

# **Impact of urbanisation on invertebrates examined using field and lab experiments: the case of Halifax Harbour**

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- Dr. Brian Petrie, BIO
- Elaine Desnoyer, s
- Dr. Ellen Kenchington, BIO...

Francis Kelly, Graphics, BIO *s: students*

## **Vision behind our work:**

**Research to increase knowledge  
to respond to the goal of Canada's Oceans Strategy**

**“to ensure healthy, safe and prosperous  
oceans for the benefit of  
current and future generations of Canadians”**

**Several projects and phases of research to**

**Assess the state of Halifax Harbour using  
mussels, sediments and seawater.**

**Compare the bioavailability of contaminants  
and health of inter-tidal mussels collected in the field**

**to that of benthic invertebrates commonly found elsewhere,  
i.e. amphipods and snails, exposed in the laboratory**

**Link chemical fate and biological effects**

**Prepare for future developments**

**Study more impacted sites  
to better understand the ecosystem**

# Urbanisation: Halifax Harbour

## Past:

Halifax founded in 1749

*20<sup>th</sup> century:*

Several studies in

Biology  
Chemistry  
Geology  
Physics

*examples*

Lobsters

Mutatox

Bacteria

Imposex

1 core: PAH

Water, sediments :  
metals

## Present:

Wastewater treatment plants  
(STP)

Mill Cove WTP: 1971

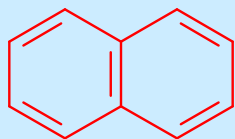
Eastern Passage WTP: 1974

21<sup>st</sup> century:

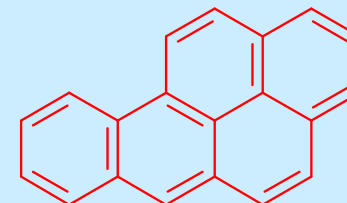
Construction of 3 STP  
since 2005 till 2011

**Need more integrated approach**

# Studied contaminants



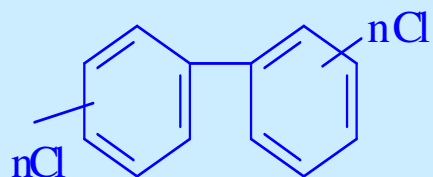
“Human introduced”  
synthetic or not



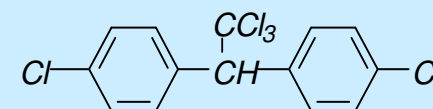
**PAH: polycyclic aromatic hydrocarbons and S compounds**

**Multiple sources:** include the burning of coal, wood, food and garbage; forest fires and volcanic eruptions; creosote; heavy industries like aluminium smelters, and the discharge of various petroleum products, <biogenic

**More than 80 family members analysed**



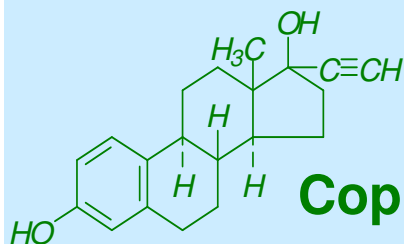
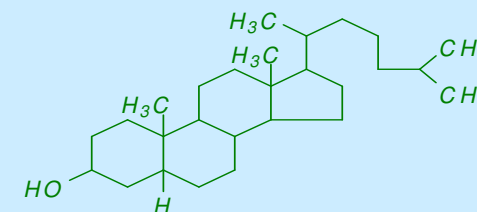
**159 PCB structures**



**6 DDT related compounds**

**12 alkanes, >12 terpanes**

**“Emerging chemicals”**



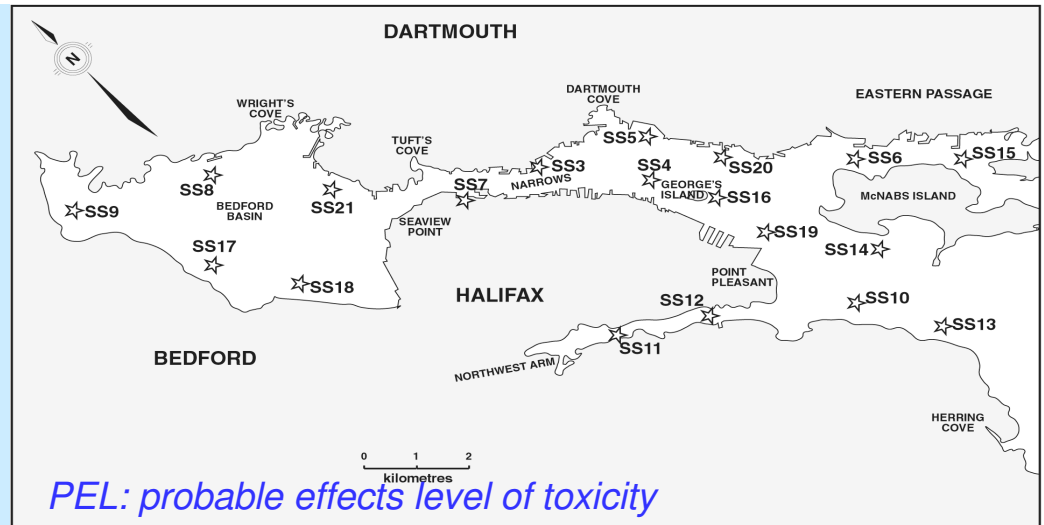
**Coprostanol, estradiol, ethynylestradiol, bisphenol A**

**Musk Fragrances**

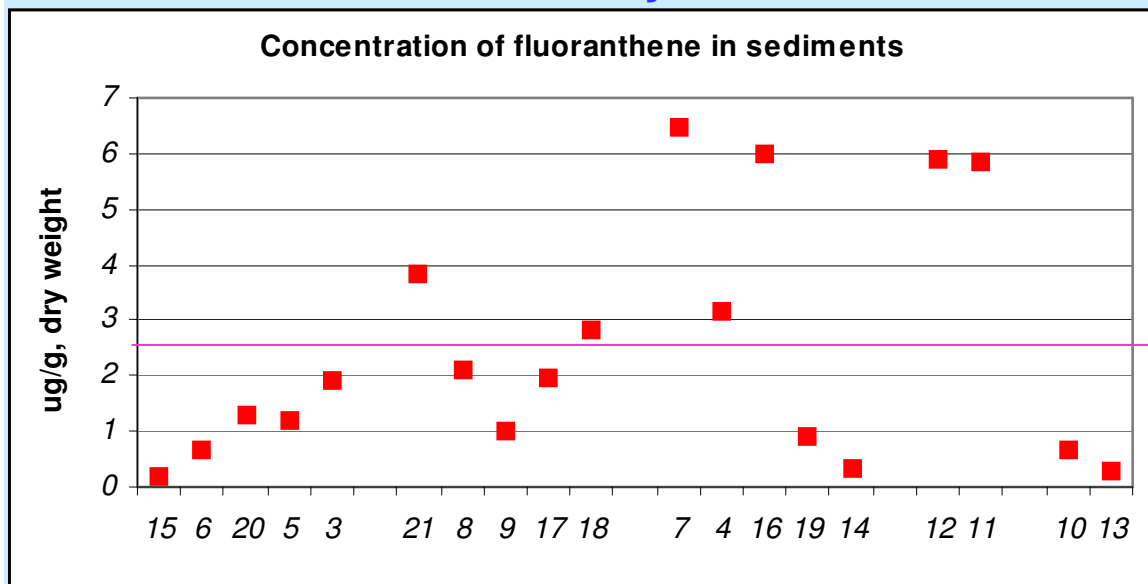
Robinson, B., Su, E., Hui, J. and Hellou, J. In Preparation.

Ricking, M., Schwarzbauer, J. Hellou, J., Svenson, A., Zitko V. 2003. Mar. Poll. Bull. 46: 410-417.

