

Knowledge Gaps: Abiotic Factors and Ecosystem-Wide Effects

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NNI USA workshop: 6-7th October 2009, Arlington, Virginia



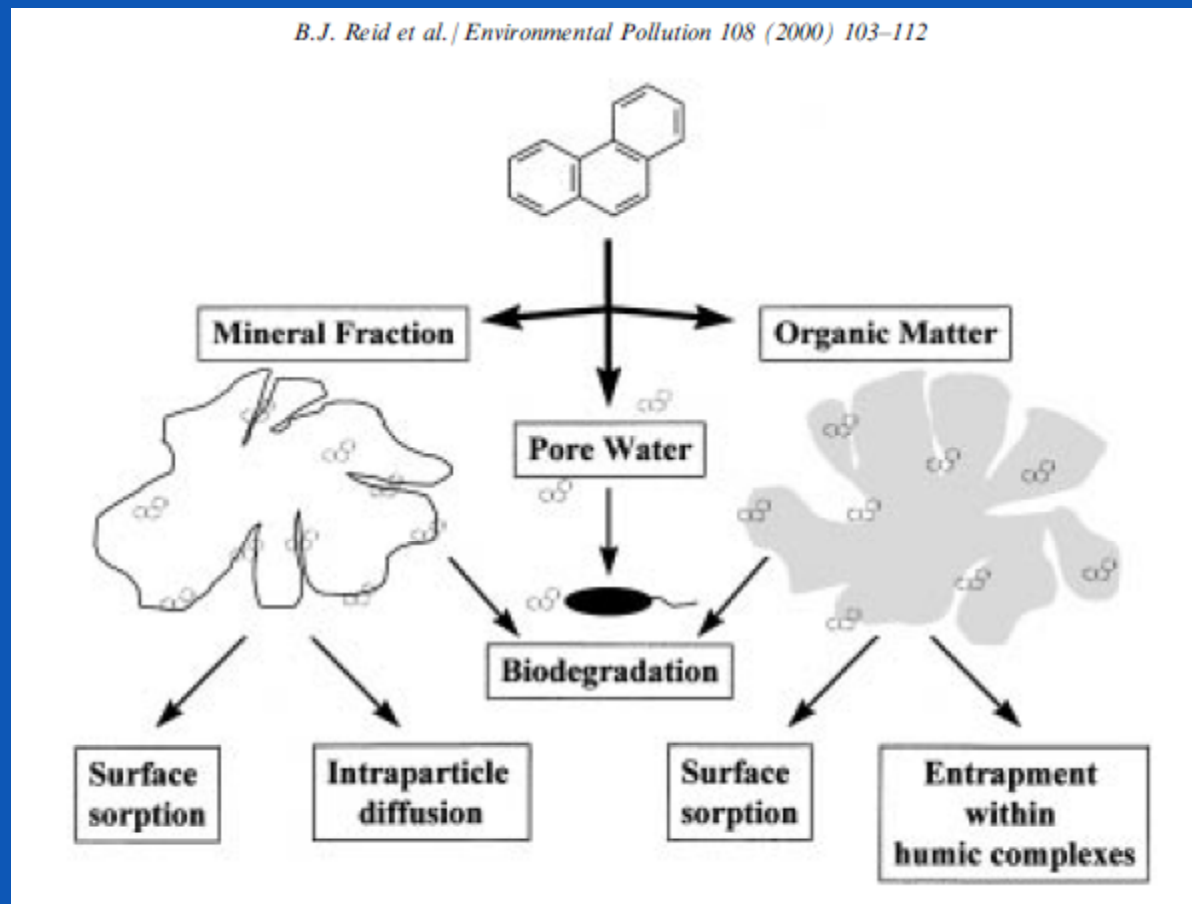
Contents

- Translating the effects on soils/sediments, primary producers, herbivores, carnivores, into a population or ecosystem level effect.
- Terrestrial and aquatic ecosystems.
- Physico-chemistry and ecosystems.
- Experimental Approaches



Soils and Sediments

- At the base of the ecosystem
- Look after the sediments/soils.



Antibacterial Activity is Inversely Related to Particle Size

Neal, *Ecotoxicology* (2008) 17:362–371

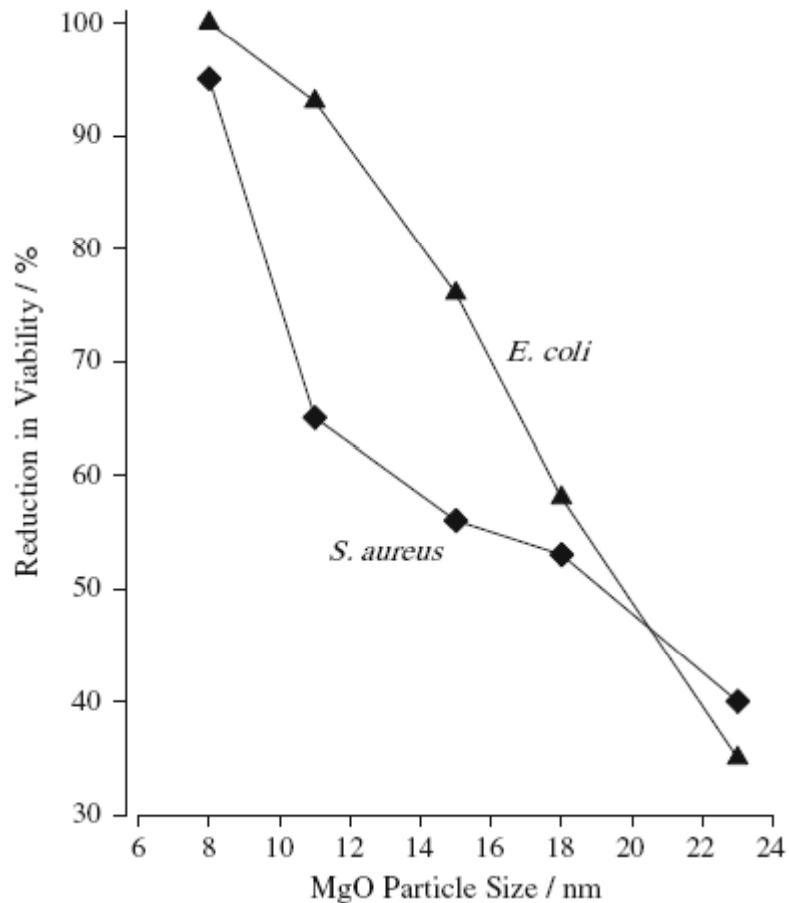


Fig. 1 Nanoparticles exhibit an inverse relationship between antibacterial activity (here expressed as a percentage reduction in viability after 4 h exposure, compared to unexposed cells) and particle size. Data are shown for the Gram-negative bacteria *Escherichia coli* and *Staphylococcus aureus* exposed to 1 mg ml^{-1} of nanoparticles in nutrient broth. (Data taken from Makhluif et al. 2005)



