sensitive
- orient in water column using light (phototaxis), gravity (gravitaxis)
- motile behaviour allows for analysis using real-time image analysis





Nikon MicroscopyU
Digital Video Gallery

Euglena rostrifera
(Protozoan)

Through the Nikon Eclipse
E600 Microscope with
Dark Field Illumination

Daphnia magna

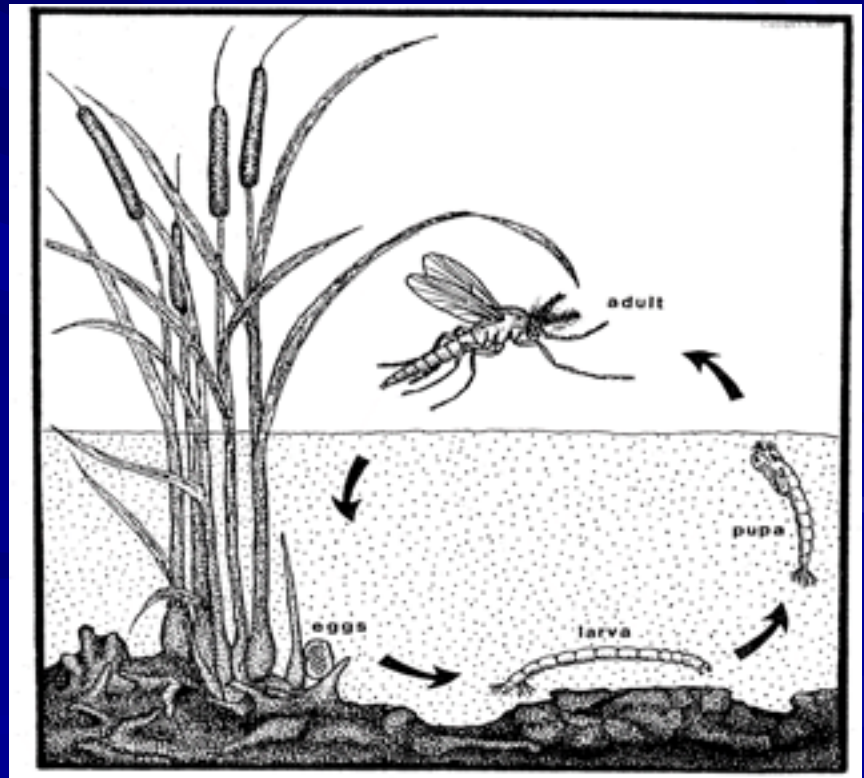
Respiration, Swimming Behaviour, Heart Rate

- C: Crustacea
- 10mm long freshwaters
- planktonic
- valuable fishfood
- consume phytoplankton
- acute /chronic / reproduction
- Env. Canada 1992



Chironomidae

- larval insects
- highly sensitive to pesticides (ex. atrazine)
- possible to monitor
 - swimming undulations
 - ventilation
 - avoidance responses



Lumbricus variegatus

- C: Oligochaeta
- 4 - 6 cm in length
- feeds on micro-organisms, organic material
- rapid asexual reproduction



Hydra viridissima

- polyp common in North American ponds
- simple animal with *Chlorella*
- catches prey with stinging tentacles
- when stressed, retracts into small “blob”





Nikon MicroscopyU
Digital Video Gallery

Hydra
(Coelenterata)

Through the Nikon SMZ1500
Microscope with
Oblique Illumination

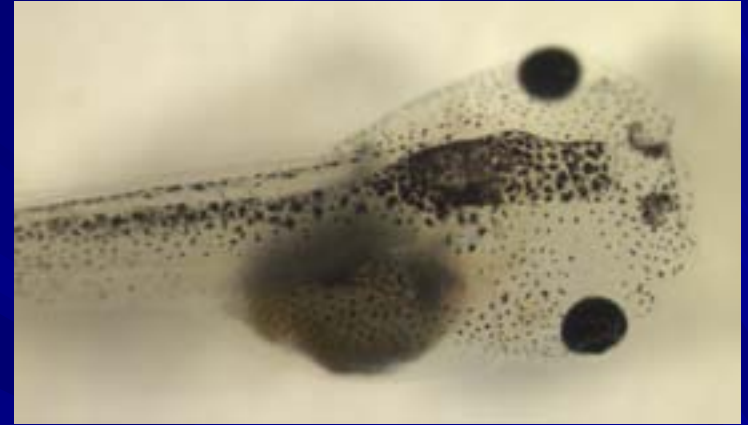
Anadonta grandis

- bivalves common in North America
- burrow into substrate with posterior margins exposed
- incurrent/excurrent apertures close quickly upon stress



