Ecotoxicologie des nanoparticules en milieu marin

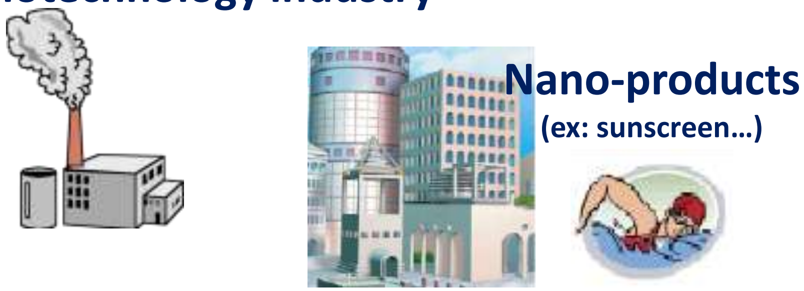
C. Mouneyrac,

http://www.uco.fr

Atelier thématique « nano-environnement » CNRS 19 novembre 2013

ENMs in estuaries and coastal waters: Relevance?

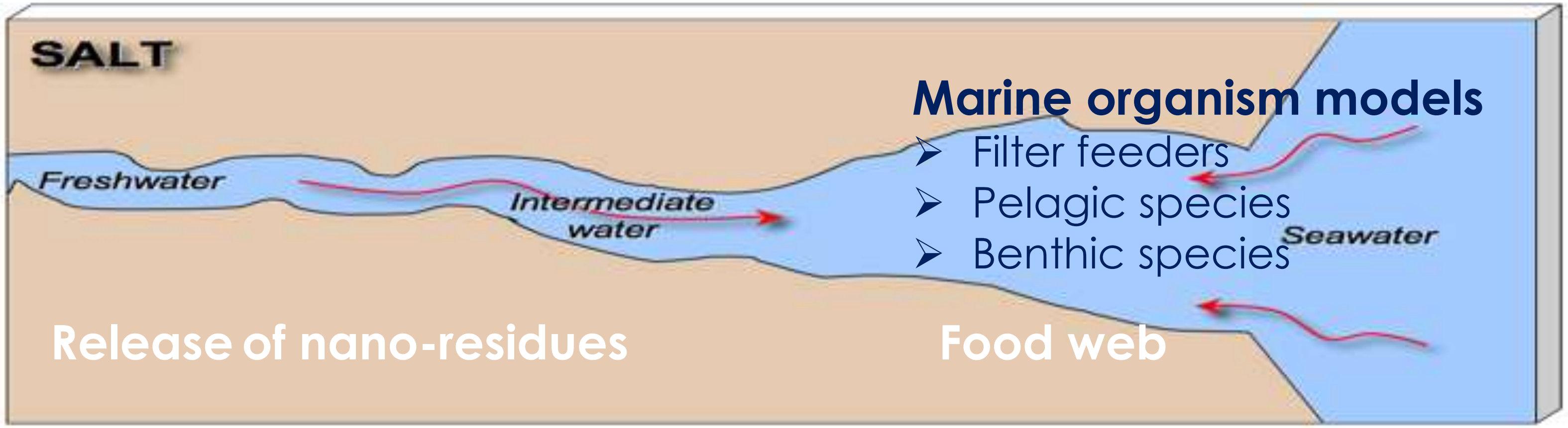
Nanotechnology industry



Nano-products
(ex: sunscreen…)

Three main sources of ENPs in marine ecosystems:

- ➢ Personal care products
- ➢ Urban and seawage plants
- ➢ Antifouling and paints



Marine organism models

- ➢ Filter feeders
- ➢ Pelagic species
- ➢ Benthic species

Release of nano-residues

Food web

- ➢ 40 000 km3/year of freshwater flows into the worldwide oceans through the main rivers.
- ➢ The major part of this freshwater water is not treated before reaching the rivers.
- ➢ ENMs accidentally or not (usage, end of life) released in the environment reach estuarine or coastal areas.

Key questions ?

Transformations?
Fate and behaviour ?

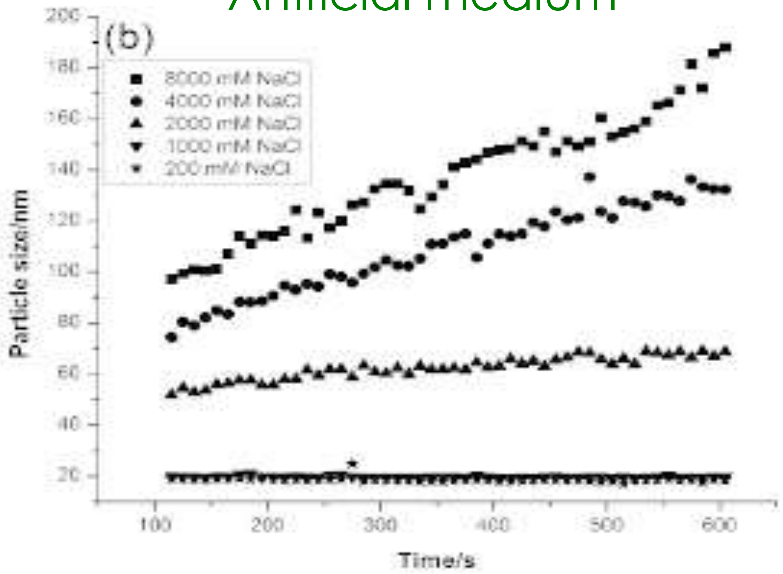
Can they be transformed?
What do they become?
Which concentrations?
Do transformations affect toxicity?

Interaction with living organisms?
Uptake?
Bioavailablility?
Bioaccumulation?
Localization?
Toxicity effects?