PAH	CCME Guidelines	Halifax H	Sites>SQG out of 21
Fl	0.144	0.03-0.73	1
AN	0.245	0.04-1.52	12
PA	0.515	0.14-5.21	13
FL	2.355	0.17-6.54	7
PY	0.875	0.14-5.30	13
CH	0.862	0.08-3.61	11
BA	0.385	0.08-3.36	12
BaP	0.782	0.04-2.83	11



**PAH are above the PEL of the Canadian Council of Ministers of the Environment Sediment Quality Guidelines at 13 out of 21 sites sampled...**



**Reference sediments have <10-100 ng/g > 100 x less PAH**

**Highest levels observed in downtown Halifax and in the NW Arm PAH bioavailability, toxicity?**

## Comparison of some PAH concentrations in sediments especially harbours ( $\mu\text{g/g}$ , dry)

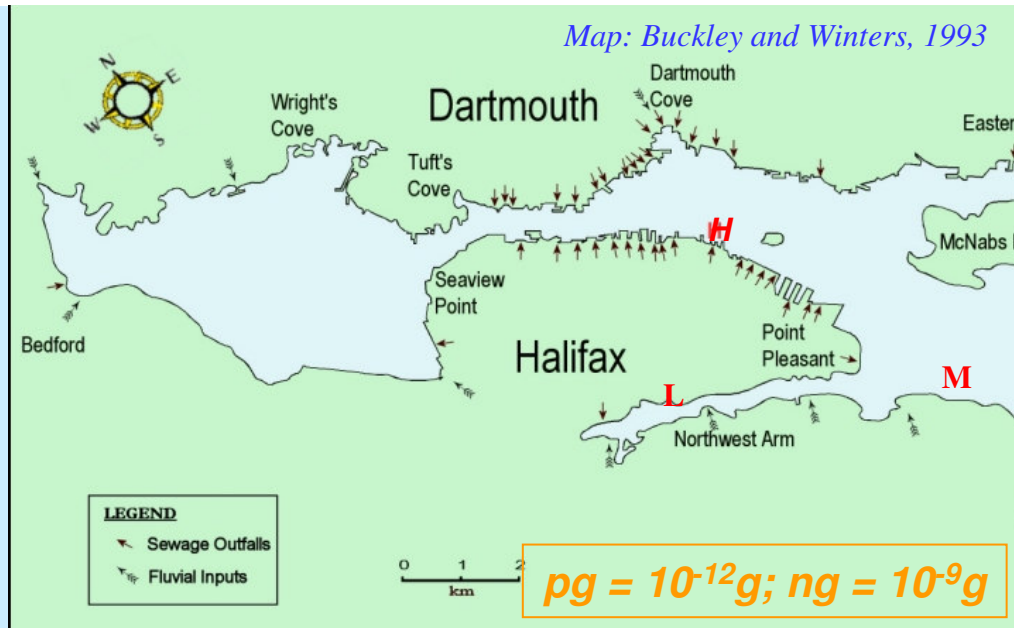
	Halifax (19 sites)	St. John <sup>1</sup>	Mediterr. <sup>2</sup>	Boston <sup>3</sup>	Sydney <sup>4</sup>
PA	0.14-5.21	0.06-1.5	0.0003-7.77	0.05-63.7	1.11(1.34)
FLU	0.08-6.11	0.06-1.2	0.0001-1.31	0.005-84.5	1.84(2.74)
PY	0.15-5.13	0.05-0.96	0.00009-1.15	0.20-66.8	1.53(2.32)
CH	0.08-3.61	0.04-0.44	0.0001-0.83	0.04-364.7	1.34(1.95)
BaP	0.04-1.75	0.03-0.08	0.00007-0.61	0.06-95.0	2.39(3.58)

### Relatively high concentrations within other observations

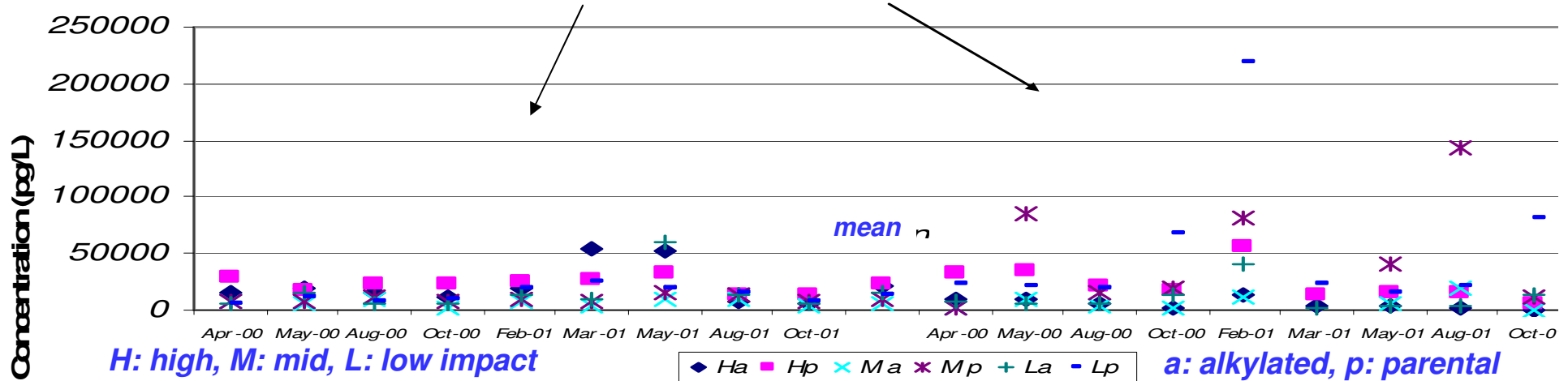
1. Zitko (1999); 2. Baumard et al (1998a) 3. Range of concentrations, Shiaris and Jambard-Sweet (1986) BaA is analysed along with CH and T; 4. average and (standard deviation), JWEL-IT (1995).

**Halifax Harbour:**  
dynamic, changing state  
Analysed H<sub>2</sub>O at 3 sites,  
9 times, over 18 months

**Reference site:**  
**<1,000pg/L or <1ng/L**



**Soluble and particulate PAH**



**Water vs sediments**

pg/ml or pg/g water compared to ug/g sediment: 1,000,000 x higher

**Partitioning affects animals and effects to be studied**

Hellou, J., Langille, M., Leonard, J., Steller, S and Tremblay, D. 2004. Mar. Environ. Res. 59: 101-117.

## Comparing our results with other locations for the water column (ng/L)

<b>Soluble</b> <b>Halifax</b>	<b>Iceland/N. Atl.<sup>1</sup></b>	<b>Chesapeake<sup>2</sup></b> Bay	<b>Norway<sup>3</sup></b> sewage effluents
<b>PA</b> <b>0.64-11.1</b>	<b>&lt;0.005-0.03</b>	<b>2.2-4.1</b>	<b>72-1117</b>
<b>FL</b> <b>1.1-5.7</b>	<b>&lt;0.005-0.009</b>	<b>0.28-22.1</b>	<b>82-515</b>
<b>PY</b> <b>0.41-2.9</b>	<b>&lt;0.005-0.007</b>	<b>0.23-10.6</b>	<b>110-248</b>
<hr/>			
<b>Particules</b> <b>Halifax</b>	<b>Baltic<sup>4</sup></b>	<b>Greece<sup>5</sup></b>	<b>Montréal<sup>6</sup></b> sewage influent/effluent
<b>PA</b> <b>0.24-26.0</b>	<b>0.38-1.31</b>	<b>47-58</b>	<b>333/109</b>
<b>FL</b> <b>0.39-52.7</b>	<b>1.5-3.9</b>	<b>24-37</b>	<b>150/20</b>
<b>PY</b> <b>0.30-43.5</b>	<b>0.30-43.5</b>	<b>27-50</b>	<b>138/23</b>

*-1. Schultz-Bull et al. 1998; 2. Gustafson et al., 1997; 3. Kveseth et al. 1982; 4. Witt, 2002; 5. Kildikis et al. 1994; 6. Pham et al., 1997*

## In the aquatic environment, exposure is

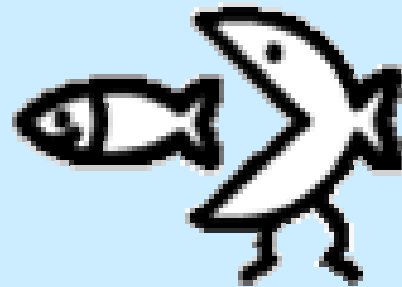
- from interstitial water and sea-surface microlayer  
more than from the water column,
- from surface sediments more than from deeper sediments

if  
H  
Y  
D  
R  
O  
P  
H  
O  
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C