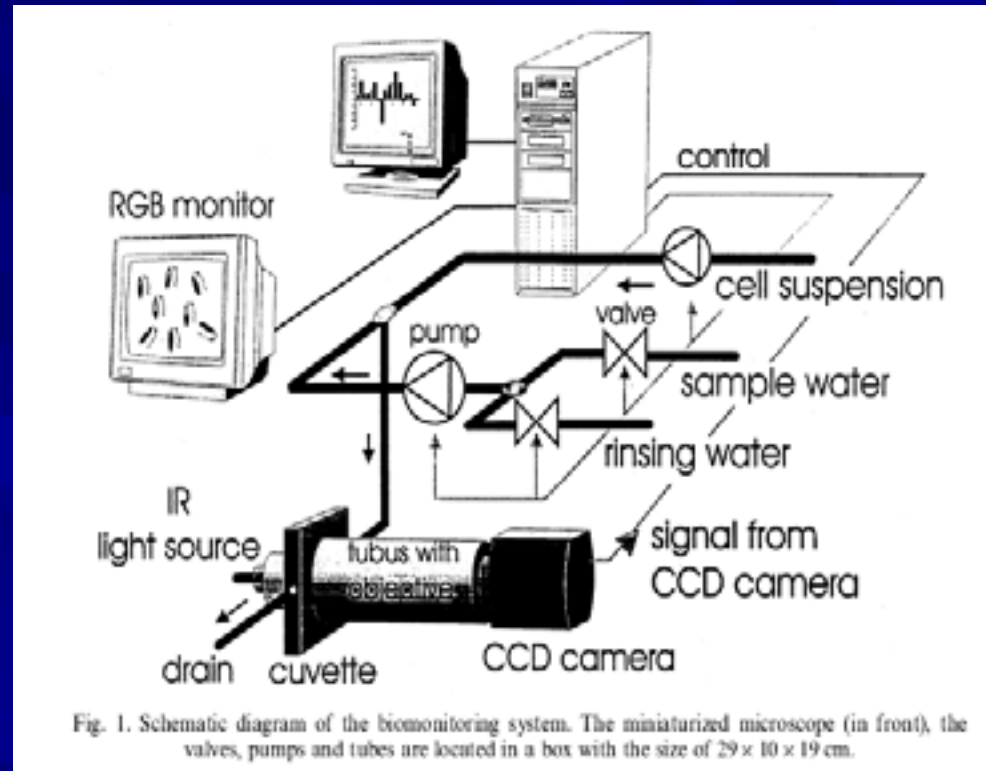
Larval Amphibians (Tadpoles)

- very sensitive to agriculture run-off, endocrine-disrupting compounds
- possible to monitor:
 - respiration
 - feeding behaviour
 - overall activity



ECOTOX Biomonitoring

- automatic, inexpensive, easy to use, real time online image analysis
- determines endpoints in behaviour
- swimming velocity, relative depth from surface, turning, preference of light/dark habitats, etc.



Multispecies Freshwater Biomonitor

- records multiple organisms' behaviour
- flow-through chamber contains two pairs of electrodes that determines changes in the electrical field delivered by the organism's movements