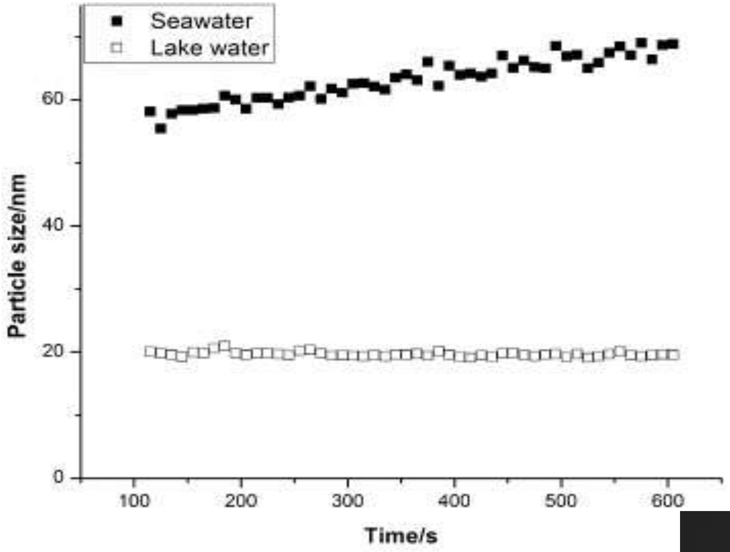
Which mechanisms ?
Transfer in the food web?

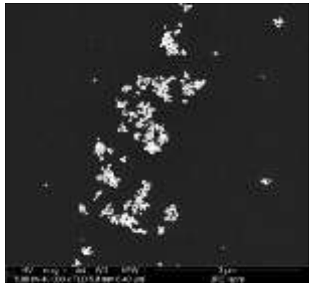
Aggregation in seawater?

Artificial medium



Natural medium





A higher aggregation rate of Ag NPs in seawater due to the reduction of electric repulsion force and energy barrier between nanoparticles. Cl^- promote bridges between NPs

Does aggregation reduces bioavailability?

Citrate capped Ag NPs

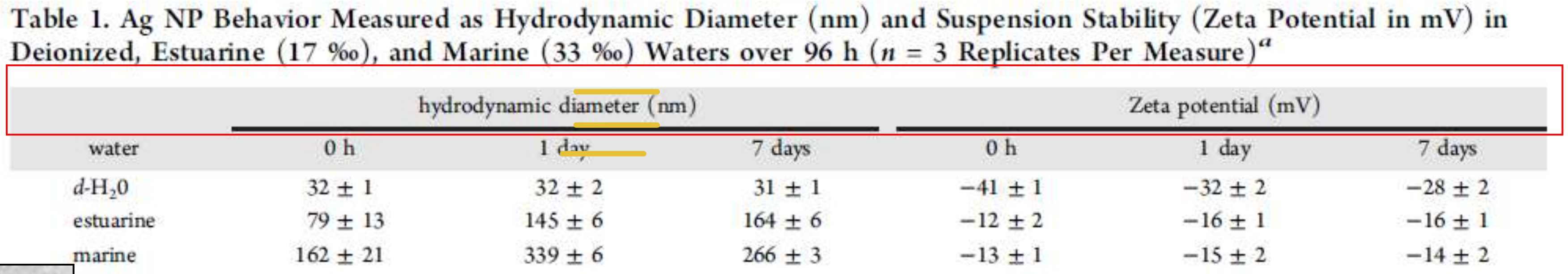
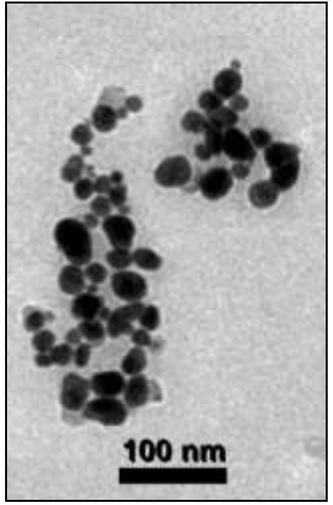
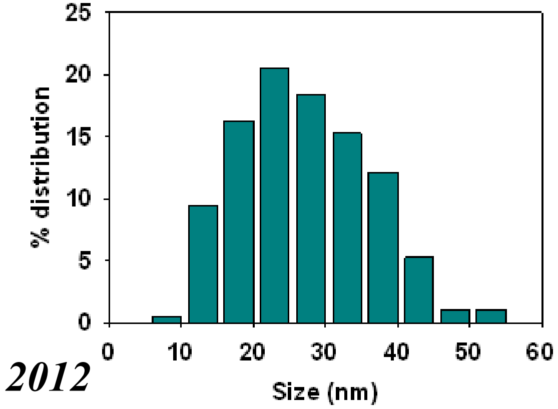


Table 1. Ag NP Behavior Measured as Hydrodynamic Diameter (nm) and Suspension Stability (Zeta Potential in mV) in Deionized, Estuarine (17 ‰), and Marine (33 ‰) Waters over 96 h (n = 3 Replicates Per Measure)[a]

	hydrodynamic diameter (nm)			Zeta potential (mV)		
water	0 h	1 day	7 days	0 h	1 day	7 days
d-$H_2$0	32 ± 1	32 ± 2	31 ± 1	−41 ± 1	−32 ± 2	−28 ± 2
estuarine	79 ± 13	145 ± 6	164 ± 6	−12 ± 2	−16 ± 1	−16 ± 1
marine	162 ± 21	339 ± 6	266 ± 3	−13 ± 1	−15 ± 2	−14 ± 2

Increase in aggregation at higher salinities





Khan et al., 2012

Solubility study

No detectable release of dissolved Ag over 24hr solubility in 1mM NaNO3.

