

# Pollutant effect biomarkers in aquatic organisms

Main sub-lethal effect parameters and biomarkers  
(applications, discriminatory power, pros and cons, price of analysis, analytical complexity level).

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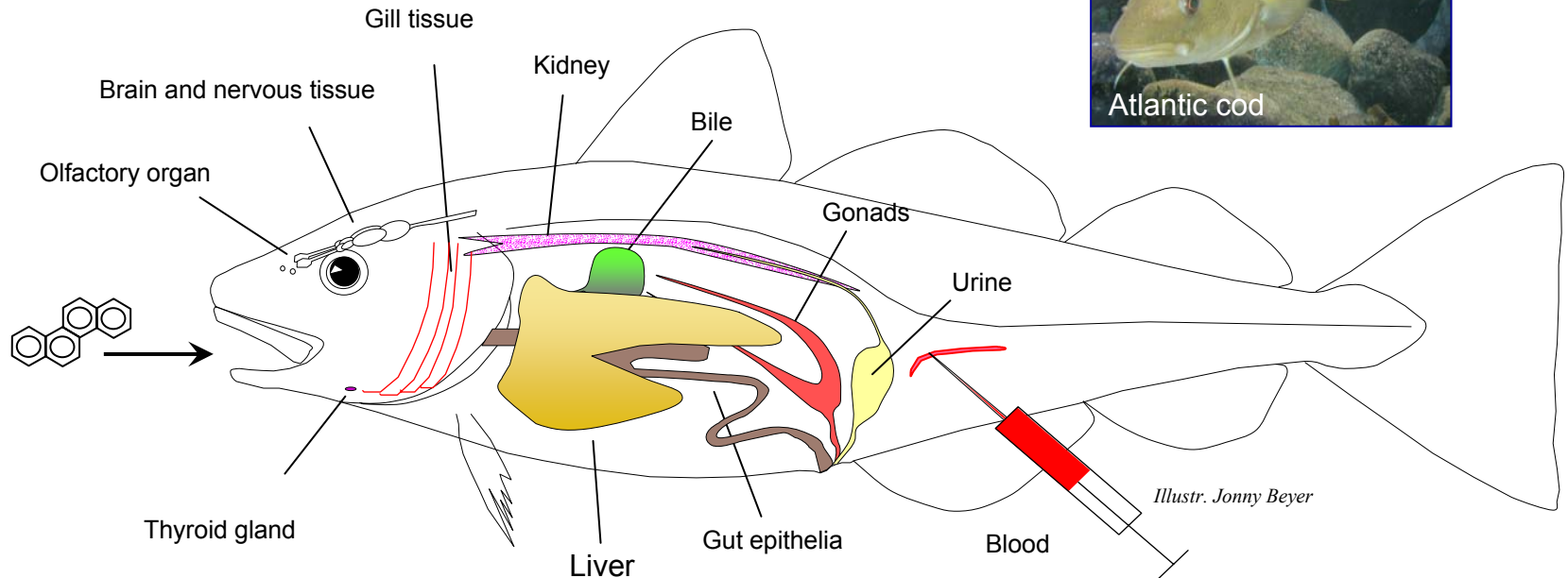
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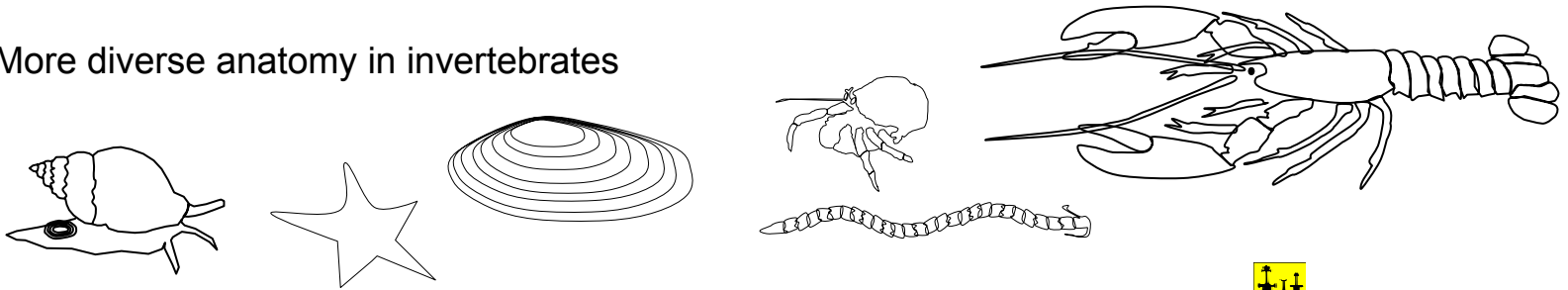
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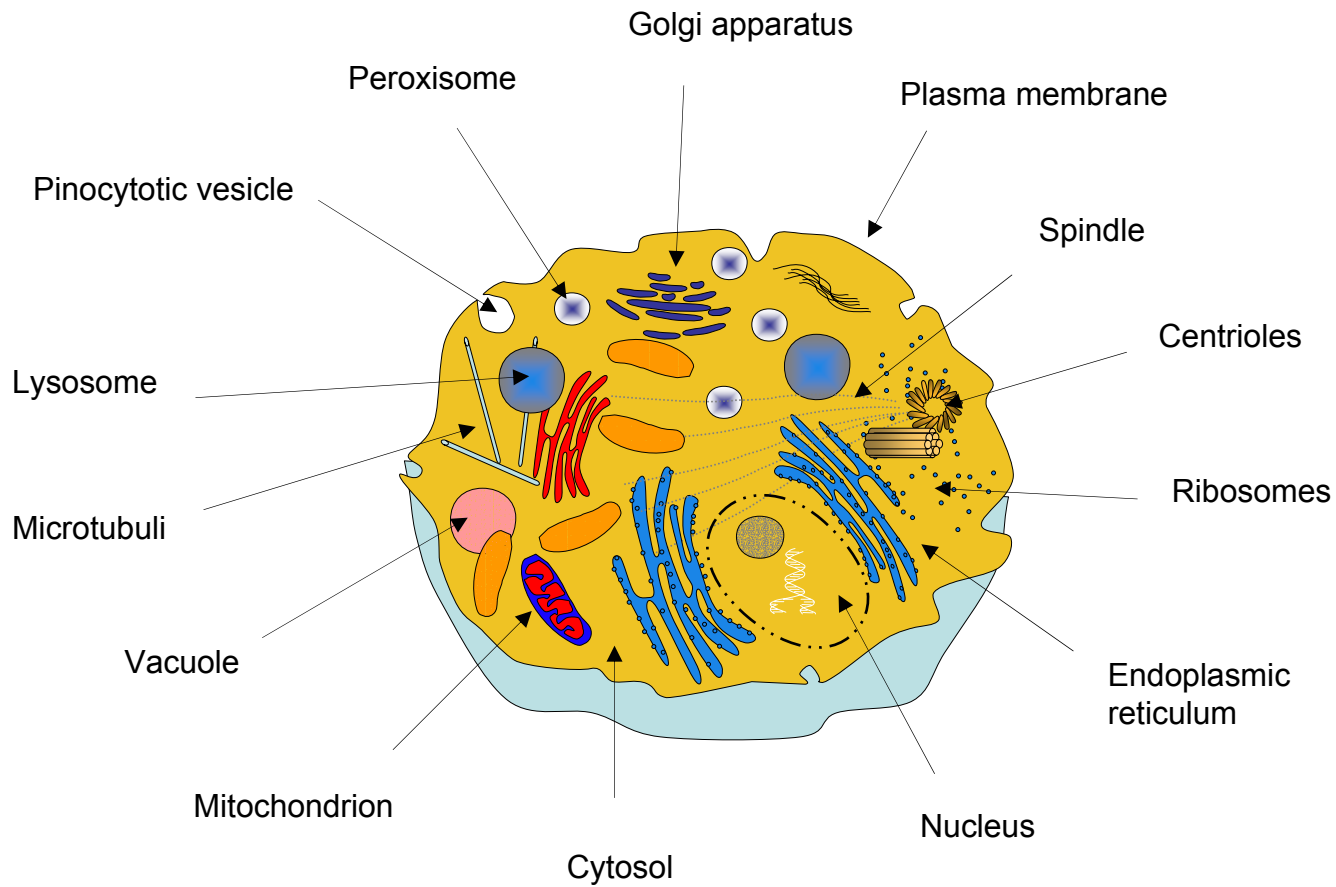
# Animals and organs for biomarker studies



More diverse anatomy in invertebrates

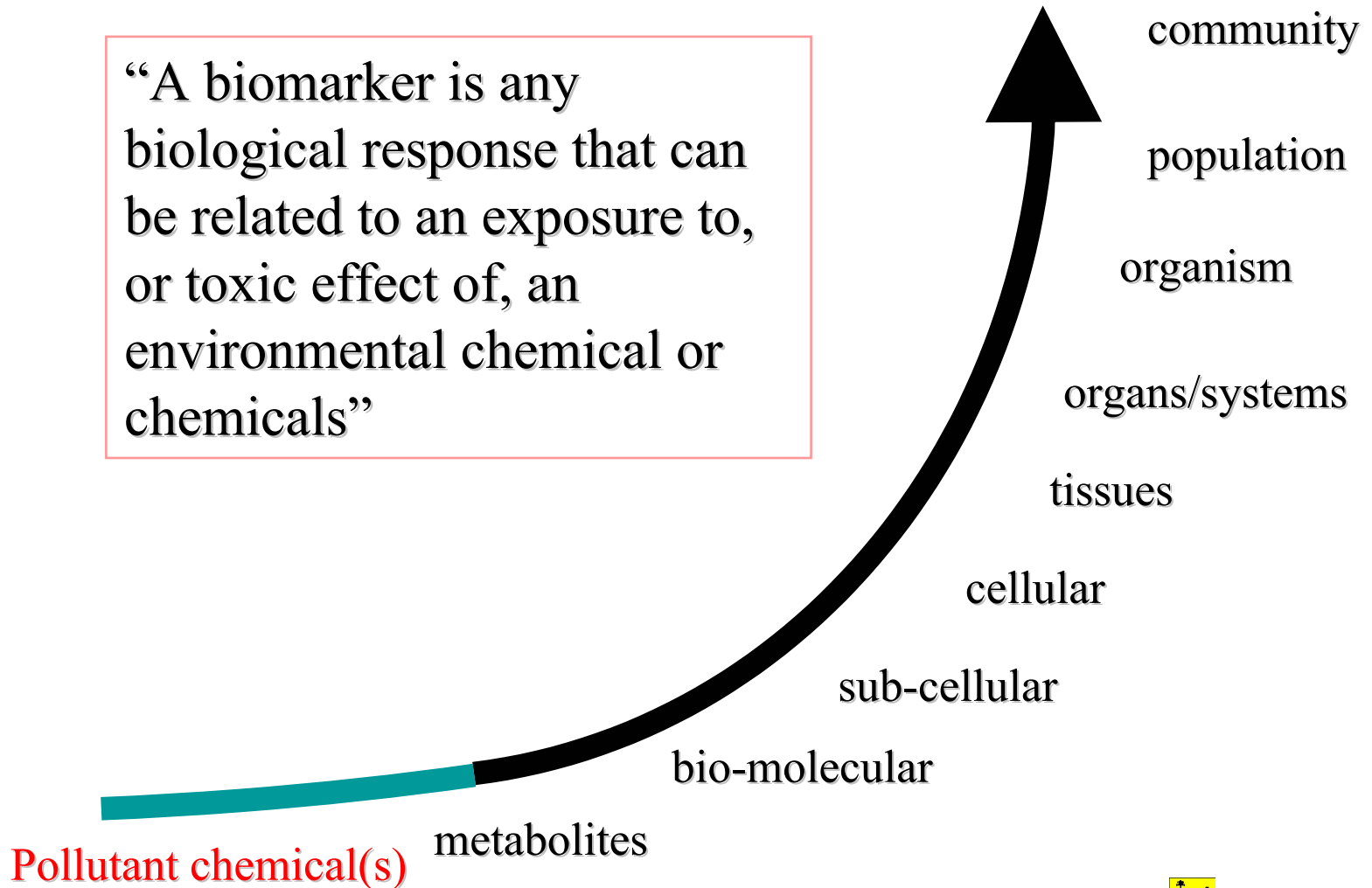


# The eukaryotic cell



# A pollutant effect is a hierarchical row of events

“A biomarker is any biological response that can be related to an exposure to, or toxic effect of, an environmental chemical or chemicals”

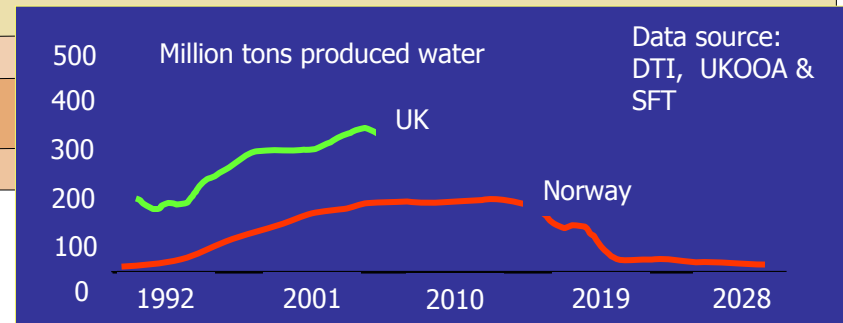
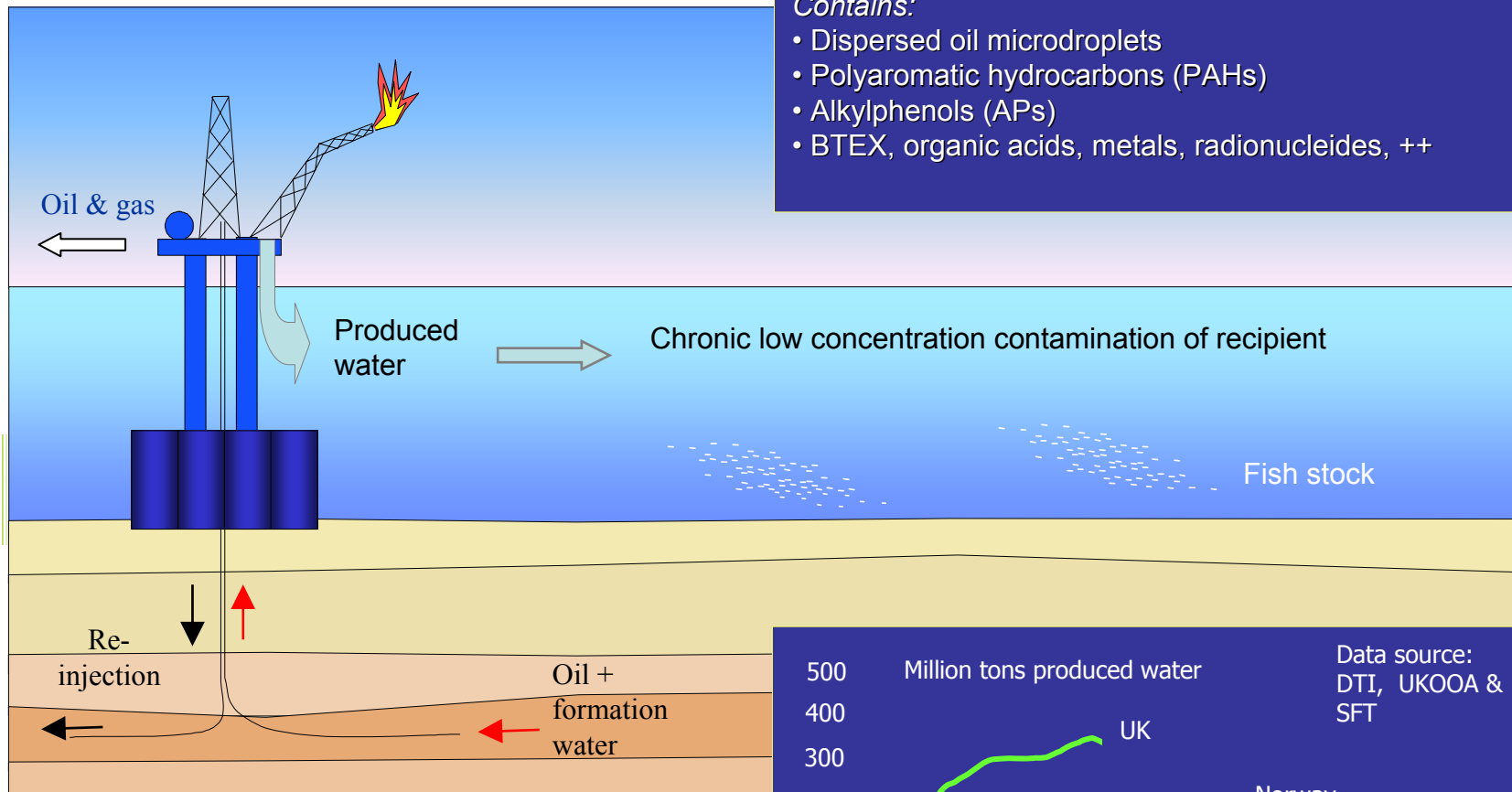


# Offshore oil and gas E&P (2): Produced water

A complex and variable aqueous mixture of chemicals

*Contains:*

- Dispersed oil microdroplets
- Polyaromatic hydrocarbons (PAHs)
- Alkylphenols (APs)
- BTEX, organic acids, metals, radionuclides, ++



# ***Applications related to offshore E&P: the different approaches in field studies of pollutant effects***

***“The chemical detection approach”***

What is the pollutant concentration?