Silver NPs: Behaviour in Natural Estuarine Sediments

Bradford et al. (2009) ES&T

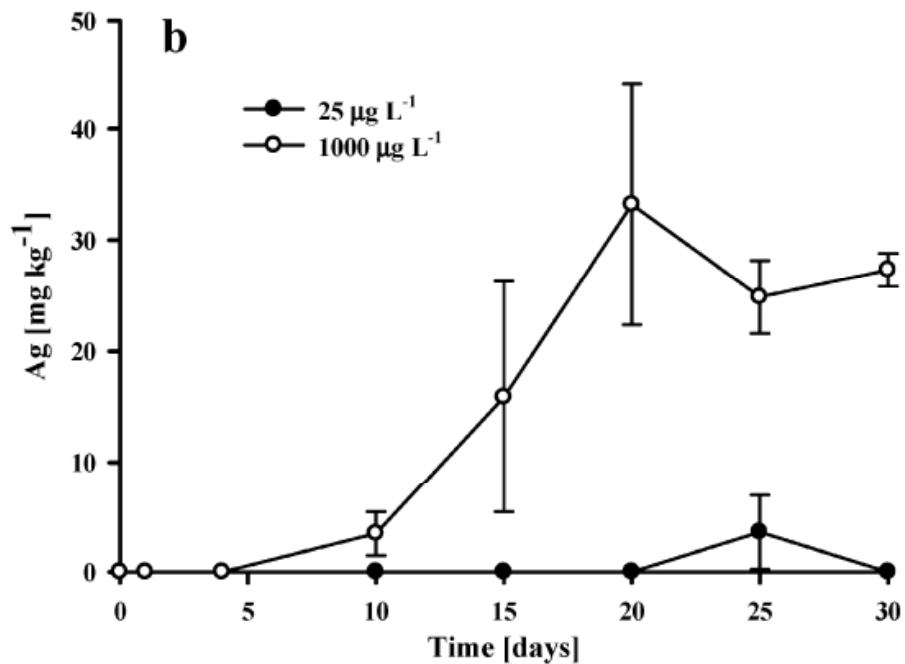
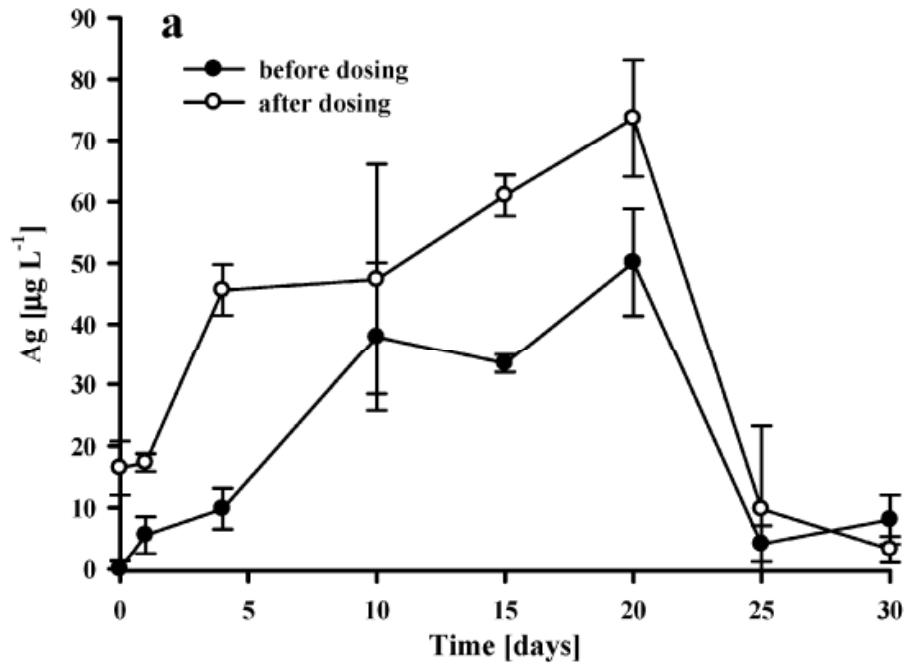


FIGURE 1. Ag concentrations in the overlying estuary water (a) and the surface layer of the sediment core (b) as determined by ICP-OES (mean \pm S. E. M, $n = 3$ tanks). Ag concentrations in the water were measured before and directly after dosing, but were below detection limit (approximately ≤ 1 ng) in the control and 25 $\mu\text{g L}^{-1}$ treatments throughout the time course. Data in panel (a) is for the 1000 $\mu\text{g L}^{-1}$ treatment.

Silver NPs: No Effect on Natural Microbes in Estuarine Sediments

Table 1

Bacterial counts in form of colony forming units (cfus) on medium containing cycloheximide and eight different antibiotics. The experiments were carried out in triplicate (control/0 $\mu\text{g l}^{-1}$ overlaying estuary water: microcosms 1, 2, 3; 50 $\mu\text{g l}^{-1}$: microcosms 4, 5, 6; 2 mg l^{-1} : microcosms 7, 8, 9).

Antibiotic ^a	Microcosm →								
	1	2	3	4	5	6	7	8	9
Cycloheximide	108	65	100	130	142	100	105	97	250
Erythromycin ^b	0	0	0	0	0	0	0	0	0
Oxytetracycline ^b	6	9	3	5	5	5	4	0	9
Amoxicillin ^b	1	15	2	9	5	13	0	0	3
Ceftazidime ^c	6	15	11	8	6	5	8	4	17
Sulfadiazine ^c	24	44	17	34	17	17	23	11	27
Trimethoprim ^c	38	24	46	94	48	17	22	28	27
Lincomycin ^c	28	51	23	76	27	37	25	14	23
Vancomycin ^c	34	42	10	49	185	17	17	5	8

Table 2

Results from permutation-based Analysis of Variance tests for differences among groups of samples, using Type III sums of squares based on 999 permutations of residuals under a reduced model. The factors are antibiotic and Ag-NPs (SS = square sum, MS = medium square, F = ratio of SS/MS).

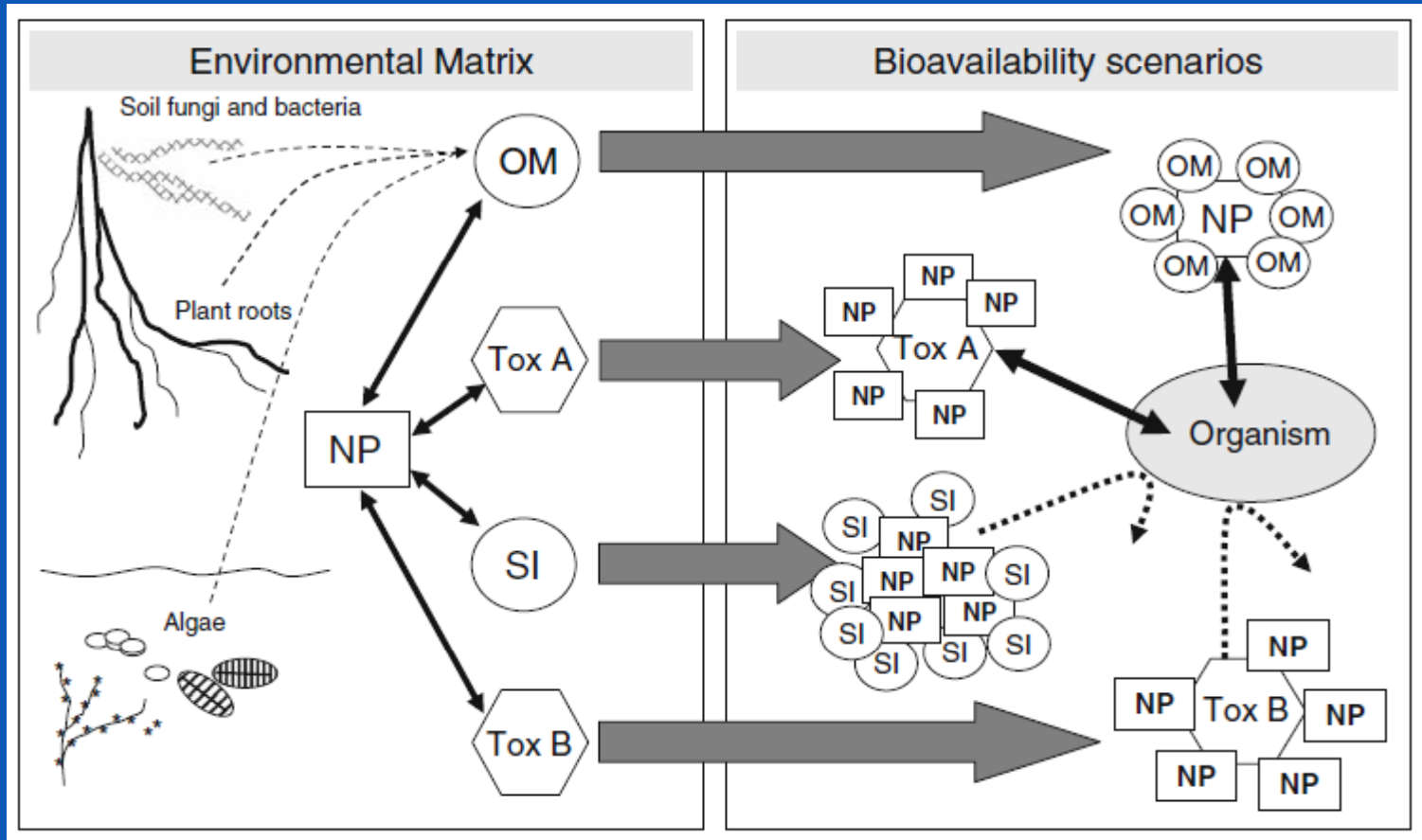
Source	df	SS	MS	F	p
Antibiotic	7	91,580	13,083	15.9	0.001
Ag-NPs	2	2962	1481	1.8	0.164
Antibiotic \times Ag-NPs	14	13,591	971	1.2	0.264
Residual	48	39,565	824		
Total	71	147,700			