Biodynamic model

Biodynamic parameters

Peringia ulvae

Lymnaea stagnalis

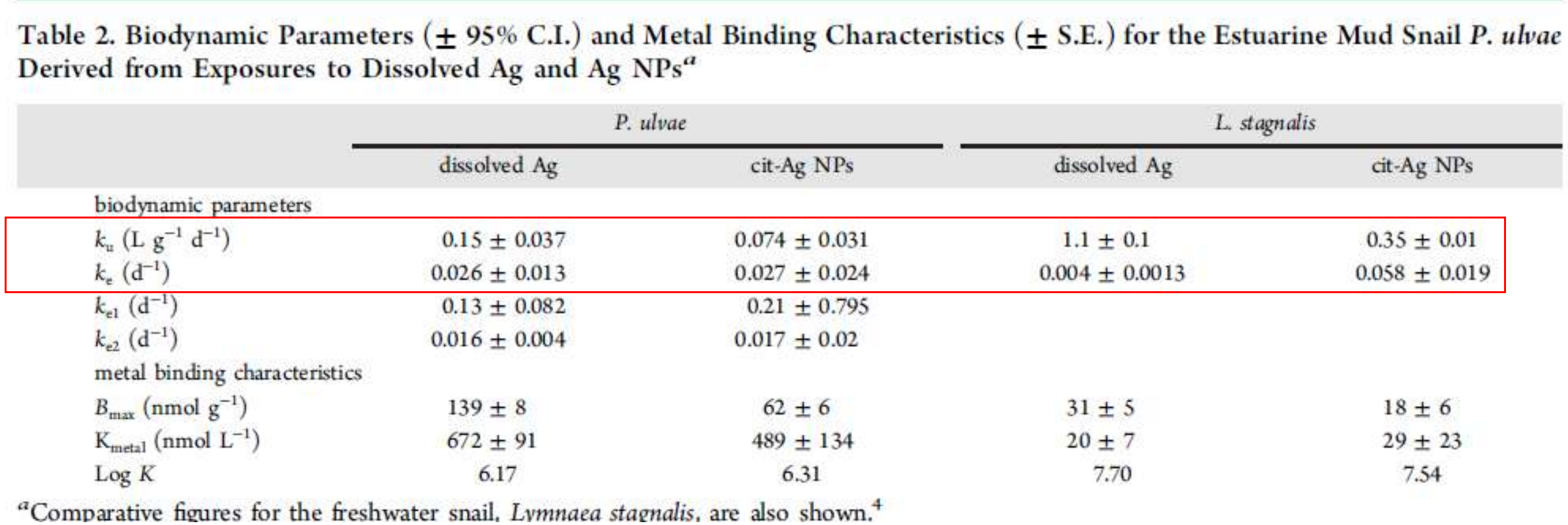


Table 2. Biodynamic Parameters (± 95% C.I.) and Metal Binding Characteristics (± S.E.) for the Estuarine Mud Snail *P. ulvae* Derived from Exposures to Dissolved Ag and Ag NPs[a]

	P. ulvae		*L. stagnalis*	
	dissolved Ag	cit-Ag NPs	dissolved Ag	cit-Ag NPs
biodynamic parameters				
k_u (L g^{-1} d^{-1})	0.15 ± 0.037	0.074 ± 0.031	1.1 ± 0.1	0.35 ± 0.01
k_e (d^{-1})	0.026 ± 0.013	0.027 ± 0.024	0.004 ± 0.0013	0.058 ± 0.019
k_{e1} (d^{-1})	0.13 ± 0.082	0.21 ± 0.795		
k_{e2} (d^{-1})	0.016 ± 0.004	0.017 ± 0.02		
metal binding characteristics				
B_{max} (nmol g^{-1})	139 ± 8	62 ± 6	31 ± 5	18 ± 6
K_{metal} (nmol L^{-1})	672 ± 91	489 ± 134	20 ± 7	29 ± 23
Log *K*	6.17	6.31	7.70	7.54

[a] Comparative figures for the freshwater snail, *Lymnaea stagnalis*, are also shown.[4]

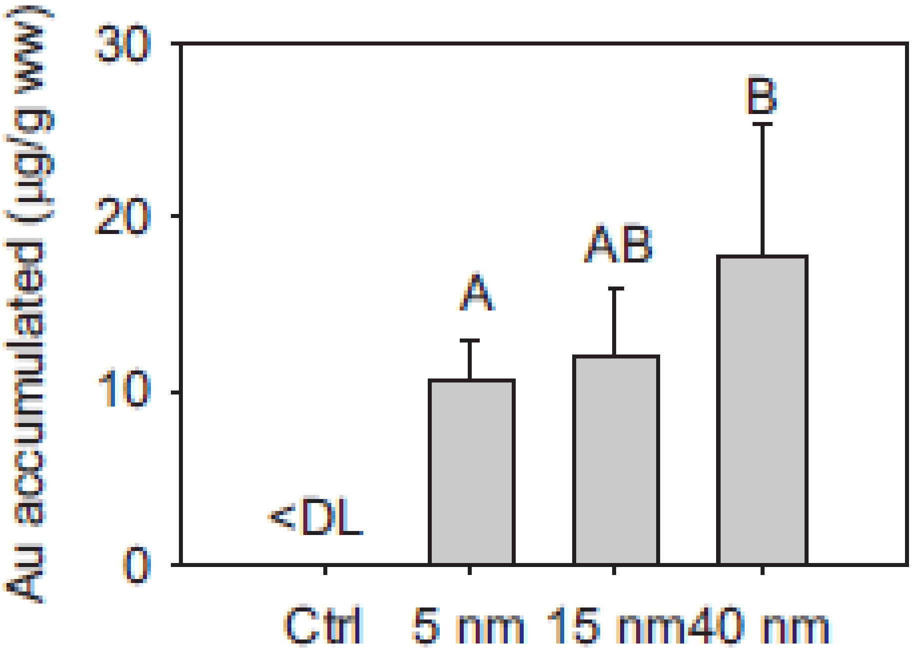
- Dissolved Ag is more available than Ag NPs in both species
- Both forms are more bioavailable in freshwater
- Ag bioavailability is reduced by greater complexation of Ag^+ in seawater and increased agregation

Khan et al., 2012

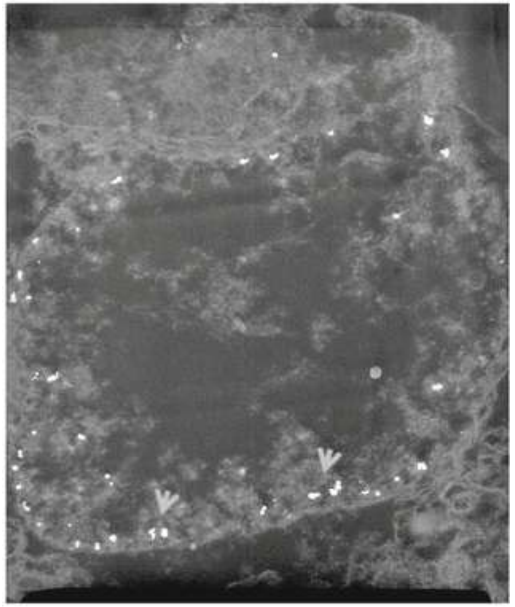
NanoReTox

Do NPs can be incorporated in biological tissues?