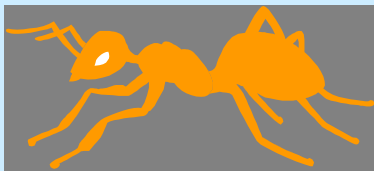
-from food: *ecosystem*

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-from dermal contact

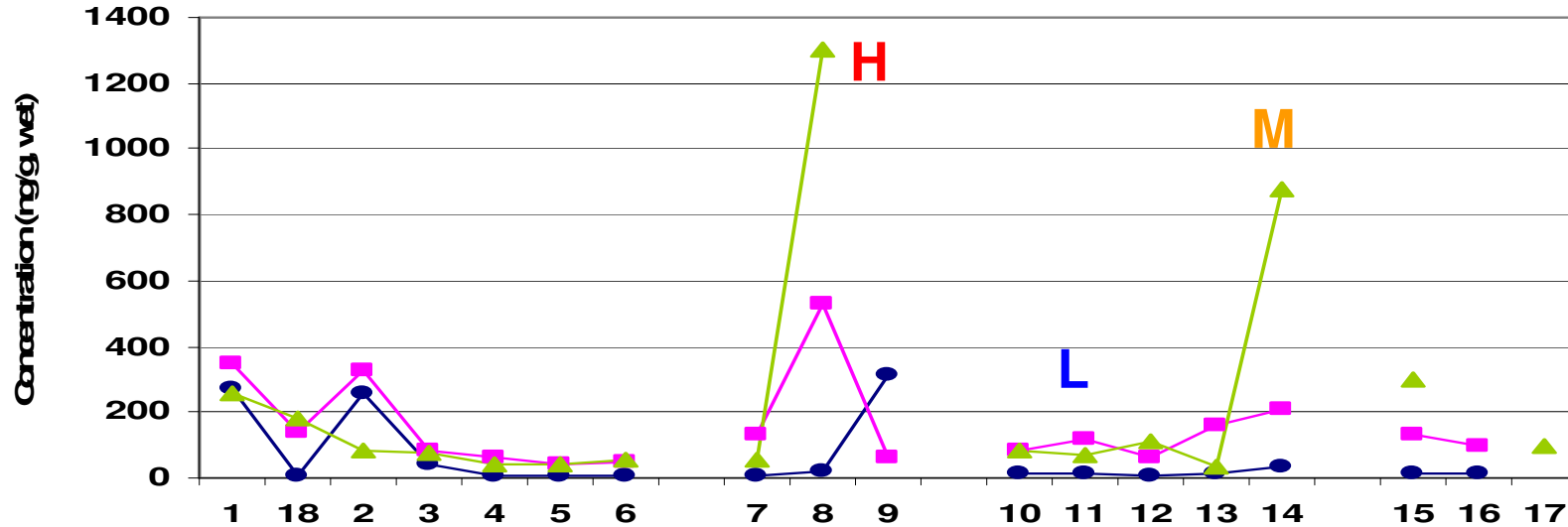


-from breathing



## Total PAHs observed over three seasons

*Mussels*



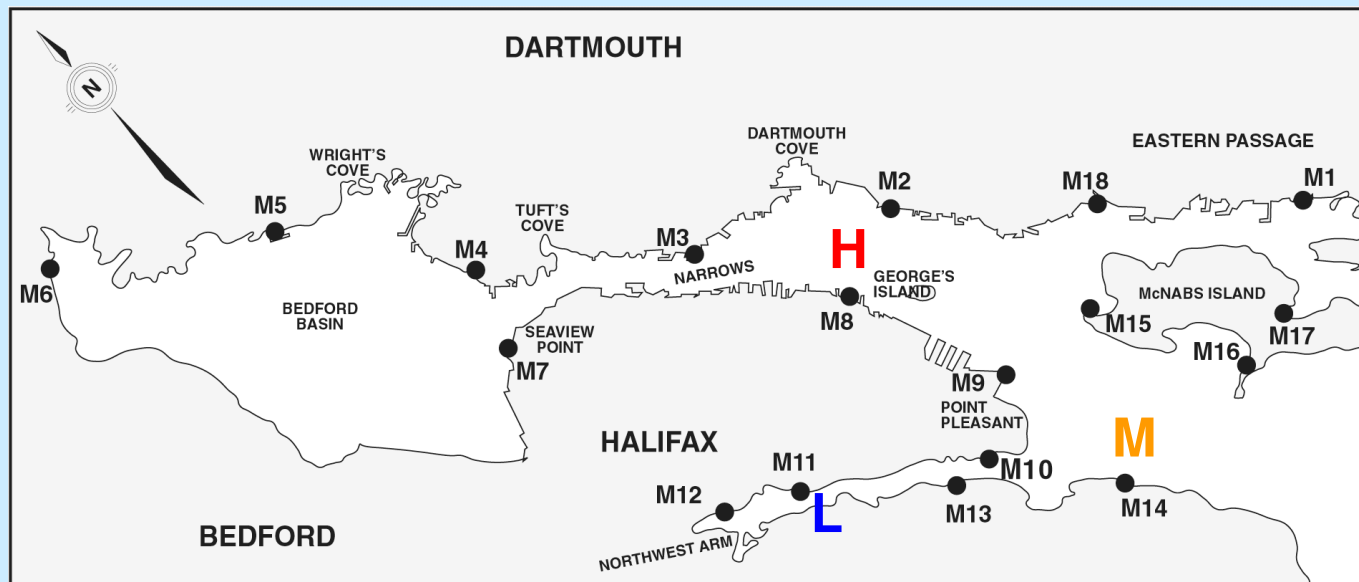
July  
Nov.  
April

Impact  
H: high  
M: mid  
L: low

Hellou, J., King T. and Willis, D. 2000. *Int. J. Polycyclic Aromatic Compounds*. 20: 21-38.

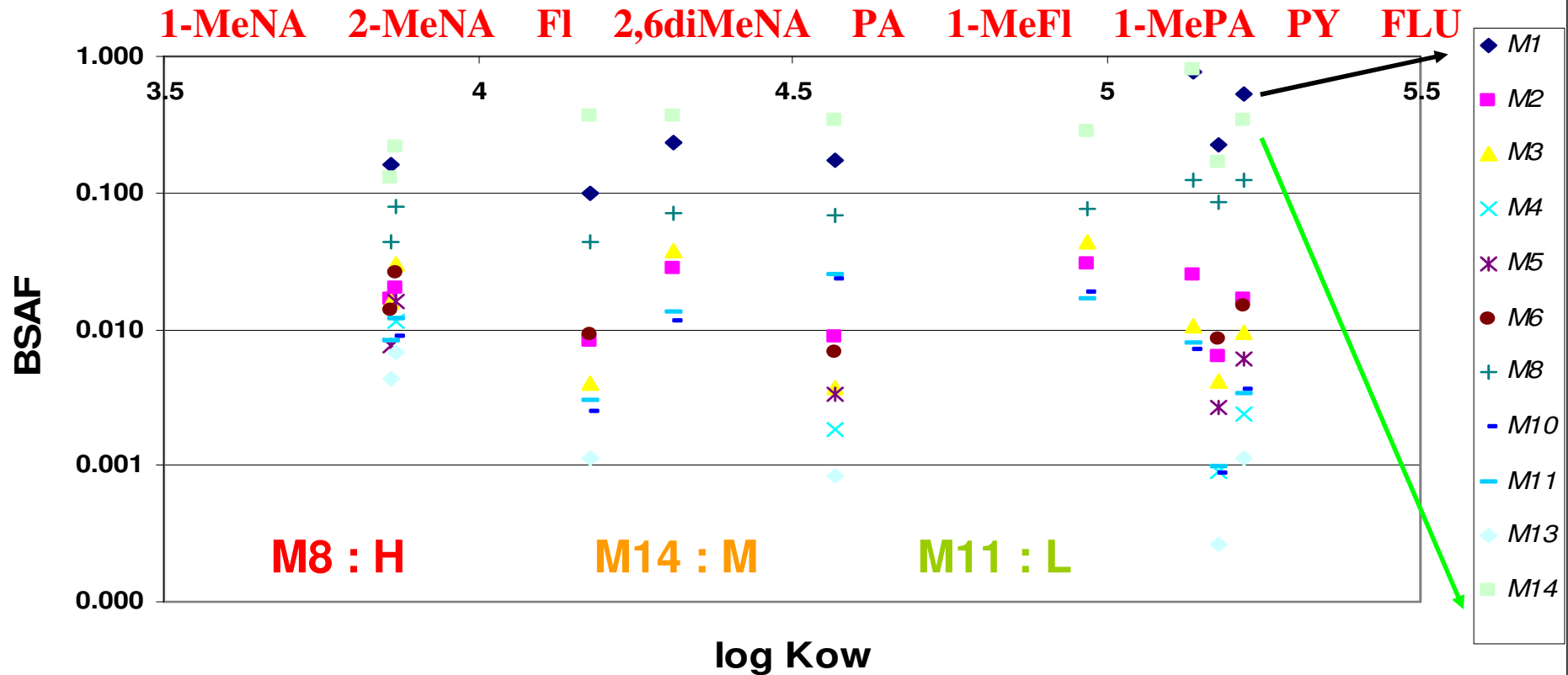
Many variables affect PAH levels in mussels: currents, dredging,...

