evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Lemnaceae;

model species, toxicological guinea-pig or …

……fast growing food!

Tato akce se koná v rámci projektu:
Vybudování vědeckého týmu environmentální metabolomiky a ekofyziologie a jeho zapojení do mezinárodních sítí (ENVIMET; r.č. **CZ.1.07/2.3.00/20.0246**) realizovaného v rámci Operačního programu Vzdělávání pro konkurenceschopnost.

Model species

- What organism should one use?
- What is the question you are asking?
- Different model organisms have different strengths and weaknesses

Arabidopsis

Small genome & Rapid growth

Nicotiana tabacum

Tissue culture & Genetic engineering

Rice

Real crop

Poplar

Real crop

Spinach

Chloroplasts

Lemnaceae; what are they?

Lemnaceae; the model of the 1960 and 1970s

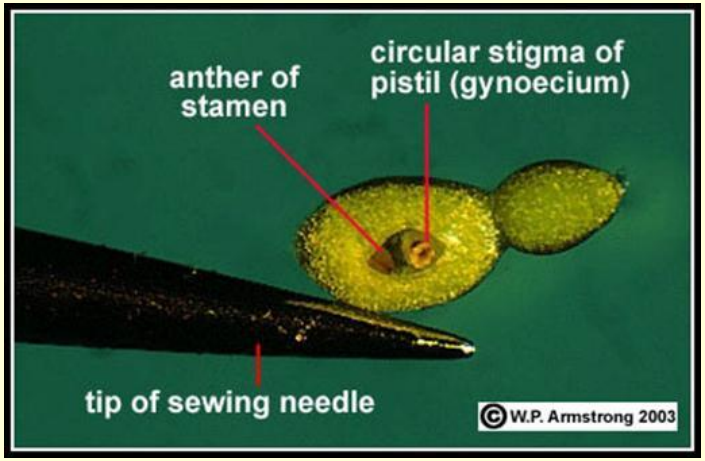
Lemnaceae; the guinea-pigs of the plant kingdom

Lemnaceae; the cleaners of the plant kingdom

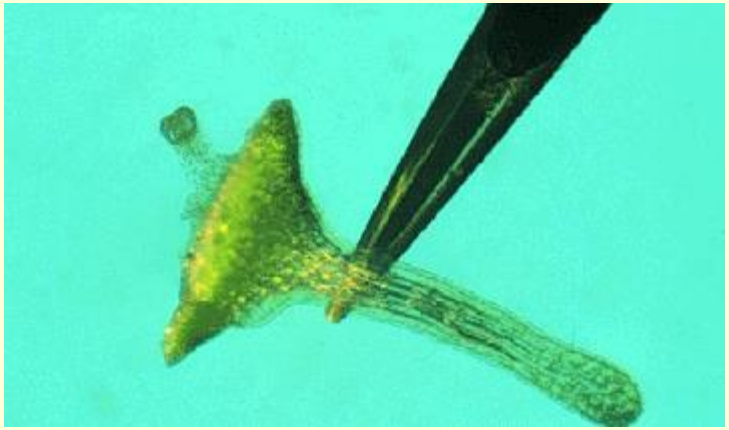
Lemnaceae; Invasive aliens?

Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels

Smallest flowering plants



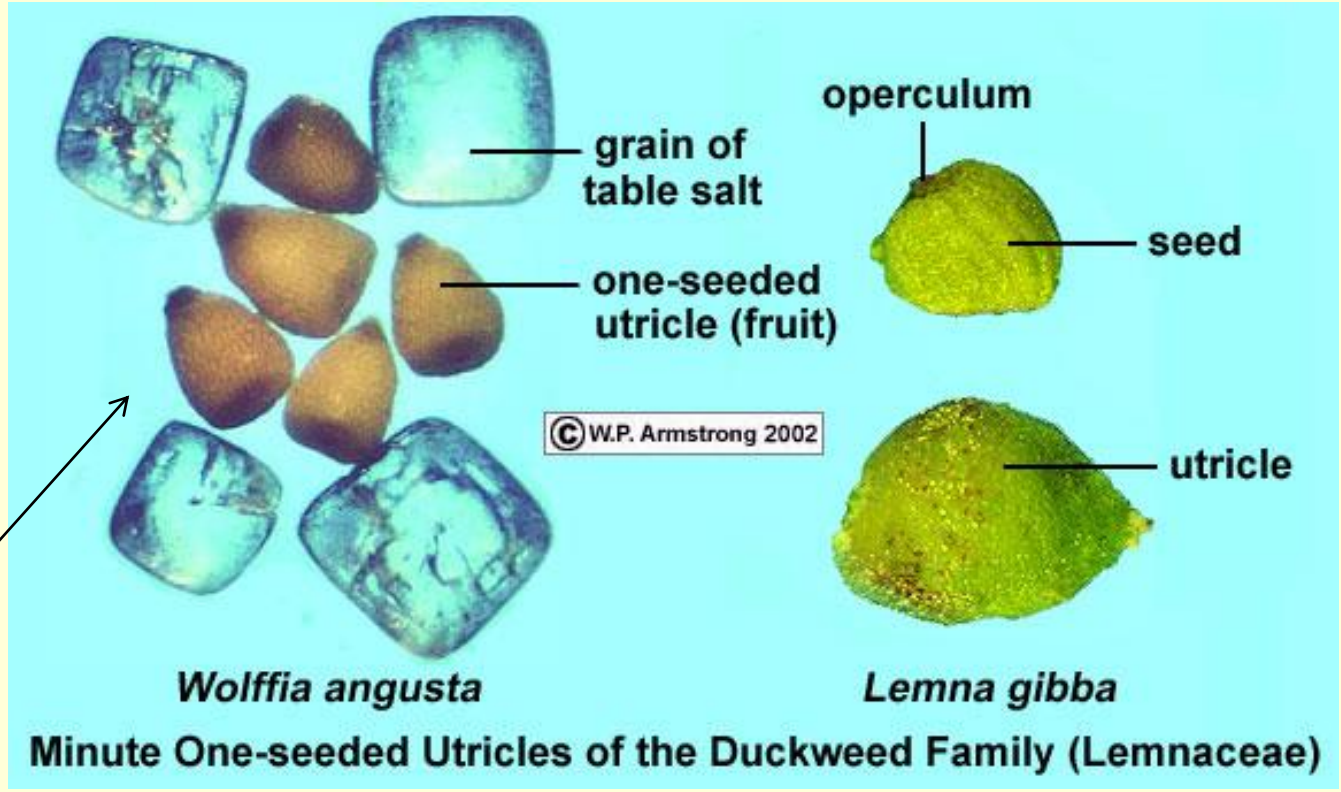
http://waynesword.palomar.edu



http://waynesword.palomar.edu

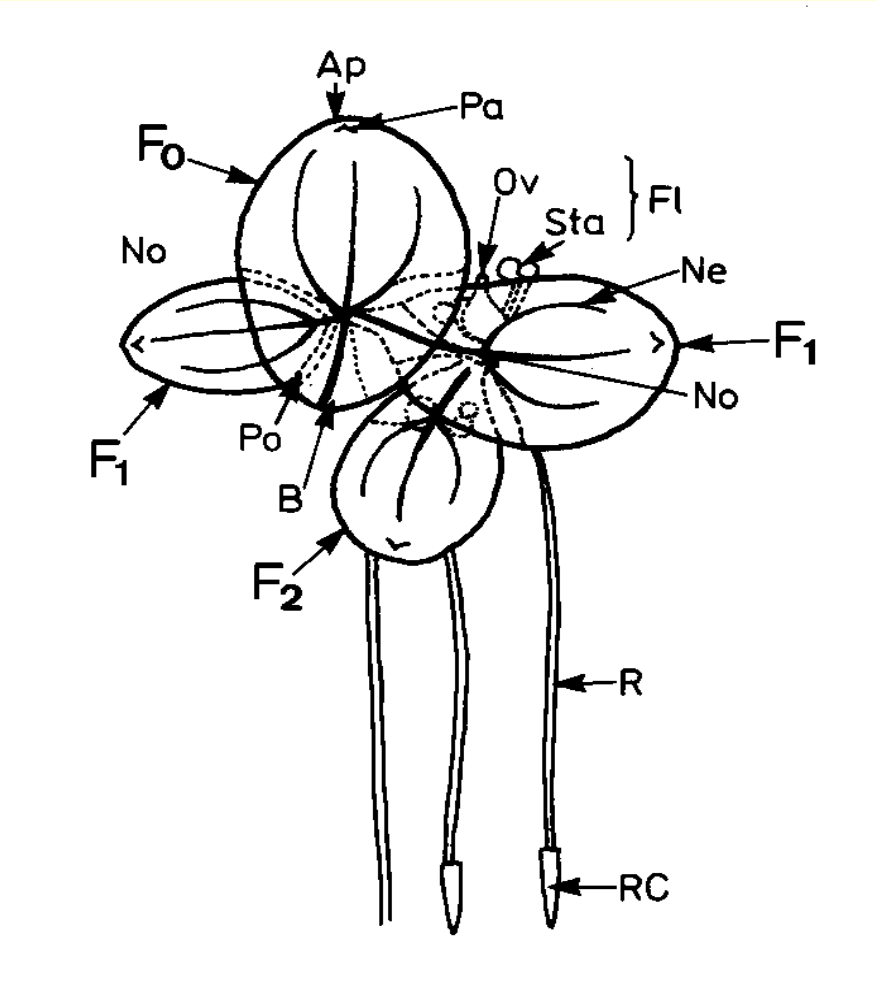
http://world-flowers-plants.blogspot.com/2011/04/smallest-crop-in-world.html

Smallest flowering plants



0.2-0.3 mm

Lemna aequinoctialis