Muhling et al. (2009) Marine
Environmental Research



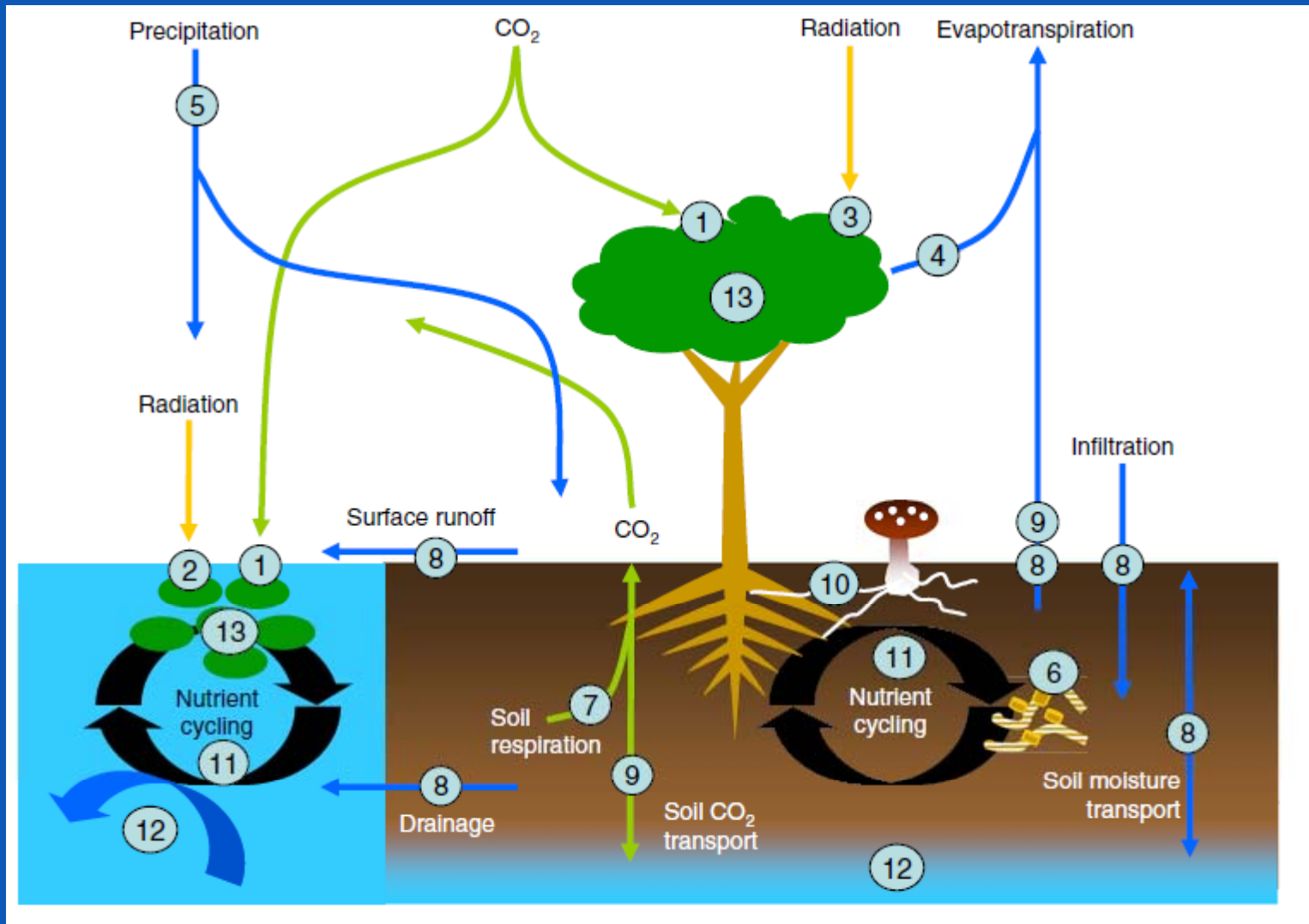
Algae, Fungi, Terrestrial Plants

Navarro et al. (2008) *Ecotoxicology*, 17:372–386



Unknown Effects on Nutrient Cycling, Water Depuration & Biomass Production

Navarro et al. (2008) *Ecotoxicology*, 17:372–386



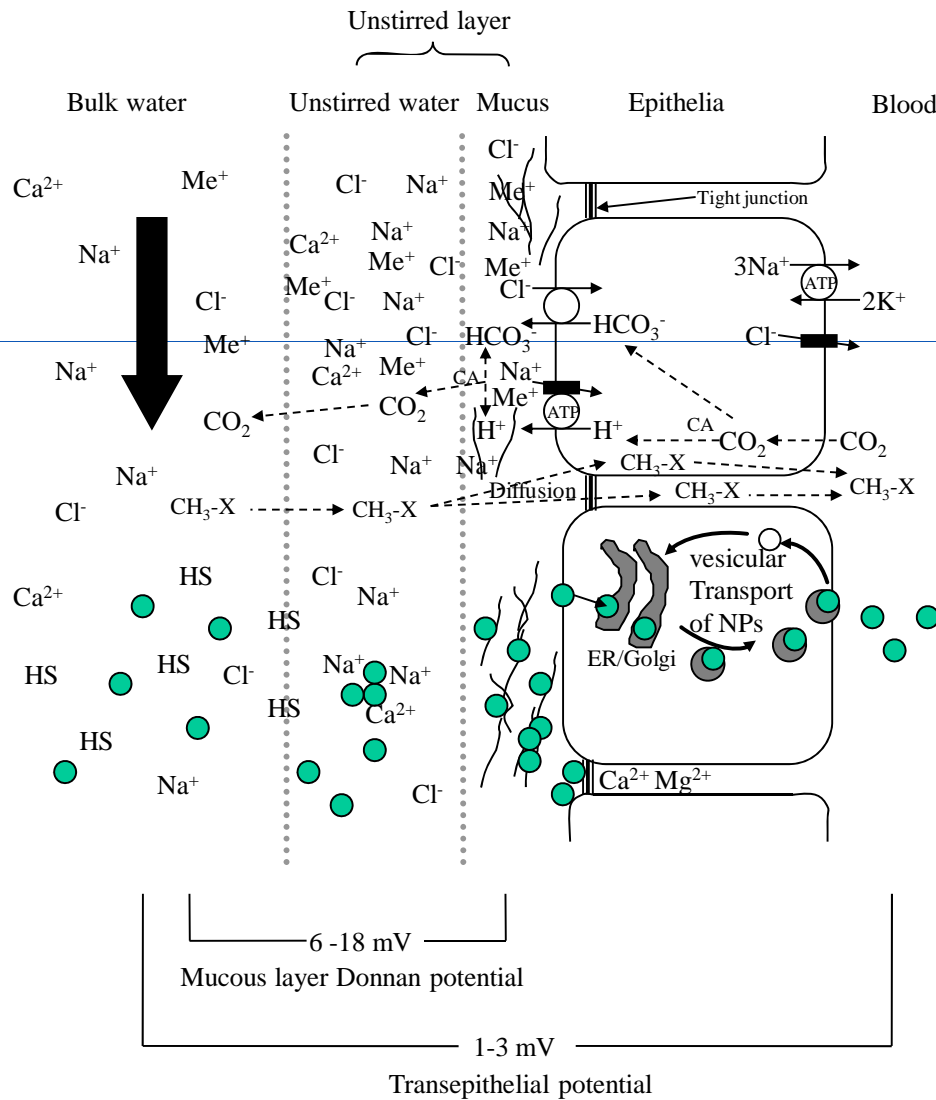
Dietary Exposure

- Dietary bioavailability and uptake measurements are needed.
- Gut chemistry and NP adsorption onto epithelia.
- Relate by feeding habit and gut anatomy, not by particle chemistry alone.
- Herbivores, carnivores, tropical, temperate.
- Food webs: species sensitivity with/without biomagnification.
- Gut function is about energy acquisition by animals.
- Use bioenergetics to link individual with population level effects.
- Trade offs-animals preserve growth at the expense of locomotion/behaviour and reproduction.