**Reference mussels have very few ng/g of PAH**



# Comparison of some BSAF

*Biota-sediment  
accumulation factors*



**Mussels collected every 2 km around the harbour,  
when accessible from land**

**Bioavailability can not be predicted readily  
It also varies with the reproductive cycle**

# Biological effects: many to chose from

- Chemical and biochemical,
- Sub-cellular and cellular,
- Tissue or organ,
- Individual,
- Population and community (survival, growth, reproduction)

*“Milder”*



*“Worse”*

## Toxic effects examined:

**At first, lipid content (LC) and condition indices (CI) give and more effects were added with time**

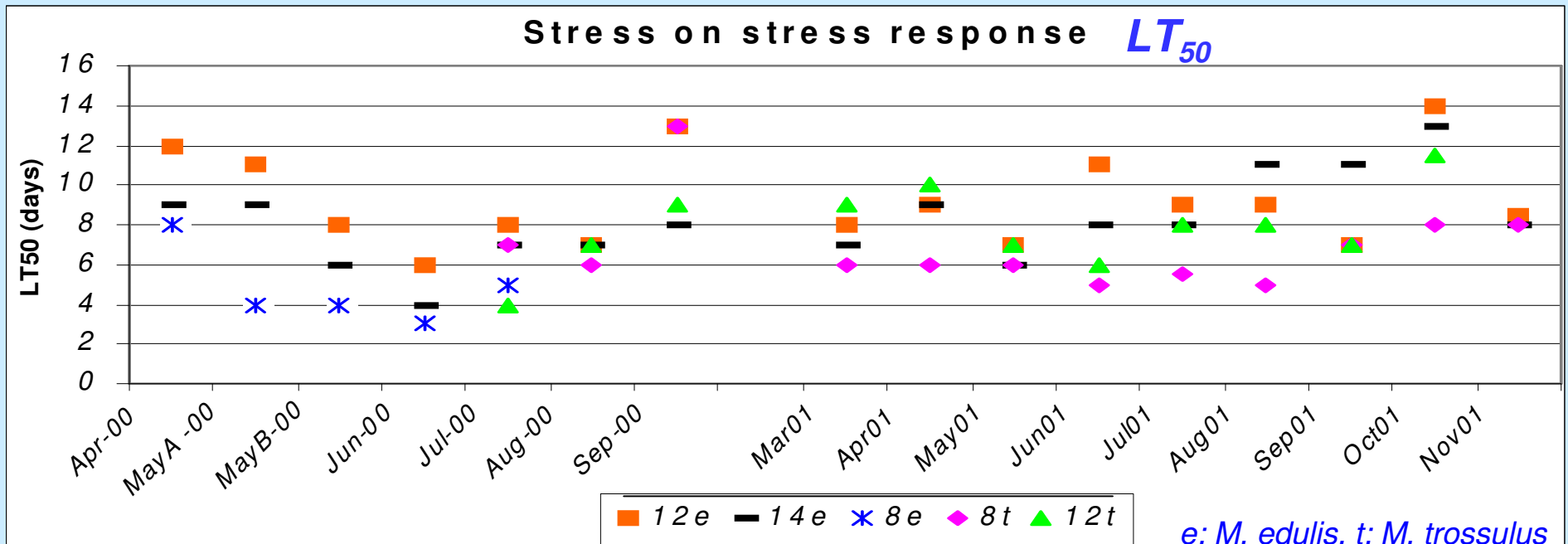


- Gonad index
- Lipid content of more defined parts
- Sex ratio, reproductive cycle, Vtg
- Survival in air : immune response
- Many biochemical markers

**Toxicity was expected to reduce CI and increase LC: not quite**  
**Chemical results were relied on to pursue our assessment work**

