

Metal toxicity in water

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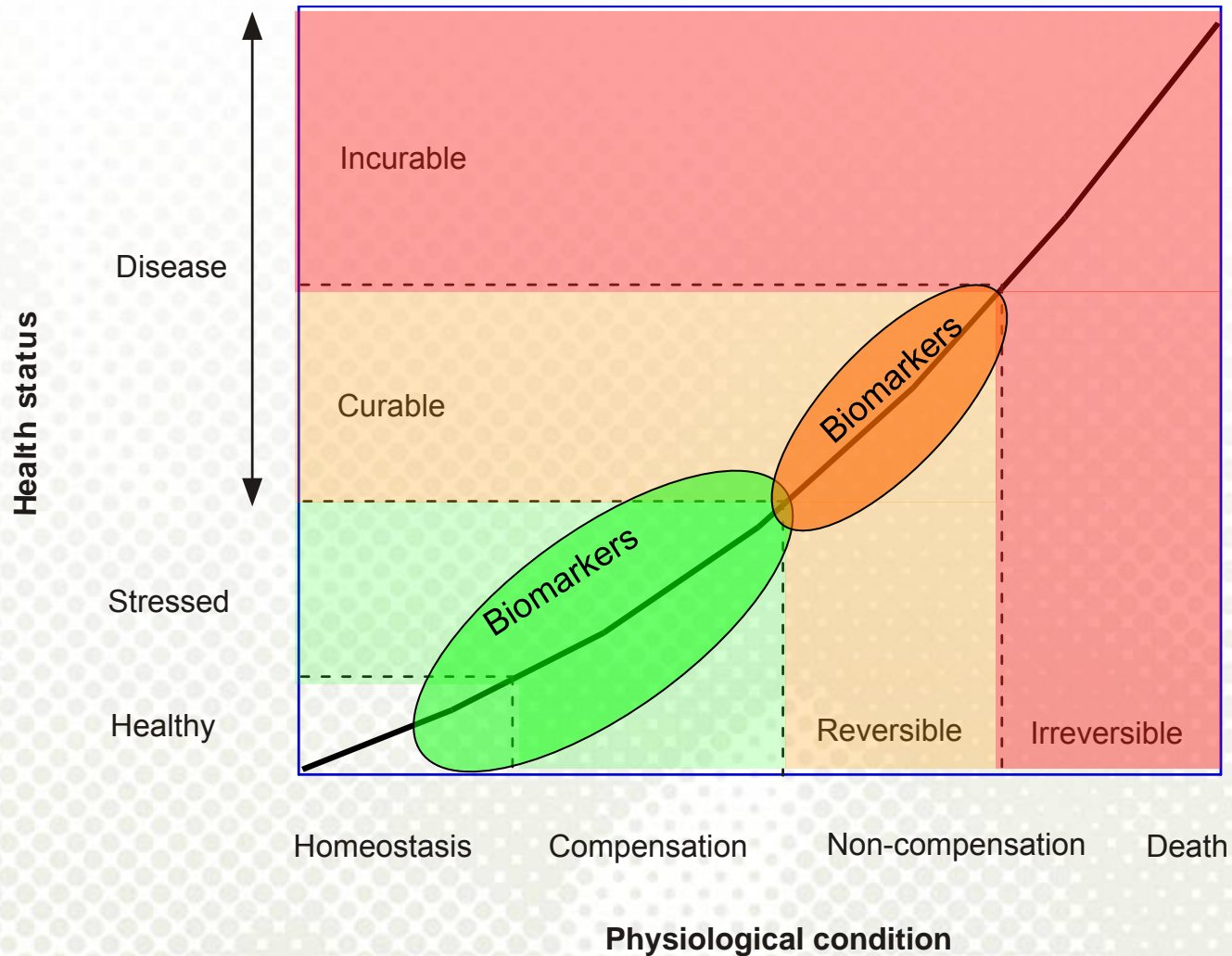
²Norwegian Institute of Water Research (NIVA)



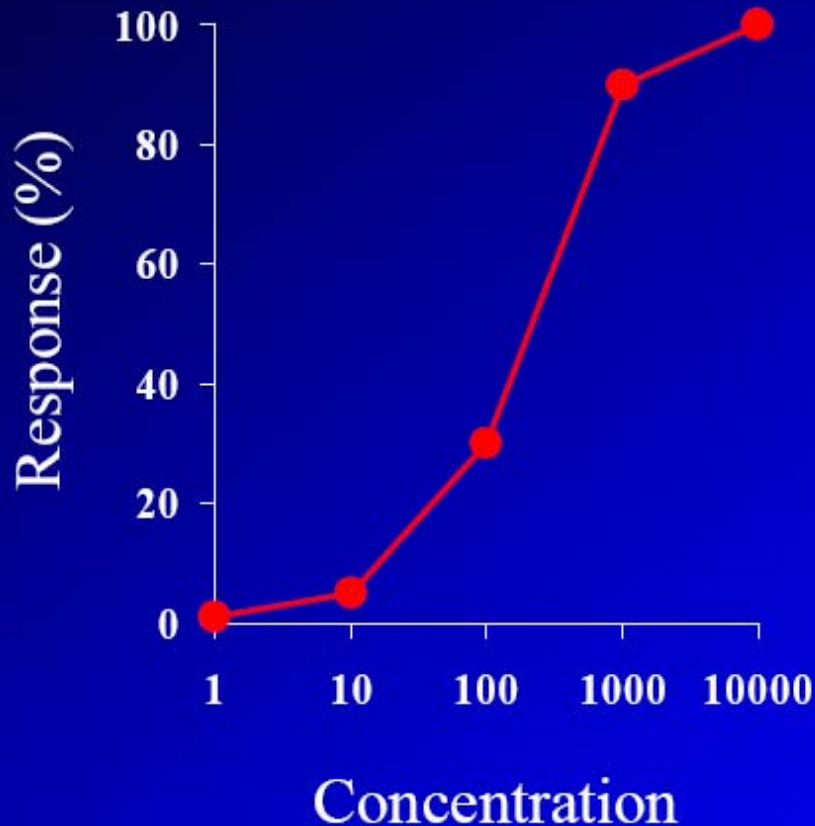
Photo: B.O. Rosseland



Use of biomarkers to study metal toxicity to aquatic organisms



What is a good biomarker?



Criteria

- Sensitive – early warning function
- Feasible & available
- Reliable & reproducible
- Ecologically relevant dose-response
- Specificity – integrative
- Quantitative
- Fully validated

➔ Response related to adverse effect in the organism

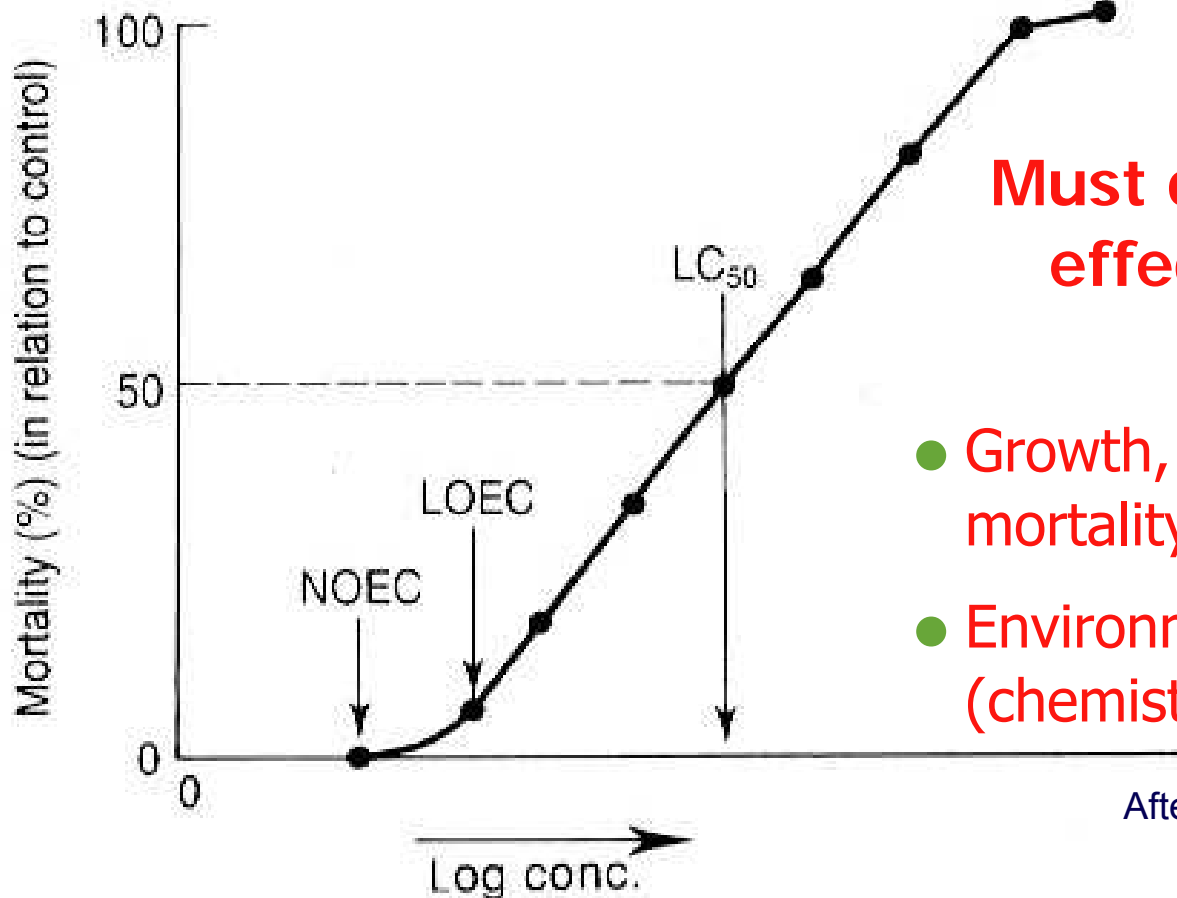
How do we express toxicity?

NOEC - No Effect Concentration

LOEC - Low Effect Concentration

LC50 – Lethal Concentration, 50% mortality

LT50, LD50



Must define type of effect for NOEC & LOEC:

- Growth, behaviour, mortality etc.
- Environmental factors (chemistry, temp. etc.)

After: Walker et al. 2001

How do we test? Single- and Multiple stressors Dose - response



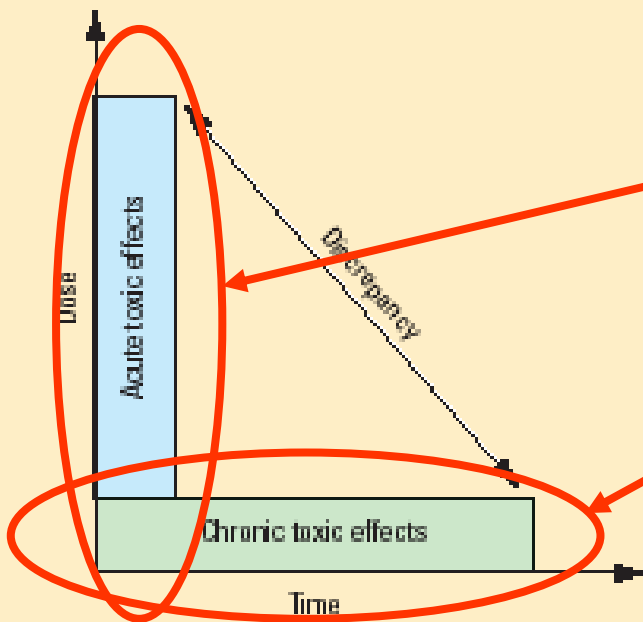
FIGURE 2

Relationship between dose and exposure time

Current ecotoxicological effect assessment tools require waiting for long time periods until visible and significant reactions in ecosystems occur. High dose and short exposure times are the most common ecotoxicological tests in practice, whereas low dose and long exposure times would be the more realistic exposure scenario.

Challenge 4: Multiple stressor effects

We believe the best way to address this issue is to use a systematic approach: First, we must understand the mechanisms for the effects of each stressor alone, and then we can increase the complexity by combining stressors.