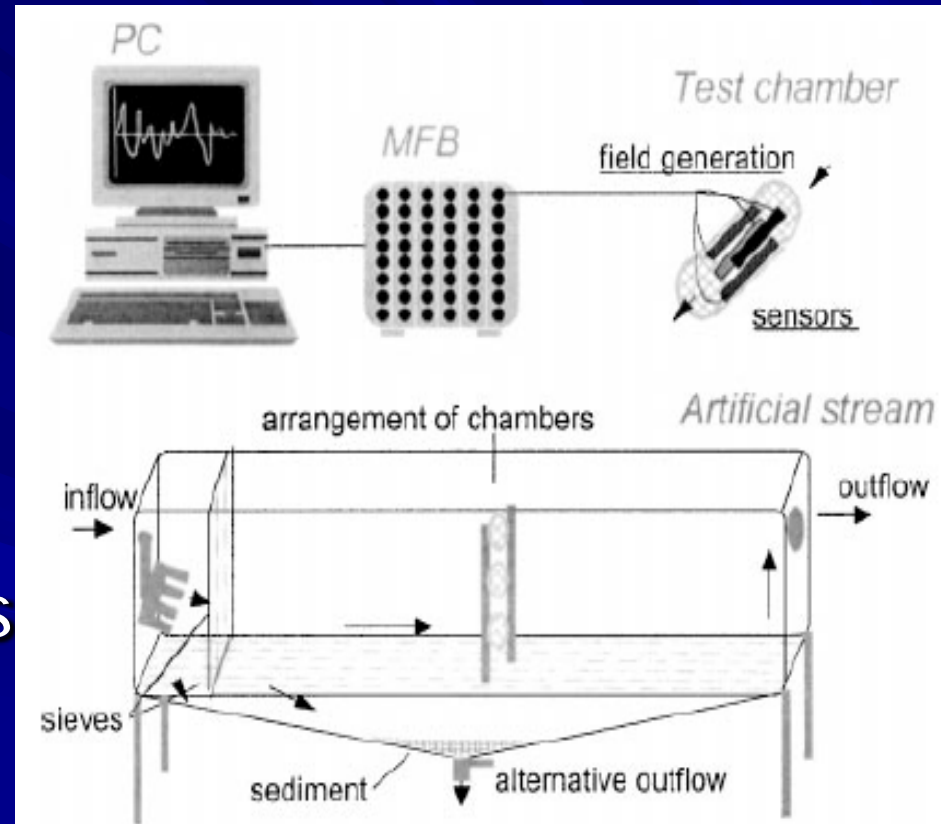


Figure 1. Experimental setup for laboratory acid pulses and *in situ* long-term experiments consisting of a PC, the Multispecies Freshwater Biomonitor (MFB) and test chambers arranged in an artificial stream.

Biological Early Warning System

Black Box



Hyallela azteca



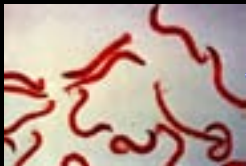
Lemna minor



Anodonta spp.



Euglena gracilis



Chironomus tentans



Hydra viridissima



Pseudokirchneriella subcapitata



Daphnia magna



Lumbriculus variegatus



Elodea densa



Rana catesbeiana



Preliminary Results - Behaviour

Daphnia magna

■ Swimming Behaviour in Reference Water



Daphnia magna

- Swimming behaviour in 25 ug/L TBT



Hyalella azteca

- Swimming behaviour in reference water



Hyalella azteca

- Swimming behaviour in 25 ug/L TBT



Chironomus tentans

- Burrowing behaviour in reference water



Chironomus tentans

- Burrowing behaviour in 10 ug/L TBT



Lumbricus variegatus

- Grouping Behaviour

- Normal behaviour: *L. variegatus* will form colonies, resulting in a “clump” of organisms



Lumbriculus variegatus

- Grouping Behaviour

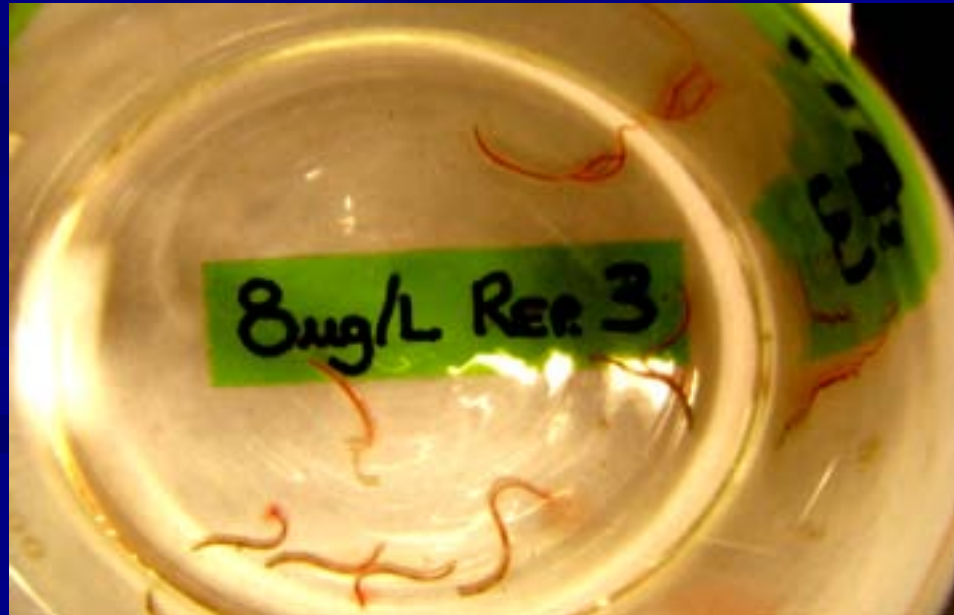
- With each TBT concentration, decreases clumping behaviour were observed