# Survival of mussels in air at 15°C, immune response nearly repetitively at each sampling time

$$H < M < L$$



**Mussel species**

## M/F sex ratio: summer of 2000

Site Mean  $\pm$  standard deviation

**M8:H 1.92  $\pm$  0.38**

**M12:L 0.83  $\pm$  0.03**

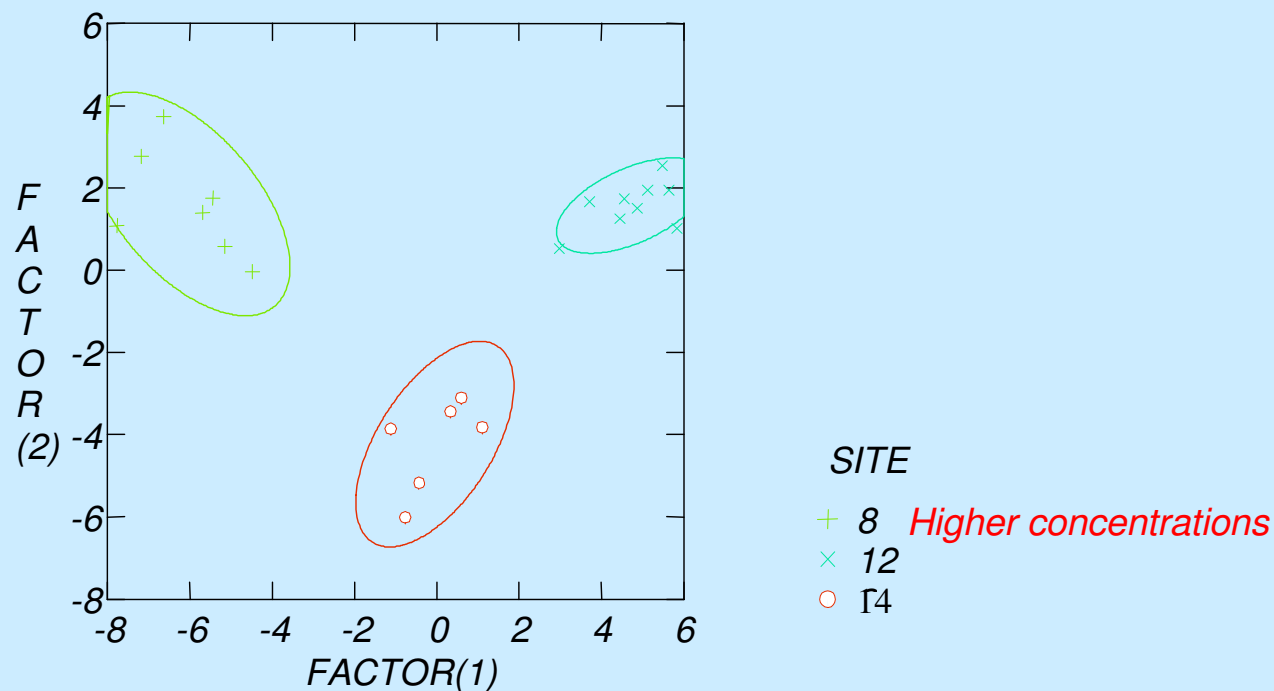
**M14:M 0.57  $\pm$  0.13**

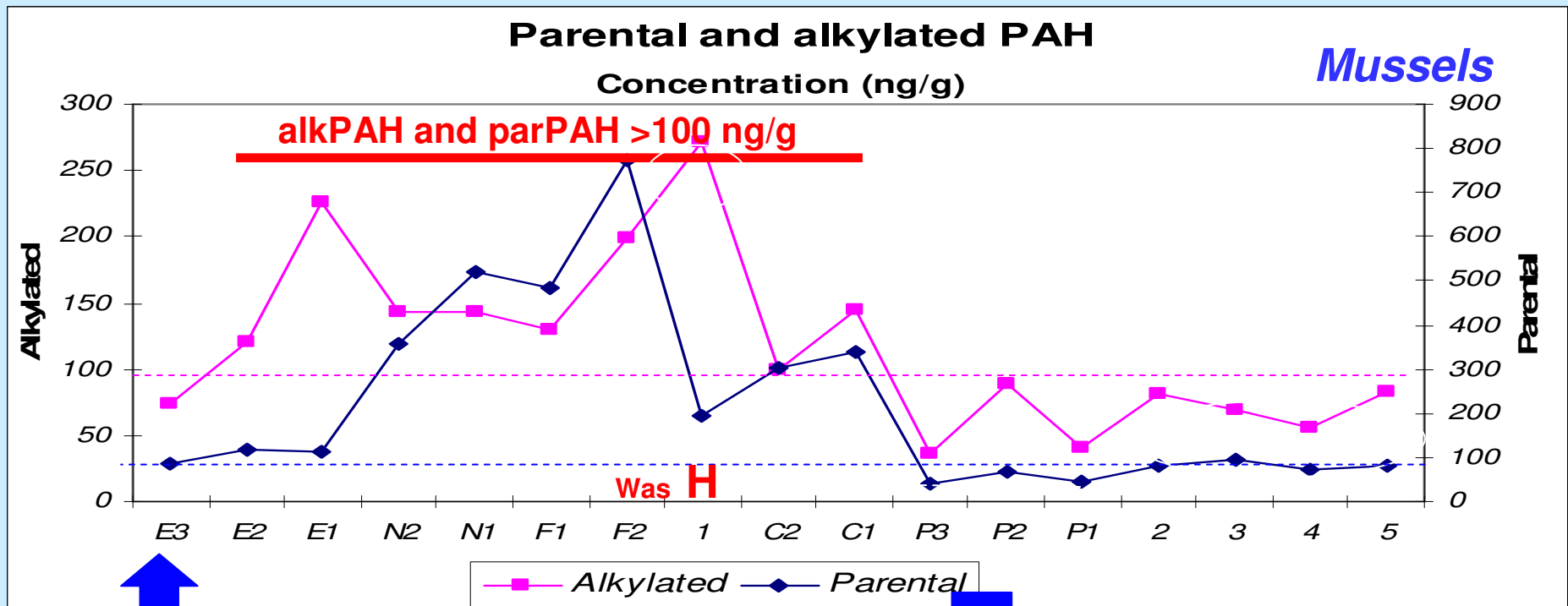
**2 x more M**  
**less variability**  
**1.7 x more F**

# Discriminant analysis using PAH, PCB, coprostanol, metals, condition and gonad indices, sex ratio, lipid and oALP as variables shows

that the three chosen sites are distinctly different.

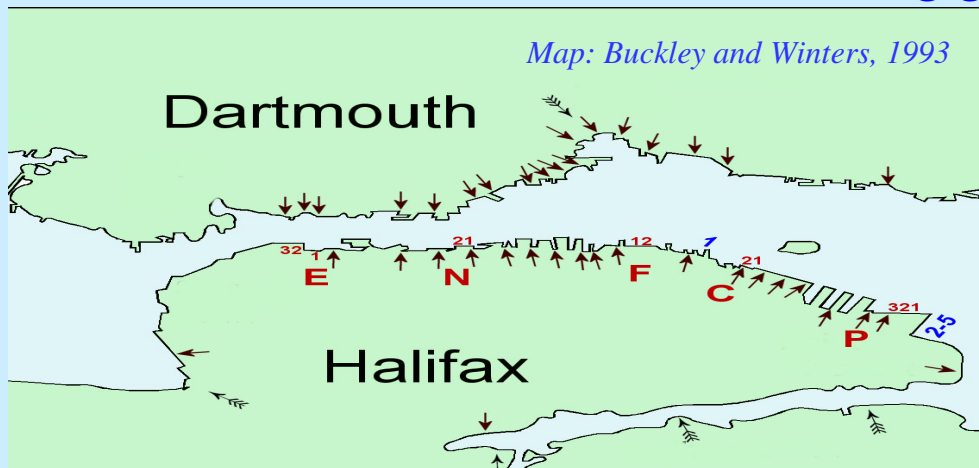
*Canonical Scores Plot*





at E3 and from P3 eastward,  
concentrations of PAH are lowest <100ng/g

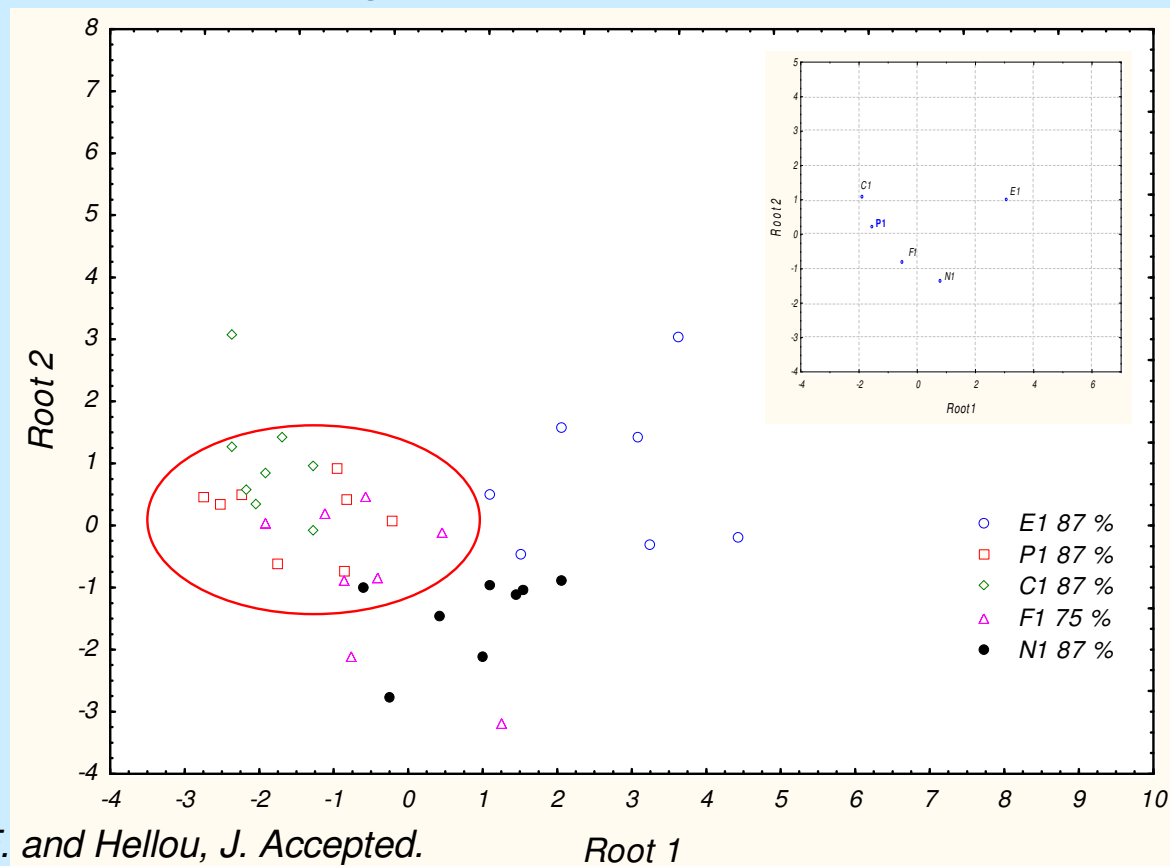
Background



- E:** sewage effluents
- N:** old navy shipyard
- F:** ferry + ships
- C:** creosote pilings
- P:** containers pier