***“The benthos community approach”***

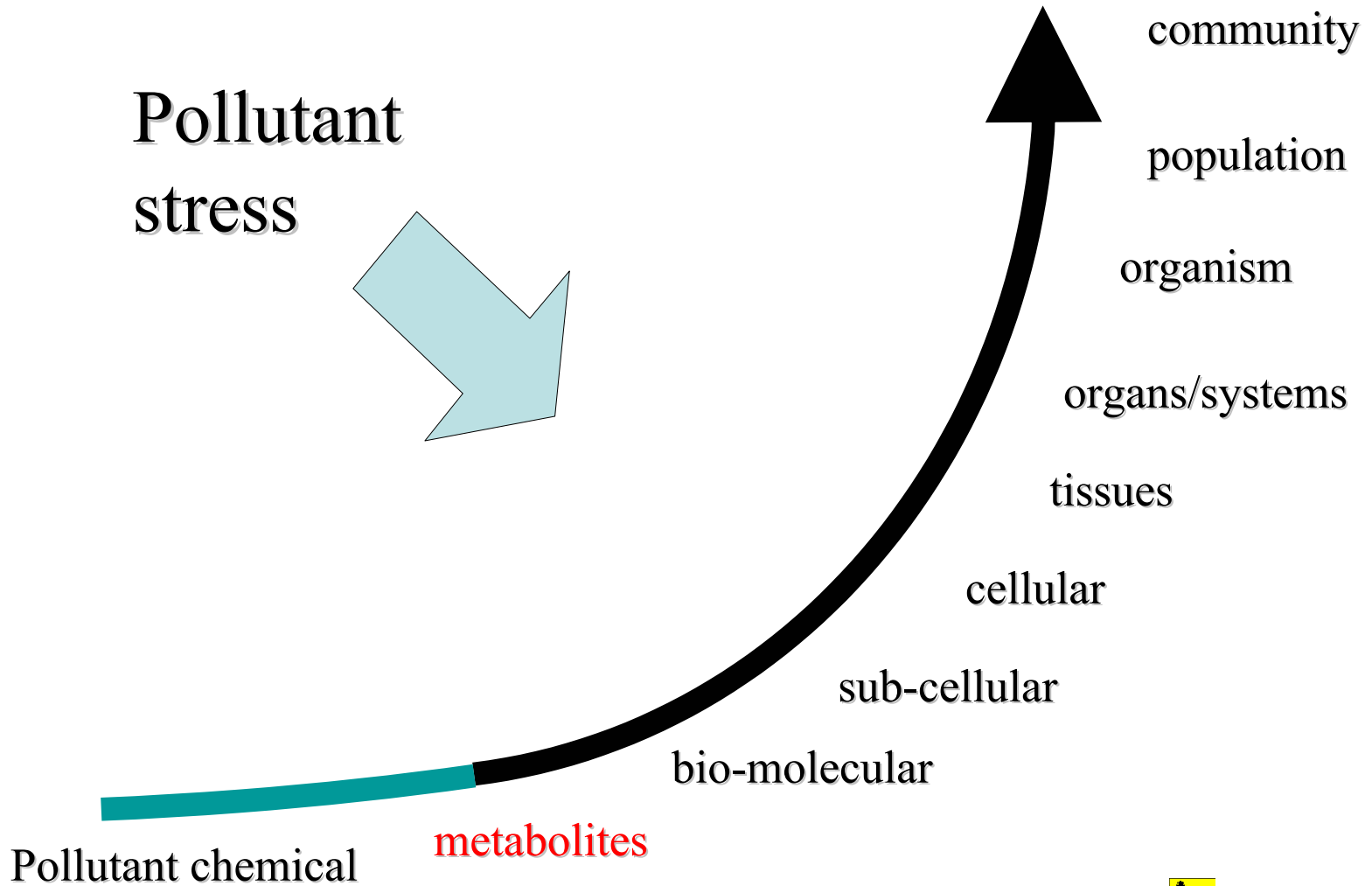
Is there a change in invertebrate populations?

***“The biomarker approach”***

Is there a detectable effect in organisms?



# A pollutant effect is a hierarchical row of events



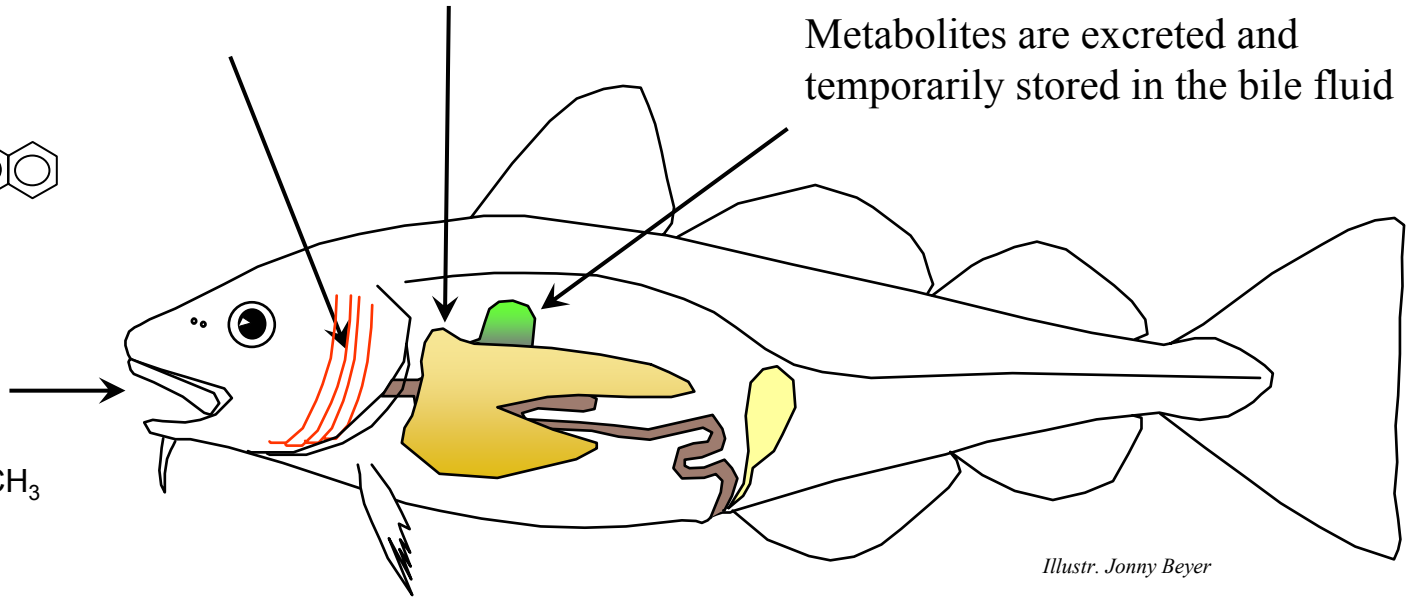
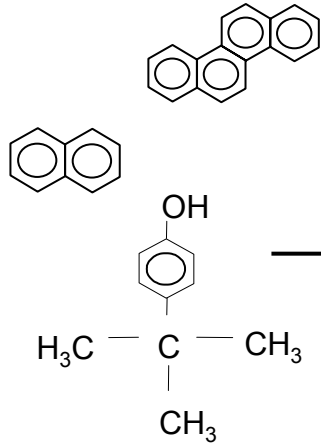


Main uptake route

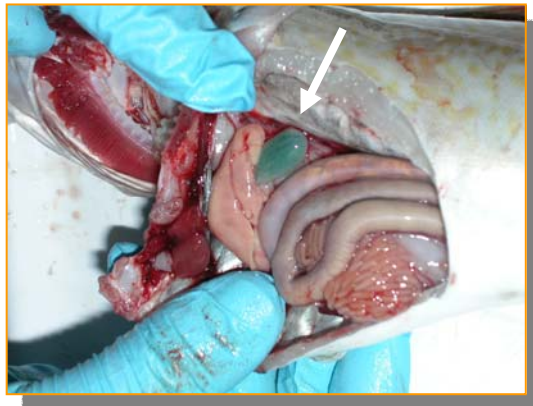
Pollutants are metabolised in the liver

Metabolites are excreted and temporarily stored in the bile fluid

Pollutant exposure



Illustr. Jonny Beyer

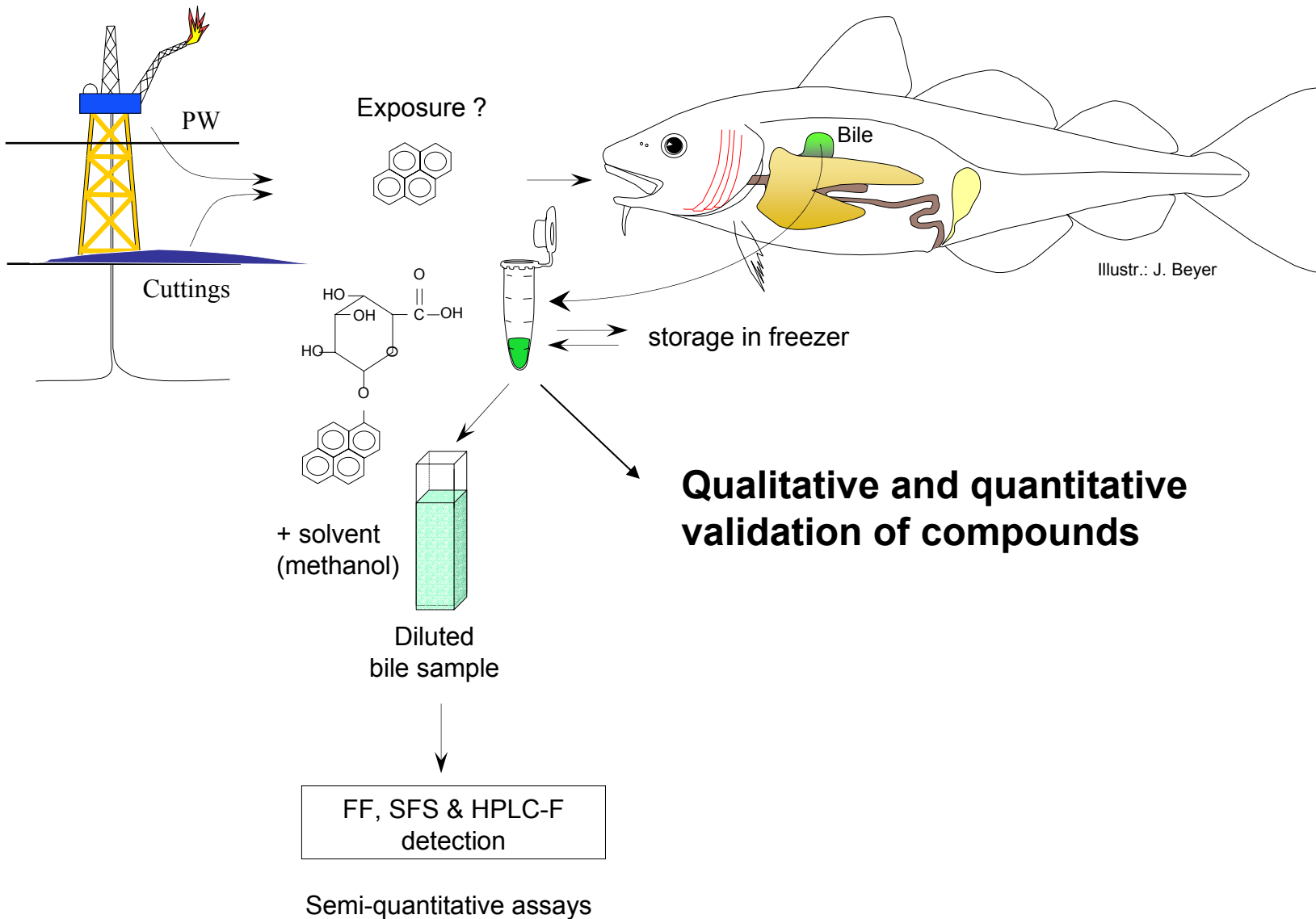


Fish bile is a “natural extract”- reflecting recent and ongoing exposure to PAHs and alkylphenols

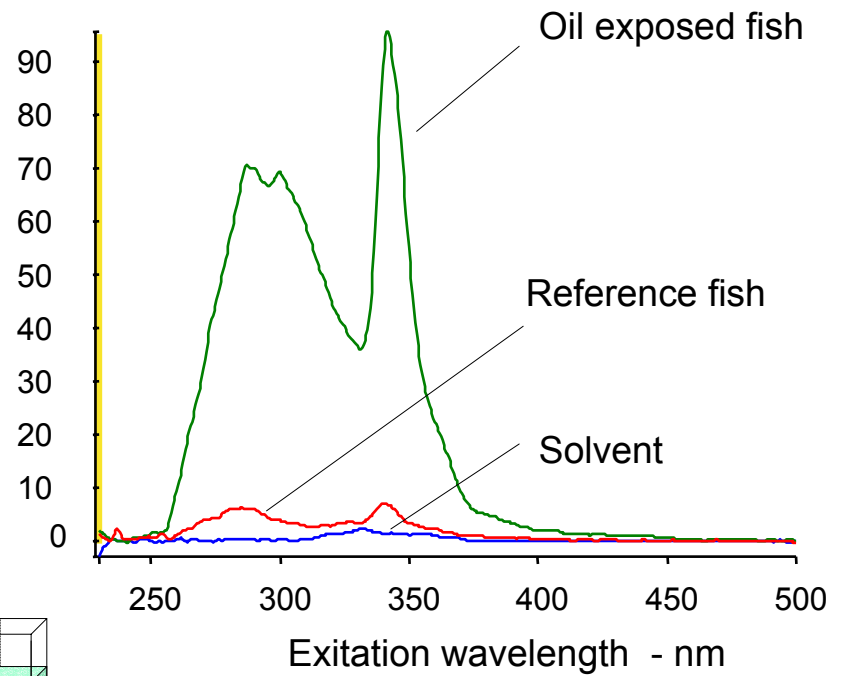
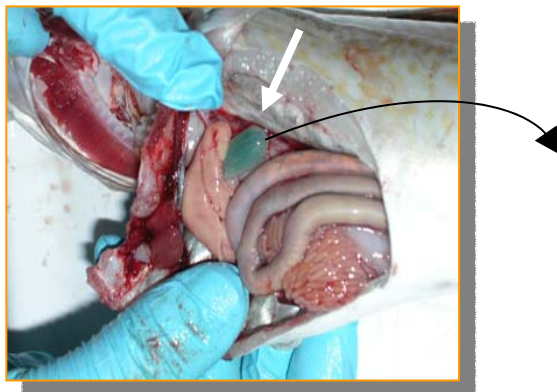