Lumbriculus variegatus

- Grouping Behaviour

- After 4 hours, death of organisms occurred at highest concentrations, haemorrhaging observed



Anodonta grandis



Anondonta – Valve Opening

O=Open, C=Closed

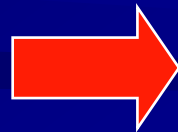
Reference			DMSO			0.5 µg/L			2.0 µg/L			4.0 µg/L				
Mussel			Mussel			Mussel			Mussel			Mussel				
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Time	Valve		Time	Valve		Time	Valve		Time	Valve		Time	Valve			
5m	O	O	O	5m	C	O	O	O	5m	O	O	O	5m	O	C	C
10m	O	O	O	10m	O	O	O	O	10m	O	O	O	10m	C	O	O
15m	O	O	O	15m	O	O	O	O	15m	O	C	O	15m	C	O	C
30m	O	O	O	30m	O	O	O	O	30m	C	C	O	30m	C	C	C
45m	O	O	O	45m	O	O	O	O	45m	C	C	C	45m	C	C	C
1h	O	O	O	1h	O	O	O	O	1h	C	C	C	1h	C	C	C
2h	O	O	O	2h	O	O	O	O	2h	C	C	C	2h	C	C	C
4h	O	O	O	4h	O	O	O	O	4h	O	C	C	4h	C	C	C
6h	O	O	O	6h	O	O	O	O	6h	O	C	O	6h	C	C	C