The “normal way” of testing a single substance: High dose-short time (24-, 48- or 96-HrLT50)

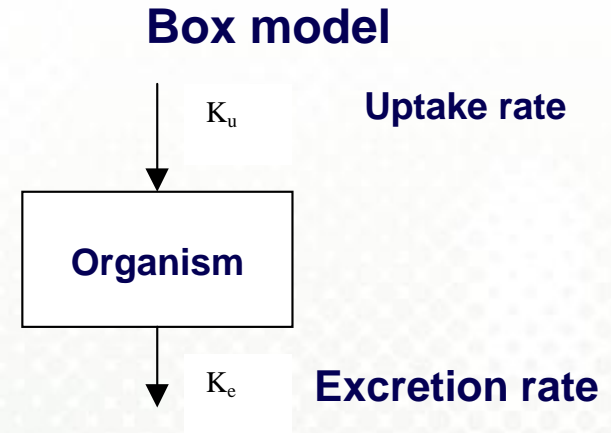
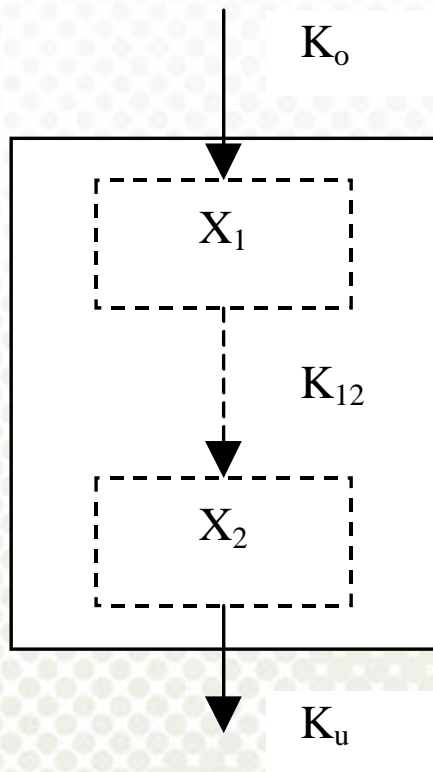
The “best way” of testing a single substance: Low dose-long time



Bio-concentration and bio-accumulation

- **Bio Concentration Factor BCF:**

$$\text{BCF} = \frac{\text{Concentration in organism } (X_{\text{org}})}{\text{Concentration in water } (X_w)}$$



Steady state:

$$C_{\text{max}} = K_{\text{uptake}} / K_{\text{excretion}}$$

Box model with Semi Markov process

Do we know when we sample in nature that we have a "steady state"?

Metal binding components in organisms

Strategy of inactivation

- **Metallothioneins (MT)** – low molecular compounds - contains cystein
- Three main types of metallothioneins spread among animal and plants (MT I-III)
- Specific binding of metals: Cu, Zn, Cd, Zn/Cd and Hg
 - Gills, liver, kidney, muscle

Other binding sites

- ✦ Binding to mucus
- ✦ Binding to chorion (egg membrane) on fish eggs
- ✦ Binding to calciferous structures (invertebrates)

Heavy metals – effects on fish

General effects:

- Formation of “Free radicals” = “Reactive Oxygen substances” (ROS)
- Reduced enzyme-activity
- Increased deformities (development of eye - Hg sensitive)
- Failure in heart development
- Reduced hatching (reduced embryo activity)
- Reduced yolk sac absorption
- Affect behaviour
- Affect metabolism (reduced growth)
- Critical levels depends on pre-history exposure
- Species and strain differences in sensitivity

Vital organs in fish which can be affected by pollutants in the environment

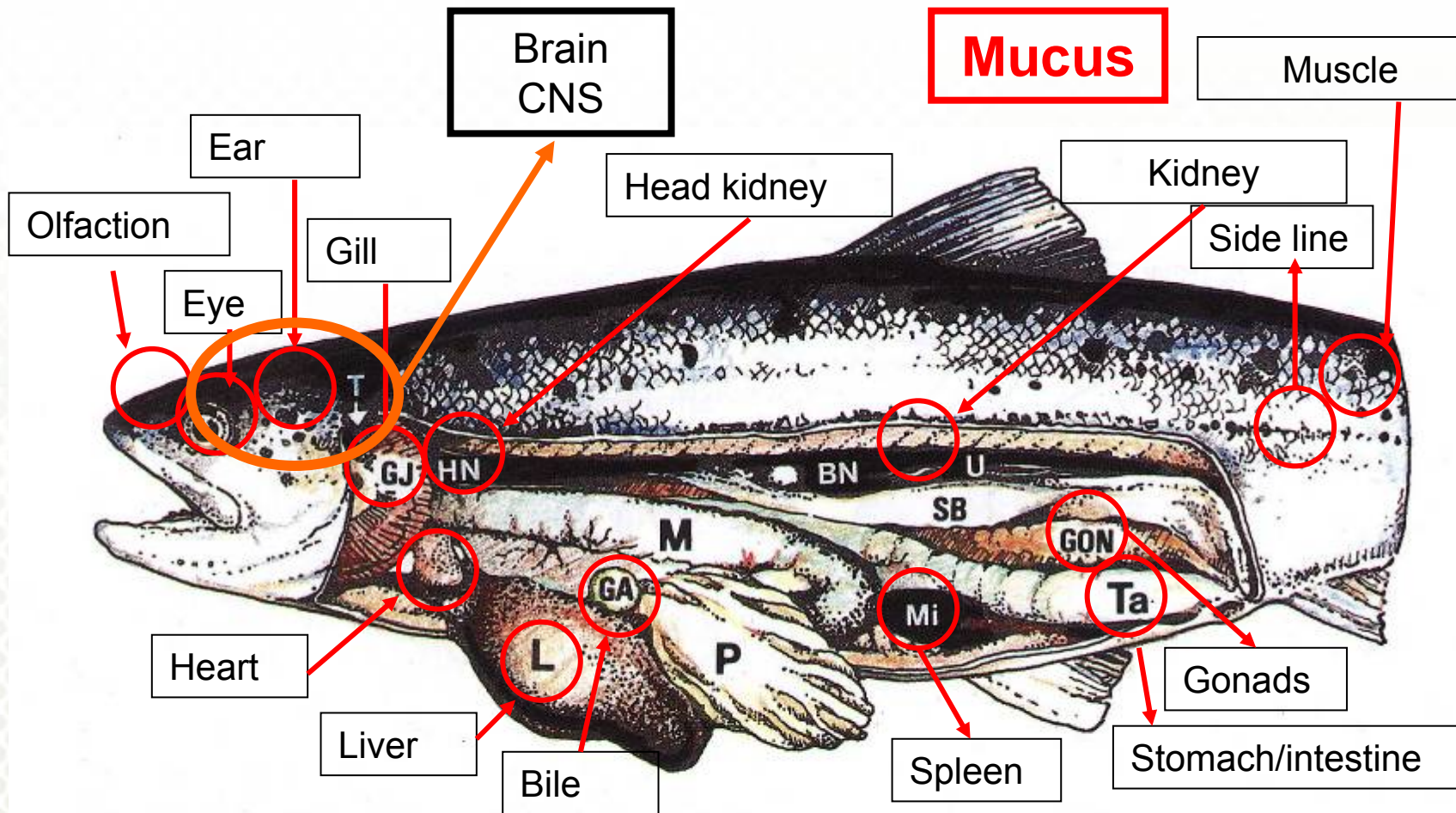


Fig. 1.4 Laksefisk med venstre bukside fjernet for å vise de indre organer. T: Thymus, GJ: Gjeller, HN: Hodenyre, BN: Baknyre, U: Urinleder, GON: Gonade, Ta: Tarm, Mi: Milt, M: Magesekk, P: Pylorusblindsekker, L: Lever, GA: Galleblære. Hjertet sees mellom lever og gjeller. Et stanniuslegeme sees foran BN i nyren.

Modified after Kryvi 1992



Organs for study of metal toxicity



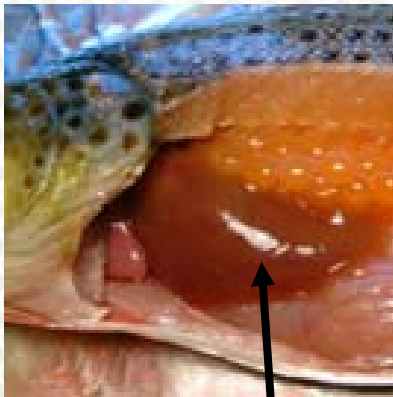
Skeletal



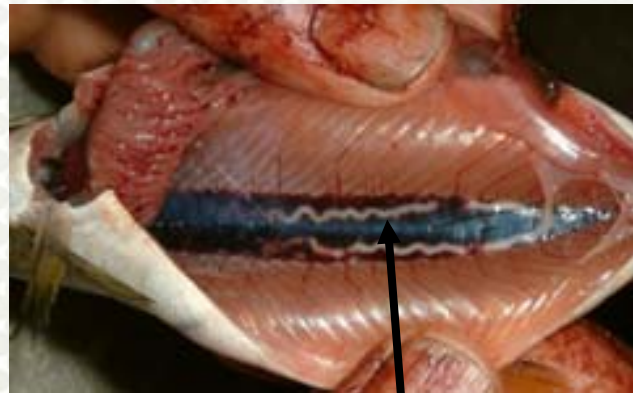
Muscle & fin



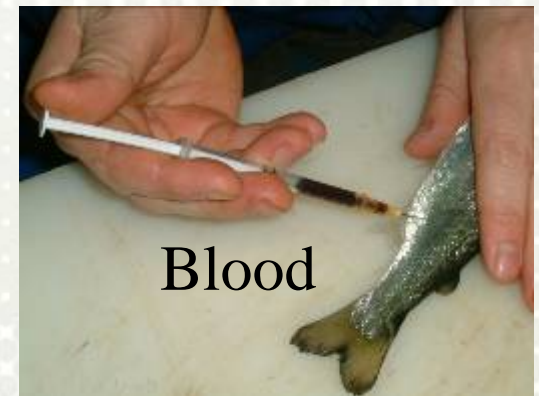
Gill



Liver



Kidney



Blood

Photos: B.O. Rosseland



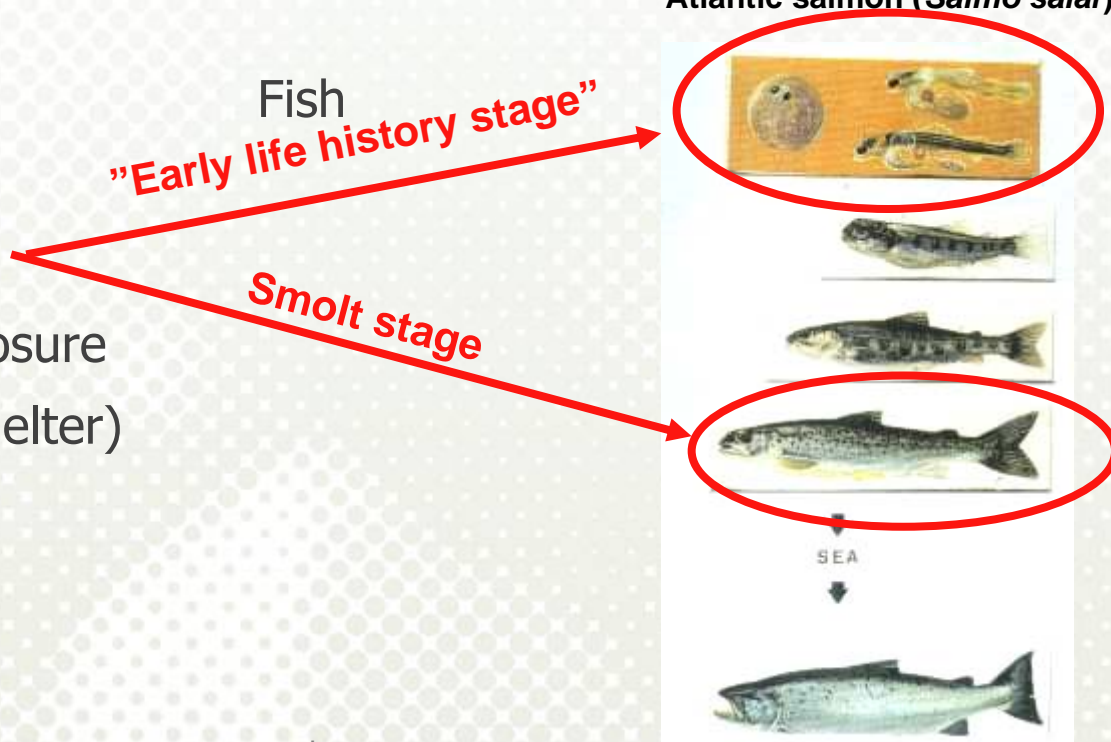
How to link a specific metal to fish toxicity

Water:

- Total conc. of little value
- Metal species being reactive to biological membranes/organs
 - Depends on: pH, chelators (TOC, Si), temperature
- Other metals interacting: additive, synergistic or antagonistic
- Ameliorating factors: Ca, Ion strength or Conductivity

- Fish species
- Life history stage
- Pre history of exposure
- Habitat (hiding, shelter)