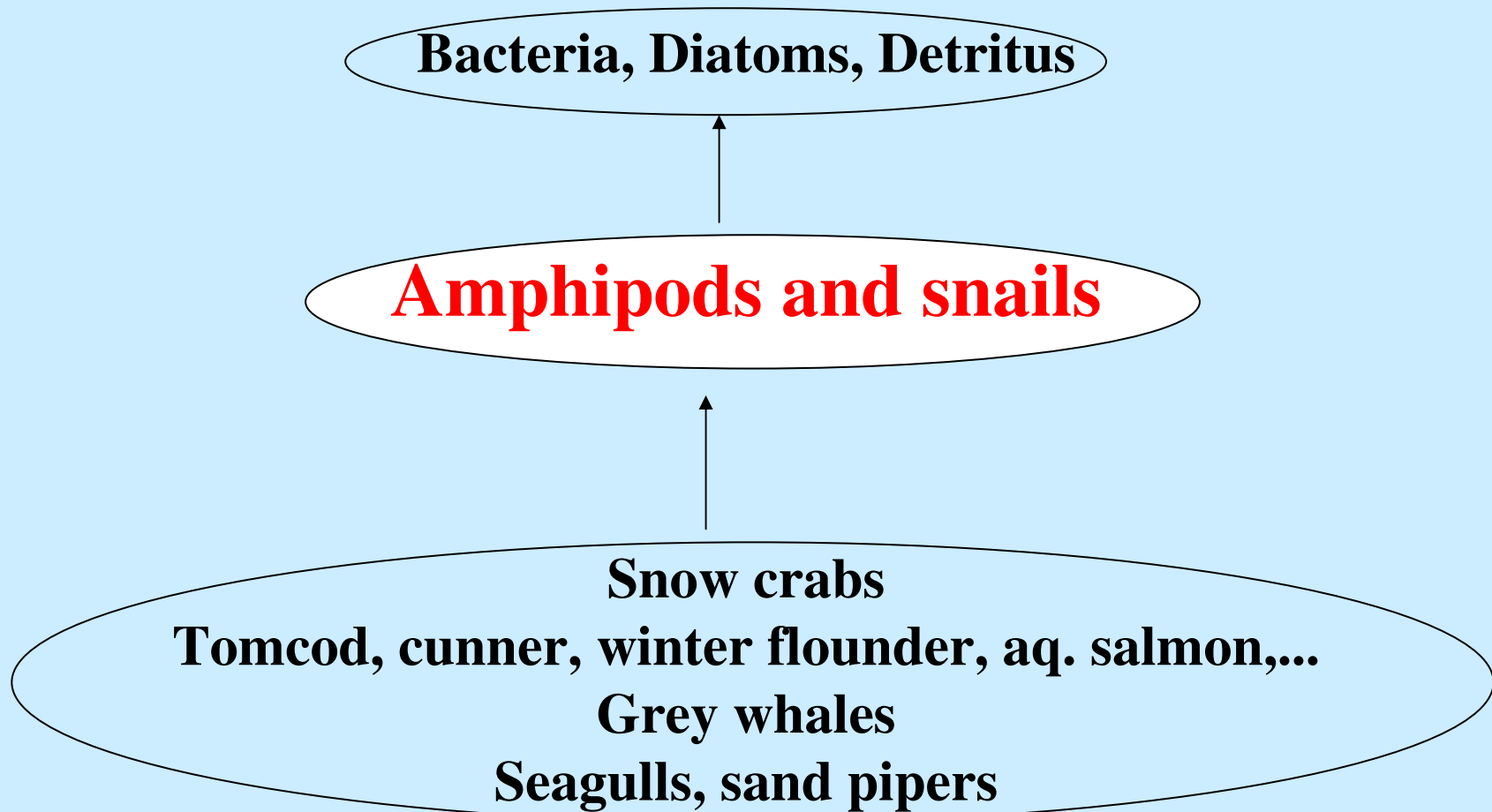
Ag and male/female ratio decreased from E to P  
while Cd, Sn and LT<sub>50</sub> showed the inverse.  
The PAH concentrations were higher centrally  
With more alkylated PAH or fresh oil at E1

The distributions of Ag, Sn, PAH and coprostanol were uncoupled  
Discriminant analyses of biomarkers response indicated:  
**P1 < C1 < F1 < N1 << E1**



# Aquatic food chain: questions on benthic component

Chose abundant, available, representative lower links



**Amphipods : standard toxicity test**  
**Lethal concentration to 50% (LC<sub>50</sub>) of the animals**  
**during 2 or 10 days exposures**

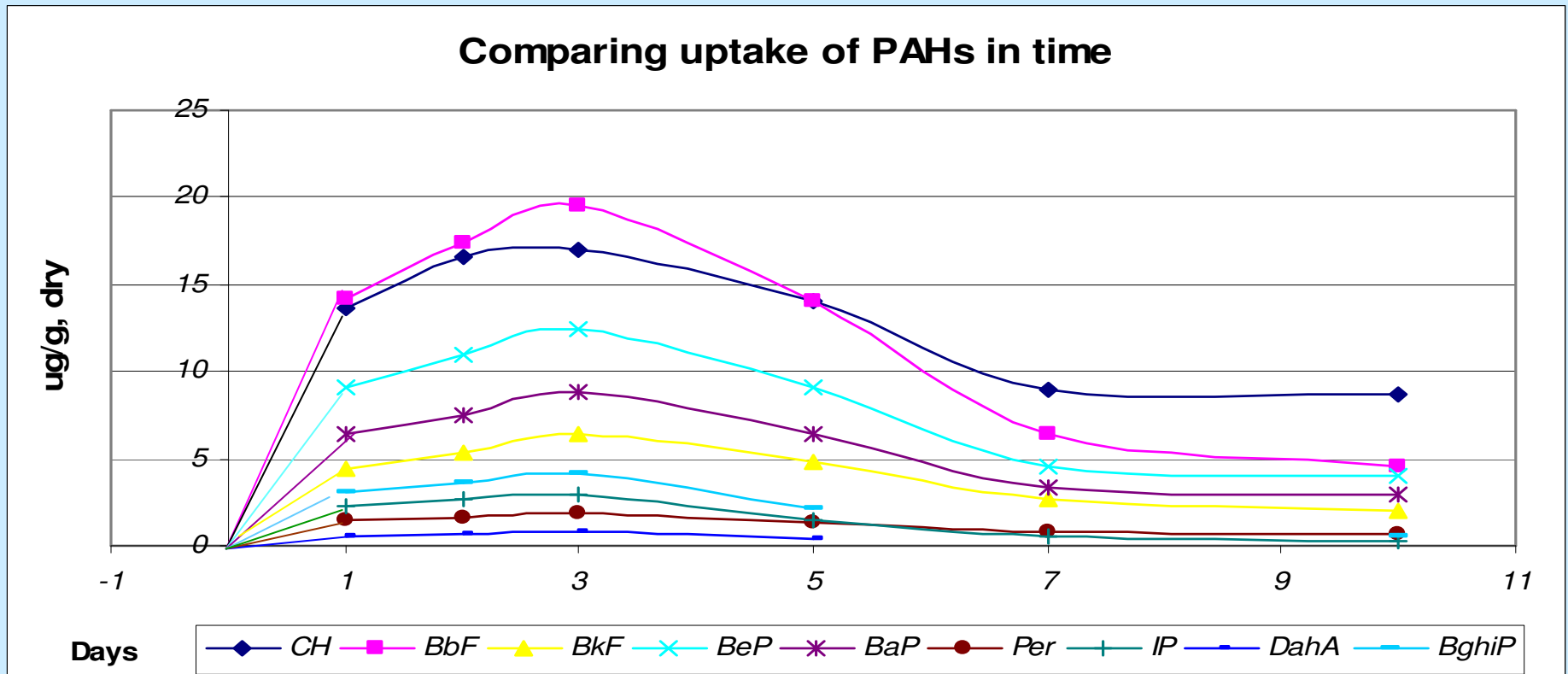
**Using harbour sediments**  
**all animals survived >80-90%**

**Except once:**  
**black visceral mass, as the sediments,**  
**sum of PAH = 0.75, 0.91 and 1.1 mmole/kg**

**McCarthy and Mackay Environ. Sci. Technol. (1984); McCarthy, Mackay et Sci. Tot. Environ. 109-110: 515-525 (1991)**  
**1-5 mmole/kg associated with lethality**  
**depending also on the lipid content of the animals**