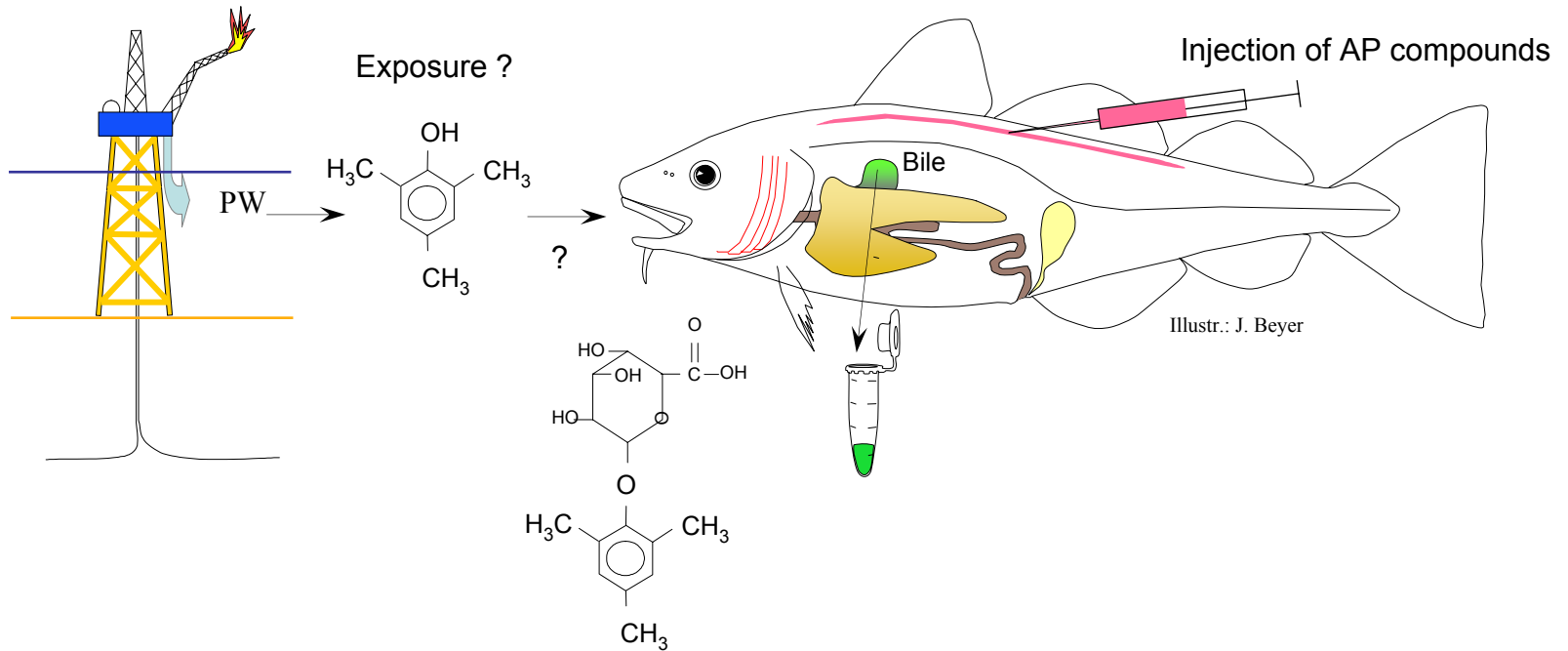
# 3 Methods for bile PAH detection



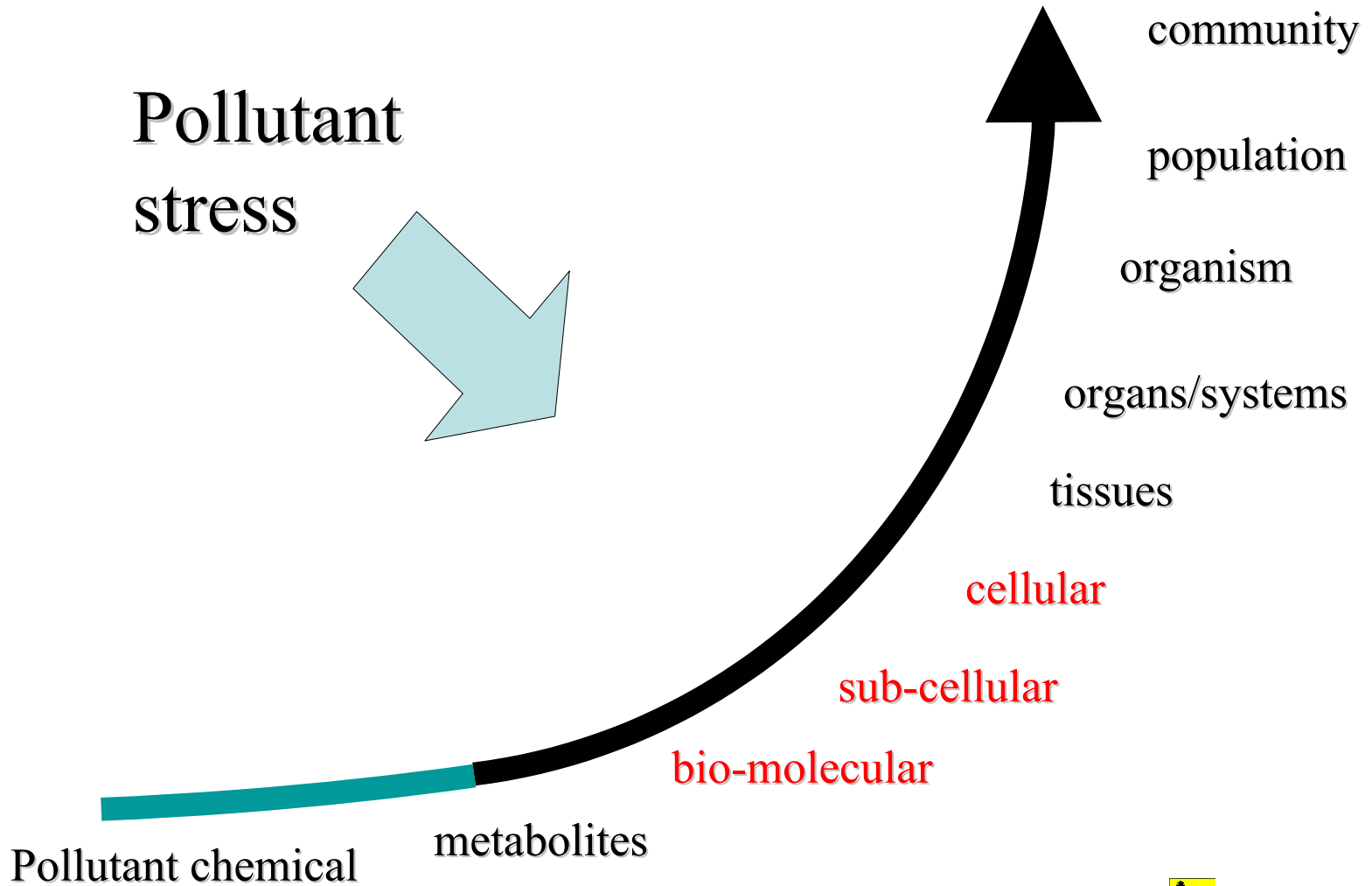
# Bile FAC - PAH screening



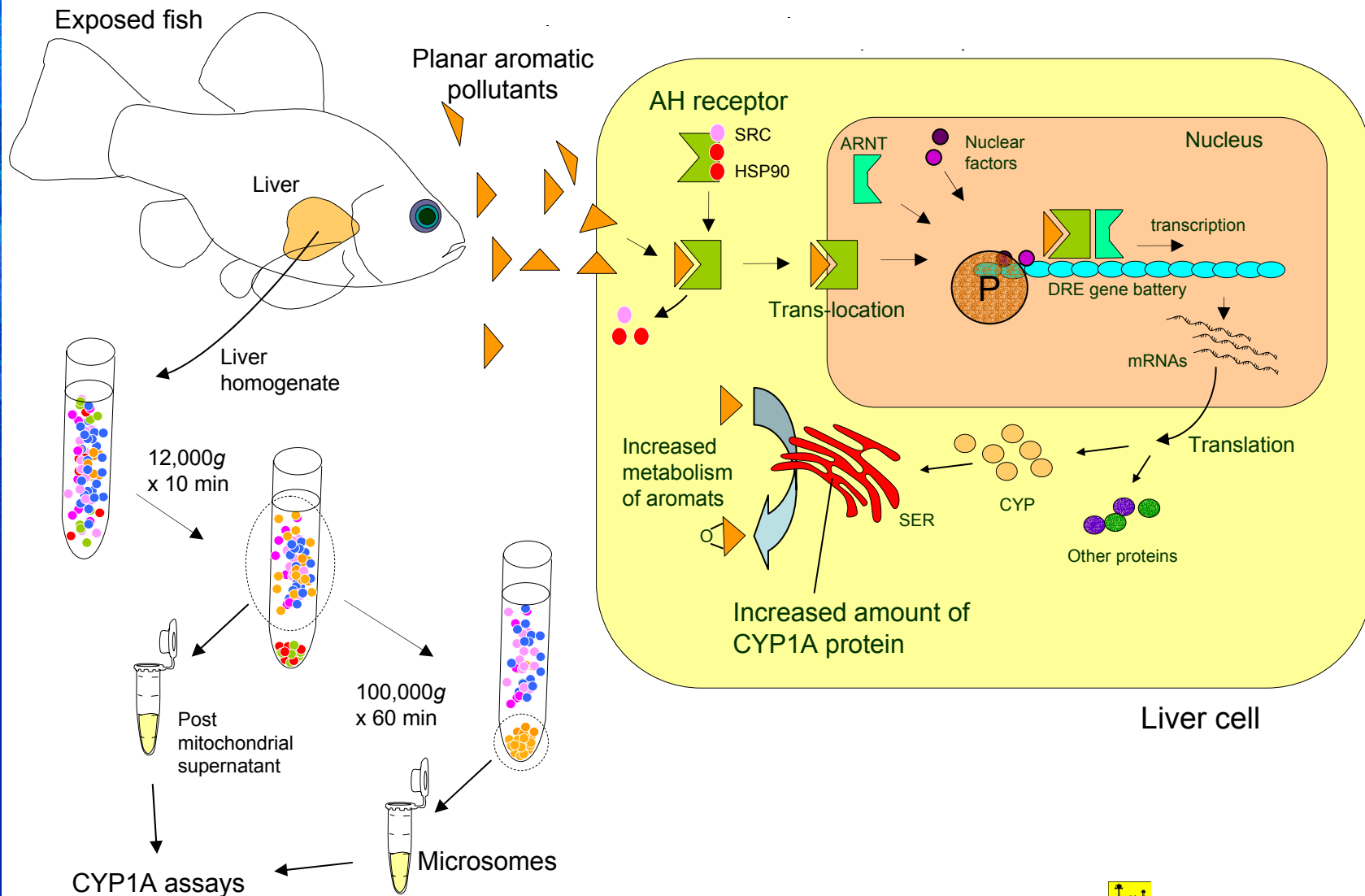
# Alkylphenol biomonitoring – method development



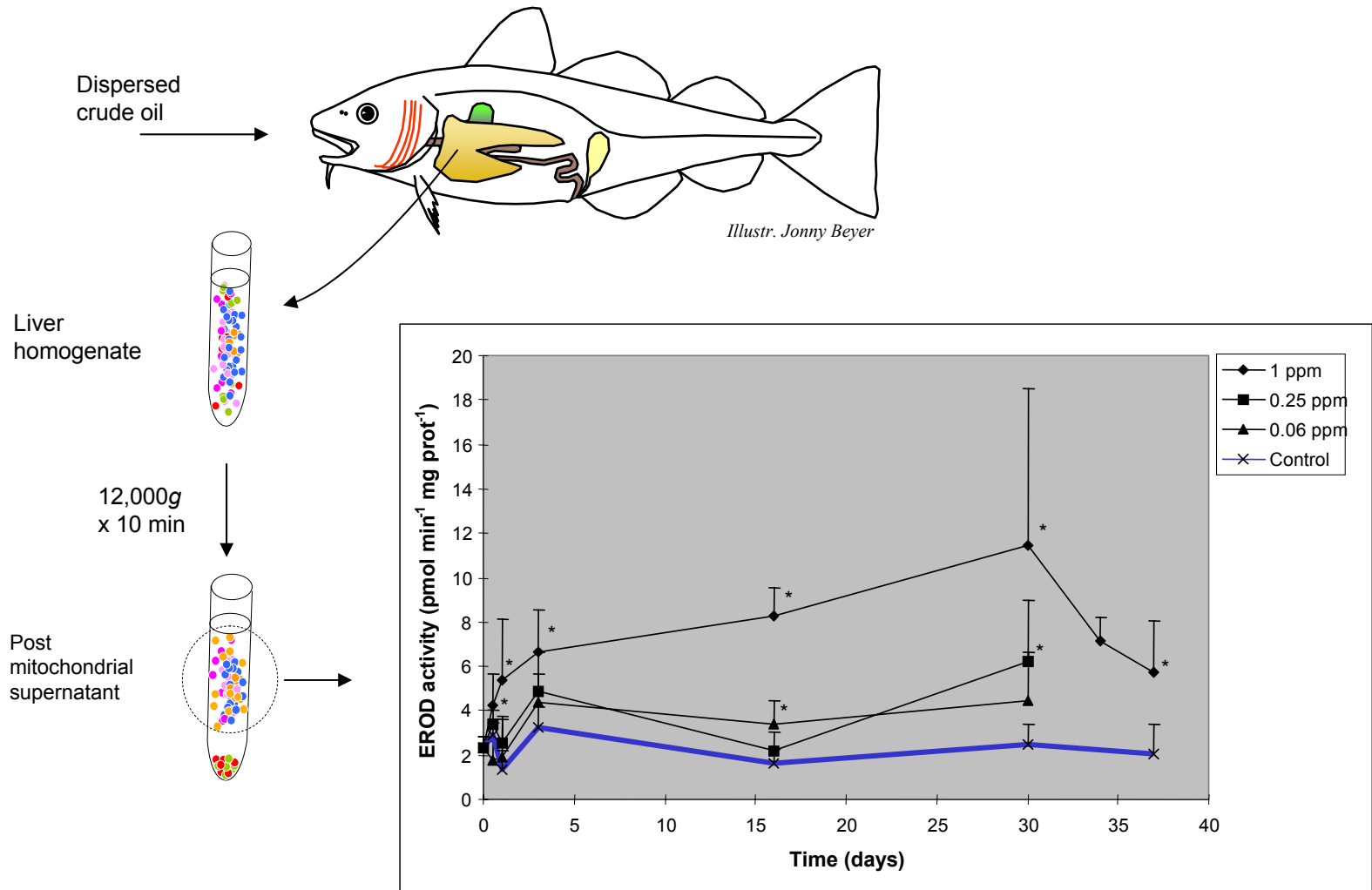
# A pollutant effect is a hierarchical row of events