Bioaccumulation of gold nanoparticles in the clam *Scrobicularia plana*



Pan et al., 2012, Environmental Pollution



Localization within the nuclei
colocalized with DNA

Joubert et al., 2013
Gold Bulletin

NanoReTox

Uptake and localization of TiO_2 NPs on mussels?

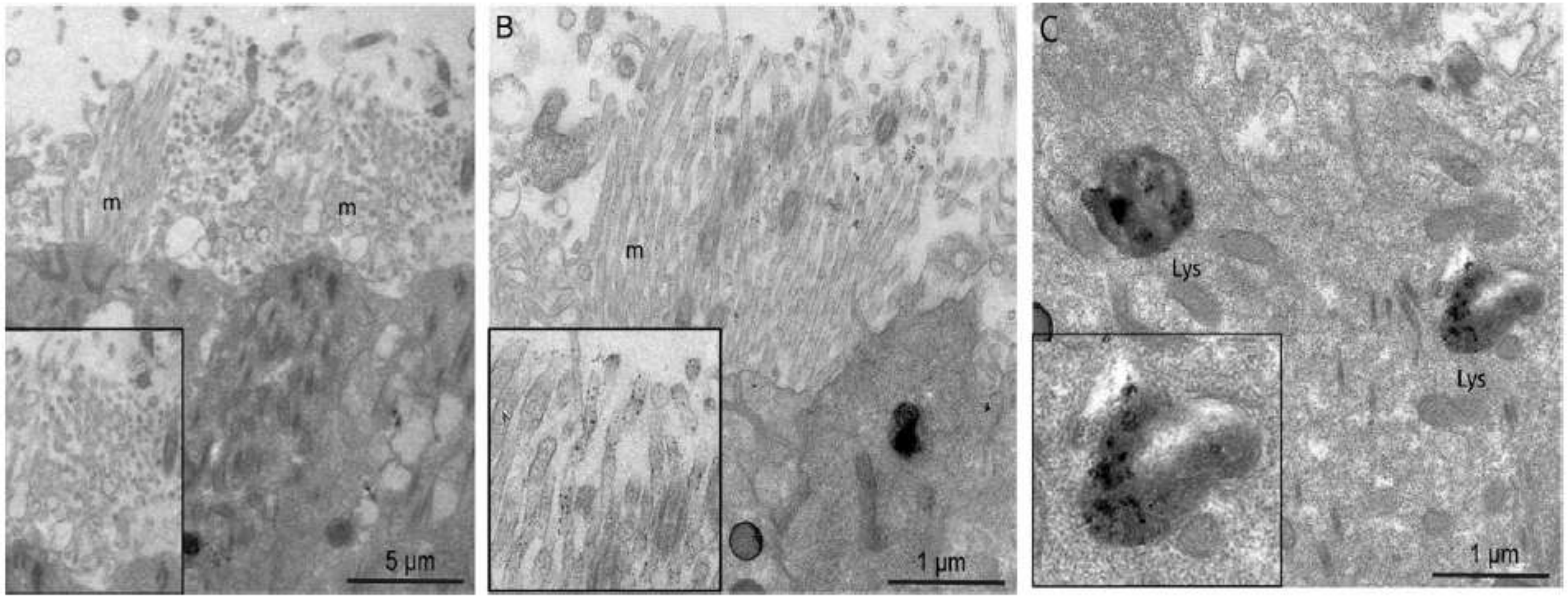


Fig. 5. Electron microscopy of *M. galloprovincialis* digestive glands. Representative images obtained from control (A) and n-TiO_2-exposed mussels (100 µg L^{-1}, 96 h) (B and C). (A) Low magnification image of the epithelium of a digestive tubule from control digestive gland. Digestive epithelium shows the typical features of polarised epithelia. The apical surface is decorated with several microvilli, which represent extensions of the apical plasma membrane towards the lumen of the tubule. Microvilli increase the membrane surface for nutrient uptake. The cytoplasm is enriched with dark lysosomal granules; (B) apical surface of an epithelial cell from mussels exposed to n-TiO_2. Note several n-TiO_2 particles on microvilli (see enlargement); (C) n-TiO_2 particles within lysosomes (Lys) of epithelial cells.

Uptake of TiO2 NP
by microvilli of digestive cells,
lysosomal localization

Barmo et al., Aquat. Toxicol., 2013

Do bioaccumulated NPs have noxious effects?

Classic and recent biomarkers

- **Multi-marker approach**: Endpoints at different levels of the biological organization
  - Individual level: survival/mortalityy, growth, reproduction, behaviour (burrowing, feeding rate
  - Sub-individual level: biochemical biomarkers (oxydative stress, genortoxicity, lysosomal membrane destabilization, neurotoxicity)
- « **omics »** approaches to unravel toxicity mechanisms

Ecological Relevance

Do bioaccumulated NPs have noxious effects?