Uptake of NPs Across Epithelia

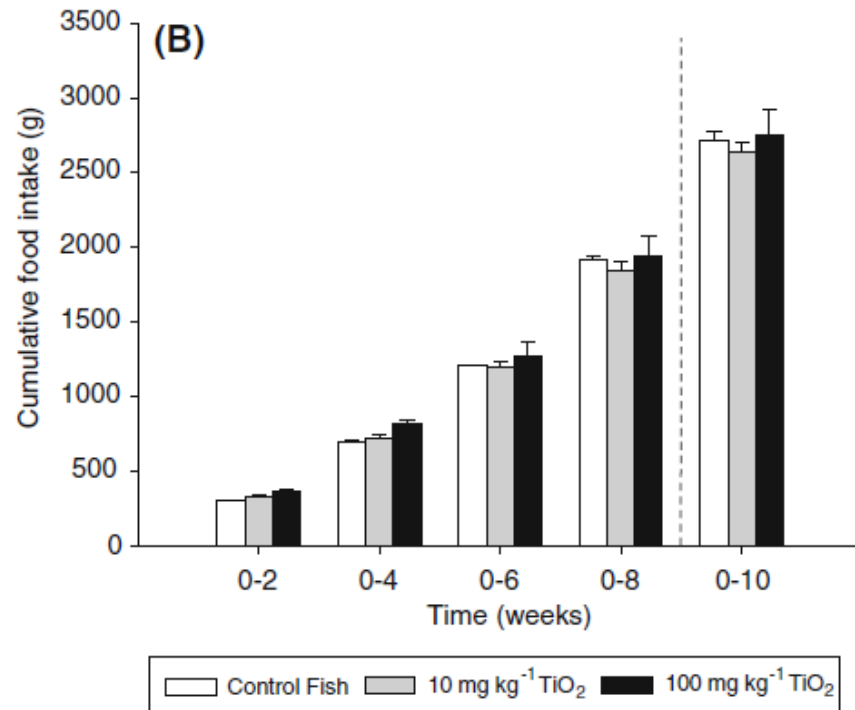
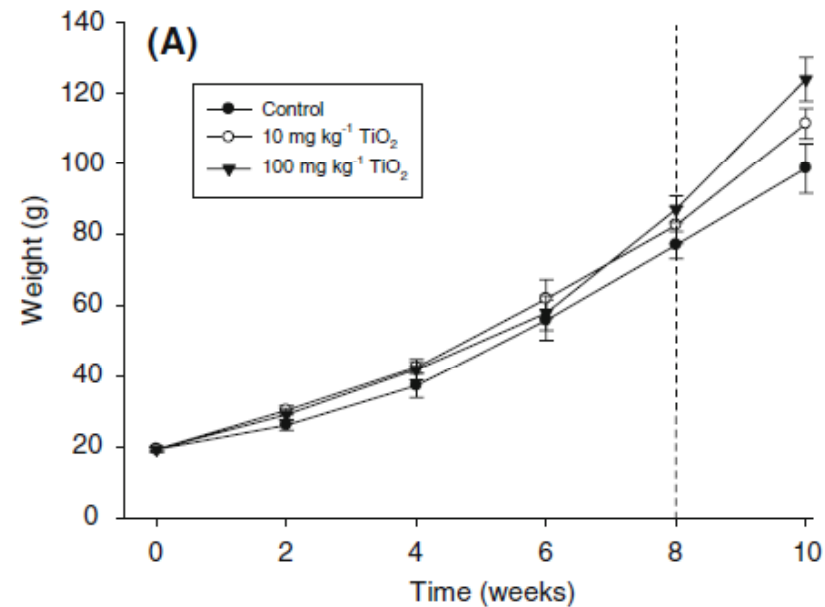
Handy et al., 2008.
 Ecotoxicology, 17, 396–409



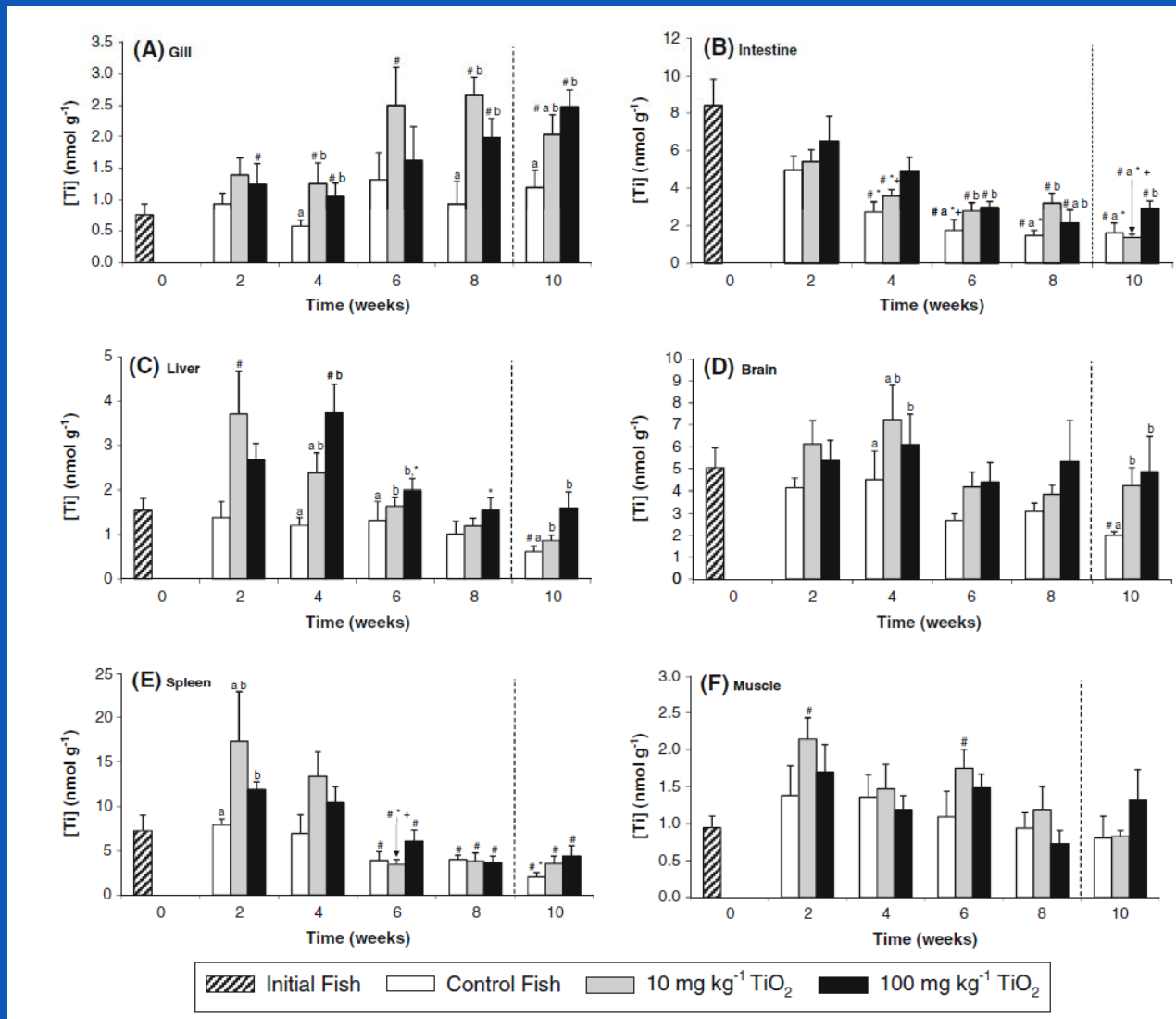
Dietary TiO₂: Growth & Food Intake by Rainbow Trout