# CYP1A1 induction - a biomarker for exposure to “dioxin-like” pollutant chemicals



# Dispersed crude oil induce EROD in cod liver



Data courtesy of Endre Aas (RF-Akvamiljø) & TOTAL AS



RF-Akvamiljø



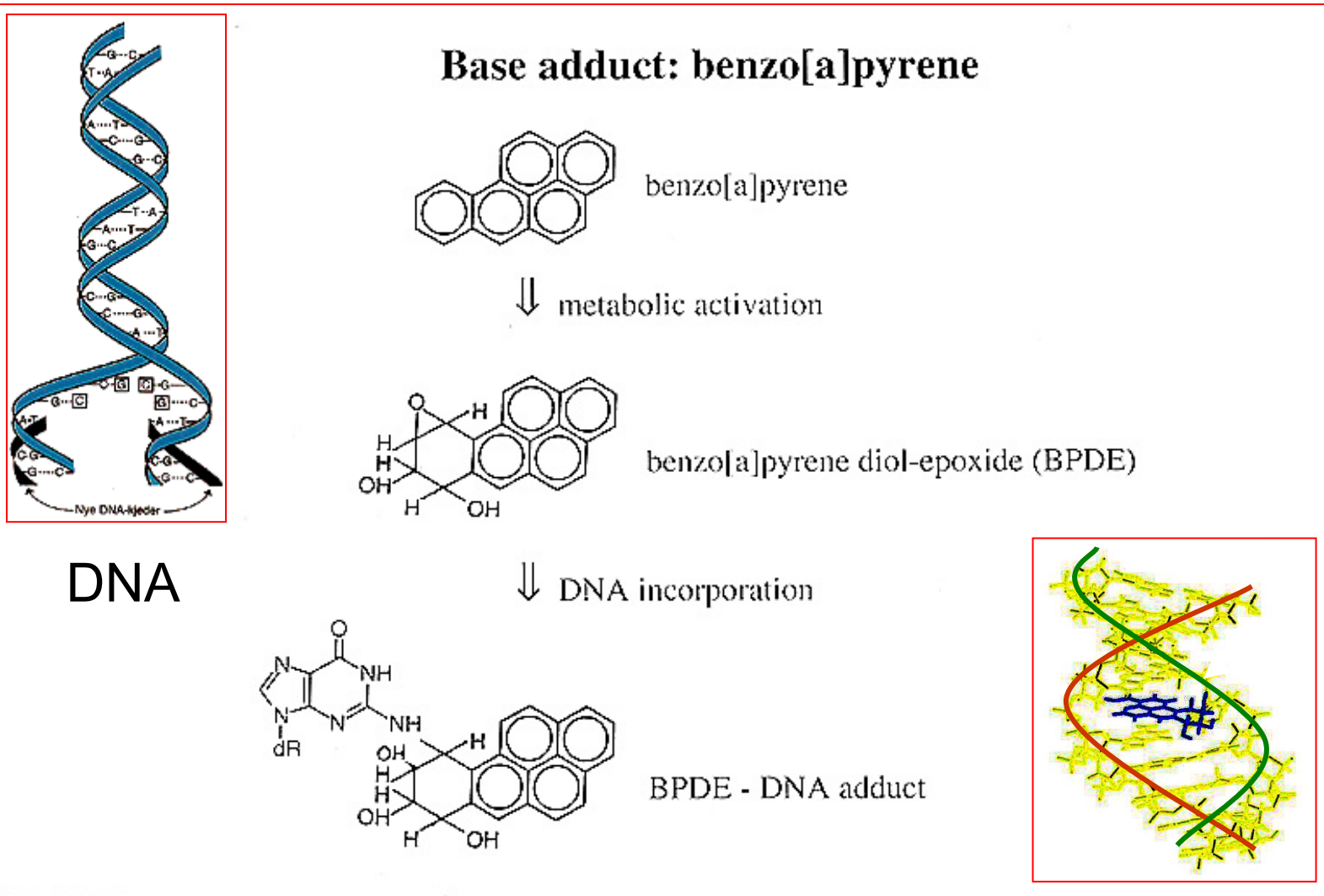


# Biomarkers of genotoxic stress

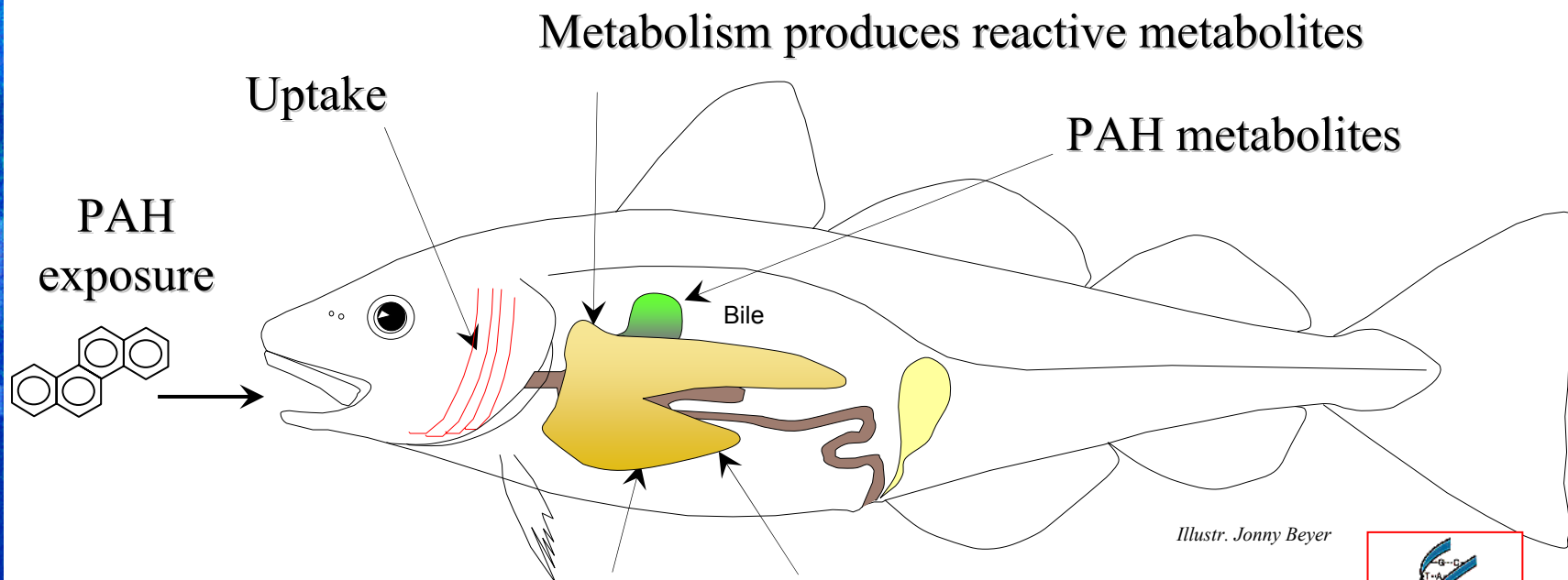
- Covalent pollutant adducts in DNA
- DNA strand breaks (alkaline unwinding and comet assay)
- Micronuclei formation
- DNA repair mechanisms



# DNA adduct formation - a biomarker of potential genotoxic effects of polyaromatic pollutants



# DNA adduct formation - a biomarker of potential genotoxic effects of polyaromatic pollutants

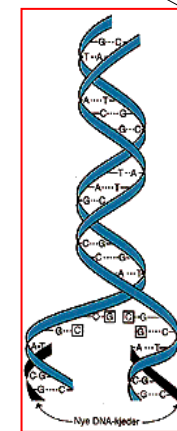
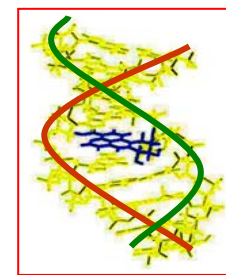
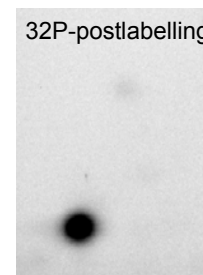
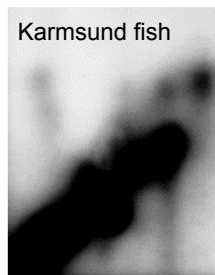


Illustr. Jonny Beyer

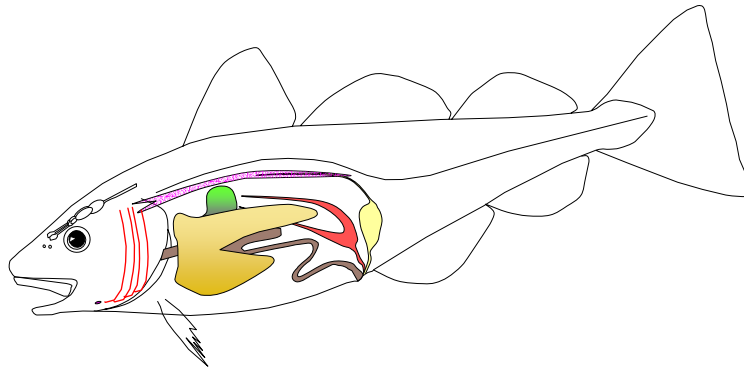
Cellular and tissue lesions



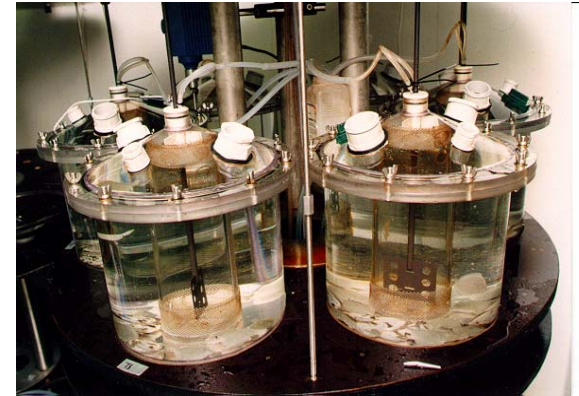
PAH-DNA adduct formation



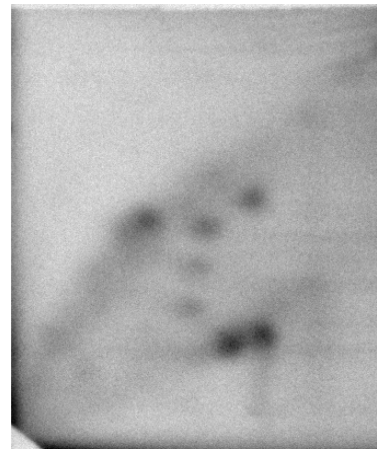
# Hepatic DNA adduct formation occur in fish exposed to dispersed crude oil



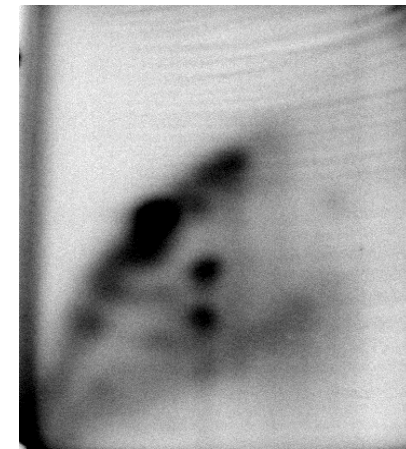
Cod exposed to dispersed crude oil in water for 30 days