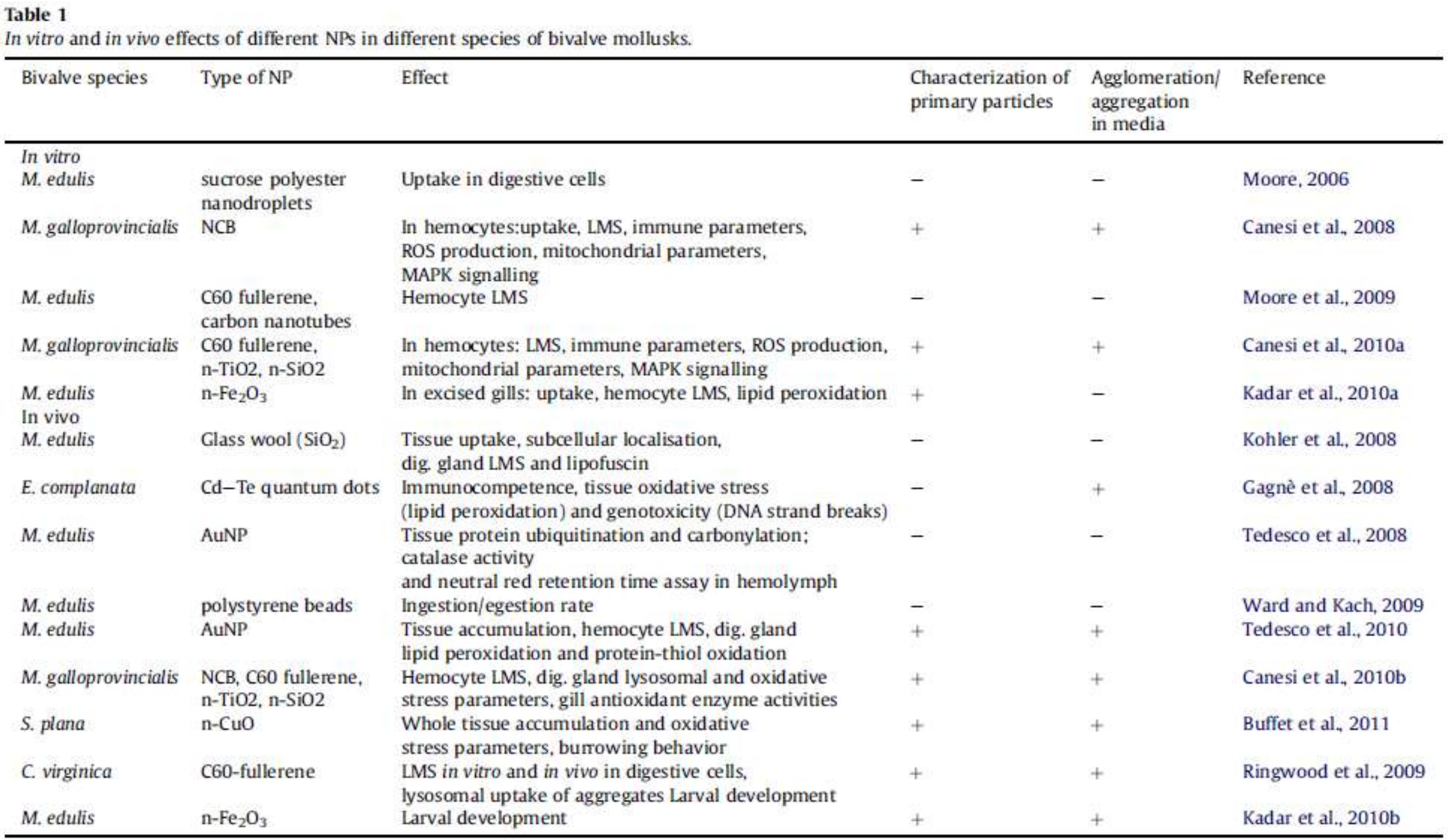


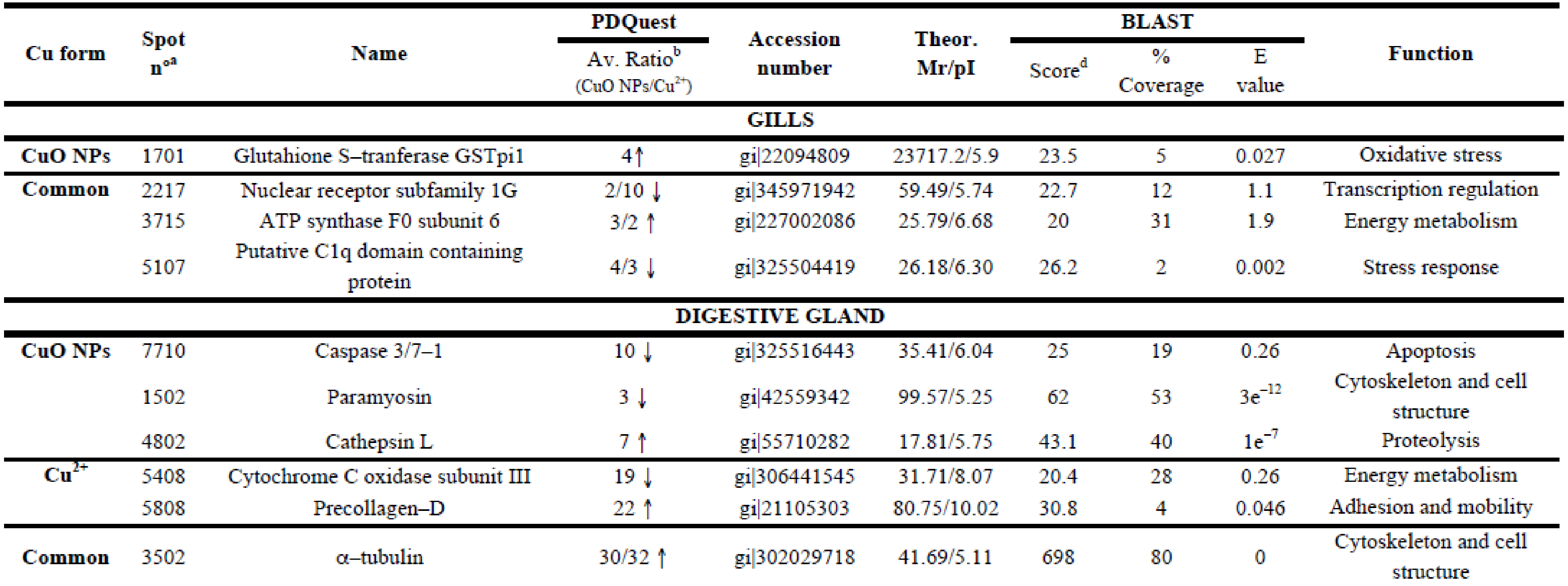
Table 1
In vitro and *in vivo* effects of different NPs in different species of bivalve mollusks.