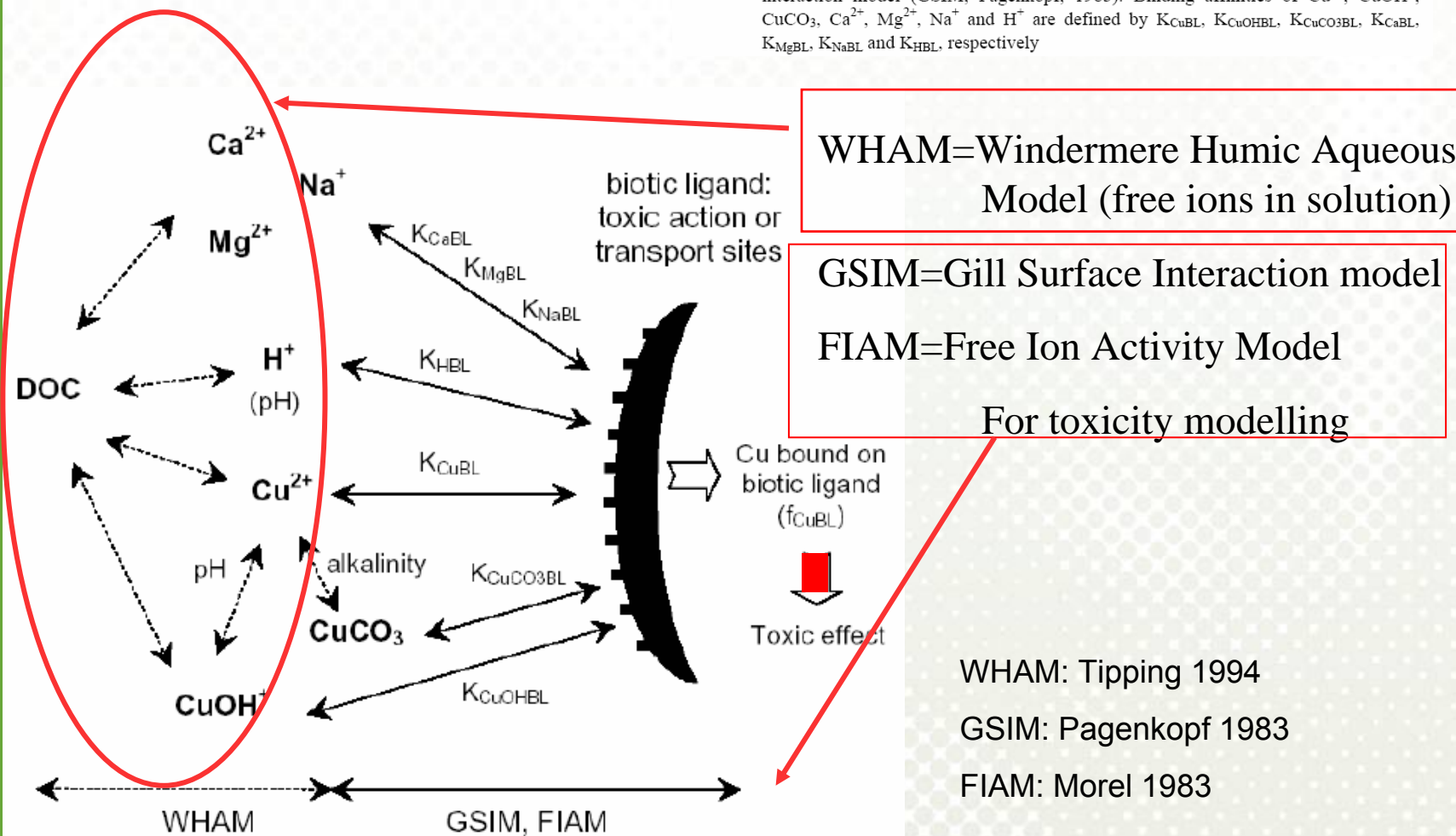
Atlantic salmon (*Salmo salar*)



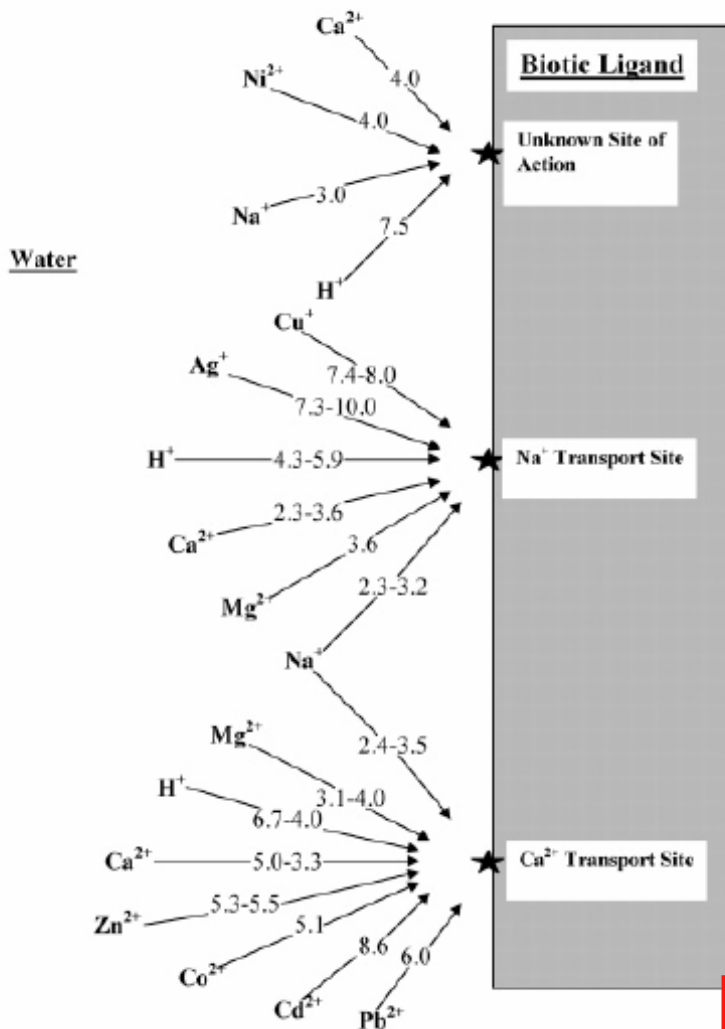
Binding models Cu



Figure 7: Schematic overview of the biotic ligand model of copper. Dashed lines represent speciation reactions (modeled by WHAM, Tipping, 1994). Solid lines represent binding to the biotic ligand (which may be toxic action sites or transport sites). The free copper ion (Cu^{2+}) forms complexes with inorganic ligands such as OH^- and CO_3^{2-} (other ligands not shown). The concentration of OH^- and CO_3^{2-} is determined by pH and alkalinity. Cu^{2+} and CuOH^+ also form complexes with dissolved organic carbon (DOC). Ca^{2+} , Mg^{2+} and H^+ (also determined by pH) competes with copper for binding sites on DOC. Cu^{2+} , CuOH^+ and CuCO_3 bind to the BL and the Cu-concentration bound to the BL determines the toxic effect. The latter concentration is assumed to be constant for a given effect size (e.g. 50% mortality). Ca^{2+} , Mg^{2+} , Na^+ and H^+ can compete with copper for BL-sites thus decreasing the amount of Cu bound to the BL (and thus the toxicity) at a given dissolved copper concentration. This concept was first formulated in the free ion activity model (FIAM, Morel, 1983) and the gill surface interaction model (GSIM, Pagenkopf, 1983). Binding affinities of Cu^{2+} , CuOH^+ , CuCO_3 , Ca^{2+} , Mg^{2+} , Na^+ and H^+ are defined by K_{CuBL} , K_{CuOHBL} , $K_{\text{CuCO}_3\text{BL}}$, K_{CaBL} , K_{MgBL} , K_{NaBL} and K_{HBL} , respectively



Binding affinities (log K) of free ions onto gills



Blood

Biotic Ligand Model, a Flexible Tool for Developing Site-Specific Water Quality Guidelines for Metals

SOUMYA NIYOGI AND CHRIS M. WOOD*

6178 ■ ENVIRONMENTAL SCIENCE & TECHNOLOGY / VOL. 38, NO. 23, 2004

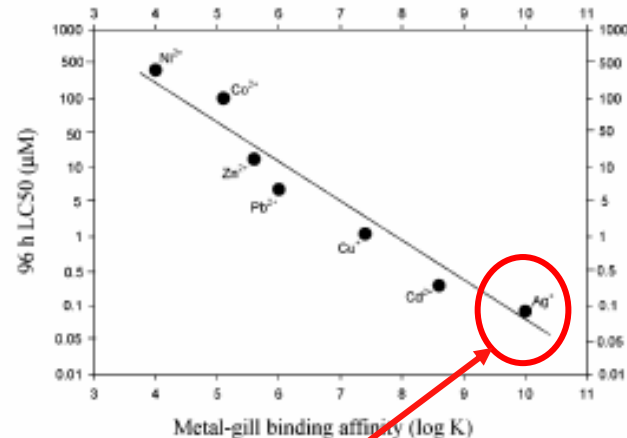


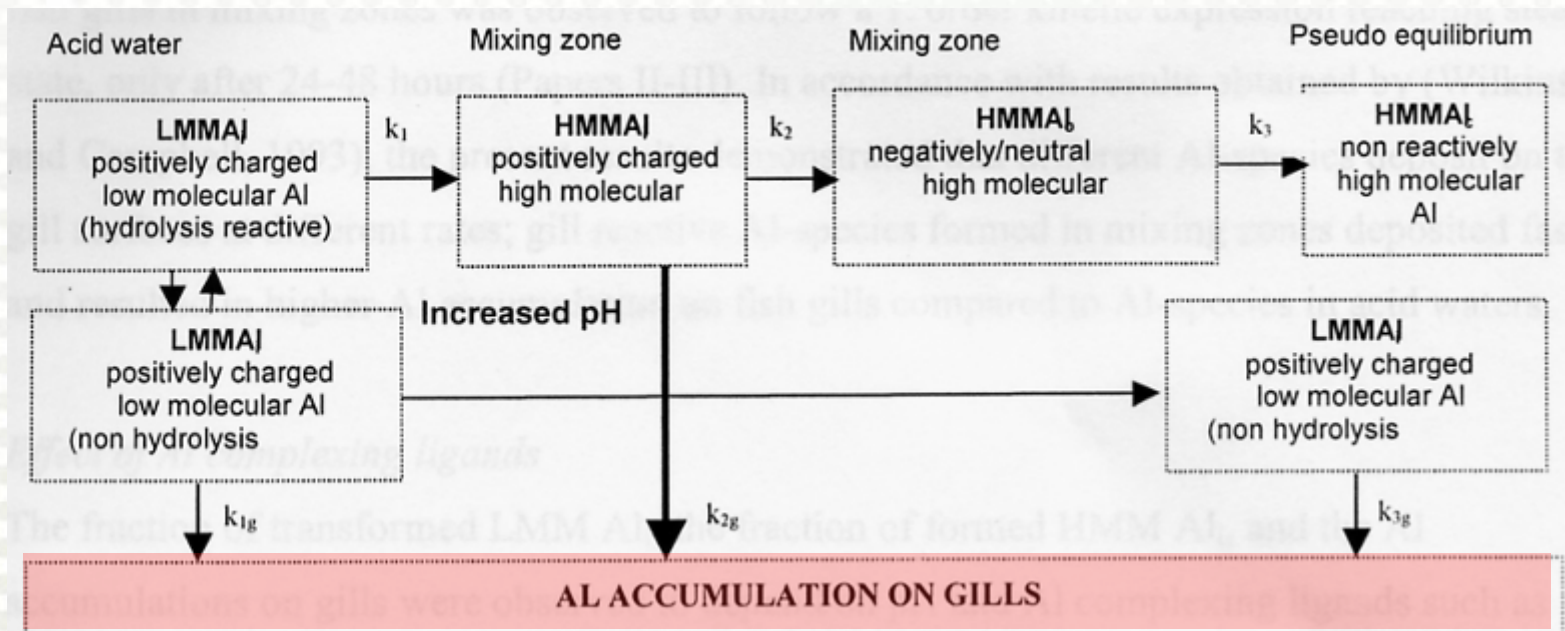
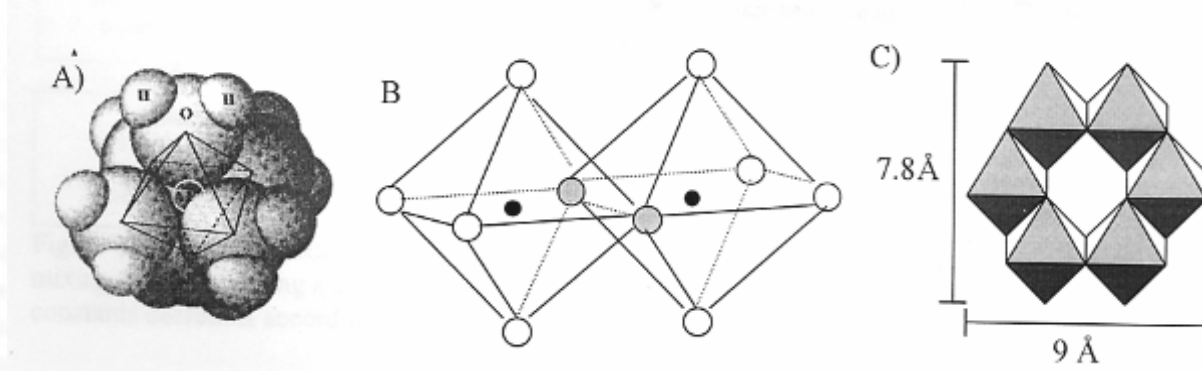
FIGURE 2. Relationship between gill-metal binding affinity (log K) and acute toxicity (96 h LC₅₀) in juvenile rainbow trout in the laboratory water (moderately hard, from Lake water). The log K data for Ag⁺, Cu⁺, Cd²⁺, Pb²⁺, are taken from the cited literature: refs 61, 60, 62, respectively. The 96 h LC₅₀ data for the same on the cited literature: refs 13, 75, 73, 173, 77, 176, and 160, respectively. The Y-axis of the plot is in log scale.

Higher affinity = higher toxicity

Niyogi and Wood (2004).