Ramsden et al (2009)
Ecotoxicology, 18:939-951

No statistical differences
between treatments
(ANOVA, $P > 0.05$)

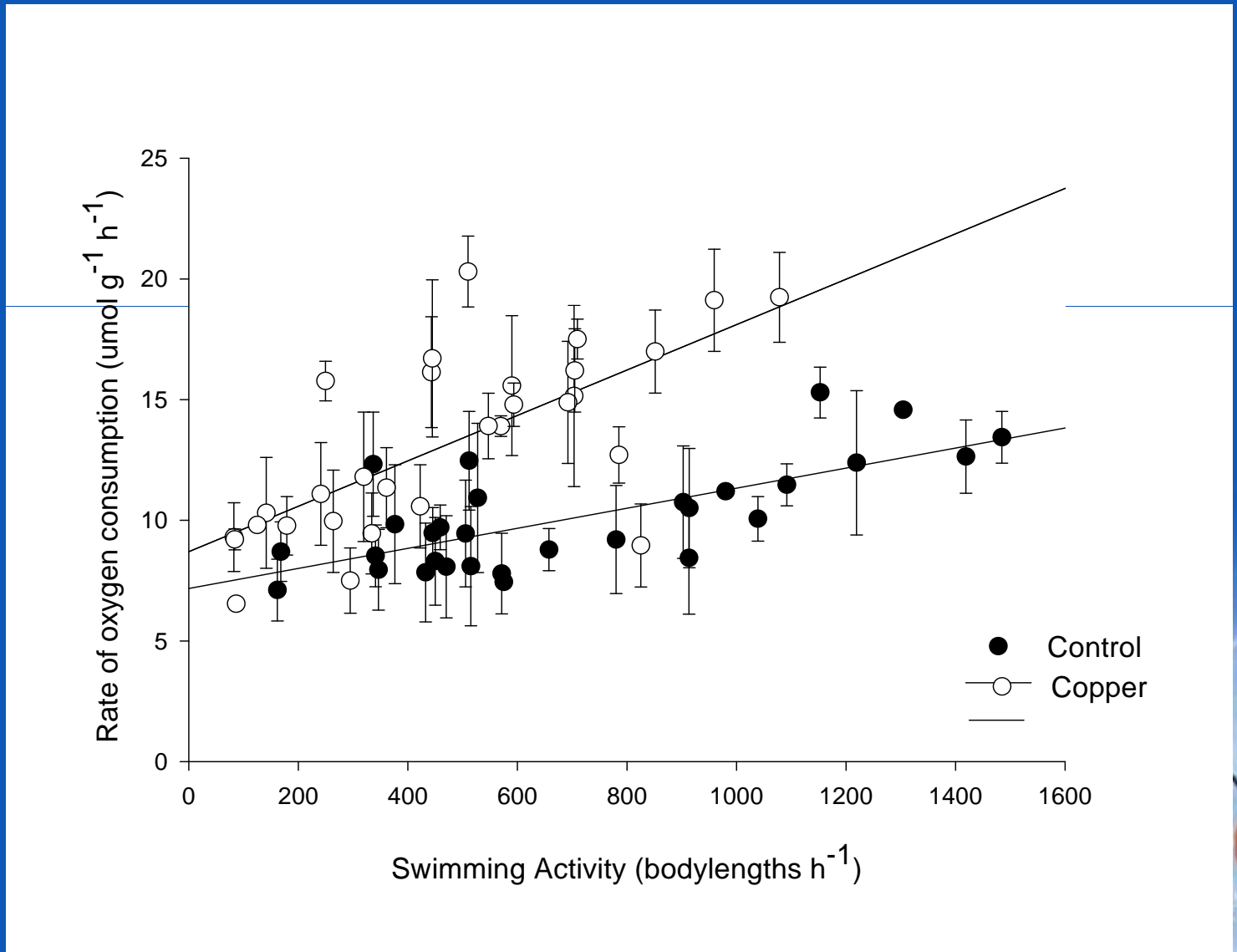


Dietary TiO₂: Titanium Increases in Some Internal Organs-But Dynamic.



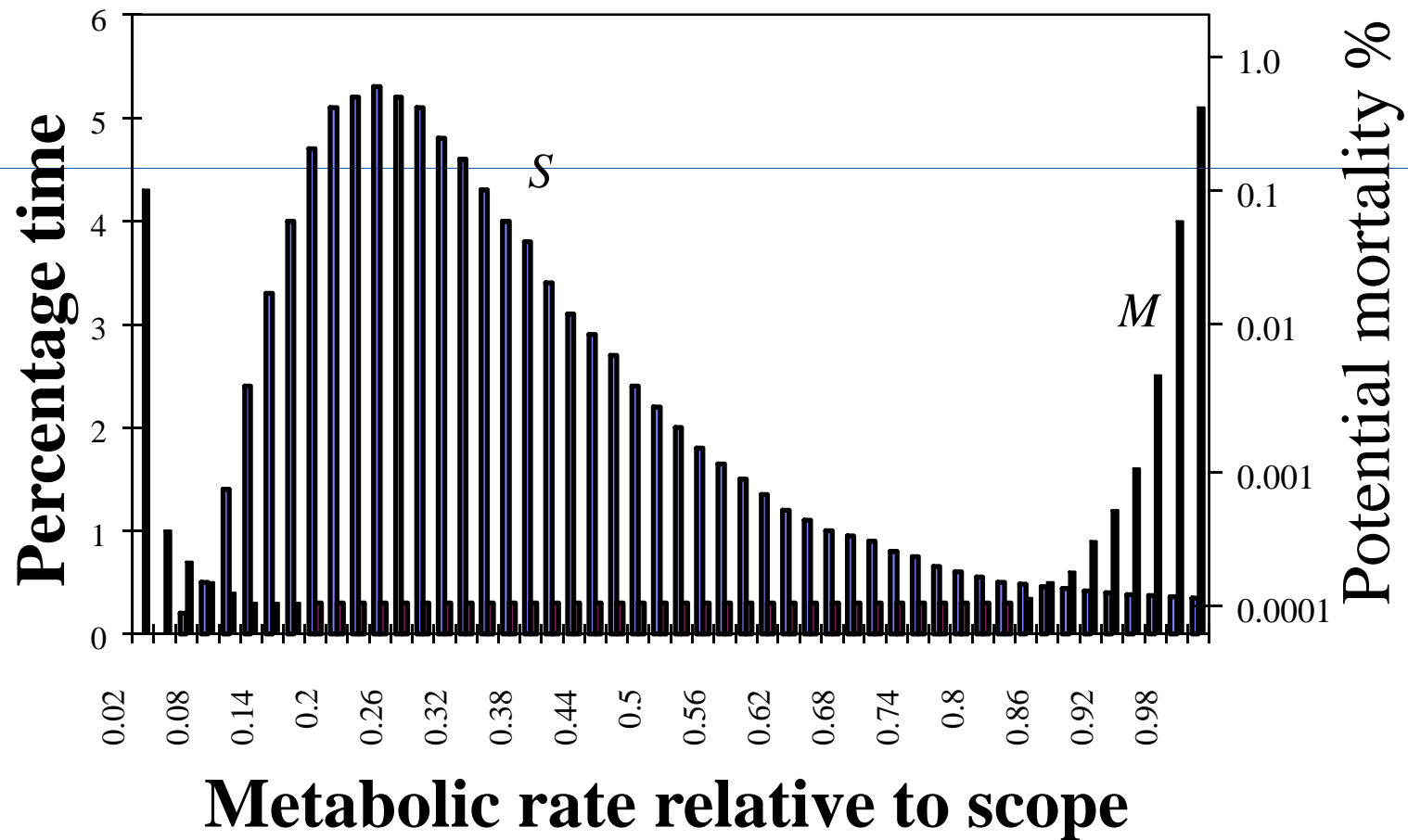
Effect of Dietary Copper on Metabolic Rate of Trout