Bivalve species	Type of NP	Effect	Characterization of primary particles	Agglomeration/ aggregation in media	Reference
In vitro					
M. edulis	sucrose polyester nanodroplets	Uptake in digestive cells	–	–	Moore, 2006
M. galloprovincialis	NCB	In hemocytes:uptake, LMS, immune parameters, ROS production, mitochondrial parameters, MAPK signalling	+	+	Canesi et al., 2008
M. edulis	C60 fullerene, carbon nanotubes	Hemocyte LMS	–	–	Moore et al., 2009
M. galloprovincialis	C60 fullerene, n-TiO2, n-SiO2	In hemocytes: LMS, immune parameters, ROS production, mitochondrial parameters, MAPK signalling	+	+	Canesi et al., 2010a
M. edulis	n-Fe_2O_3	In excised gills: uptake, hemocyte LMS, lipid peroxidation	+	–	Kadar et al., 2010a
In vivo					
M. edulis	Glass wool (SiO_2)	Tissue uptake, subcellular localisation, dig. gland LMS and lipofuscin	–	–	Kohler et al., 2008
E. complanata	Cd–Te quantum dots	Immunocompetence, tissue oxidative stress (lipid peroxidation) and genotoxicity (DNA strand breaks)	–	+	Gagnè et al., 2008
M. edulis	AuNP	Tissue protein ubiquitination and carbonylation; catalase activity and neutral red retention time assay in hemolymph	–	–	Tedesco et al., 2008
M. edulis	polystyrene beads	Ingestion/egestion rate	–	–	Ward and Kach, 2009
M. edulis	AuNP	Tissue accumulation, hemocyte LMS, dig. gland lipid peroxidation and protein-thiol oxidation	+	+	Tedesco et al., 2010
M. galloprovincialis	NCB, C60 fullerene, n-TiO2, n-SiO2	Hemocyte LMS, dig. gland lysosomal and oxidative stress parameters, gill antioxidant enzyme activities	+	+	Canesi et al., 2010b
S. plana	n-CuO	Whole tissue accumulation and oxidative stress parameters, burrowing behavior	+	+	Buffet et al., 2011
C. virginica	C60-fullerene	LMS *in vitro* and *in vivo* in digestive cells, lysosomal uptake of aggregates Larval development	+	+	Ringwood et al., 2009
M. edulis	n-Fe_2O_3	Larval development	+	+	Kadar et al., 2010b

Oxydative stress, lipid peroxydation, genotoxicity, lysosomal membrane destabilization, immunotoxicity, inflammation,neurotoxicity, behaviour

Canesi et al., 2012, MER

Proteomic analysis of mussels exposed to CuO NPs



Cu form	Spot n^{oa}	Name	PDQuest Av. Ratio^{b} (CuO NPs/Cu^{2+})	Accession number	Theor. Mr/pI	BLAST Score^{d}	BLAST % Coverage	BLAST E value	Function
GILLS									
CuO NPs	1701	Glutahione S–tranferase GSTpi1	4 ↑	gi|22094809	23717.2/5.9	23.5	5	0.027	Oxidative stress
Common	2217	Nuclear receptor subfamily 1G	2/10 ↓	gi|345971942	59.49/5.74	22.7	12	1.1	Transcription regulation
	3715	ATP synthase F0 subunit 6	3/2 ↑	gi|227002086	25.79/6.68	20	31	1.9	Energy metabolism
	5107	Putative C1q domain containing protein	4/3 ↓	gi|325504419	26.18/6.30	26.2	2	0.002	Stress response
DIGESTIVE GLAND									
CuO NPs	7710	Caspase 3/7–1	10 ↓	gi|325516443	35.41/6.04	25	19	0.26	Apoptosis
	1502	Paramyosin	3 ↓	gi|42559342	99.57/5.25	62	53	3e^{-12}	Cytoskeleton and cell structure
	4802	Cathepsin L	7 ↑	gi|55710282	17.81/5.75	43.1	40	1e^{-7}	Proteolysis
Cu^{2+}	5408	Cytochrome C oxidase subunit III	19 ↓	gi|306441545	31.71/8.07	20.4	28	0.26	Energy metabolism
	5808	Precollagen–D	22 ↑	gi|21105303	80.75/10.02	30.8	4	0.046	Adhesion and mobility
Common	3502	α–tubulin	30/32 ↑	gi|302029718	41.69/5.11	698	80	0	Cytoskeleton and cell structure

- CuO NPs altered the expression of three proteins involved in apoptosis(caspase 3/7–1), oxidative stress responses (GST) and proteolysis (cathepsin L),
- Cu2+ increased the expression of one protein related to adhesion and cell mobility (precollagen–D).

Gomes et al., 2013, PhD

Combine effects of NMs and other pollutants ?