Cationic Al-species transform to transient, more "gill-reactive" Al-species during the initial step of polymerisation

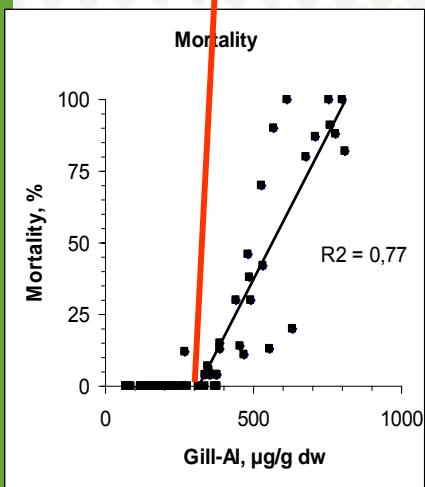
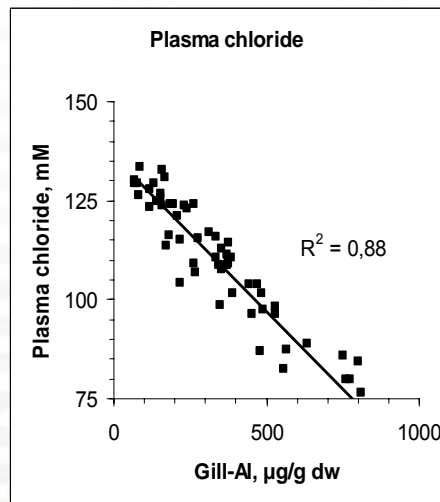
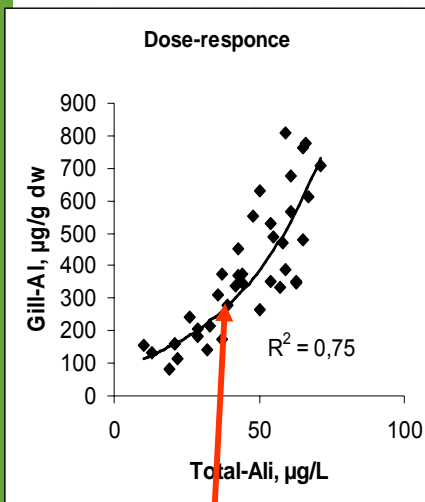


Teien et al. 2004

Al_i water - Al_{gills} - osmoregulation-toxicity



Photo: B.O. Rosseland



Mortality in freshwater at: 300 µgAl g⁻¹dw

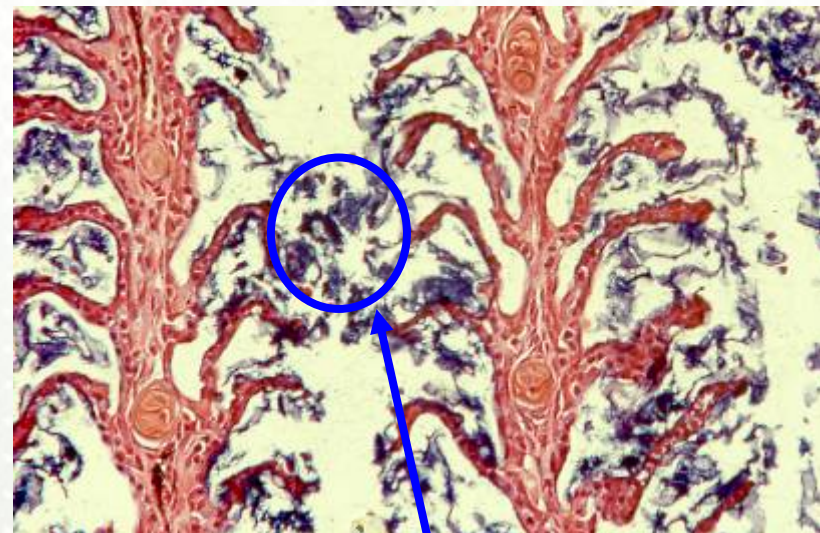


Photo: D. Oughton

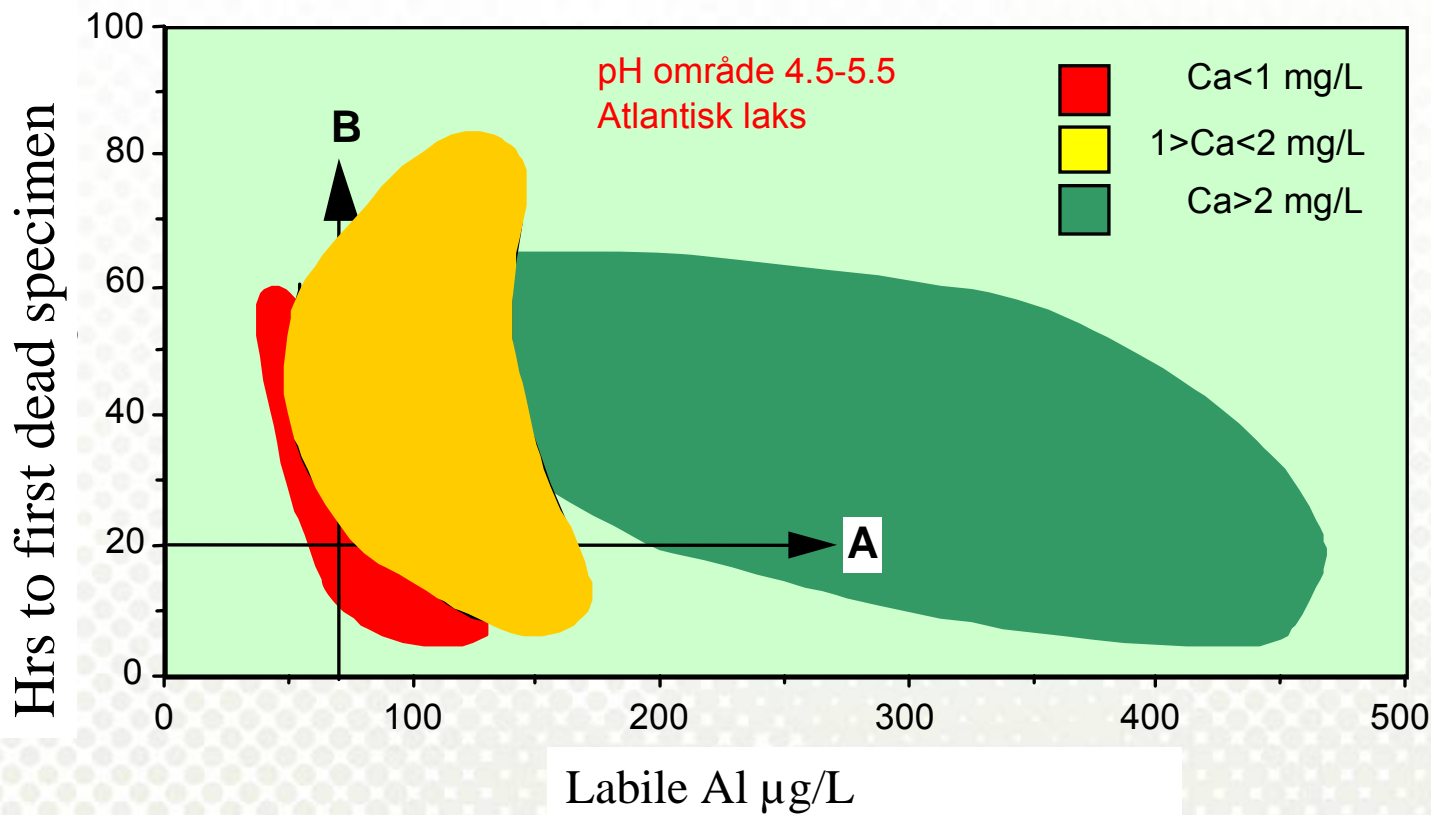
Al deposits

Gill-Al becomes THE Biomarker!



Ca/Al-toxicity model – pH 4.5 - 5.5 Labile Al and time (Hrs) to death

Atlantic salmon (*Salmo salar*)



Etter: Rosseland 1989.

Ca is extremely important in modulating the toxicity of Al (and other metals)!

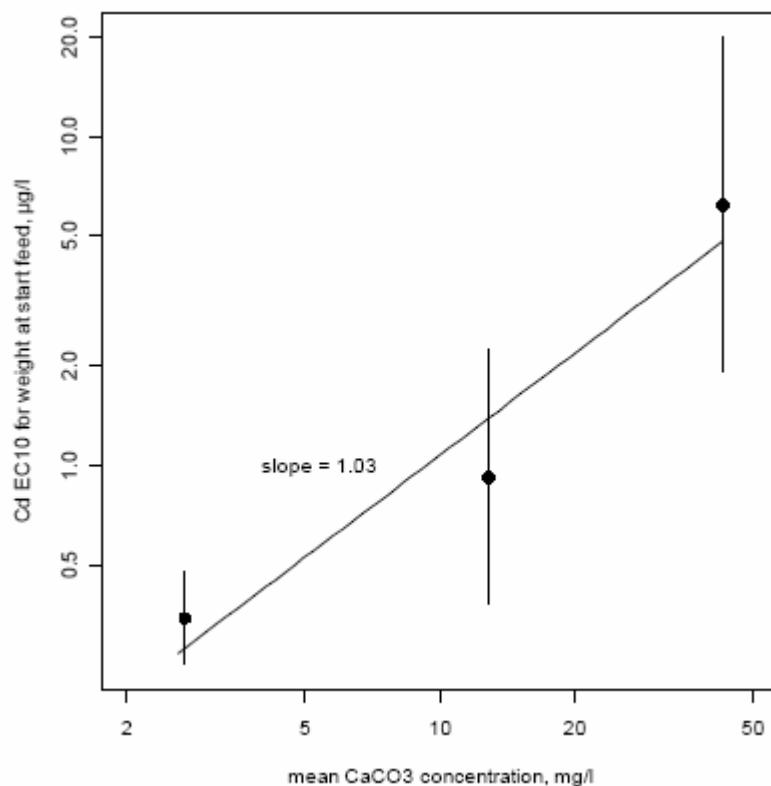
Brown trout Cd EC₁₀ for weight of start feeding



REPORT SNO 5468-2007

Study of the effect of cadmium on the early life stages of brown trout (*Salmo trutta*) at different levels of water hardness

Cd EC10



Thron O. Haugen, Torstein Kristensen, Frode Kroglund, Jarle Håvardstun and Einar Kleiven 2007

CaCO₃ mg/l

- Owing to reports on pronounced survival and growth effects of cadmium at post startfeeding life stages soft-water studies on these life stages should be performed in the future





**We need background data to establish the link
from laboratory to nature**

Photo: B.O. Rosseland

EMERGE

Metal concentration in salmonides

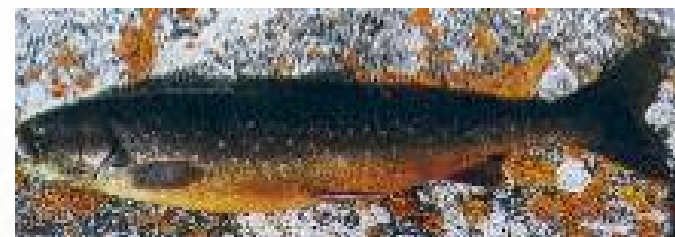
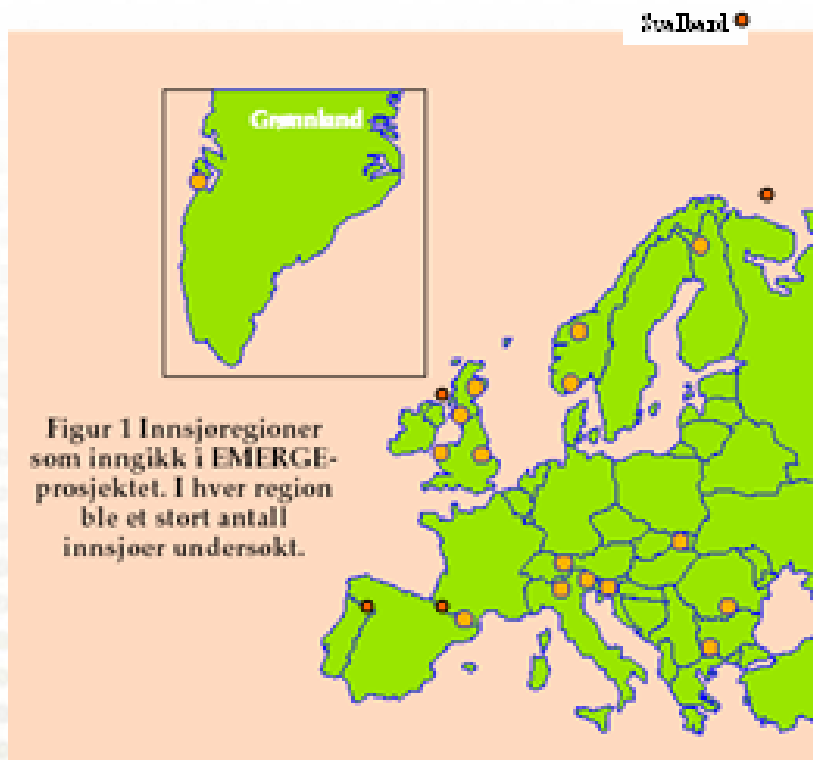