Campbell et al. (2002) CJFAS



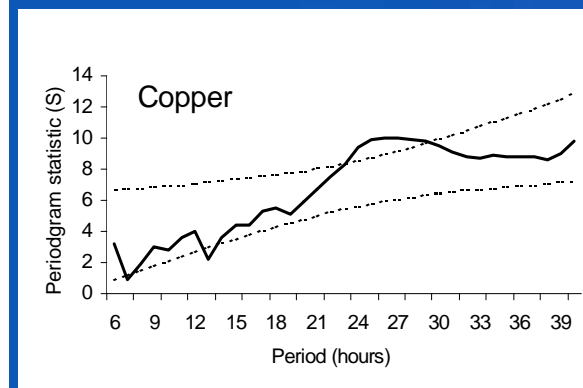
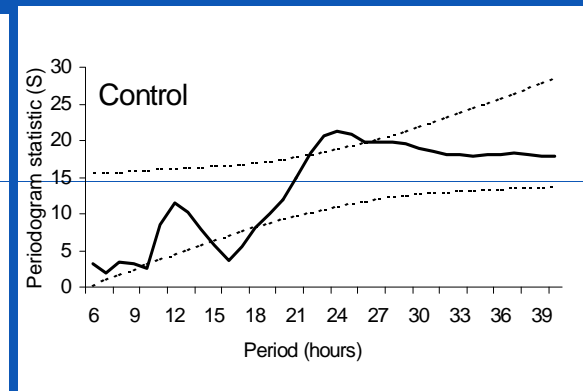
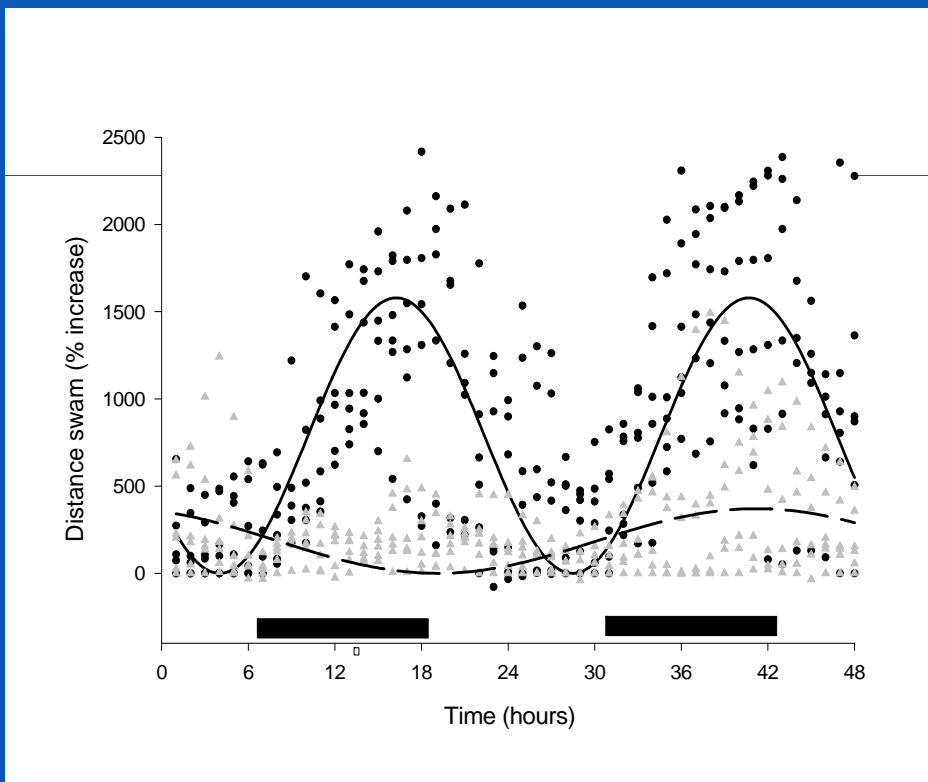
Relationship Between Activity Level and Potential Mortality

(after Priede 1977)



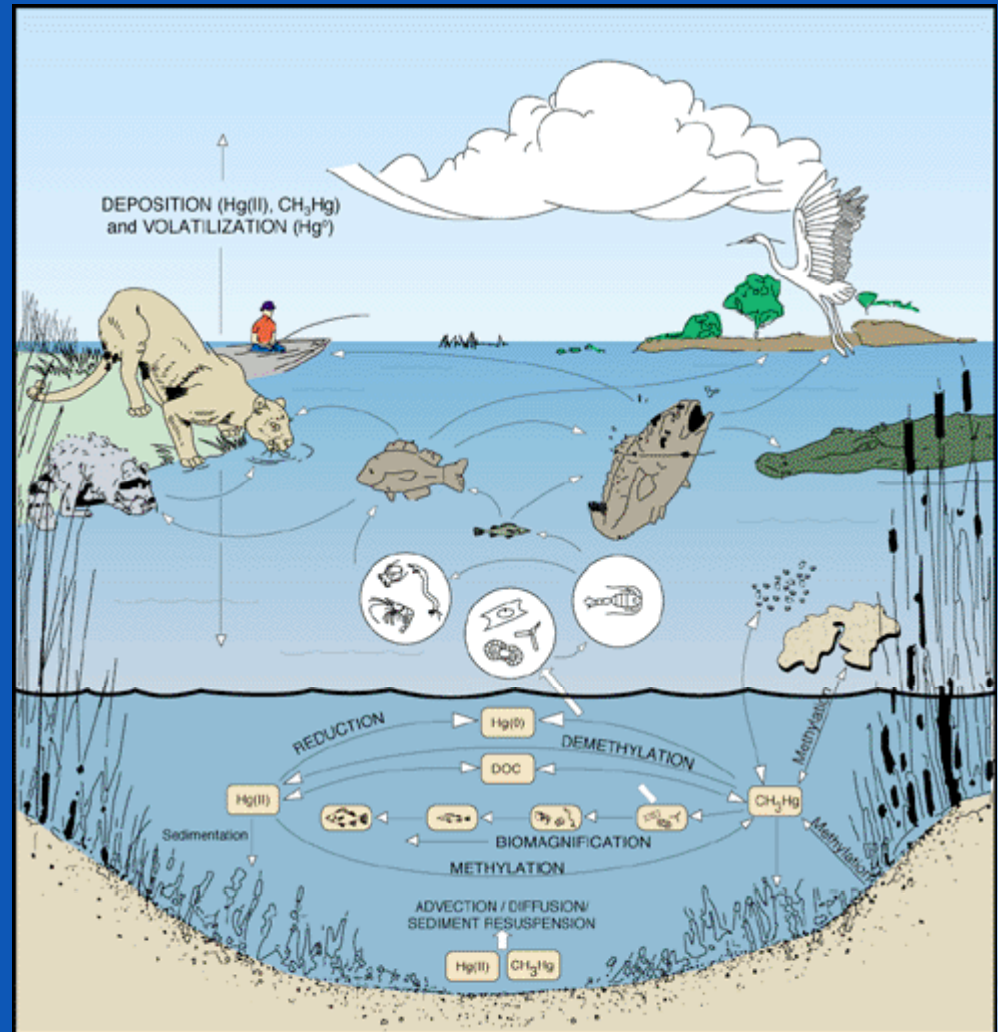
Space and Time Effects in Ecosystems: Loss of Biological Clock in Trout After Dietary Copper.