Preliminary Results - Physiology

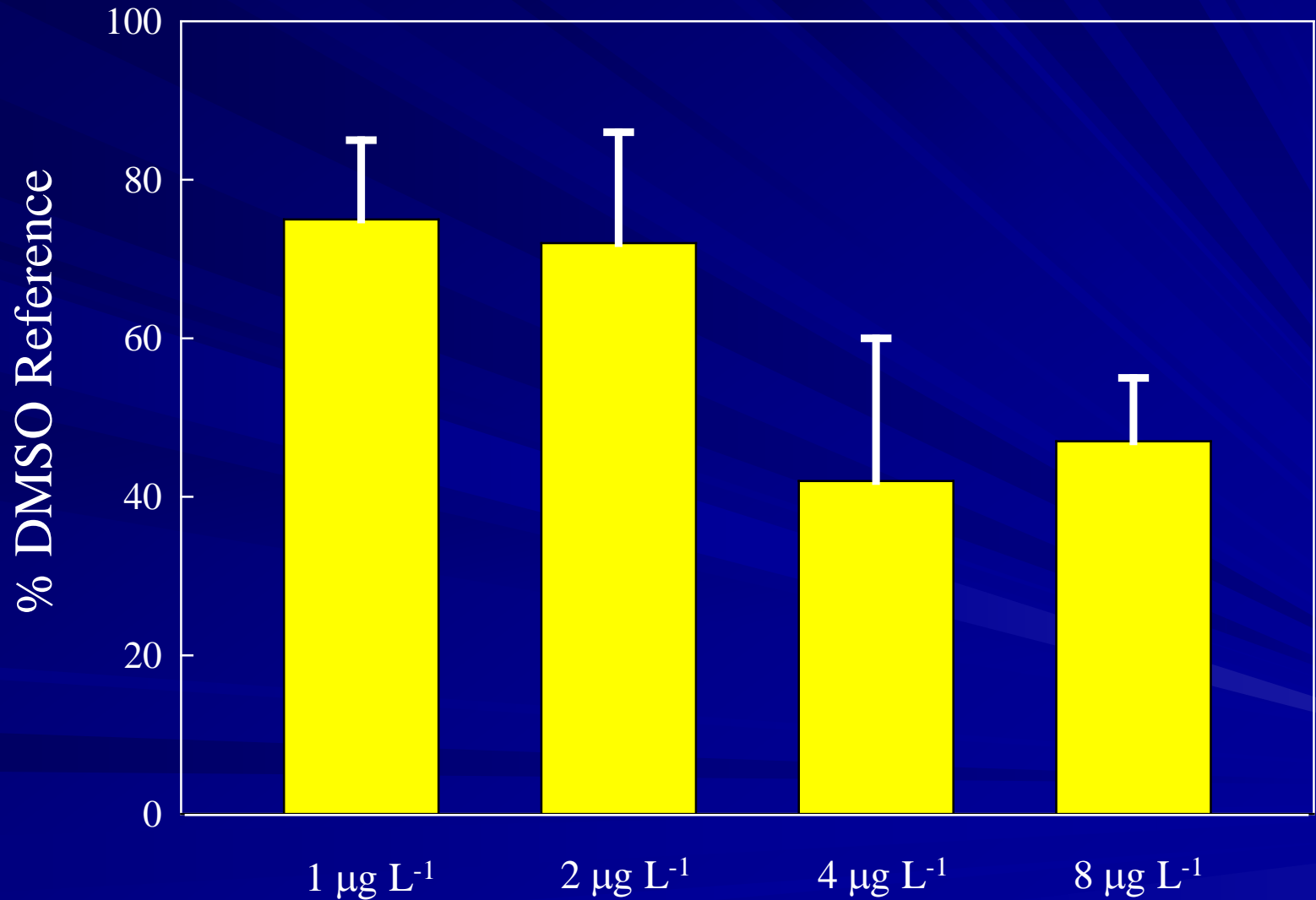
E. gracilis: Shape Change



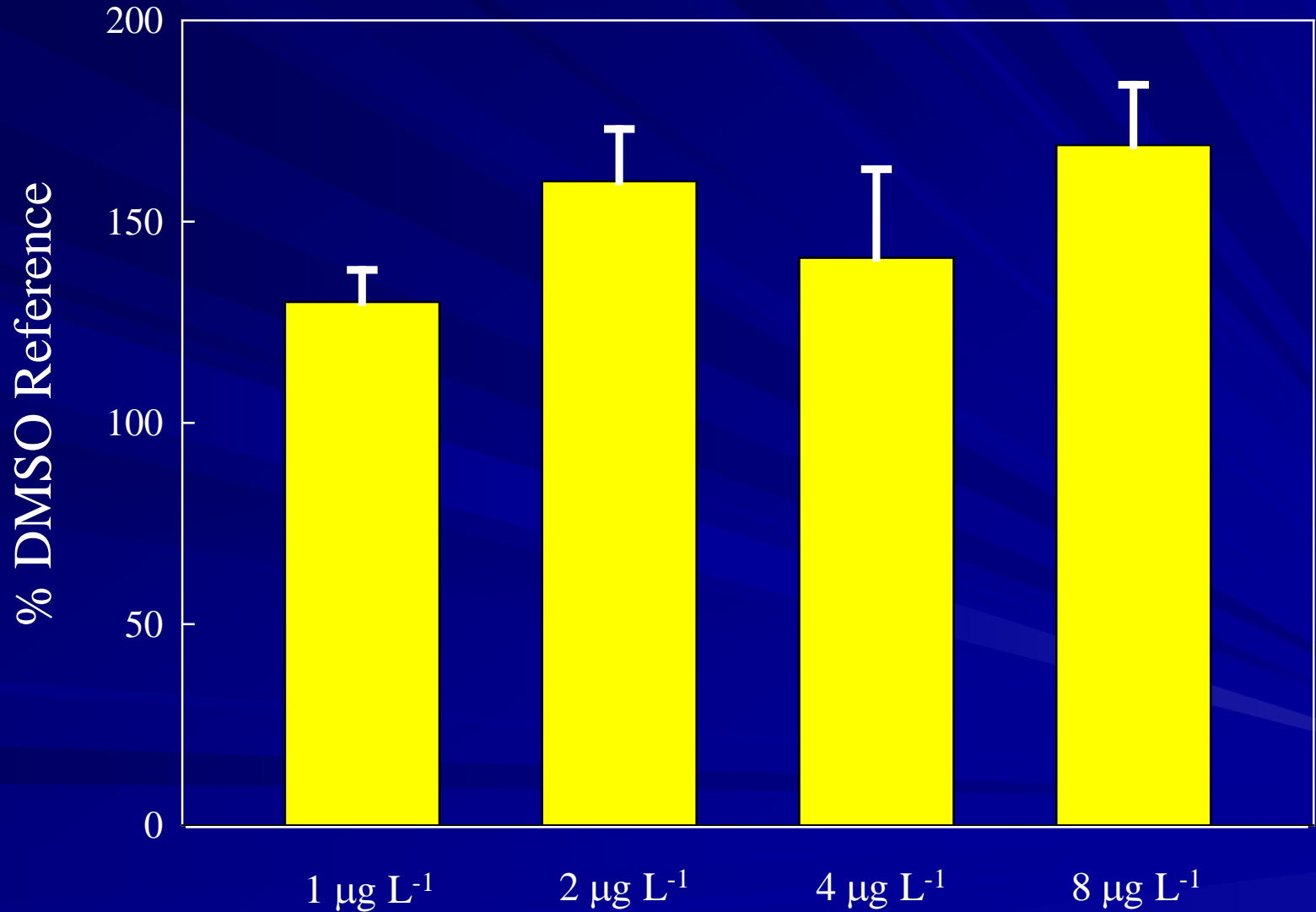
50um



Chironomus Respiration

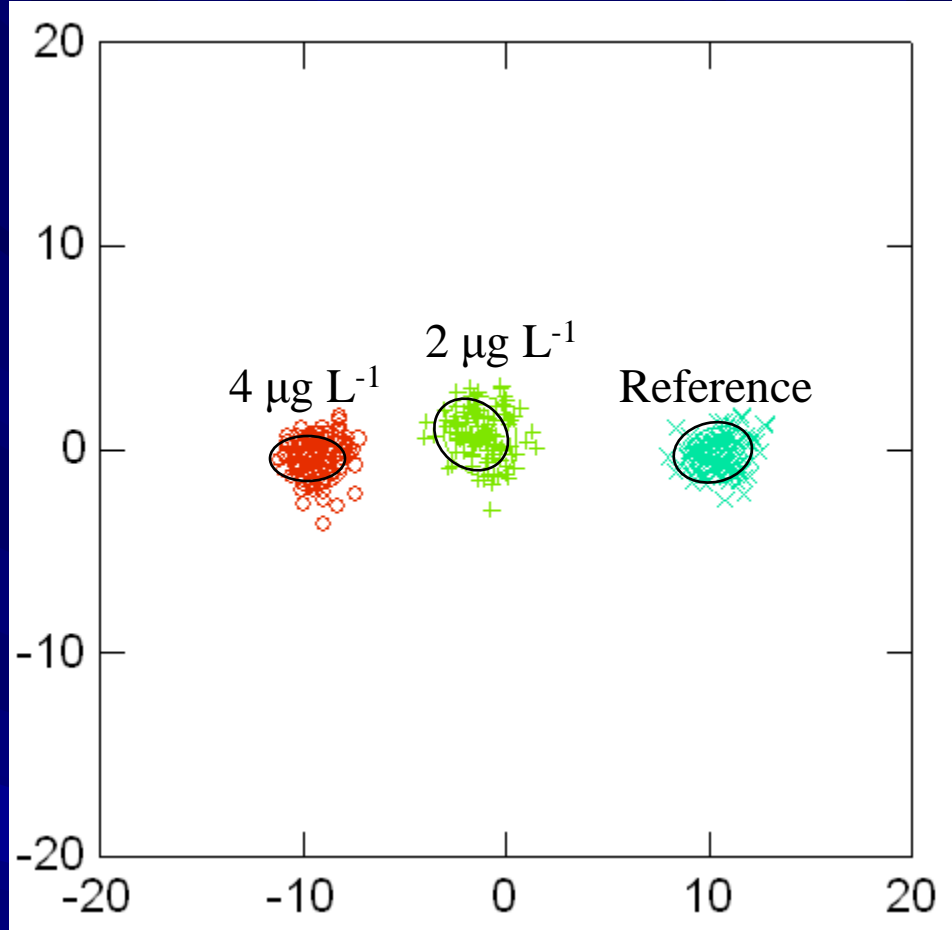


Lumbriculus Respiration



Discriminant Analysis

Factor 2



Factor 1

	Reference	2 μg L ⁻¹	4 μg L ⁻¹	% correct
Reference	112	0	0	100
2 μg L ⁻¹	0	112	0	100
4 μg L ⁻¹	0	0	112	100
Total	112	112	112	100

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