Photo: B.O. Rosseland

Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Zn

Gill

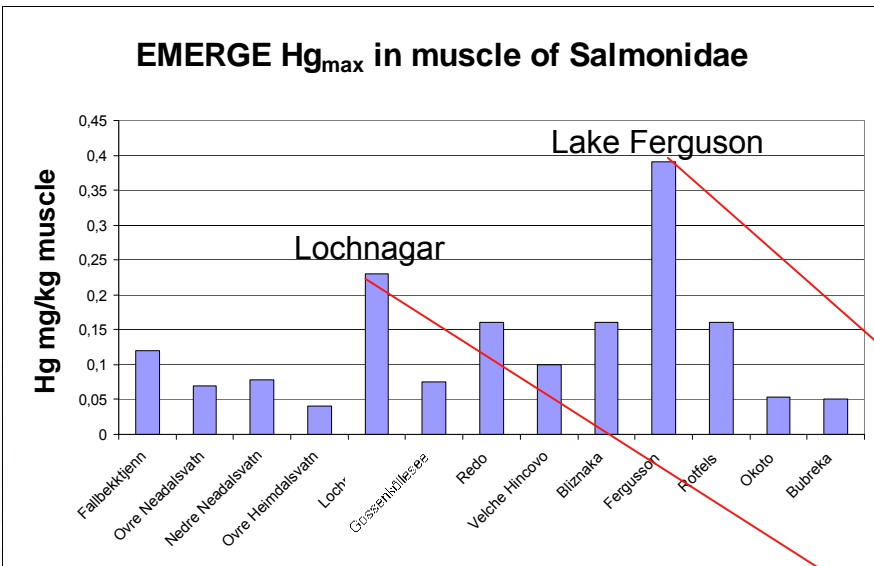
Lake Name	Country	Species	n	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
Bliznaka	Bulgaria	Salmo trutta	15	2.28 ± 1.09	0.01 ± 0.00	0.08 ± 0.01	0.67 ± 0.06	0.39 ± 0.02	37.25 ± 3.25	1.76 ± 0.16	0.02 ± 0.01	0.09 ± 0.01	0.87 ± 0.04	72.64 ± 5.39
Fallbekk	Norway	Salmo trutta	20	5.73 ± 0.42	0.01 ± 0.01	0.42 ± 0.02	0.14 ± 0.01	0.39 ± 0.01	58.80 ± 2.75	2.72 ± 0.18	0.04 ± 0.01	0.28 ± 0.03	1.95 ± 0.07	37.20 ± 3.03
Gossenköllesee	Austria	Salmo trutta	14	1.86 ± 0.50	0.34 ± 0.26	0.23 ± 0.01	1.13 ± 0.09	0.49 ± 0.06	55.06 ± 3.25	5.49 ± 0.71	0.03 ± 0.01	1.66 ± 0.25	1.85 ± 0.11	29.14 ± 1.44
Lake Fergusson	Greenland	Salvelinus alpinus	10	2.84 ± 0.57	0.06 ± 0.01	0.01 ± 0.00	0.47 ± 0.03	0.39 ± 0.03	33.10 ± 3.08	1.02 ± 0.17	0.32 ± 0.02	0.00 ± 0.00	0.65 ± 0.08	16.96 ± 0.97
Lochnagar	UK	Salmo trutta	10	21.63 ± 1.51	0.17 ± 0.08	2.14 ± 0.32	0.59 ± 0.02	0.41 ± 0.02	55.70 ± 4.29	4.18 ± 0.26	0.35 ± 0.02	3.88 ± 0.22	2.38 ± 0.18	78.92 ± 4.31
Okoto	Bulgaria	Salvelinus fontinalis	7	1.97 ± 1.09	0.01 ± 0.00	0.21 ± 0.02	0.45 ± 0.06	0.39 ± 0.01	45.62 ± 6.56	3.42 ± 0.60	0.02 ± 0.00	0.25 ± 0.05	1.32 ± 0.14	20.18 ± 0.98
Redo	Spain	Salmo trutta	43	1.00 ± 0.11	0.02 ± 0.00	0.54 ± 0.04	0.26 ± 0.02	0.39 ± 0.01	54.76 ± 3.24	1.27 ± 0.08	0.03 ± 0.01	1.23 ± 0.97	0.97 ± 0.04	33.79 ± 2.98
Rotfelsessee	Austria	Salvelinus alpinus	21	2.04 ± 0.30	0.04 ± 0.01	0.38 ± 0.02	1.11 ± 0.05	0.32 ± 0.02	41.91 ± 1.57	1.64 ± 0.33	0.04 ± 0.00	0.52 ± 0.09	1.20 ± 0.07	21.86 ± 1.32
Velke Hincovo	Slovakia	Salmo trutta	10	5.08 ± 0.45	0.03 ± 0.02	0.71 ± 0.03	0.26 ± 0.02	0.38 ± 0.02	52.75 ± 2.42	2.01 ± 0.18	0.05 ± 0.02	1.22 ± 0.20	1.61 ± 0.13	30.38 ± 3.11
Øvre Helmdalsvann	Norway	Salmo trutta	10	1.75 ± 0.42	0.02 ± 0.01	0.31 ± 0.02	0.93 ± 0.04	0.53 ± 0.06	45.60 ± 3.65	1.80 ± 0.12	0.32 ± 0.02	0.12 ± 0.07	1.08 ± 0.06	110.64 ± 6.13
Øvre Neådalsvann	Norway	Salmo trutta	16	1.68 ± 0.27	0.00 ± 0.00	0.19 ± 0.02	0.31 ± 0.06	0.45 ± 0.02	52.11 ± 7.53	2.77 ± 0.12	0.09 ± 0.02	0.06 ± 0.02	1.84 ± 0.15	79.99 ± 4.30

wt weight (ww) in salmonid fish from 11 high mountain lakes. Values are mean ± 1 SEM



Mercury Hg, EMERGE Lakes

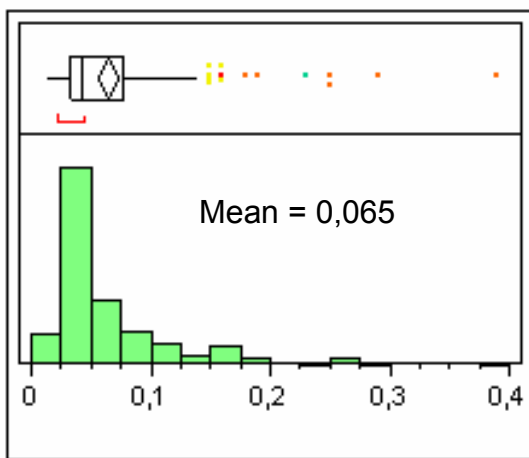
Distribution of muscle- and kidney-Hg concentration



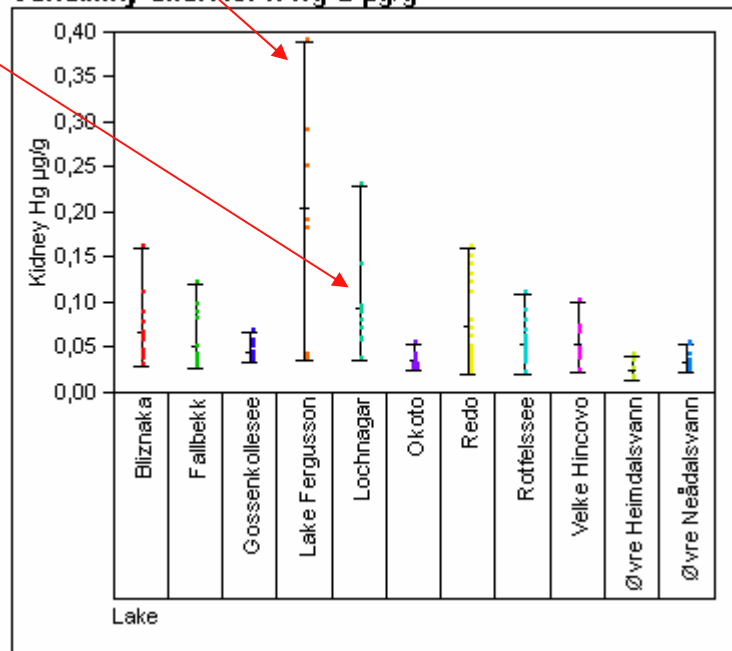
Muscle

Rosseland et al. 2003

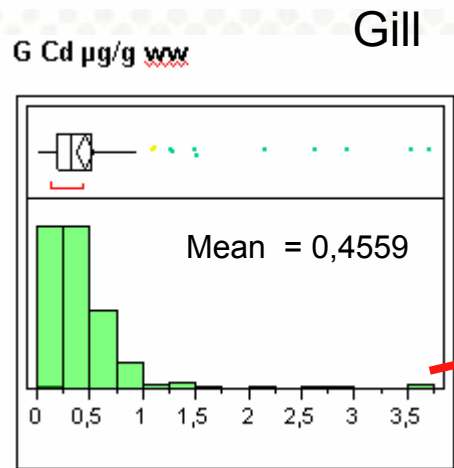
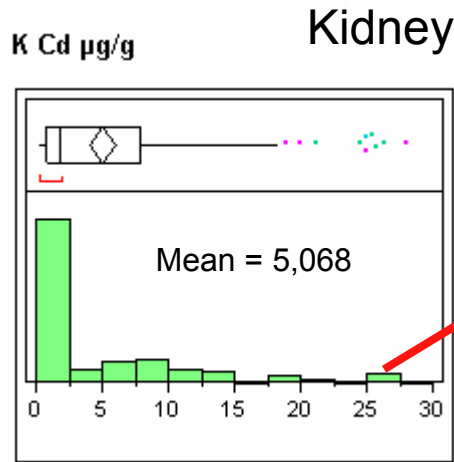
Distributions K Hg-B µg/g **Kidney**



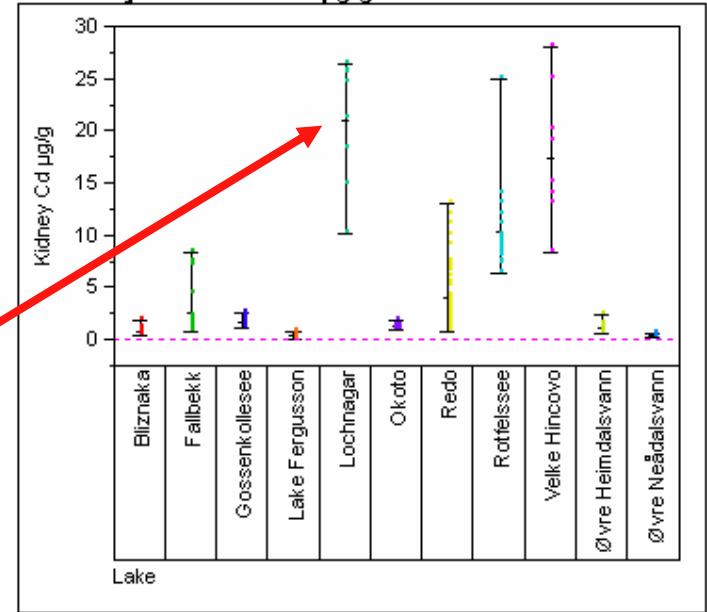
Variability Gage **Variability Chart for K Hg-B µg/g** **Kidney**