# Were PAH bioavailable? yes

Uptake takes place rapidly over the first day of exposure, continues to increase, then decreases and reaches a steady state within a week



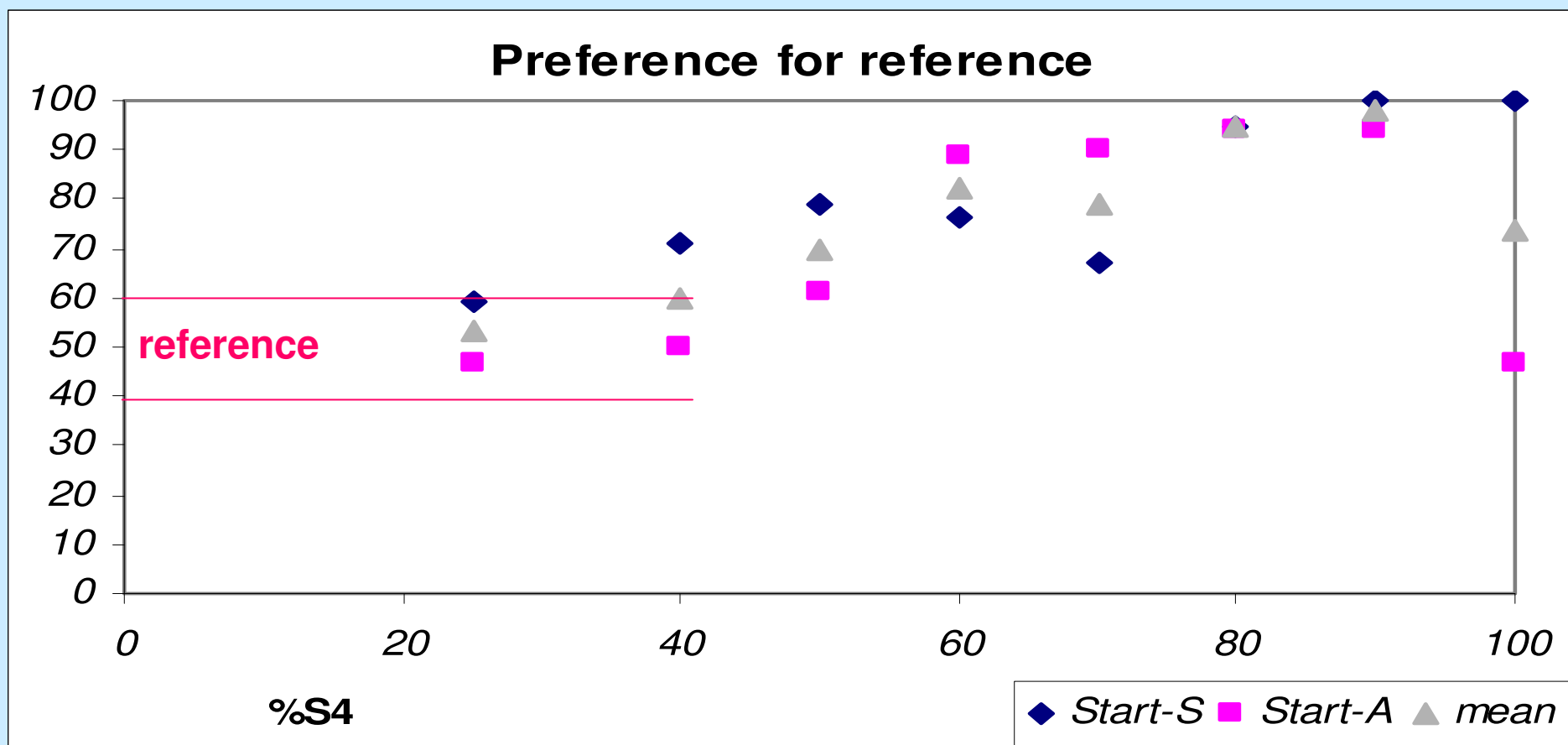
Reference amphipods only have 1 PAH detectable (20 ng/g, dry weight)  
Biological indicators of health in mussels were difficult to interpret in amphipods  
In exposures  $\leq 10$  days  
Tried lipid content, weight, reproduction, but focused on Behaviour

Hellou J, Johnston D, Cheeseman K, Gronlund A, Desnoyers E, Leonard J and Robertson S. In Press

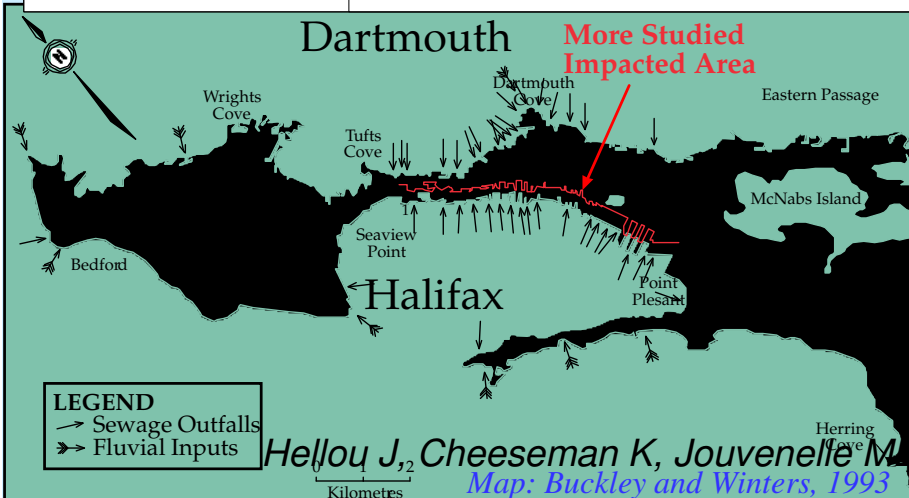
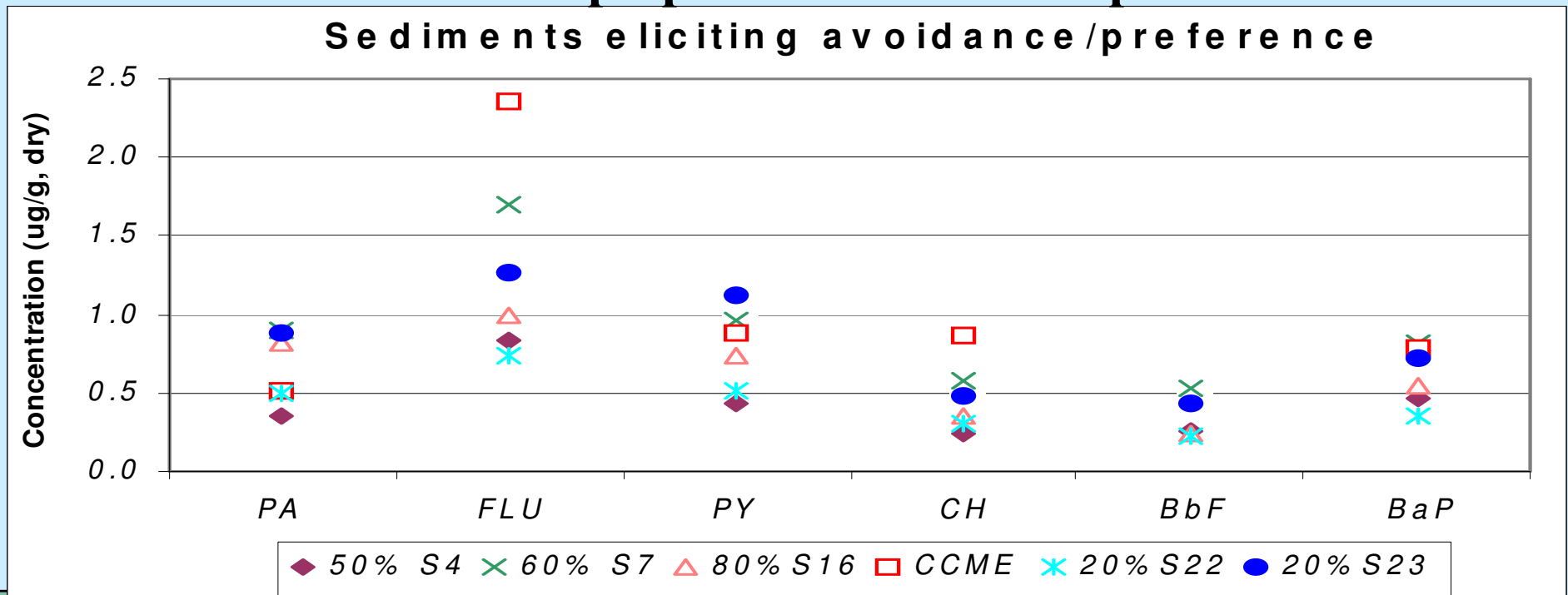
## Behavioural effects: avoidance/preference