Control



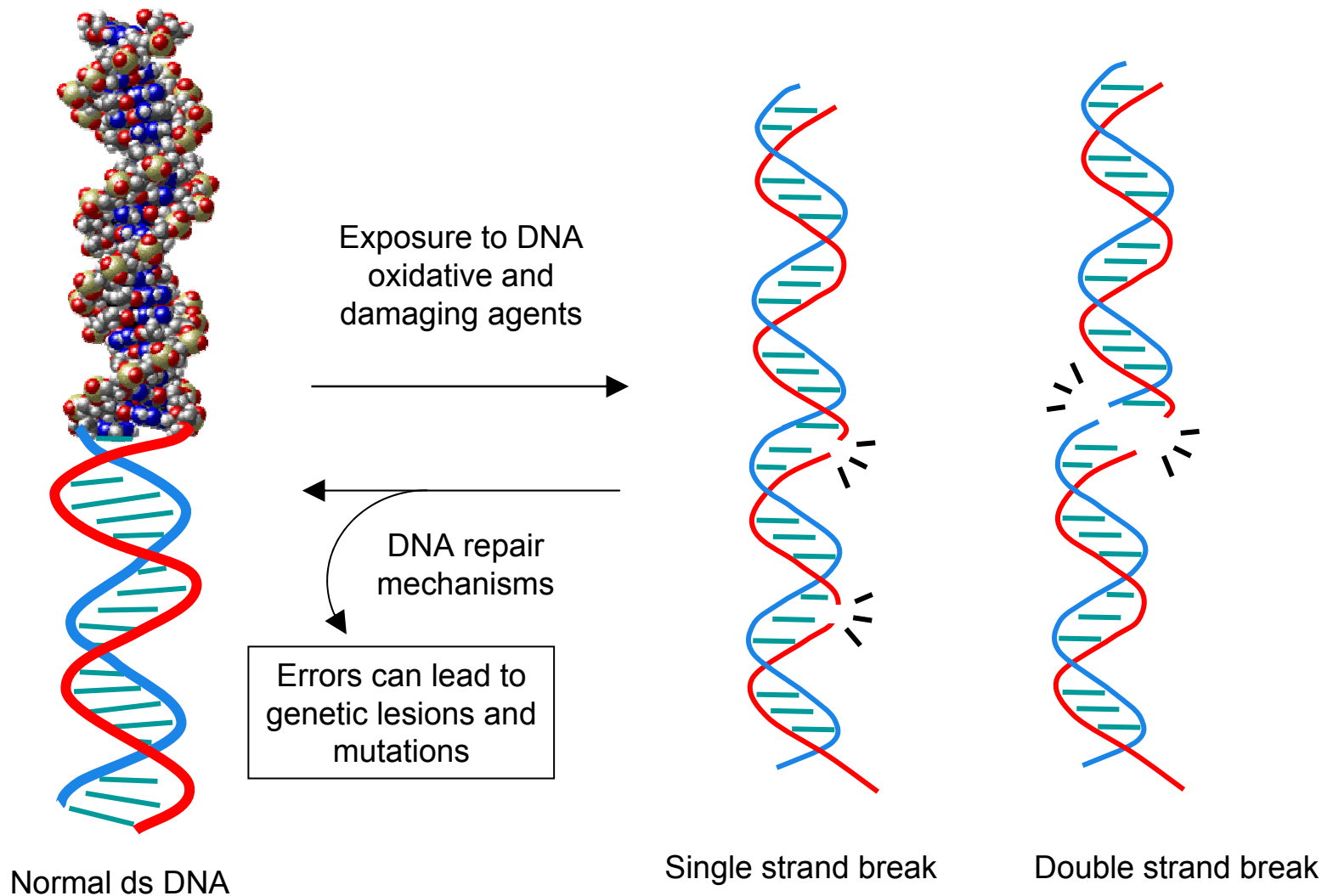
0.04 ppm



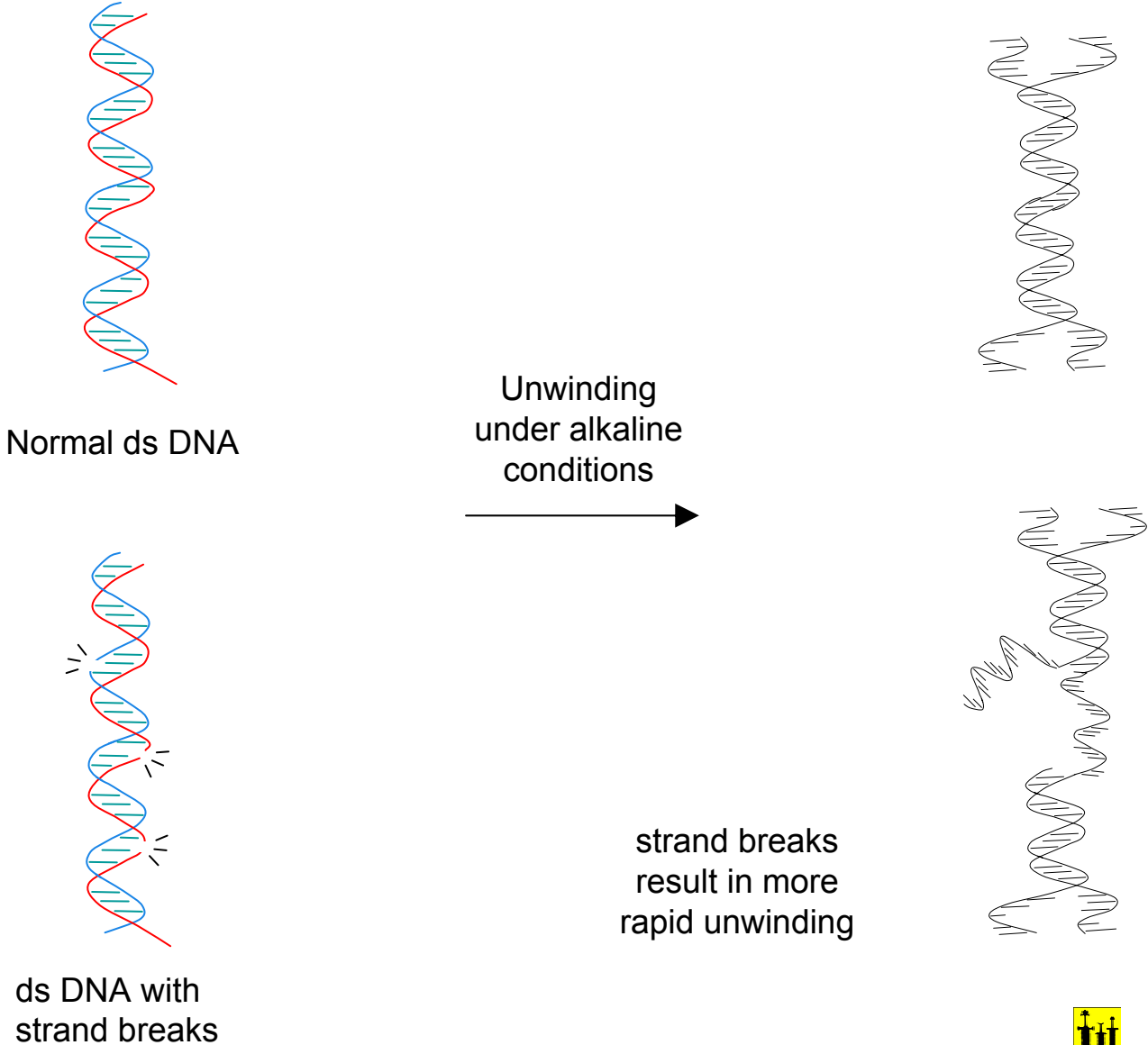
1 ppm



# DNA damage by strand breaks



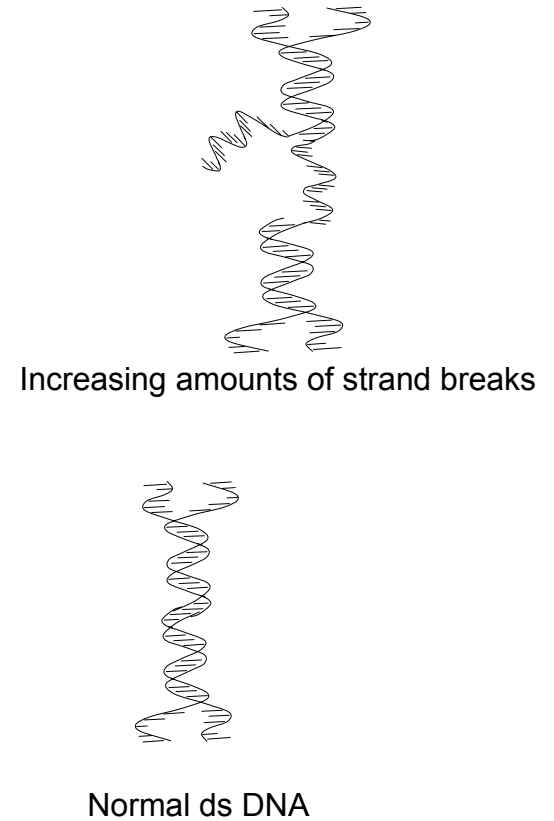
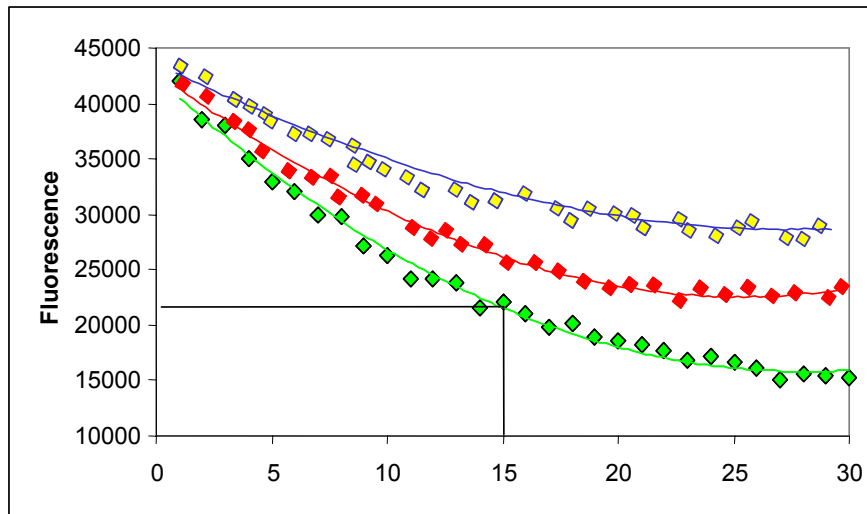
# Alkaline unwinding speed – a biomarker of strand breaks



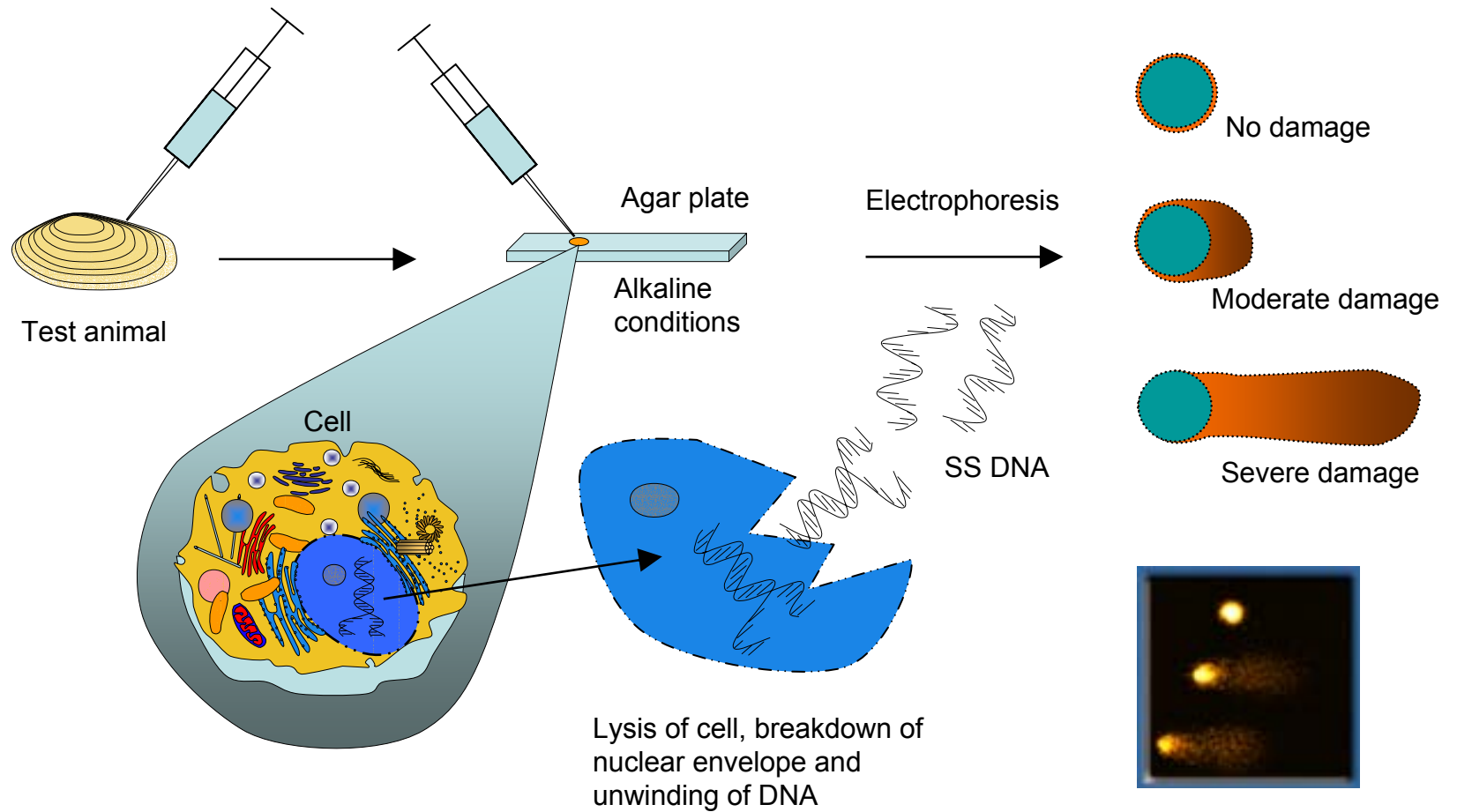


# Alkaline unwinding speed – a biomarker of strand breaks

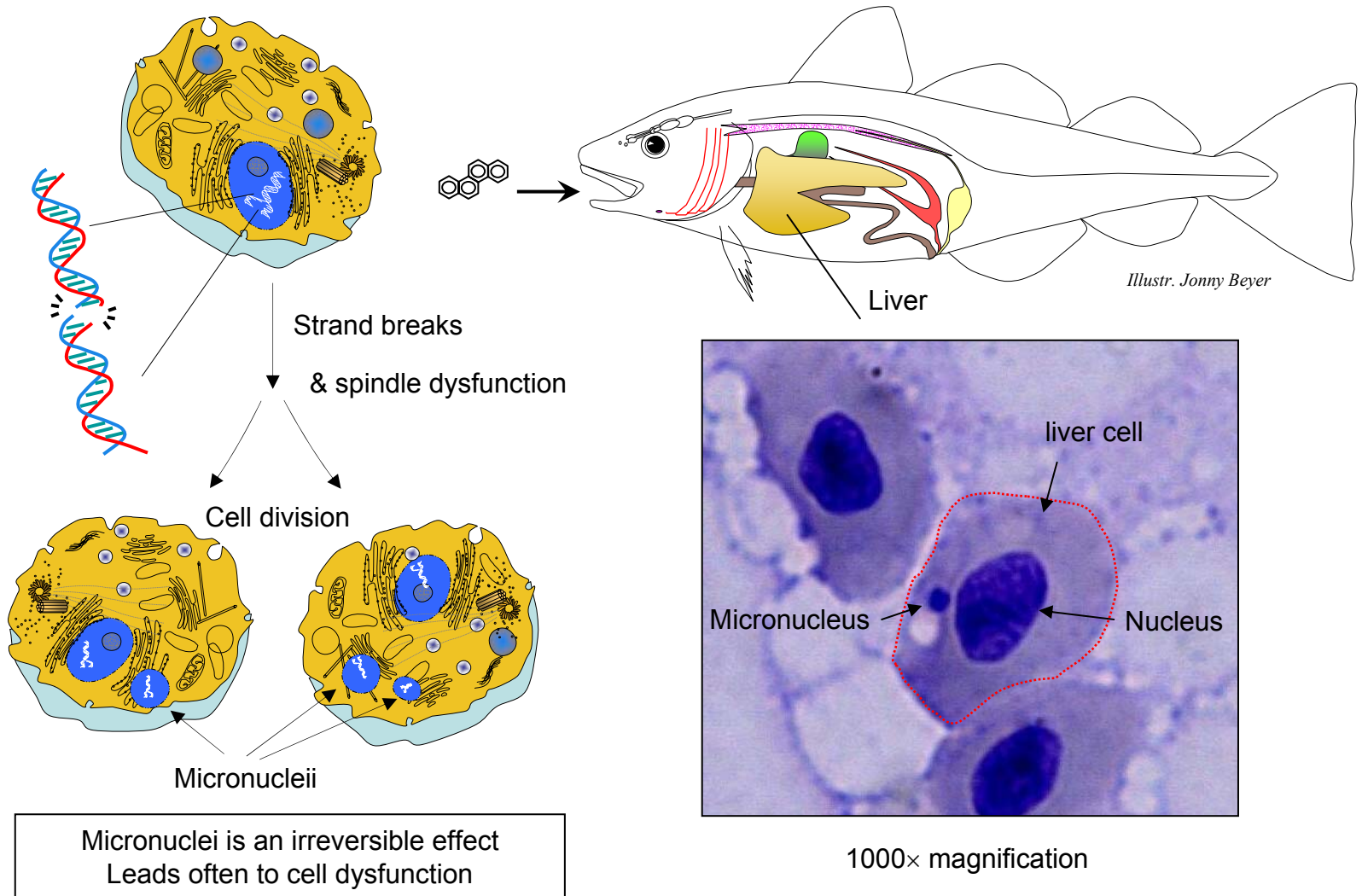
PicoGreen dye assay



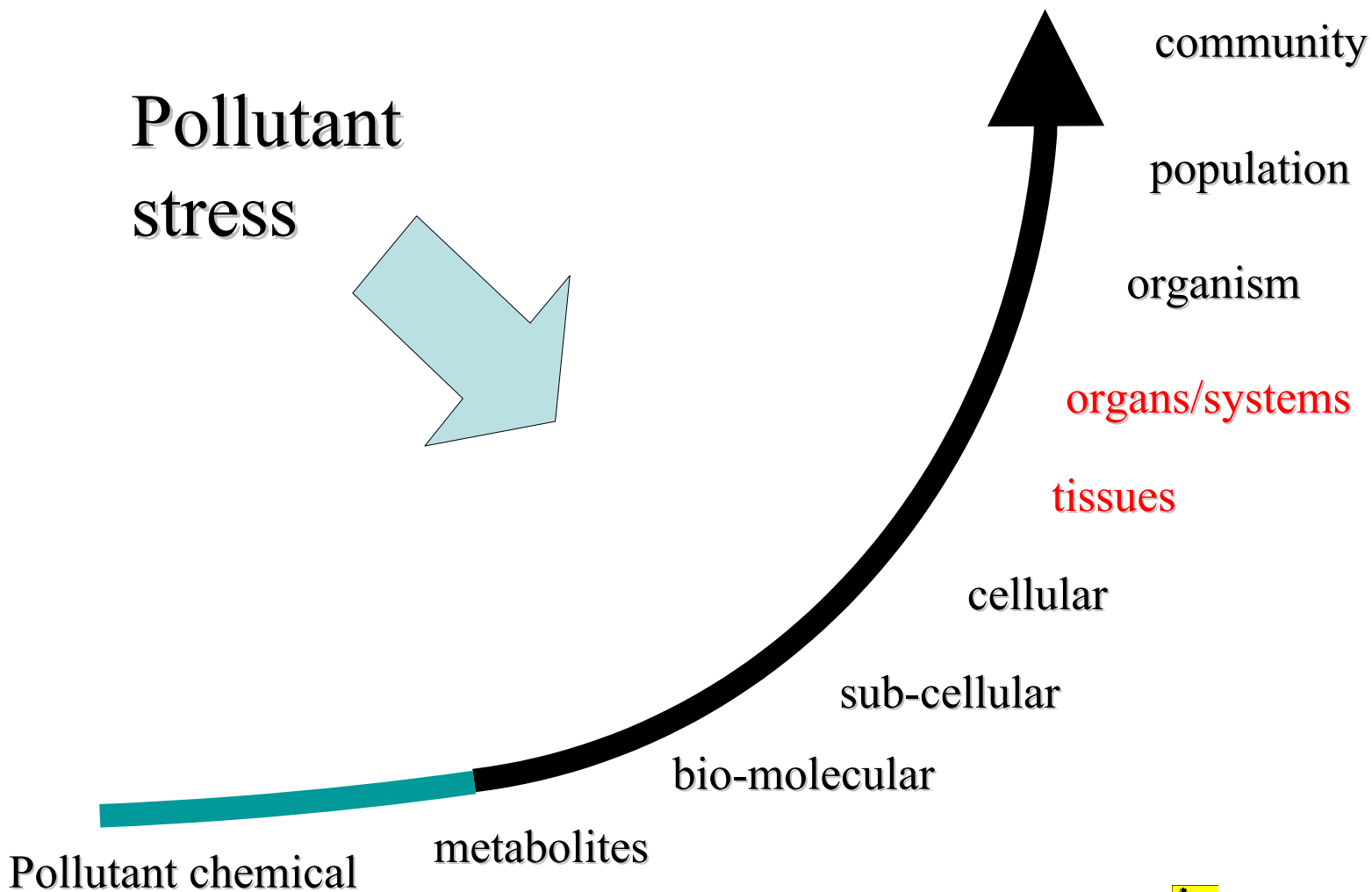
# Strand breaks – single cell comet assay