EMERGE Lakes Distribution of kidney- and gill-Cd concentration

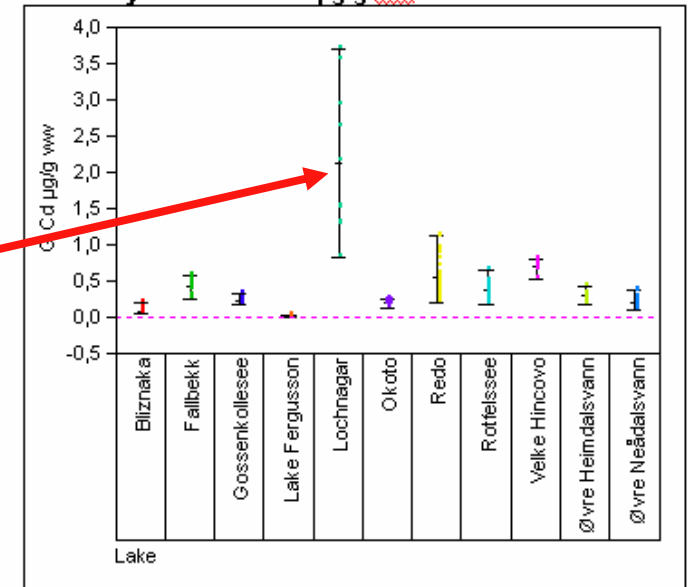


Variability Gage
Variability Chart for K Cd $\mu\text{g/g}$



Lochnagar

Variability Gage
Variability Chart for G Cd $\mu\text{g/g ww}$

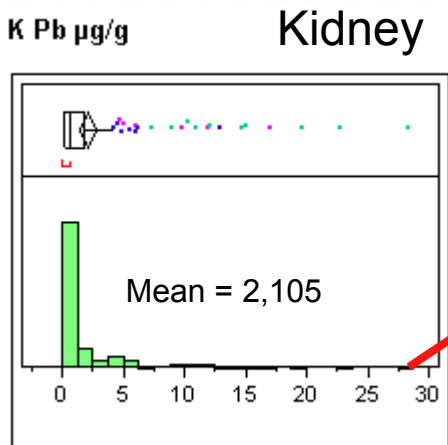


Gill

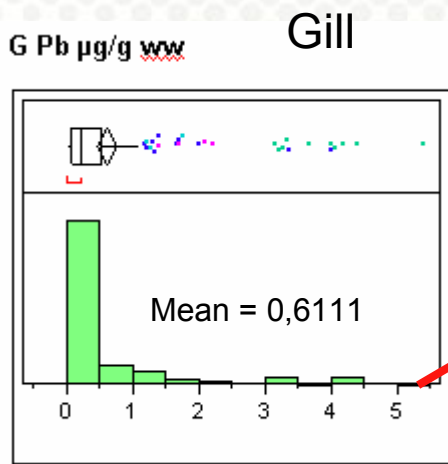
Lochnagar

Rosseland et al. 2003

EMERGE Lakes Distribution of kidney- and gill-Pb concentration



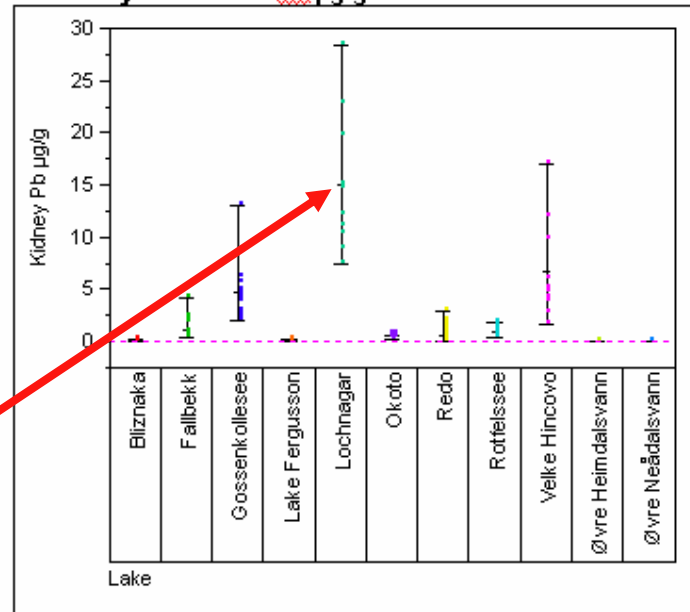
Lochnagar



Lochnagar

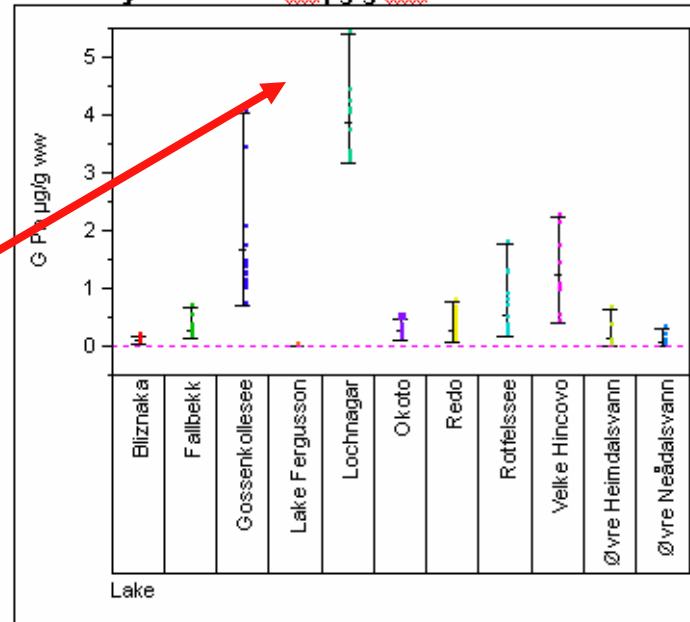
Variability Gage
Variability Chart for K Pb $\mu\text{g/g}$

Kidney



Variability Chart for G Pb $\mu\text{g/g ww}$

Gill



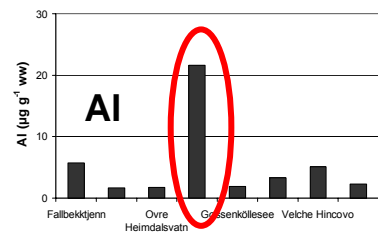
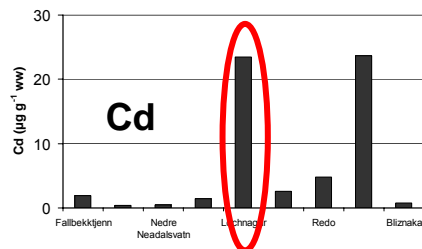
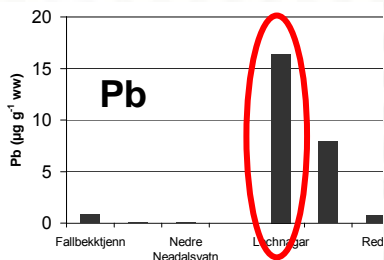
Rosseland et al. 2003



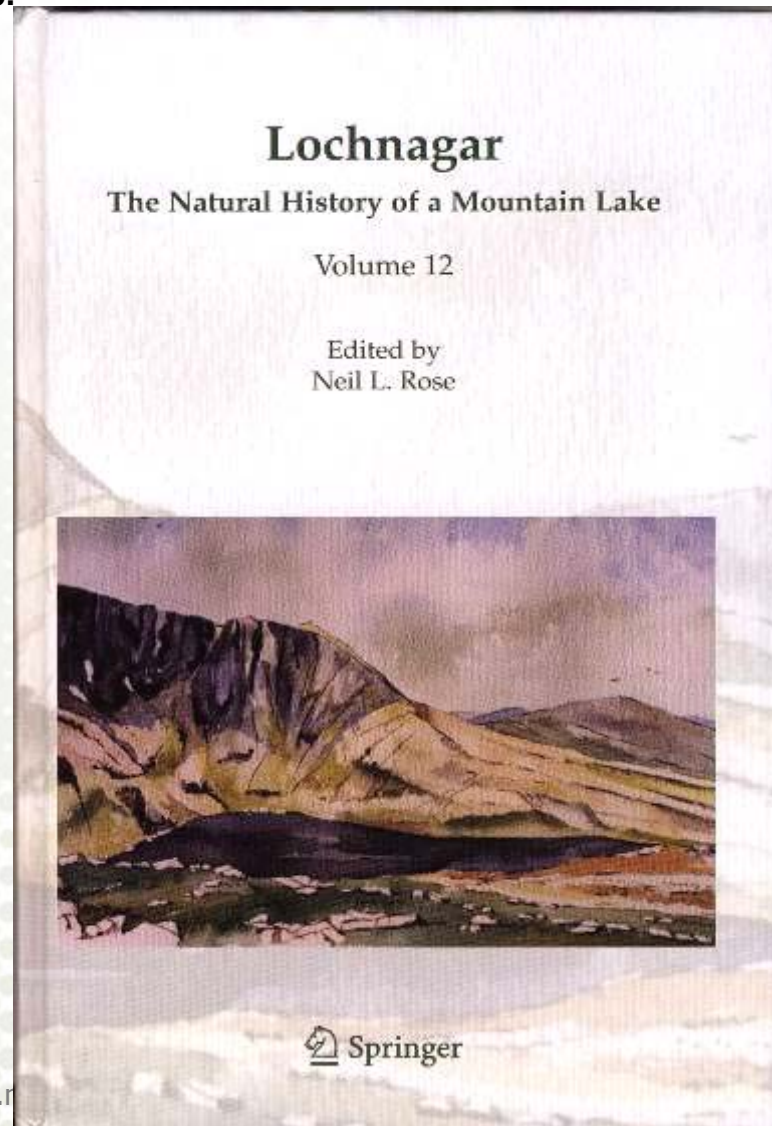
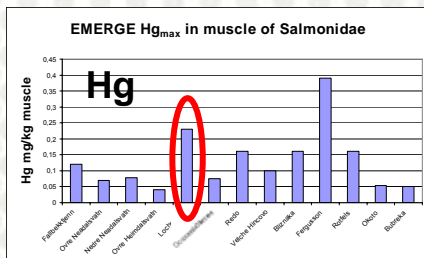
A book about Lochnagar in 2007

- The "Royal Lake" has the highest Pb, Cd, Al, Hg, PBDE etc level of the non-piscivorous trout populations found in European high mountain lakes.

Rosseland, B.O., Rognerud, S., Collen, P., Grimalt, J., Vives, I., Massabuau, J-C., Hofer, R., Lackner, R., Fjellheim, A., Harriman, R., and Pina, B. 2007. Brown trout in Lochnagar. Population and contamination by metals and organic micropollutants. In: Rose, N. (Ed.): Lochnagar: The Natural History of a Mountain Lake. Developments in Paleoenvironmental Research (DPER) Volume 12, 247-280.

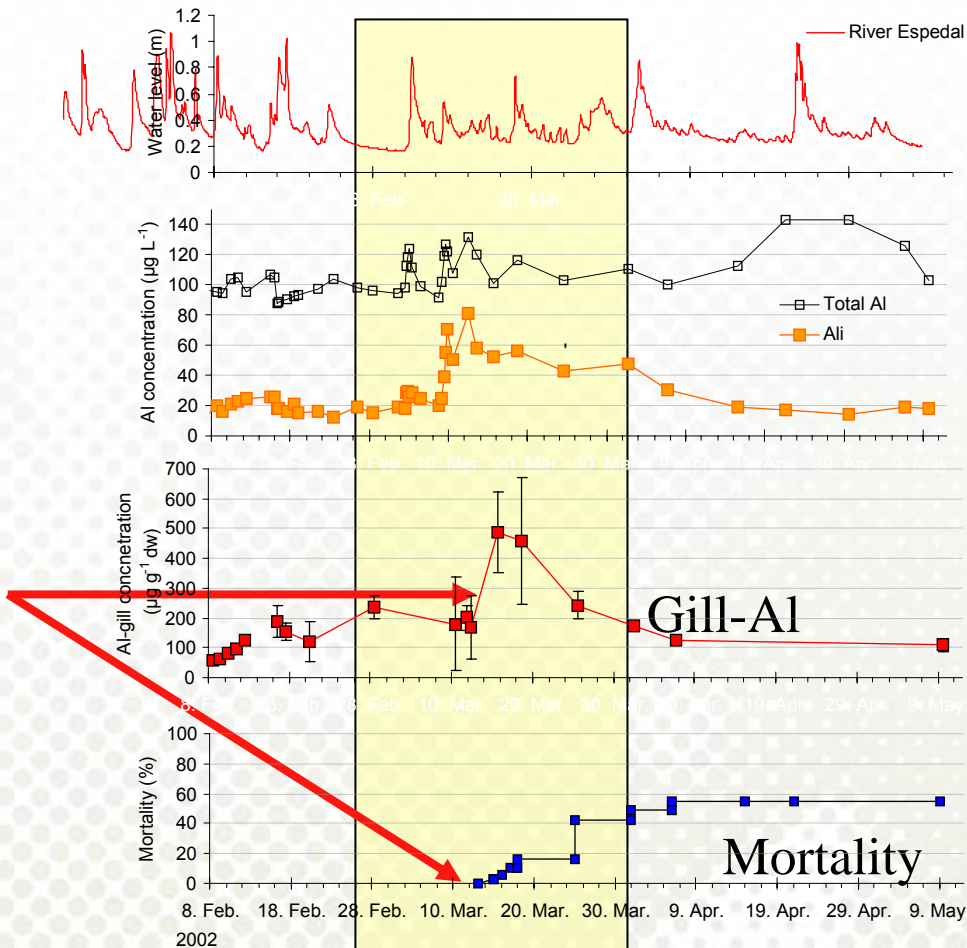


The chapter contain many data on metals in organs of brown trout



How much gill-Al causes mortality in freshwater?

Flood and seasalt episodes on the Norwegian West Coast in 2002



- Deposition of seasalts
- Mobilization of Al from soil
- Increased Ali in water
- Increased gill-Al

• Fish kill when gill-Al > 300 µg/g gill d.w.