More subtle end-point, non-lethal for tiered approach

Includes survival



**For 5 out of 7 harbour sediments, a relationship was observed between the level of PAH in sediments (CCME) and the amphipods' avoidance response**

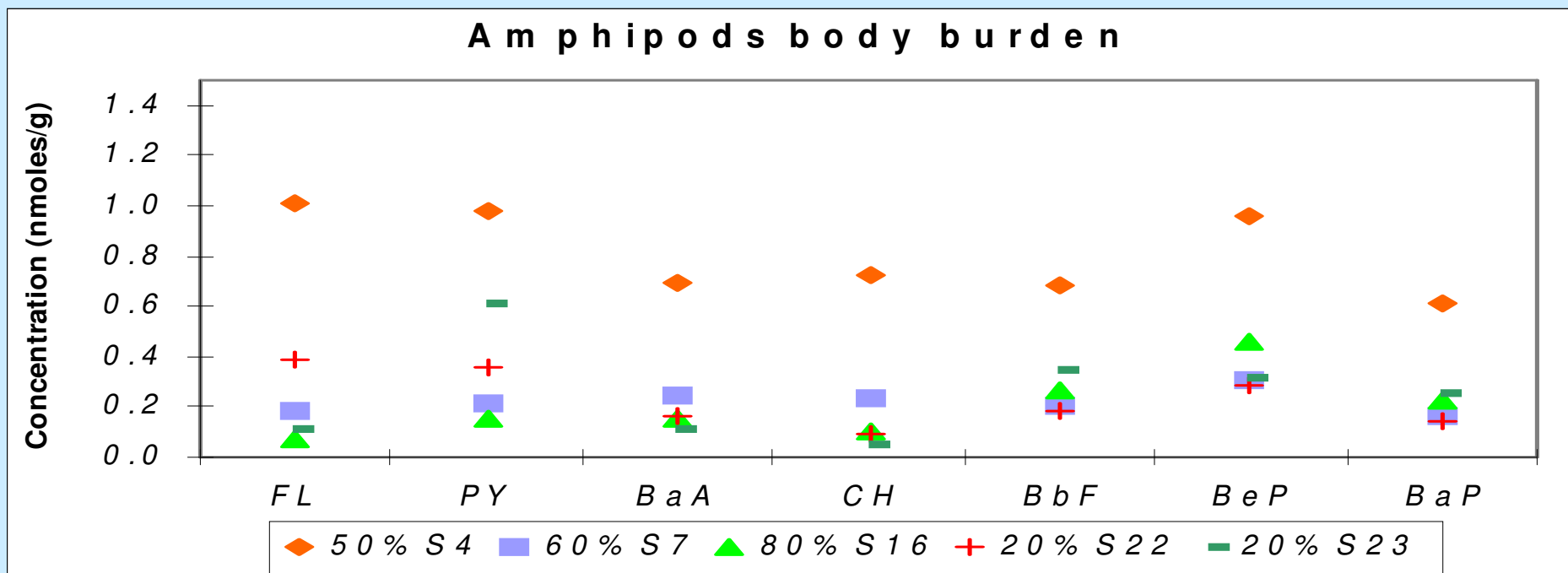


**Probable Effects Level**  
**“50% prediction of toxicity”**  
**Canadian Council of the Marine**  
**Environment (CCME)**  
**Sediment Quality Guidelines**

Robertson S. 2005. *Environ. Toxicol. Chem.* 24: 3061-3068.

**Body burden in amphipods: 1.5-5.5 nmoles/g (dry weight)  
for the sum of commonly abundant PAH.**

**Effects observed at 1,000 x lower levels of PAH  
than associated with LC<sub>50</sub>**



**Green assessment tool needing more validation**

**2 sites further away from sewage effluents  
represent an exception to the behaviour chemical link**

*Hellou J, Johnston D, Cheeseman K, Gronlund A, Desnoyers E, Leonard J and Robertson S. In Press*

**Animals exposed to PAH spiked sediments  
prefer reference sediments  
when concentrations of individual PAH > 50ng/g**

**Spiked sediments are not identical to harbour sediments...**

**Total body burden of these amphipods:  
2-7 times higher than  
those exposed to 5 harbour sediments**

**More causes than PAH are associated  
with behavioural effects**

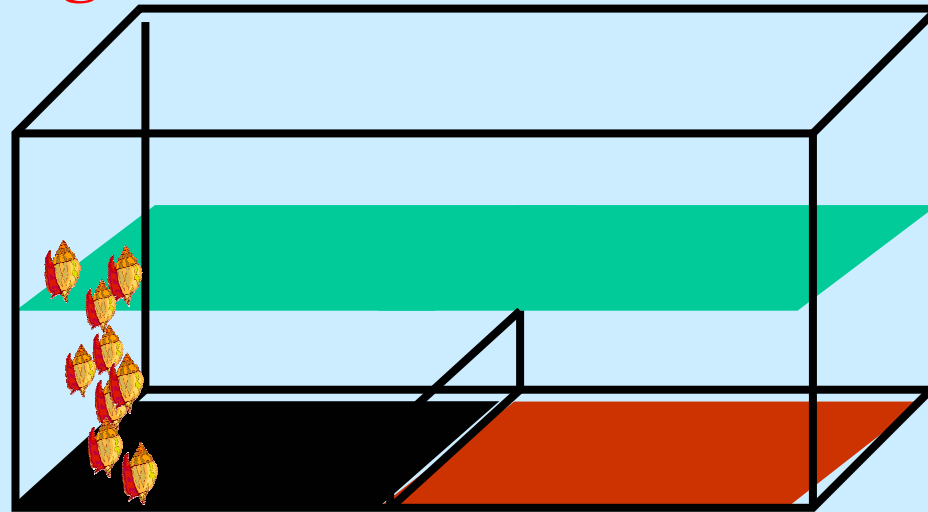
*Hellou J, Cheeseman K, Desnoyers E, Johnston D, Jouvenelle ML, Leonard J, Robertson S, Walker P.  
In Press.*

# Snails

Harbour and spiked sediments elicited an escape:  
**unambiguous behaviour**

With ref/ref exposures  
99% of snails  
remain on  
reference sediments  
in water

But not with  
Harbour sediments



Harbour  
sediment

Reference  
sediment

As well, when sediments are spiked with hexane extract:  
animals climb up the glass



Role for food? yes

Role for natural and anthropogenic products?

Tested cholesterol, coprostanol or a fatty acid methyl ester: not apparent

*Marklevitz, Almeida, Flemming, Hellou. In Preparation*



## Some of the conclusions:

Fates and effects in field mussels  
and lab exposed amphipods (and snails?)  
Presented the same ranking of sites  $\pm$  **Resilient**  
 $\pm$  **Adapt**  
 $\pm$  **Absent**

Chemistry enabled the interpretation of toxicity

**Mussels: exposure over years**  
**Amphipods, snails: exposure over 2-3 days**

PAH good markers of which other lipophilic contaminants?

**Cause-effects relationships are needed**  
**to understand, prevent and improve ecosystems health**

*Results of our assessments depend on society's values:  
Halifax Regional Municipality champions greening the environment*

# Acknowledgements: **collaborators, students**

**Funding: Toxic Chemicals Program (1997-2000)**

**ESSRF (2000-2003)**

**DFO, BIO (2003-2004)**

**NSERC, Dalhousie U. (2001-2005, 2006-2011)**

**??? \$\$ in 2004-05, 05-06, 06-07, 07-08...???**



DFO, BIO  
Dalhousie U.

**Ecosystem health needs research/knowledge**  
**Human health depends on ecosystems' health**

*Summer work of >8 Coop, research of 3 honours, 2 MSc and many colleagues*