# Micronuclei formation as genotoxic stress biomarker

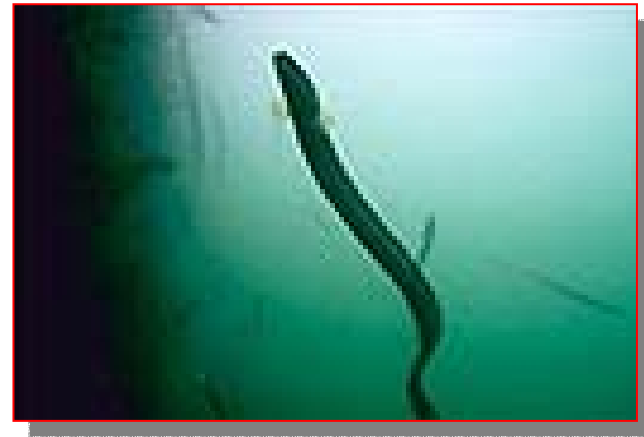


# A pollutant effect is a hierarchical row of events



# Acetylcholine esterase (AChE) inhibition in freshwater fish

A biomarker of toxic action of carbamate and organophosphate insecticides



Eel



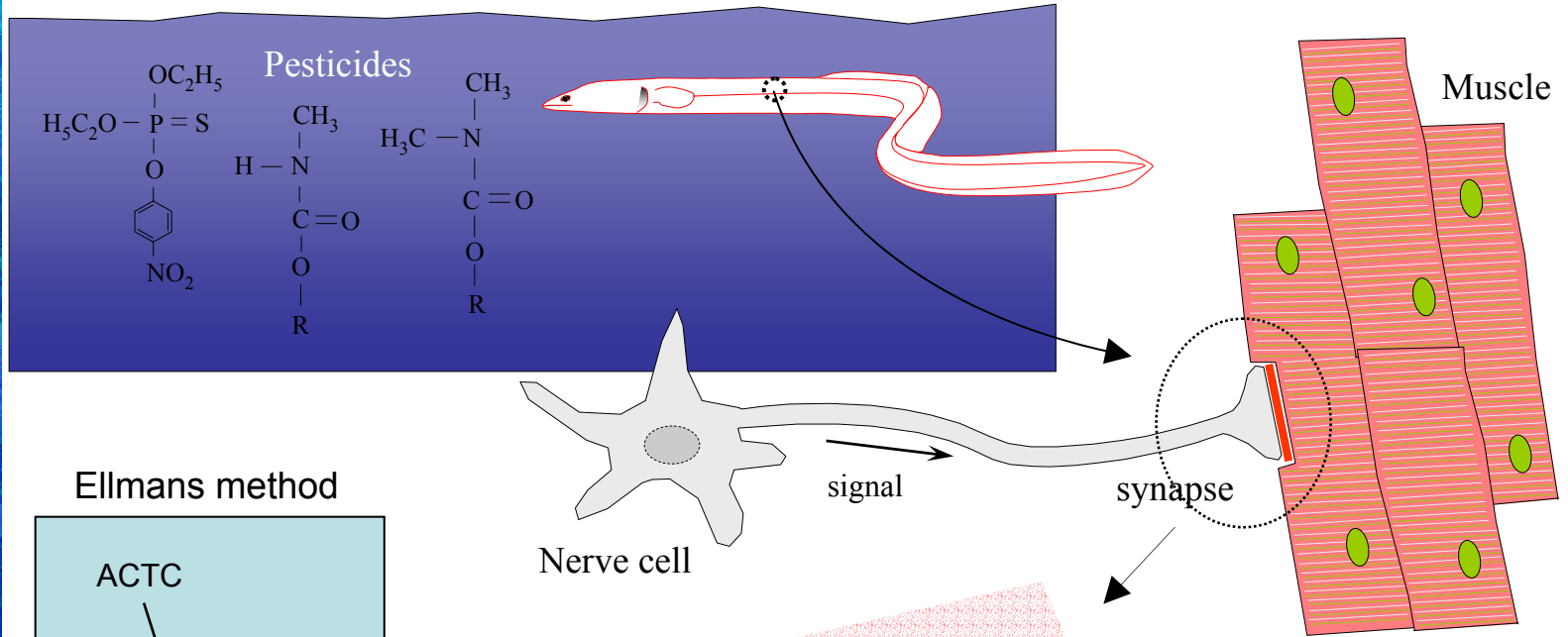
Lamprey



Sticklebacks



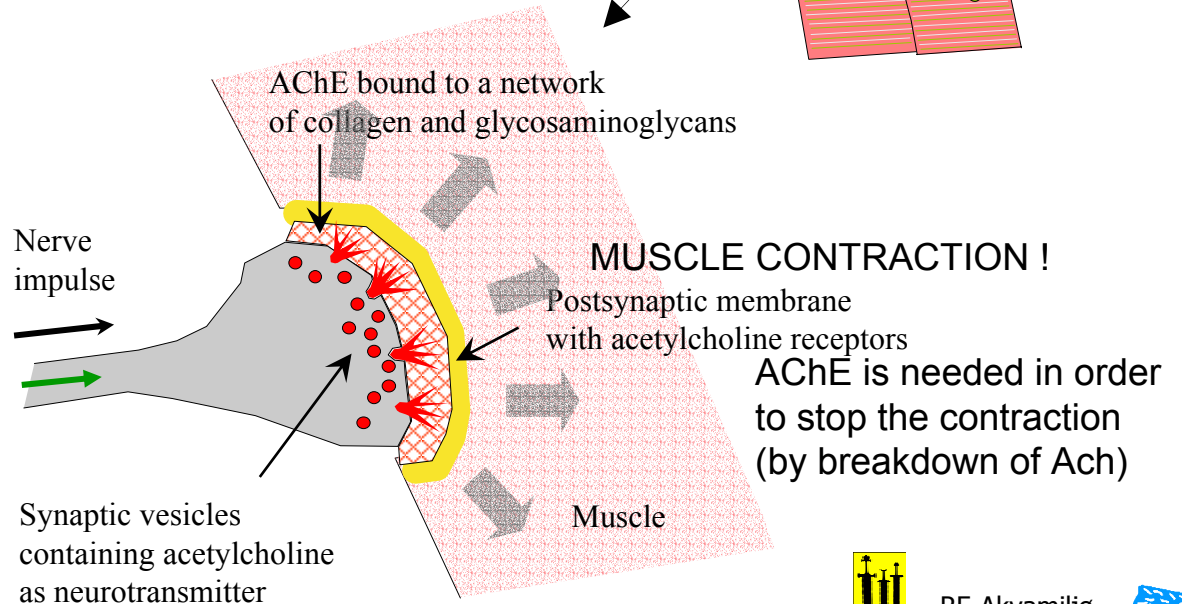
# Pesticides inhibit the function of cholinergic synapses



**Ellmans method**

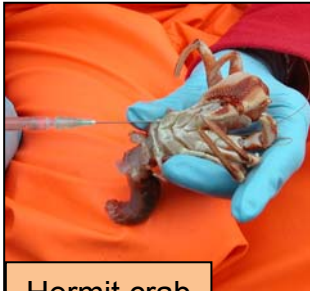
ACTC  
 $\downarrow$  AChE  
 TC + acetic acid

TC + DTNB  
 $\downarrow$  Spontaneous  
 TNB, yellow color  
 abs at 412 nm





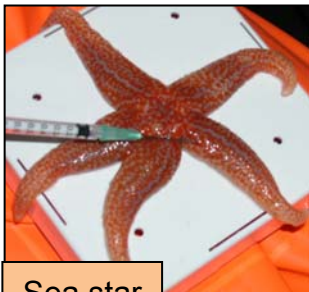
# Lysosomal stability condition in invertebrates



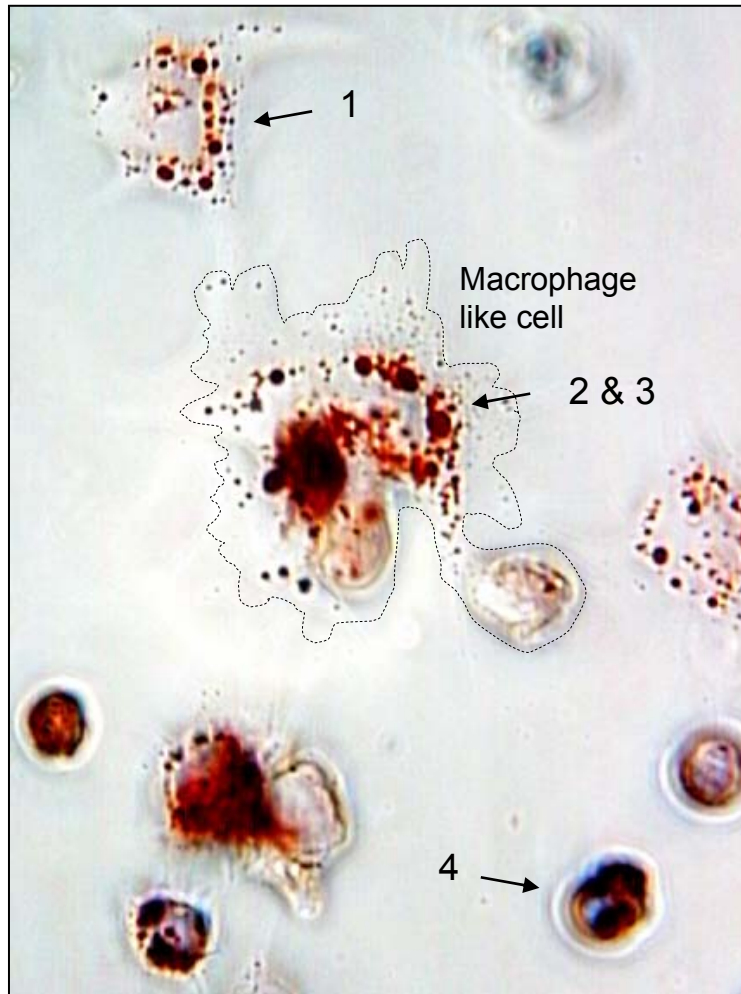
Hermit crab



Whelk

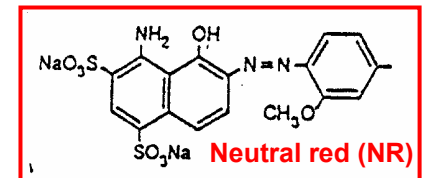


Sea star



Obtain macrophage like cells and attach them to a microscope slide

Add dye (NR) solution to cells



NR is taken up by cells & accumulates in the lysosome organelles (1)

The lysosomes swell up & the lysosome membrane becomes stressed (2)

Lysosomal membrane breaks and NR leaks into cytosol (3)

The cell rounds up (4) & subsequently dies

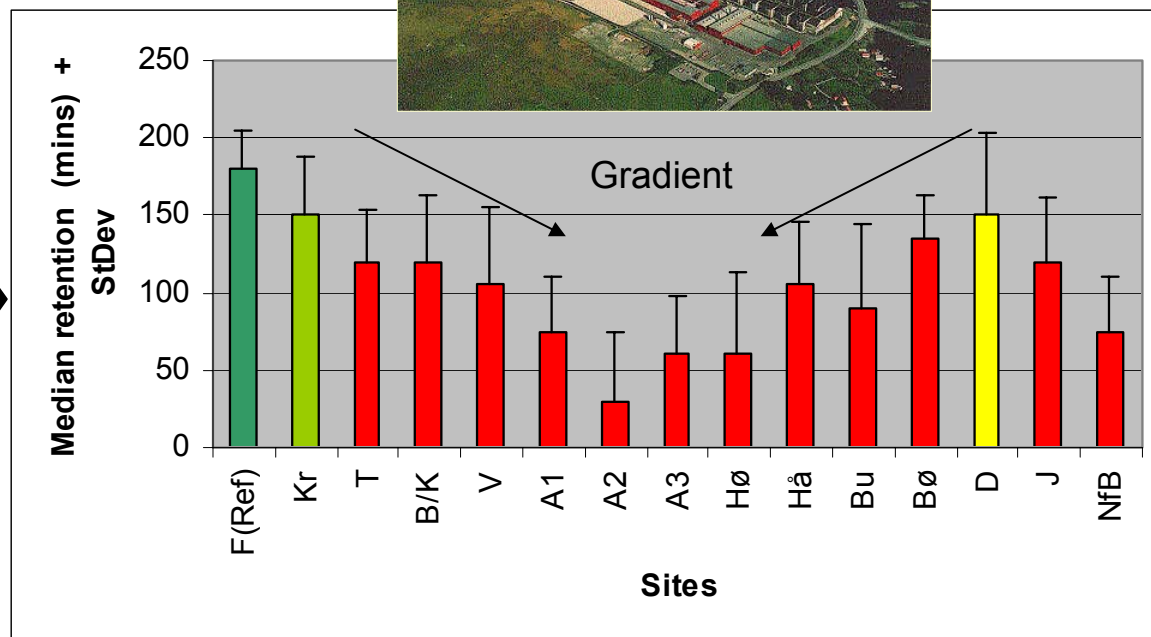
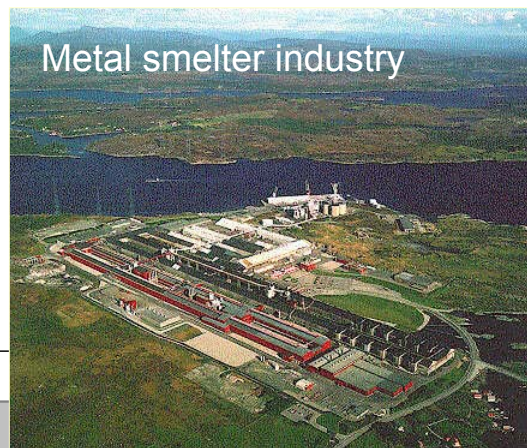
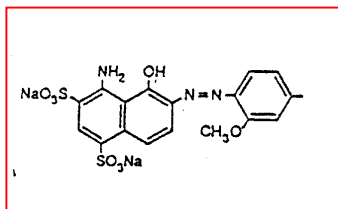
Photo: Anne Bjørnstad



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# Lysosomal stability in blue mussels from smelter recipient



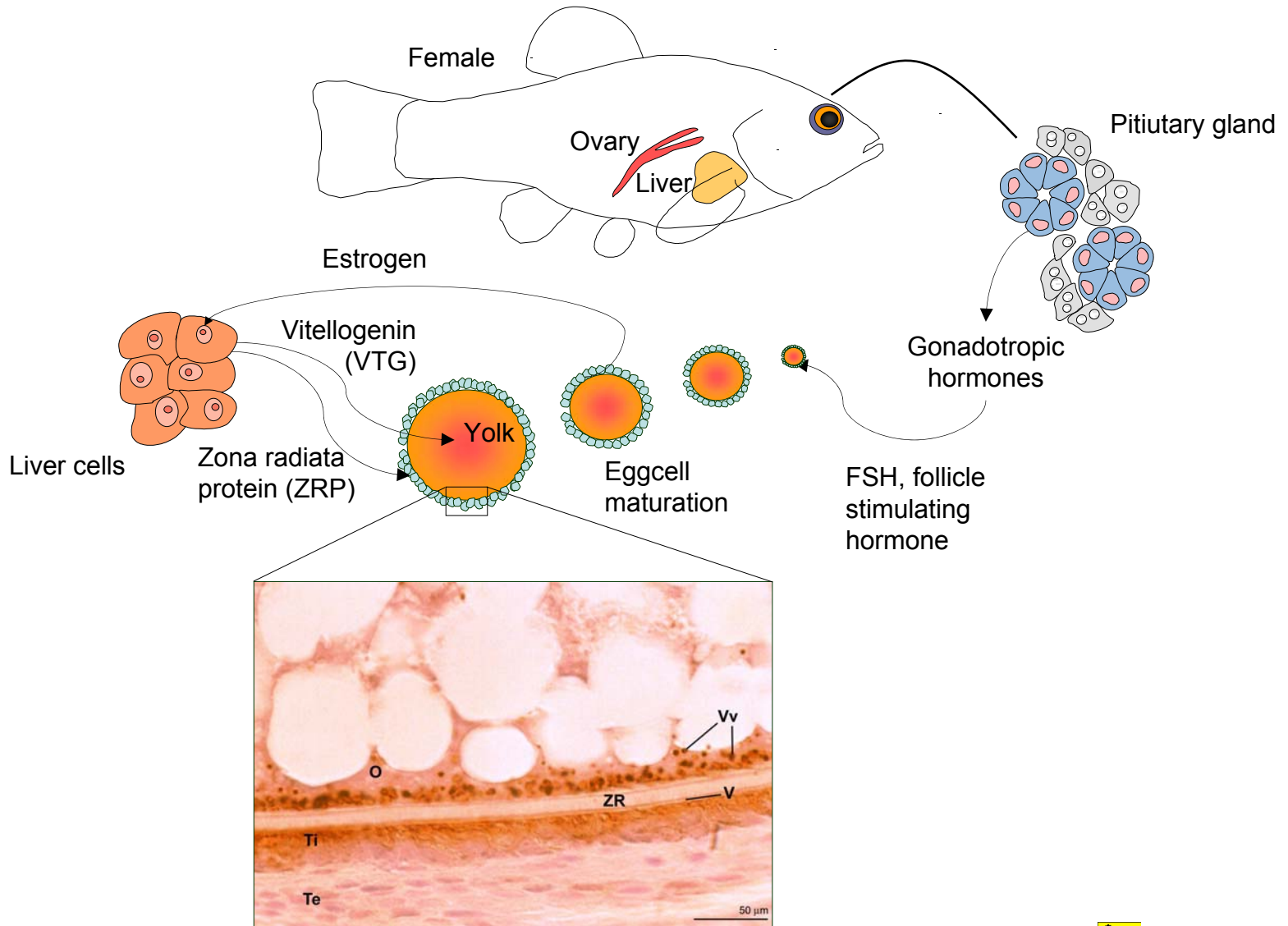
Courtesy: Anne Bjørnstad (RF-Akvamiljø)