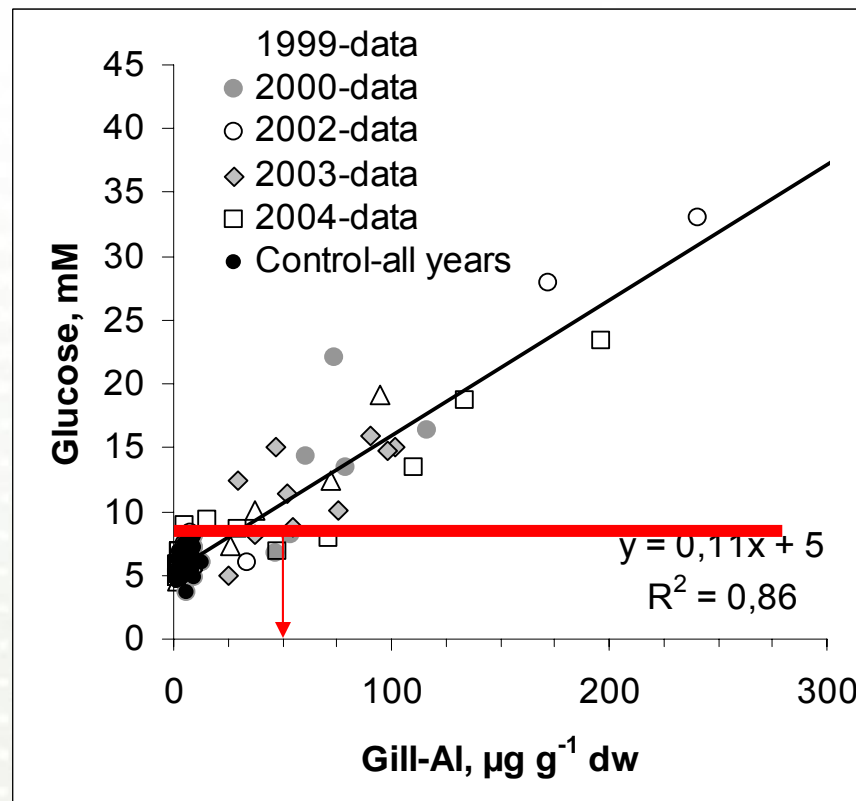
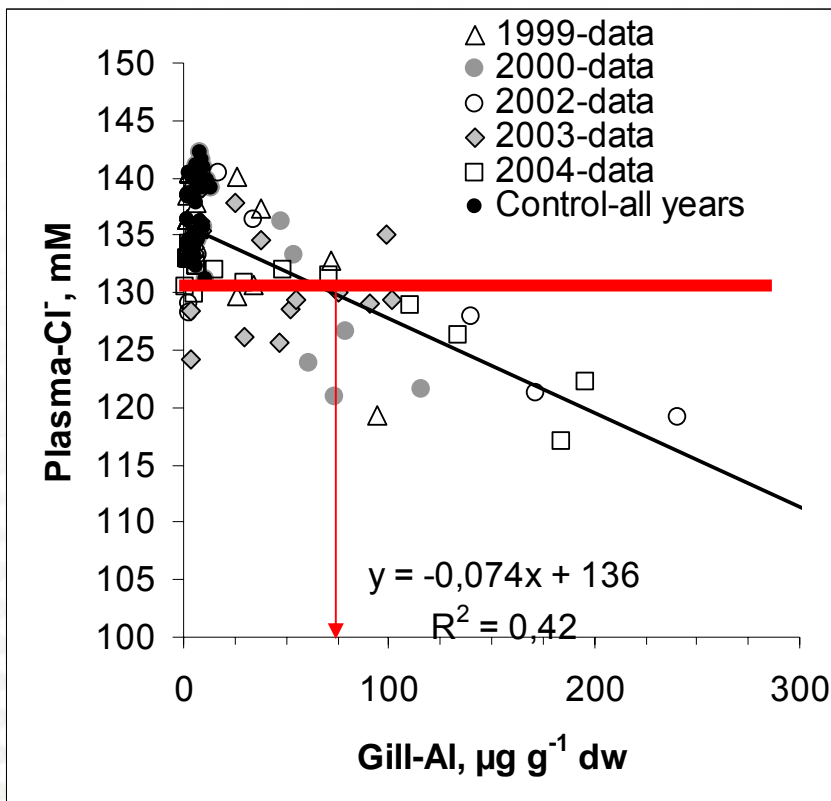
Exposure to moderate acid water and aluminum reduces Atlantic salmon post-smolt survival

F. Kroglund ^{a,*}, B. Finstad ^b, S.O. Stefansson ^c, T. Nilsen ^c, T. Kristensen ^d,
B.O. Rosseland ^e, H.C. Teien ^e, B. Salbu ^e

Can we go from a gill-metal concentration to ecologically relevant population effects?



Physiology related to gill-AI in Atlantic salmon smolt



Never normal plasma-Cl at gill-AI >75 $\mu\text{g Al/g gill d.w.}$

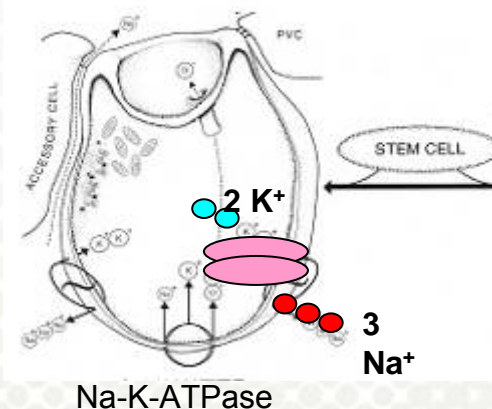
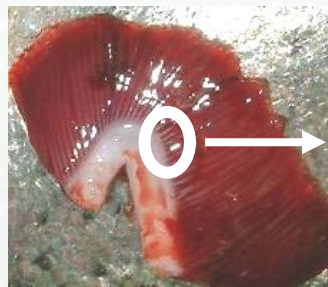
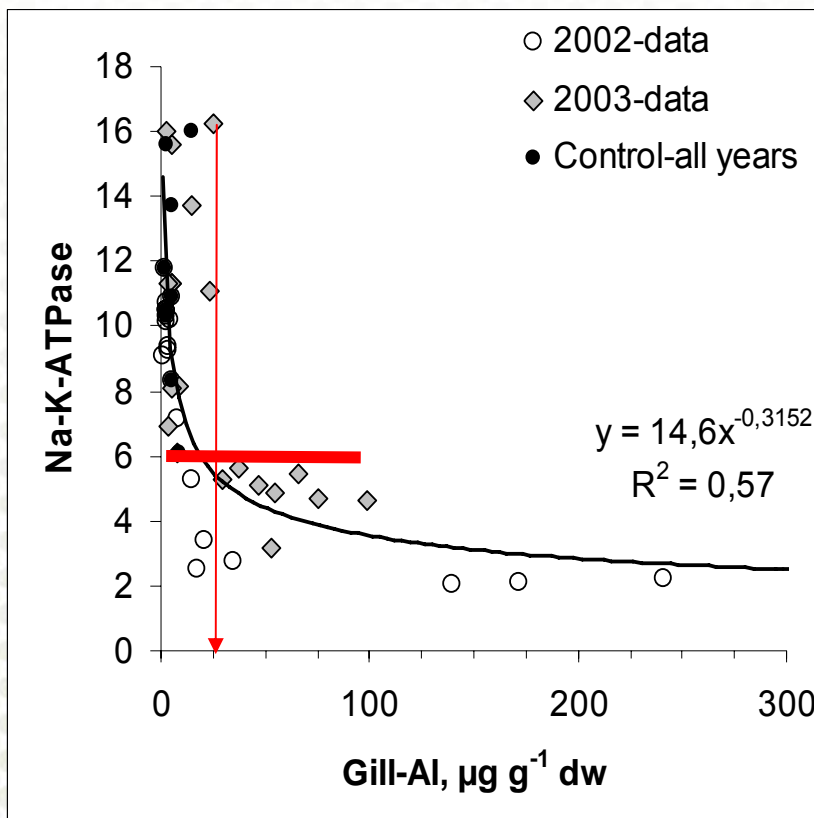
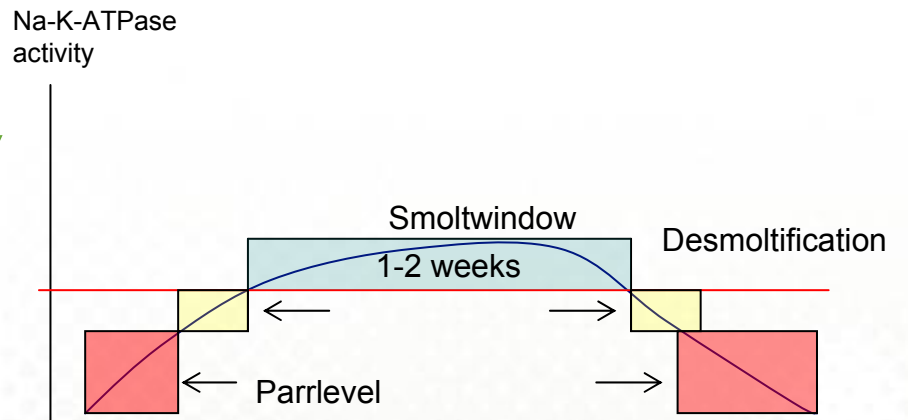
Never normal plasma-glukose at gill-AI >50 $\mu\text{g Al/g gill d.w.}$

Mortality in freshwater
at >300 $\mu\text{g g}^{-1}$ dw

Kroglund et al. 2007



Gill enzyme activity; Na⁺K⁺ATPase and gill-AI at time of "Smolt Window"



Never high enzyme activity at **gill-AI > 25 µg/g gill d.w.**

Kroglund et al. 2007



Wild salmon return from sea: Ecological implications at low gill-AI

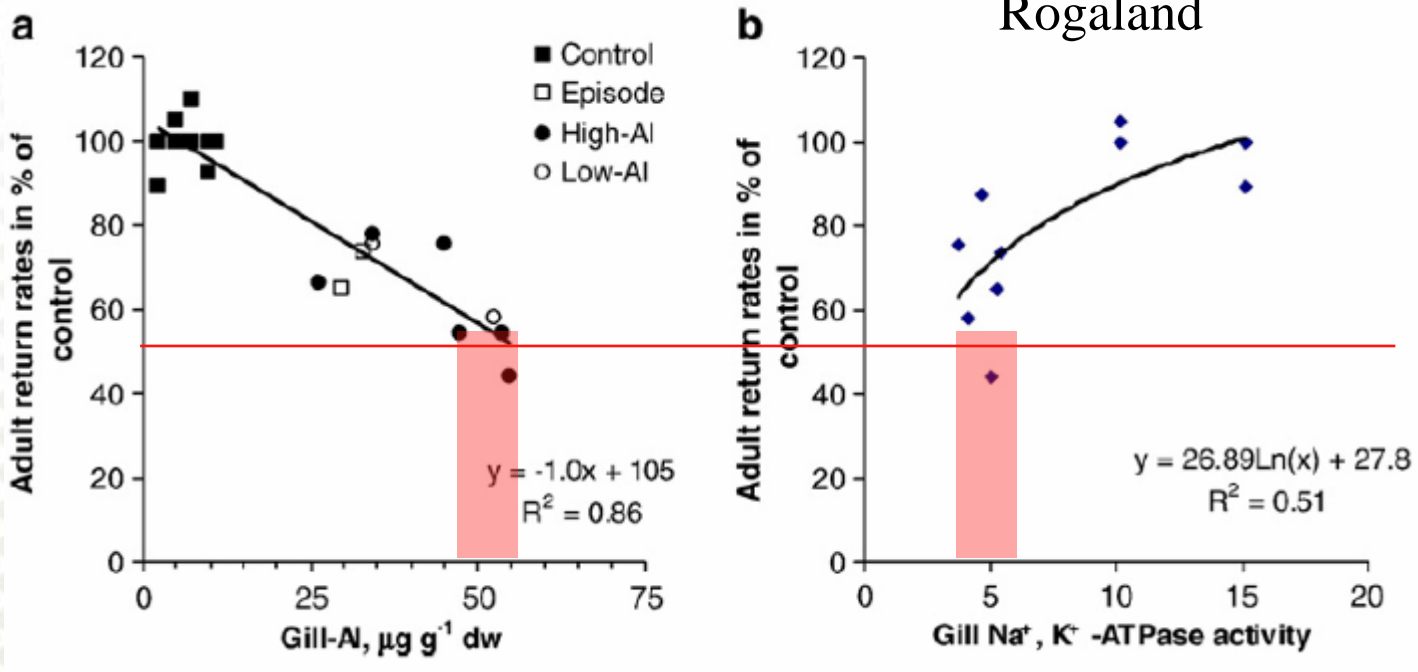


Photo: B.O. Rosseland

Carlin tagged smolt exposed to episodic acid water – **as little as 3 days exposure!**

Released from River Imsa, Rogaland

Gill-AI from 40 - 60 $\mu\text{g/g}$ gill dw and/or NaK-ATPase activity ≤ 5 , reduces return rate by 50%!