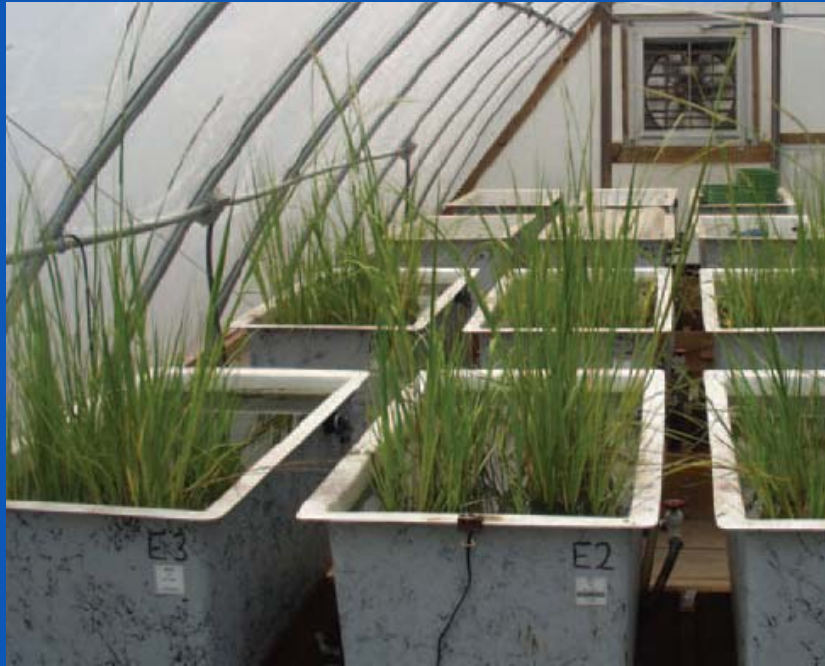
Campbell et al. (2002) CJFAS



Fate, Behaviour and Biological Effect At Ecosystem Level

- No where near enough data to make accurate models!
- Field & mesocosm experiments (top down).
- Model from component parts/organisms (bottom up).





Gold NPs in Estuarine Mesocosms

Ferry et al., 2009. Nature Nanotechnology.

Table 1 | Distribution of gold in estuarine mesocosms after aqueous introduction.

Phase (g)	Gold ($\mu\text{g kg}^{-1}$) [‡]		C_f^{\S}	Per cent recovered gold in a given phase
	0 days	12 days		
Sea water (3.66×10^5) [*]	<LOD	0.42 ± 0.22	1.00	8.61 ± 4.51
Sediment (4.91×10^4) [†]	<LOD	13.9 ± 0.7	33.1	24.5 ± 1.23
Biofilm (1.01×10^3) [*]	12.2 ± 0.8	$6.41 \pm 0.28 \times 10^3$	1.53×10^4	61.0 ± 2.65
<i>Spartina alterniflora</i> (grass, 1.50×10^3) [*]	2.68 ± 2.01	3.45 ± 1.91	8.21	0.10 ± 0.06
<i>Palaemonetes pugio</i> (grass shrimp, 15.6) [†]	0.388 ± 0.30	48.1 ± 23.0	1.15×10^2	0.03 ± 0.01
<i>Cyprinodon variegatus</i> (GI tract and organs, sheepshead minnow, 22.5) [†]	0.964 ± 0.685	$1.99 \pm 2.34 \times 10^2$	4.74×10^2	0.31 ± 0.37
<i>Ilyanassa obsoleta</i> (snail, 5.5) [*]	<LOD	70.1 ± 33.2	1.67×10^2	0.05 ± 0.02
<i>Mercenaria mercenaria</i> (juvenile clams, 10.0) [*]	<LOD	$9.57 \pm 2.44 \times 10^3$	2.28×10^4	5.79 ± 1.48

^{*}Estimated mass of a phase in grams. [†]Measured mass of a phase in grams. [‡]Gold atom content in ppb at $t = 0$ and $t = 12$ days based on dry weight for non-aqueous samples. [§]Concentration factor: $C_f = C_{\text{phase}}/C_{\text{water}}$ at $t = 12$ days. ^{||}Mass balance and relative error estimated from measured mass of water and sediment, with an assumption of 2 mm photosynthetic biofilm thickness throughout, and water contents of 36% (sediment), 67% (biofilm), 64% (*Spartina*), 80% (*Palaemonetes*), 72% (*Cyprinodon*), 36% (*Ilyanassa*) and 46% (*Mercenaria*)²⁵⁻²⁹. Limit of detection (LOD) for this method is $18.0 \pm 0.5 \text{ pg kg}^{-1}$. All concentration measurements report the grouped mean of three separate samples per tank ($n = 9$) averaged across the replicate tanks accompanied by the pooled standard deviation.

Abiotic Factors

- Abiotic factors relating to particle behaviour.
 - pH effects on point of zero charge (aggregation)
 - Ionic strength and divalent ions (“water hardness”)
 - Dissolved organic matter, ligand chemistry.
- Broader hydrological and climate issues
 - Flow dynamics of rivers, micro-environments with different chemistry that may “concentrate” NPs.
 - Topography of sea bed, river bed etc.
 - Micro-climates; tree canopy, high altitude sites vulnerable to precipitation; extreme temperatures.
 - Relationships with particle energy and temporal changes in particle chemistry, or size (dissolution/weathering).