RF-Akvamiljø



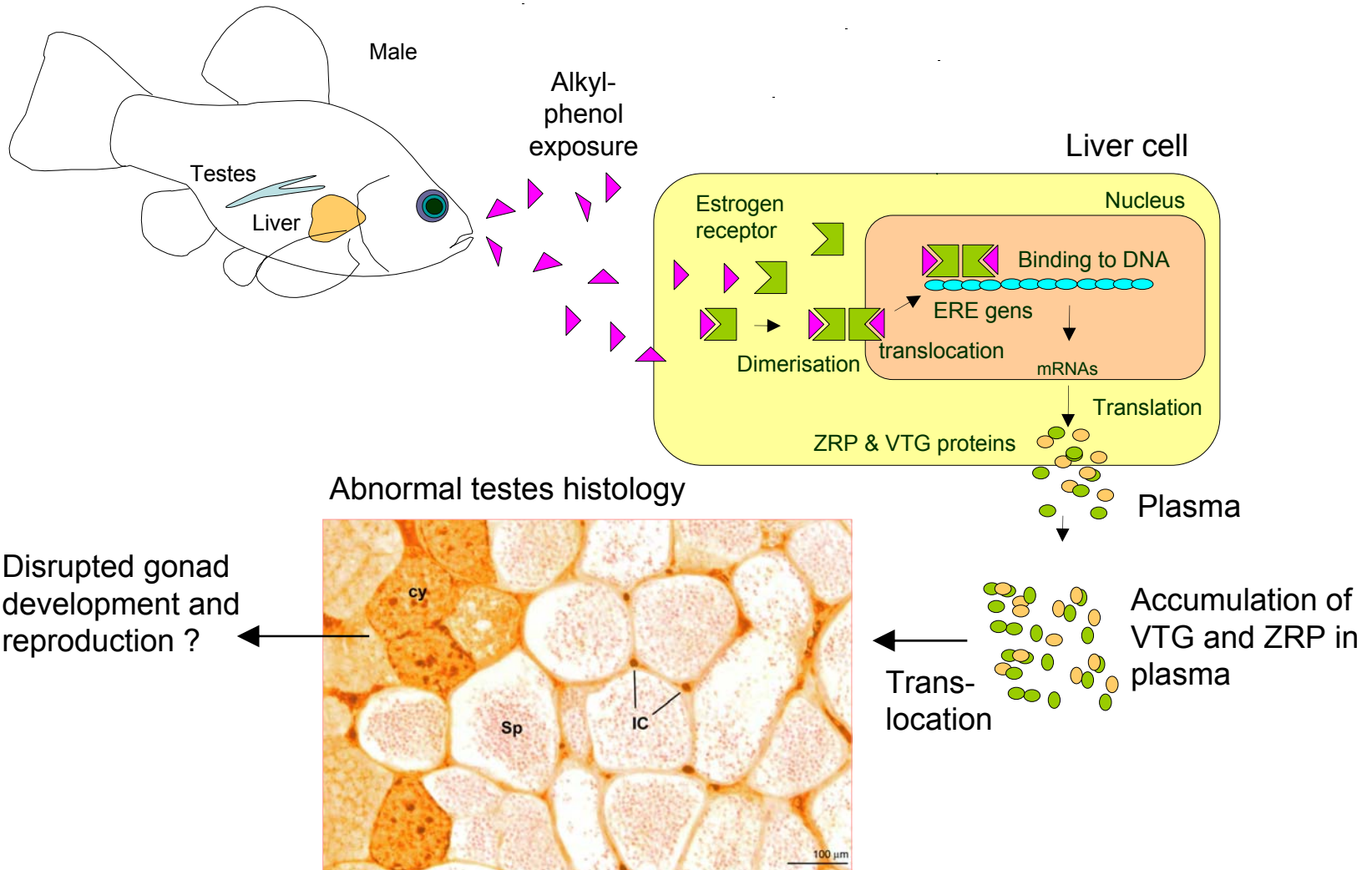
# Regulation of egg development in female fish



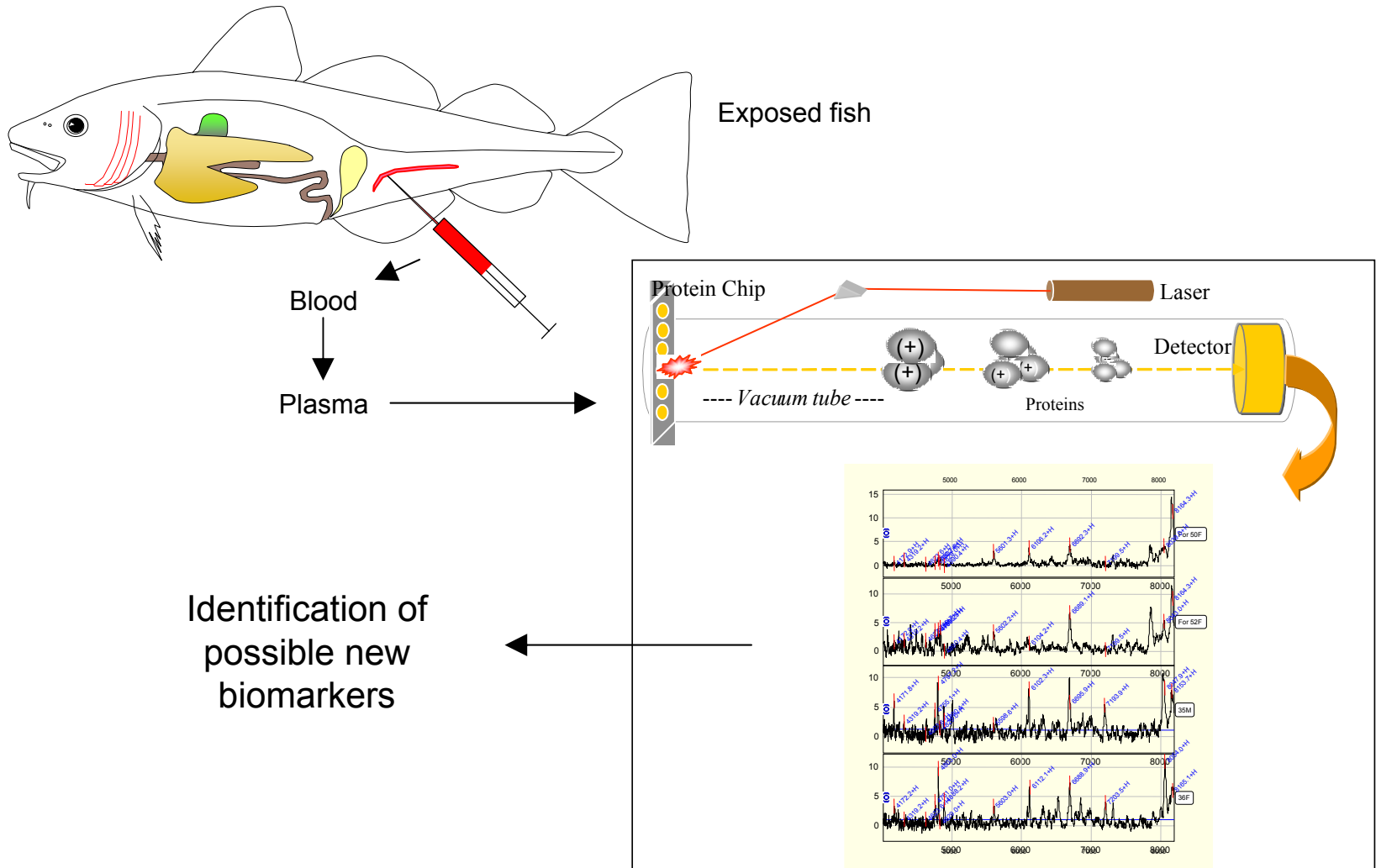


# Xeno-estrogenic effect biomarkers in male fish

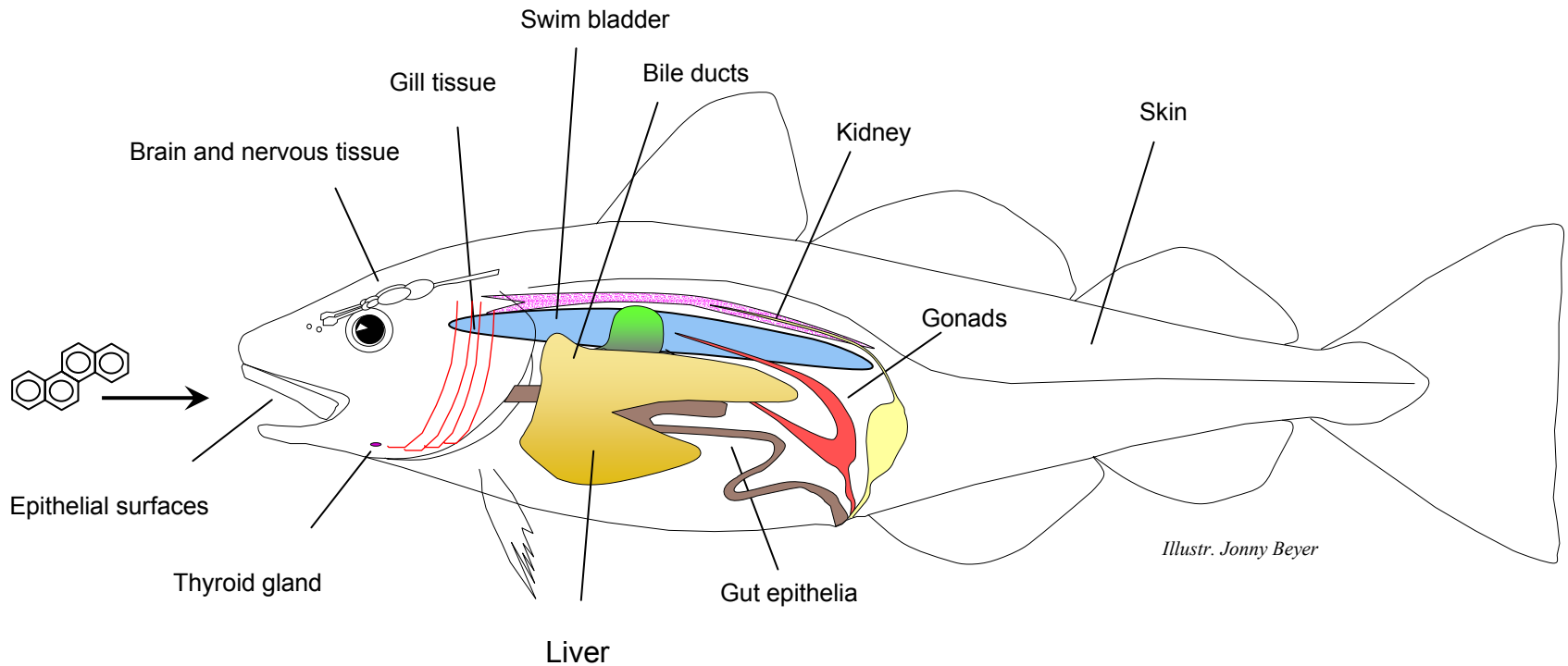
## Vitellogenin and zona radiata proteins in plasma



# Proteome Analysis – a new powerful approach



# Histopathology in fish

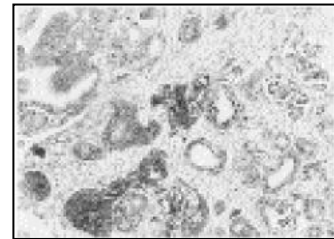




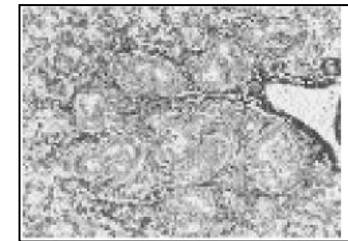
# Neoplasm histopathology in rainbow trout



papillary adenoma in the swim bladder



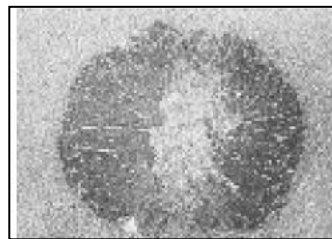
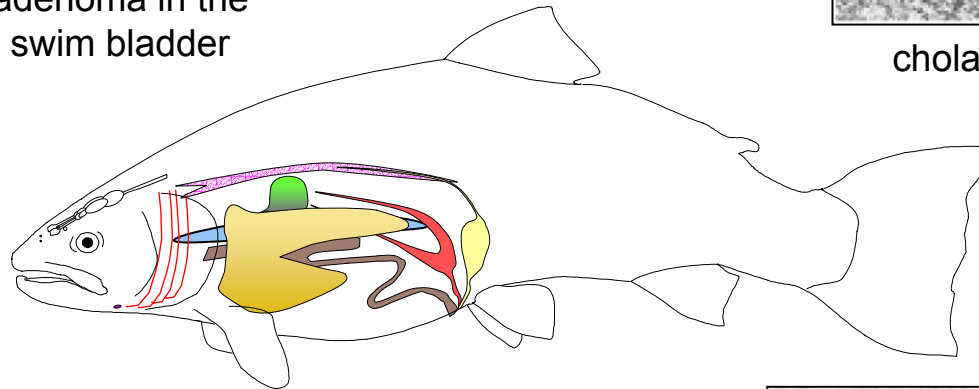
nephroblastoma



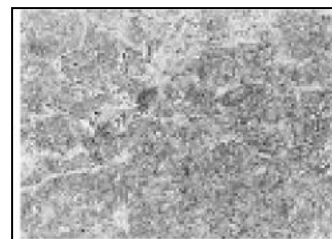
cholangioma



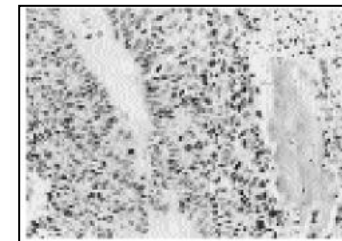
papillary adenoma of the glandular stomach



mixed carcinoma of the liver

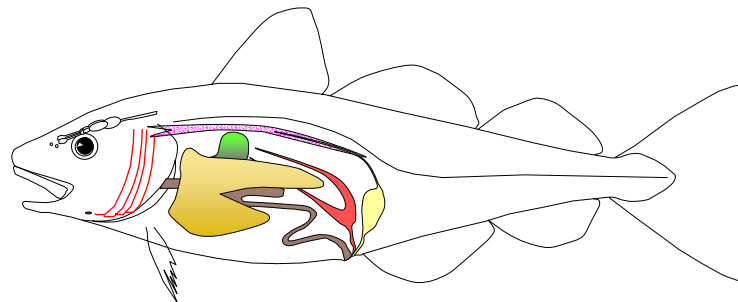
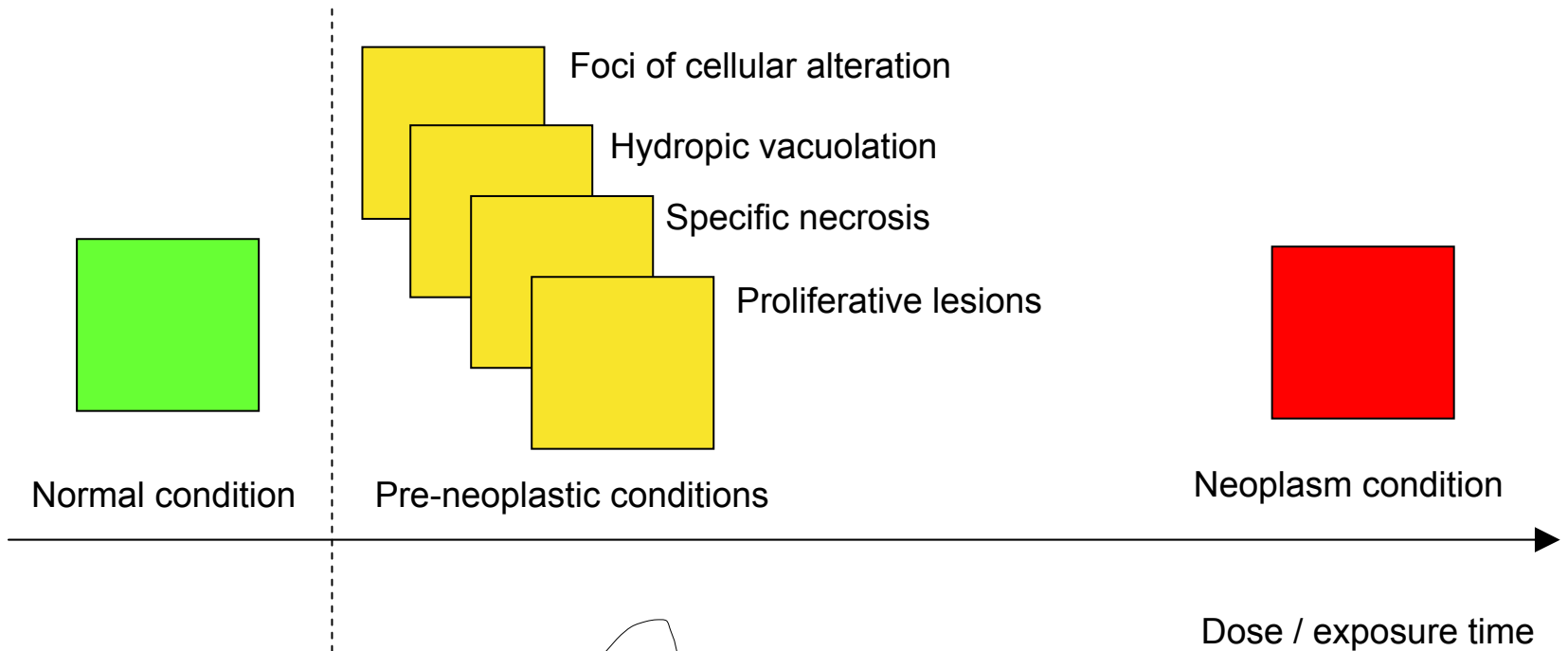