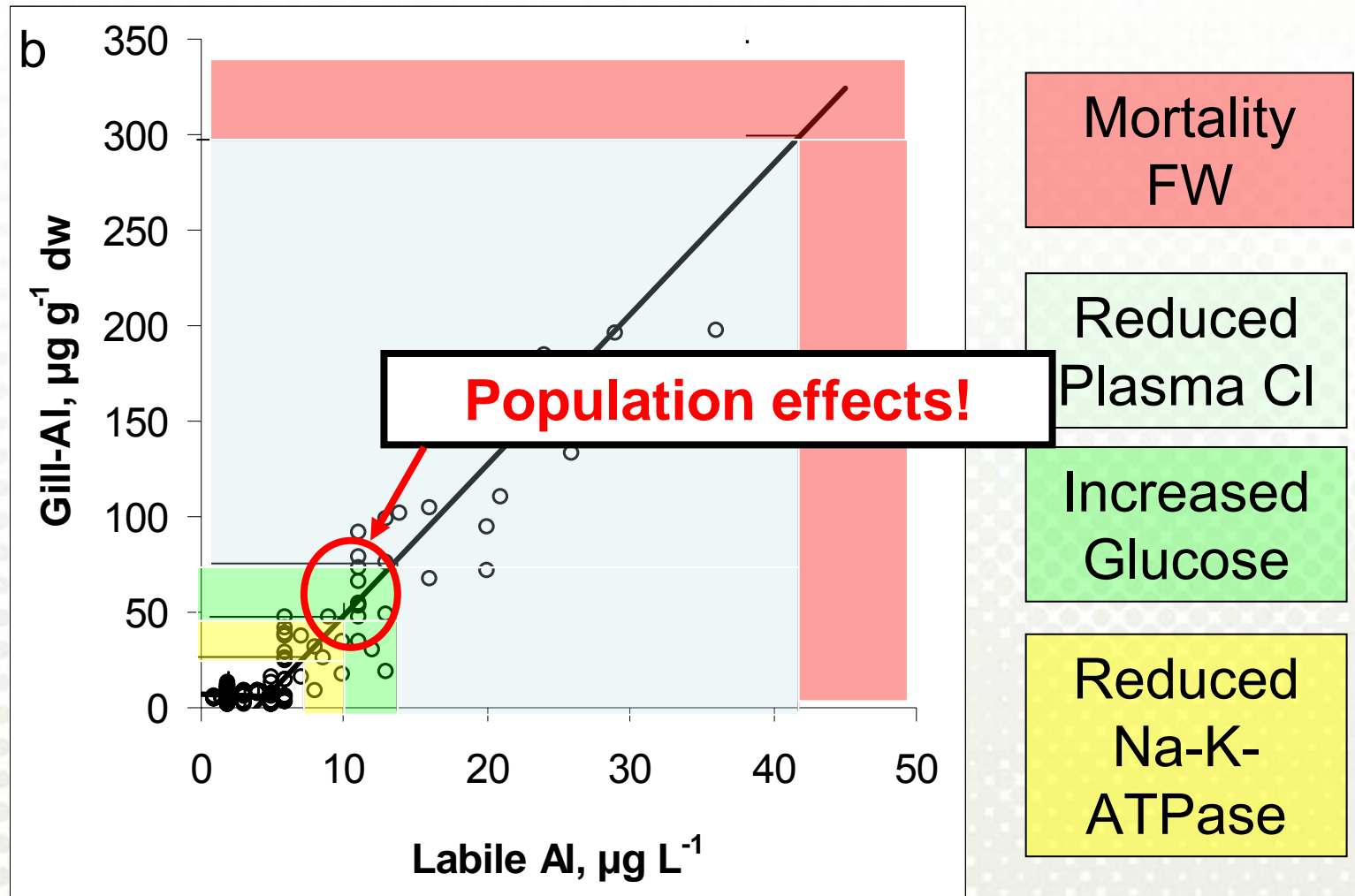


Kroglund et al. 2007



Critical levels of Al in water and gill, relative to smolts

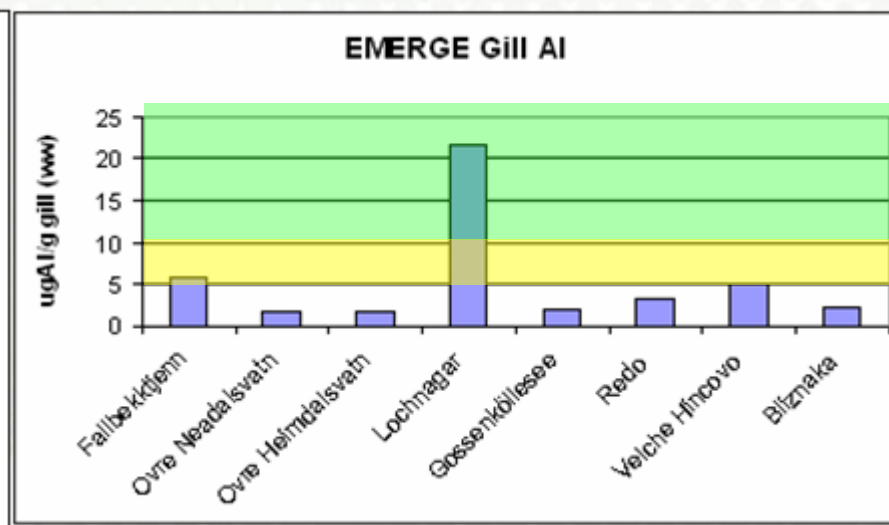
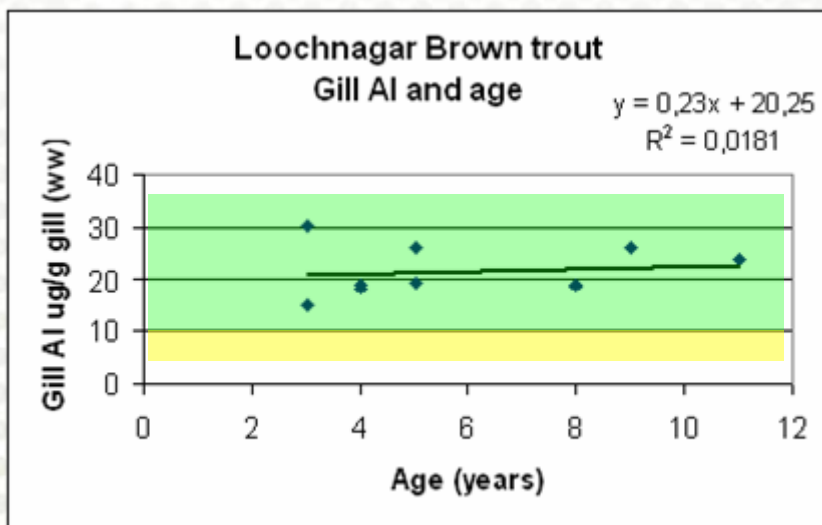
Smolt is extremely sensitive to Al during smoltification



Lochnagar Gill-Al

- Bioconcentration but no bioaccumulation of gill-Al
 - Reflecting free ion (labile Al) concentration

“Lochnagar Atlantic salmon” would have problems returning from sea



d.w. = w.w. * 5, **75 – 150 $\mu\text{gAl/g}$ gill d.w.**

Rosseland et al. 2007

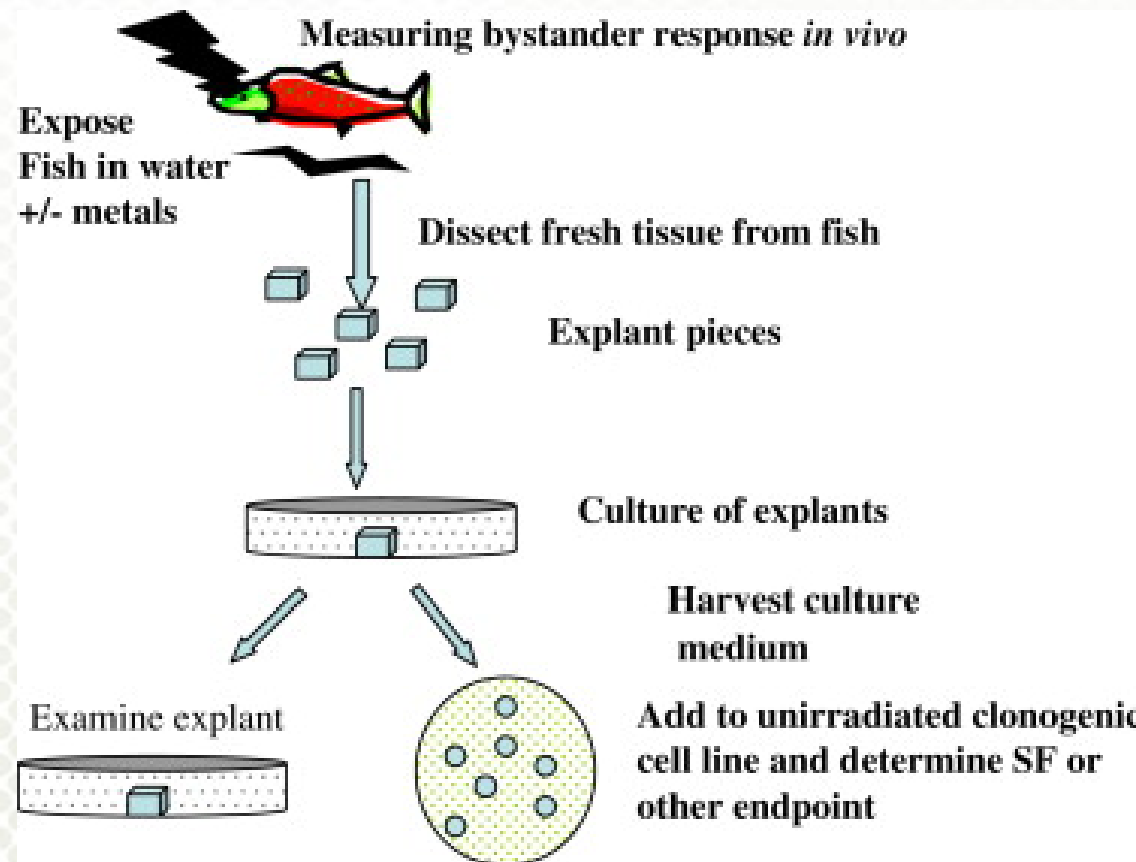
What about the Multiple stressor concept:

Al, Cd and radioactivity (γ)

"Bystandard effect"

A new tool of biomarker for early warning of effects

- Tissue from an exposed fish can through a culture medium induce apoptosis (cell death) in un-exposed cells

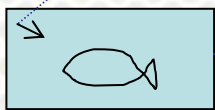


From: Mothersille et al. (2007)

Case: Salmon exposed to metals and gamma radiation, Norway and Canada

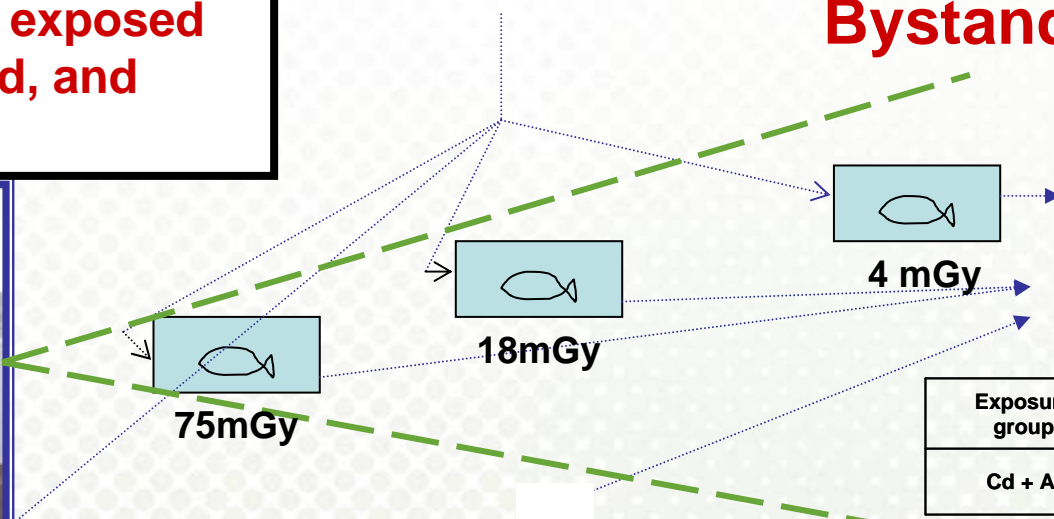
Severe multiple stressor synergism for salmon exposed to Al, Cd, and gamma

Biological endpoint: Induced genomic instability: Bystander effect

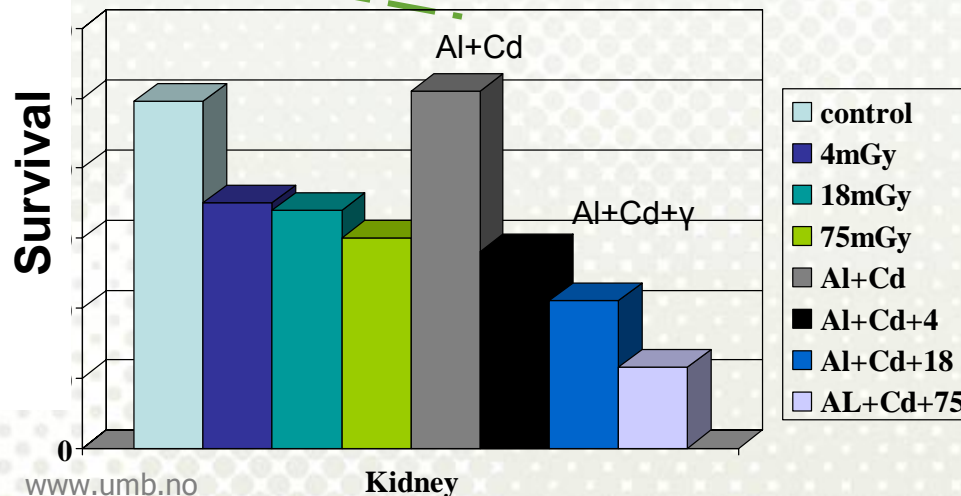


Reference (no gamma from source)

Water reservoir (aerated)



Exposure group	Cd total (µg/L)	Al total (µg/L)
Cd + Al	29	329



Conclusion

- Determination of a critical level for a single metal must include a range of data on biotic and abiotic factors
- Methods for identification of early responses in laboratory and field, must include a set of sensitive and validated biomarkers
- Critical loads and critical levels for heavy metals must take into consideration the interaction between several different stressors (“Multiple Stressors”)
- If possible, identification of “Delayed responses” should be included
- It is important to identify the **most sensitive target organisms** in actual **Eco-regions** and catchments, and establish **critical levels** based on their **most sensitive life history stages** in **low Ca waters**.