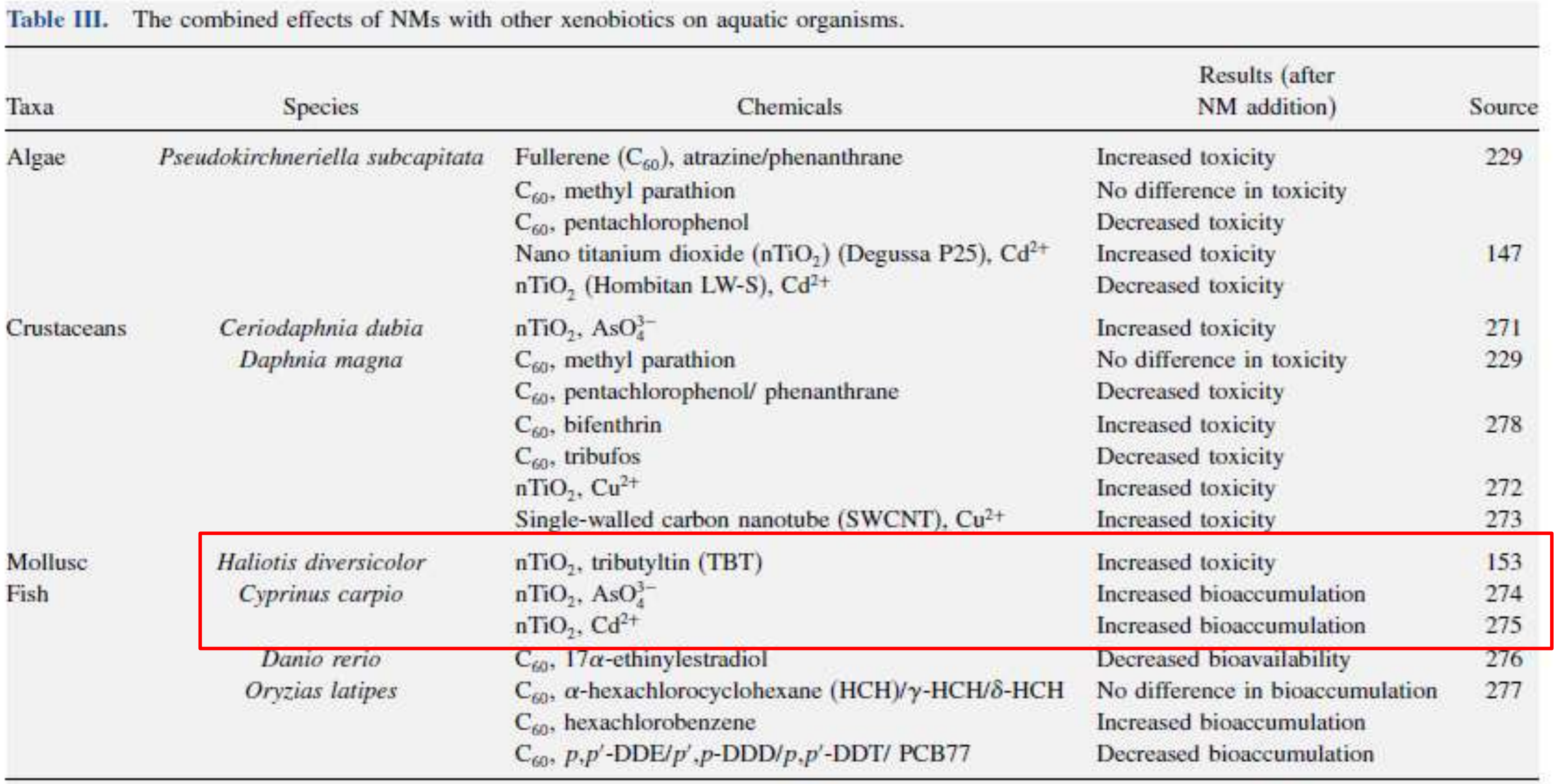


Table III. The combined effects of NMs with other xenobiotics on aquatic organisms.

Taxa	Species	Chemicals	Results (after NM addition)	Source
Algae	*Pseudokirchneriella subcapitata*	Fullerene (C_{60}), atrazine/phenanthrane	Increased toxicity	229
		C_{60}, methyl parathion	No difference in toxicity	
		C_{60}, pentachlorophenol	Decreased toxicity	
		Nano titanium dioxide ($nTiO_2$) (Degussa P25), Cd^{2+}	Increased toxicity	147
		$nTiO_2$ (Hombitan LW-S), Cd^{2+}	Decreased toxicity	
Crustaceans	*Ceriodaphnia dubia*	$nTiO_2$, AsO_4^{3-}	Increased toxicity	271
	Daphnia magna	C_{60}, methyl parathion	No difference in toxicity	229
		C_{60}, pentachlorophenol/ phenanthrane	Decreased toxicity	
		C_{60}, bifenthrin	Increased toxicity	278
		C_{60}, tribufos	Decreased toxicity	
		$nTiO_2$, Cu^{2+}	Increased toxicity	272
		Single-walled carbon nanotube (SWCNT), Cu^{2+}	Increased toxicity	273
Mollusc	*Haliotis diversicolor*	$nTiO_2$, tributyltin (TBT)	Increased toxicity	153
Fish	*Cyprinus carpio*	$nTiO_2$, AsO_4^{3-}	Increased bioaccumulation	274
		$nTiO_2$, Cd^{2+}	Increased bioaccumulation	275
	Danio rerio	C_{60}, 17α-ethinylestradiol	Decreased bioavailability	276
	Oryzias latipes	C_{60}, α-hexachlorocyclohexane (HCH)/γ-HCH/δ-HCH	No difference in bioaccumulation	277
		C_{60}, hexachlorobenzene	Increased bioaccumulation	
		C_{60}, p,p′-DDE/p′,p-DDD/p,p′-DDT/ PCB77	Decreased bioaccumulation	

- Dissimilar between species and NMs
- TiO_2 NPs enhance bioaccumulation and toxicity

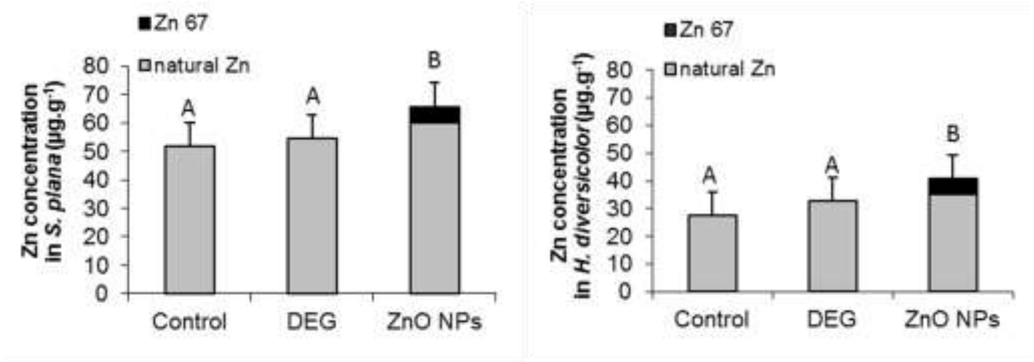
Wong et al., 2013, Reviews in Nanoscience and Nanotechnology

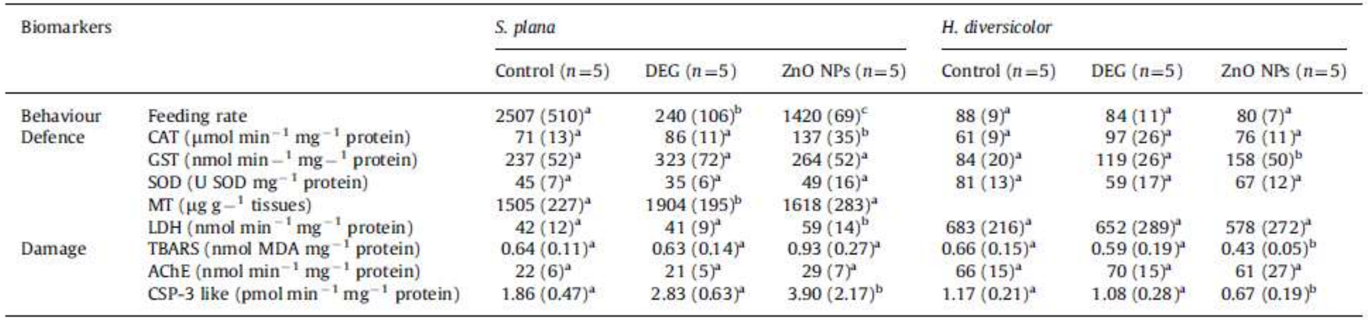
Fate and toxicity effects ^{67} ZnO NPs

Sediment Exposure: Environmental realistic dose: 3 mg.Kg^{-1} (Tiede et al. 2009)