hepatocellular carcinoma

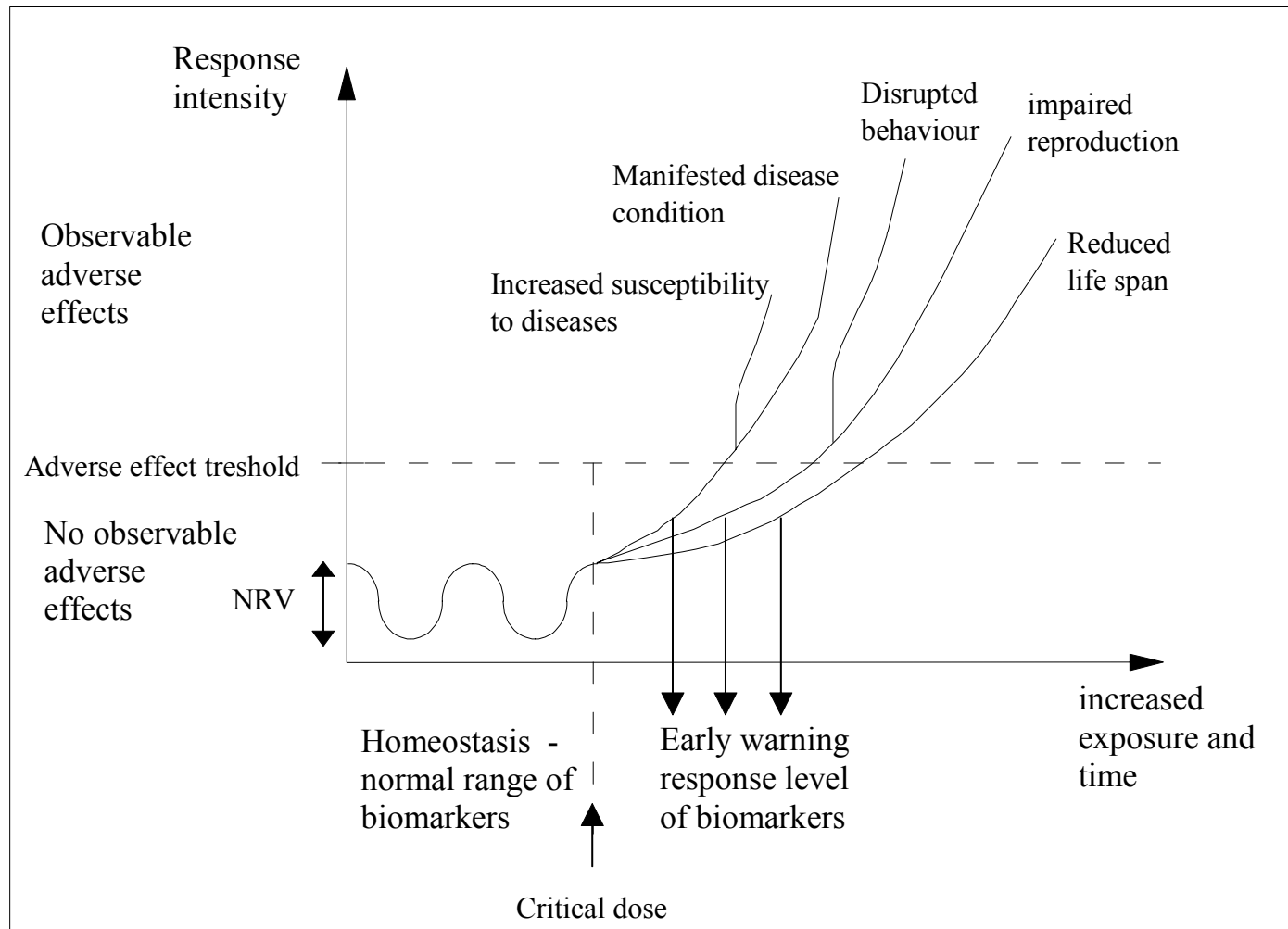


presumptive hepatoblastoma

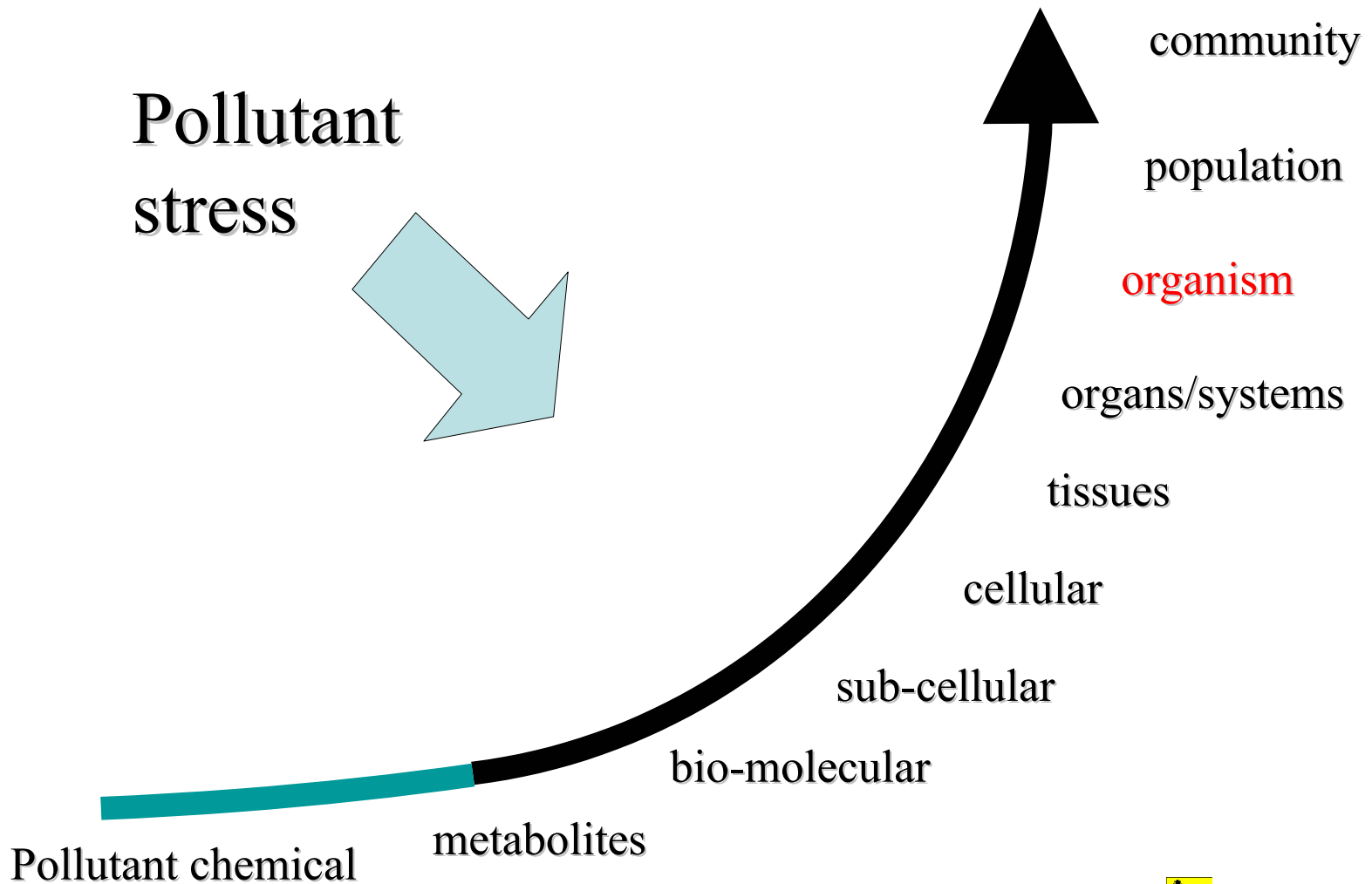
# Preneoplastic alterations vs neoplasms



# Effects vs pollutant dose and exposure time



# A pollutant effect is a hierarchical row of events



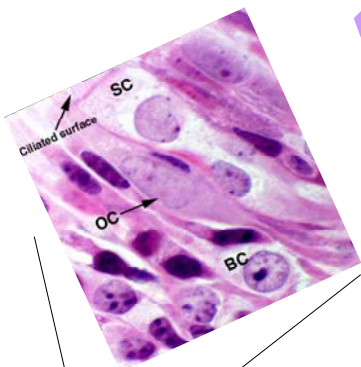
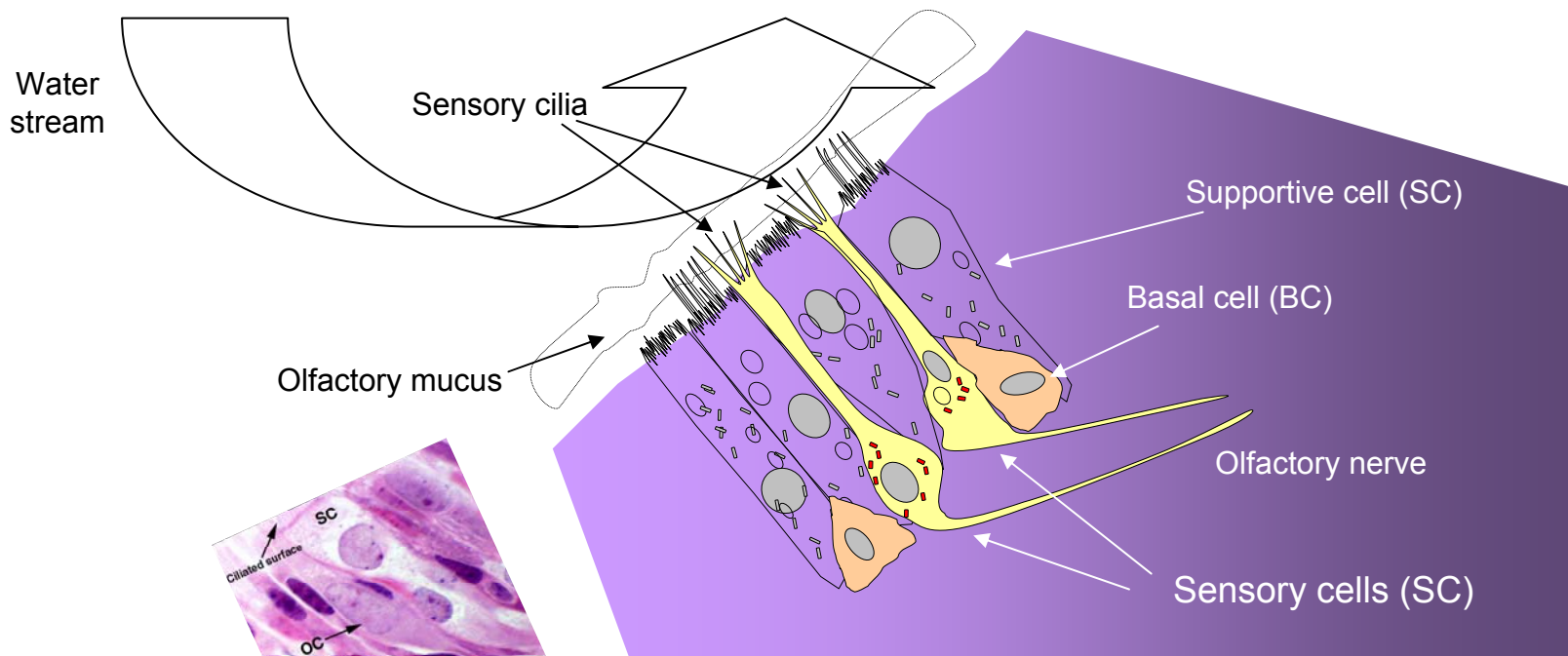


# Animal behavior and chemical signaling

A new area to search for  
biomarker tools?



# Olfaction and chemical signaling



Some pesticides impair homing success in salmon

Olfactory organ

In salmon, olfaction and chemical signals are involved during anti-predator, homing and spawning behaviors.

Ill.: Jonny Beyer





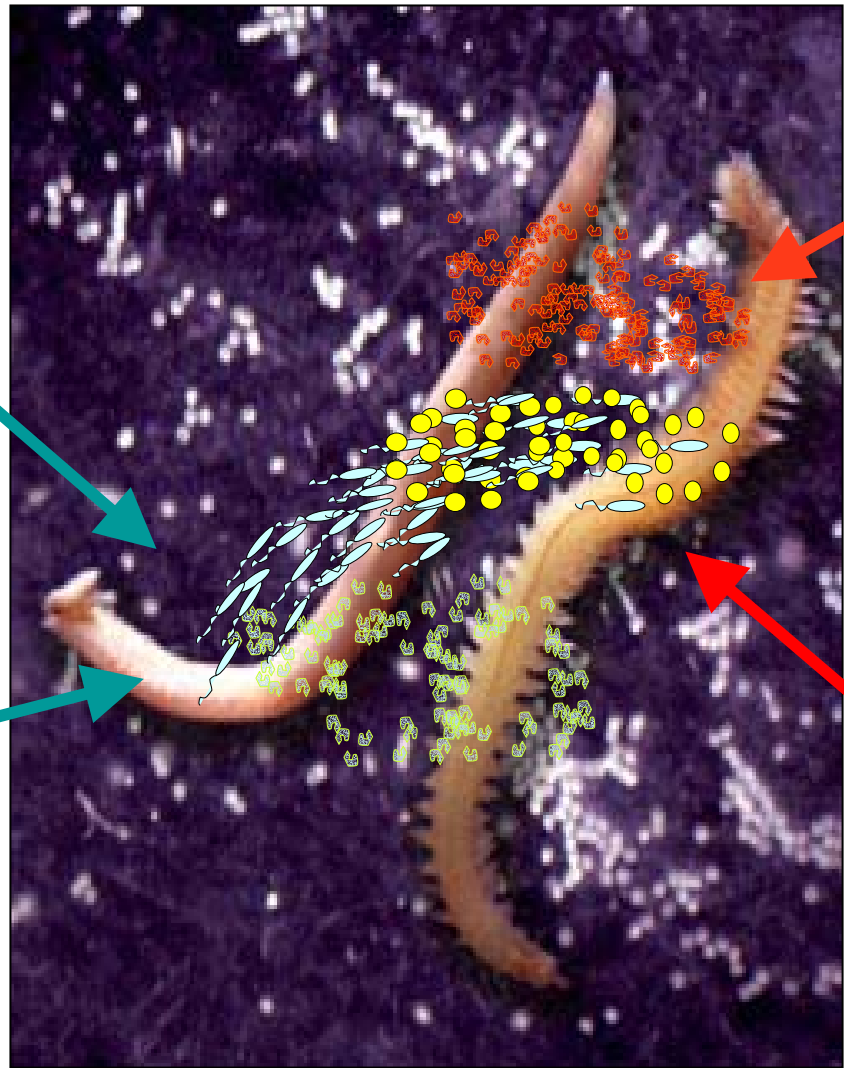
# Pheromone synchrony of invertebrate spawning

Step 1  
Male emit  
some sperm  
and egg-release  
pheromone  
(ERP)

Step 2  
Female emit  
sperm-release  
pheromone  
(SRP) and the  
eggs

Step 3  
Males  
release rest  
of sperm

Step 4  
The sperm  
fertilize the  
eggs and the  
worms dies



# Summary

- Biomarkers have gained much attention as tools for pollutant effect investigations, perhaps in particular in aquatic environments.
- Biomarker tools have been developed towards major pollutant classes and these tools represent an important supplement to chemical methods and community based methods in pollutant effect surveys.
- Biomarkers are needed in order to understand the mechanisms at which the pollutants exert their impact in animals.
- A range of biomarkers are available for detecting the effects of different pollutant chemicals at specific levels of biological organization.
- Some biomarkers are specific towards one pollutant chemical but most biomarkers are responsive towards a group of pollutants.
- Certain biomarker (e.g. PAH bile metabolites) can yield cost effective screening signals of pollutant presence or pollutant effects.
- It is necessary to obtain more information about the fitness-relevance of biomarker signals in order to enhance their descriptive power in pollutant risk and impact studies.