TiO₂ NP Aggregation in Salines

Vevers & Jha (2008) *Ecotoxicology* (2008) 17:410–420

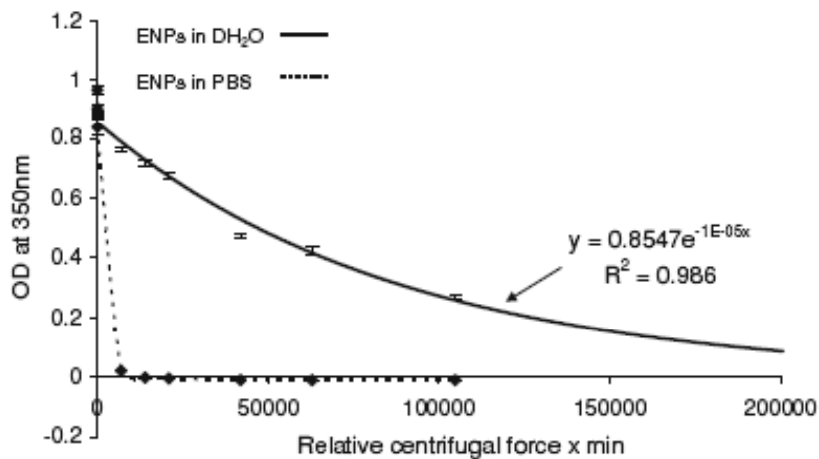
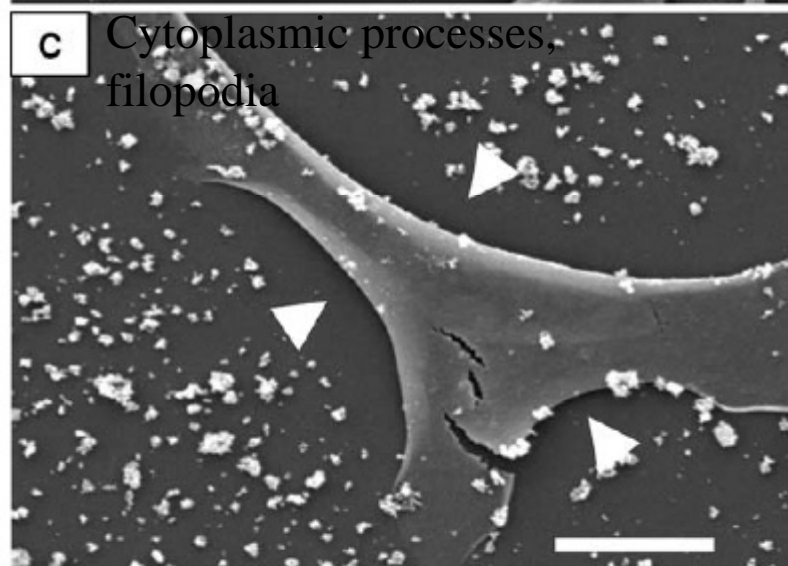
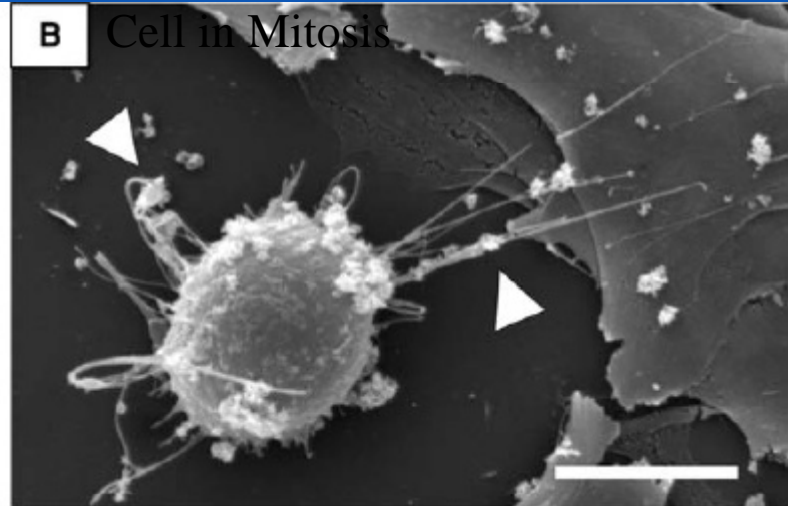
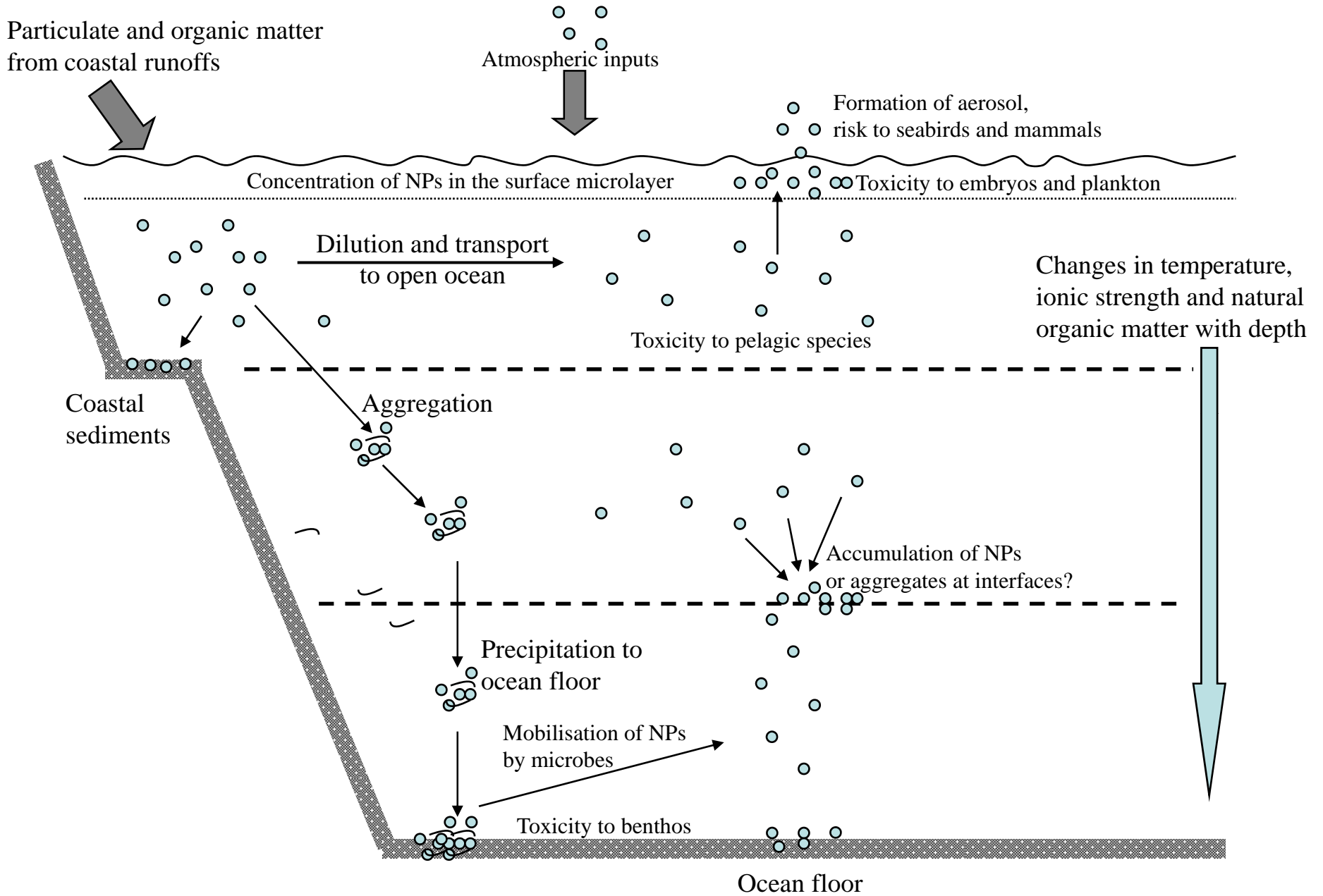


Fig. 2 A comparison of TiO₂ ENPs aggregation potential in PBS and H₂O (50 $\mu\text{g ml}^{-1}$). The optical density at 350 nm of serially sampled volumes of ENP suspension (mean \pm SE of $3 \times 200 \mu\text{l}$) is plotted against the RCF \times min to normalise the increase in RPM over the centrifugation period





Conclusions on Knowledge Gaps

- Need to employ all our experimental tools
 - Study real ecosystems, and mesocosm approaches
 - Data on the individual physical and biological components of ecosystems are also needed (reductionist approach).
 - Real wild-type organisms of ecological importance from different phyla- not just standard OECD/ISO method organisms.
- Our knowledge of the effects of NPs on plants is particularly weak compared to animal biology.
- More work on terrestrial systems.
- Dietary exposure and food chain studies are needed.
- Bioenergetics to link individuals with populations
- Abiotic factors relating to particle aggregation chemistry are not the only ones to consider.
- Micro-climate, micro-environments, hydrology etc.



Environmental Monitoring: What Compartments and Receptors (Organisms) to Prioritise?

- Uncertainty because of the knowledge gaps.
- Sediments/soil and sediment dwelling organisms a priority.
- Key stone species in food webs.
- Standard ecotoxicity test organisms
 - The consensus view is that we should continue to use these in Europe (e.g. Crane et al., 2008, Ecotoxicology, 17, 421-437)
- Whole effluent testing/Direct toxicity assessment approaches?
- Rapid Screening tools
 - Microtox, MARA, etc. Need comparing against higher tier organisms in the laboratory to validate their use with NPs.
 - Chemical methods need developing e.g., particle reactivity assays etc.
- The usual combination of chemical and biological monitoring in a tiered approach
- Review and re-visit more frequently as data on novel effects emerge.



Research Priorities

- The knowledge gaps! But which one is the most important?
- Soil and sediments.
- Food chains; sediments through to man.
- Measurement techniques for NPs in complex matrices such as soil, natural water, tissues.
- “Plan B” on measurement; antibody/bioassay approaches (endocrine disrupters & VTG assay analogy).
- Plants and terrestrial ecosystems as one of the big knowledge gaps; tree canopy effects (air) and soil contamination (root functions of plants).
- More fundamental research rather than applied science, so we don't make “wrong assumptions” in our thinking.
 - Toxic mechanisms on key biochemical pathways; photosynthesis, respiration, geochemical cycles (nitrogen, water).
 - Control systems in organisms; endocrine, immune, nervous systems.
- NPs as “delivery vehicles” for other contaminants.



Any Questions?