^{67} Zn Natural abundance: 4.1%

✓ ^{67}Zn Bioaccumulation

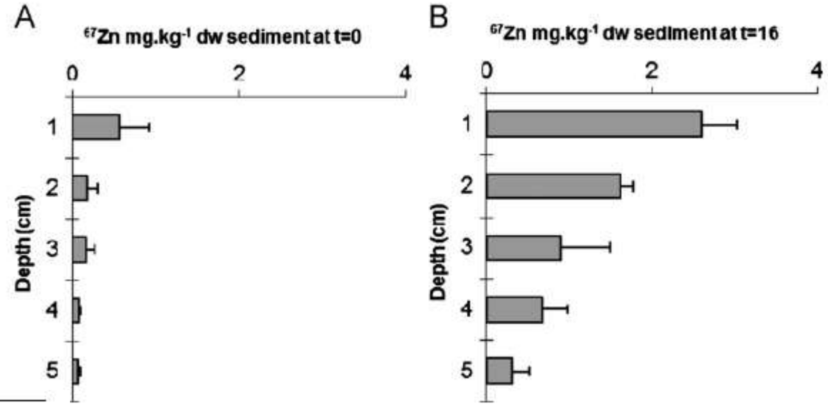




Biomarkers		*S. plana*			*H. diversicolor*		
		Control ($n=5$)	DEG ($n=5$)	ZnO NPs ($n=5$)	Control ($n=5$)	DEG ($n=5$)	ZnO NPs ($n=5$)
Behaviour	Feeding rate	2507 (510)^a	240 (106)^b	1420 (69)^c	88 (9)^a	84 (11)^a	80 (7)^a
Defence	CAT (µmol min^{-1} mg^{-1} protein)	71 (13)^a	86 (11)^a	137 (35)^b	61 (9)^a	97 (26)^a	76 (11)^a
	GST (nmol min –^{1} mg –^{1} protein)	237 (52)^a	323 (72)^a	264 (52)^a	84 (20)^a	119 (26)^a	158 (50)^b
	SOD (U SOD mg^{-1} protein)	45 (7)^a	35 (6)^a	49 (16)^a	81 (13)^a	59 (17)^a	67 (12)^a
	MT (µg g –^{1} tissues)	1505 (227)^a	1904 (195)^b	1618 (283)^a			
	LDH (nmol min^{-1} mg^{-1} protein)	42 (12)^a	41 (9)^a	59 (14)^b	683 (216)^a	652 (289)^a	578 (272)^a
Damage	TBARS (nmol MDA mg^{-1} protein)	0.64 (0.11)^a	0.63 (0.14)^a	0.93 (0.27)^a	0.66 (0.15)^a	0.59 (0.19)^a	0.43 (0.05)^b
	AChE (nmol min^{-1} mg^{-1} protein)	22 (6)^a	21 (5)^a	29 (7)^a	66 (15)^a	70 (15)^a	61 (27)^a
	CSP-3 like (pmol min^{-1} mg^{-1} protein)	1.86 (0.47)^a	2.83 (0.63)^a	3.90 (2.17)^b	1.17 (0.21)^a	1.08 (0.28)^a	0.67 (0.19)^b

Moderate toxicity effects (oxidative stress, behavior)

✓ ^{67}Zn in sediments



Scrobicularia plana

Nereis diversicolor

Labeled ^{67}Zn on sediment surface and migration in the first centimeters

Bioturbation processes operated by benthic species could contribute to this migration

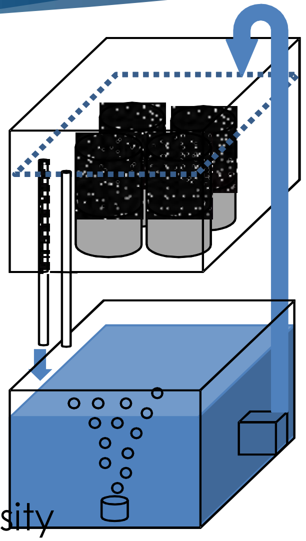
Buffet et al., 2012

NanoReTox

What happens in the real life?

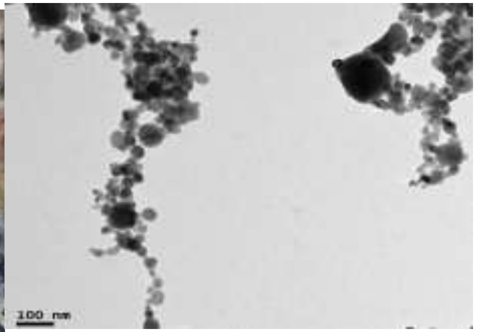
complexity,
medium/long term,
ecological relevance

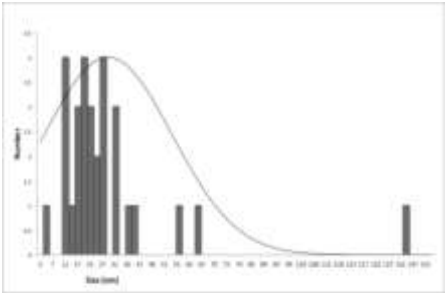
replicability, low cost



Lab conditions
- Natural sea water

Outdoor Mesocosm
- Tidal cycle
- Microalgae migrations
- Environmental animal density





Future research needs…

- The development of new and more robust methods and techniques for the detection and characterization of nanomaterials in complex environmental matrices (water, soil/sediment).

- Need of standardized protocols/ NM references

- What metric provides the best measure of dose? Progress with the relationships between NM physico-chemical properties (aggregation, surface modifications, dissolution mechanisms, redox processes) and ecotoxicological effects

- What are realistic environmental concentrations ?
  - Development and validation of exposure estimation models allowing the determination of realistic exposure scenarios (intensity/frequency) through NM life-cycle.

Future research needs…

- Toxicity effects: classical approach, new specific approach?
- Environmental modulations on toxicities of NMs
  - Ionic strength/salinity
  - Temperature
  - pH
  - UV light
  - NOM
  - Other pollutants

- Influence of ageing and non ageing of NPs on their behaviour and biological effects

- Bioaccumulation/Food chain transfer: Mesocosm approach