F_0 mother frond
F_I daughter frond 1st generation
F_2 daughter frond 2nd generation

Po pouch

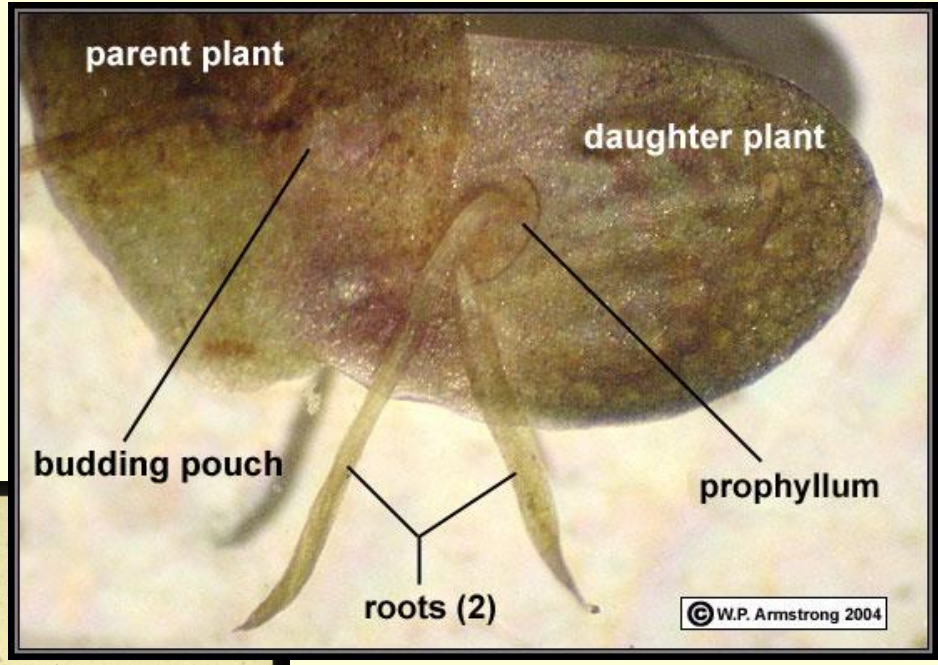
http://waynesword.palomar.edu

Fl flower
Ov ovary
Sta stamen

R root
RC root cap

Landolt, E. *The family of Lemnaceae - Monographic Study*., Vol. 1, Veroff. Geobot. Inst. ETH, Stiftung Rubel, Zurich, 71. Heft (1986)

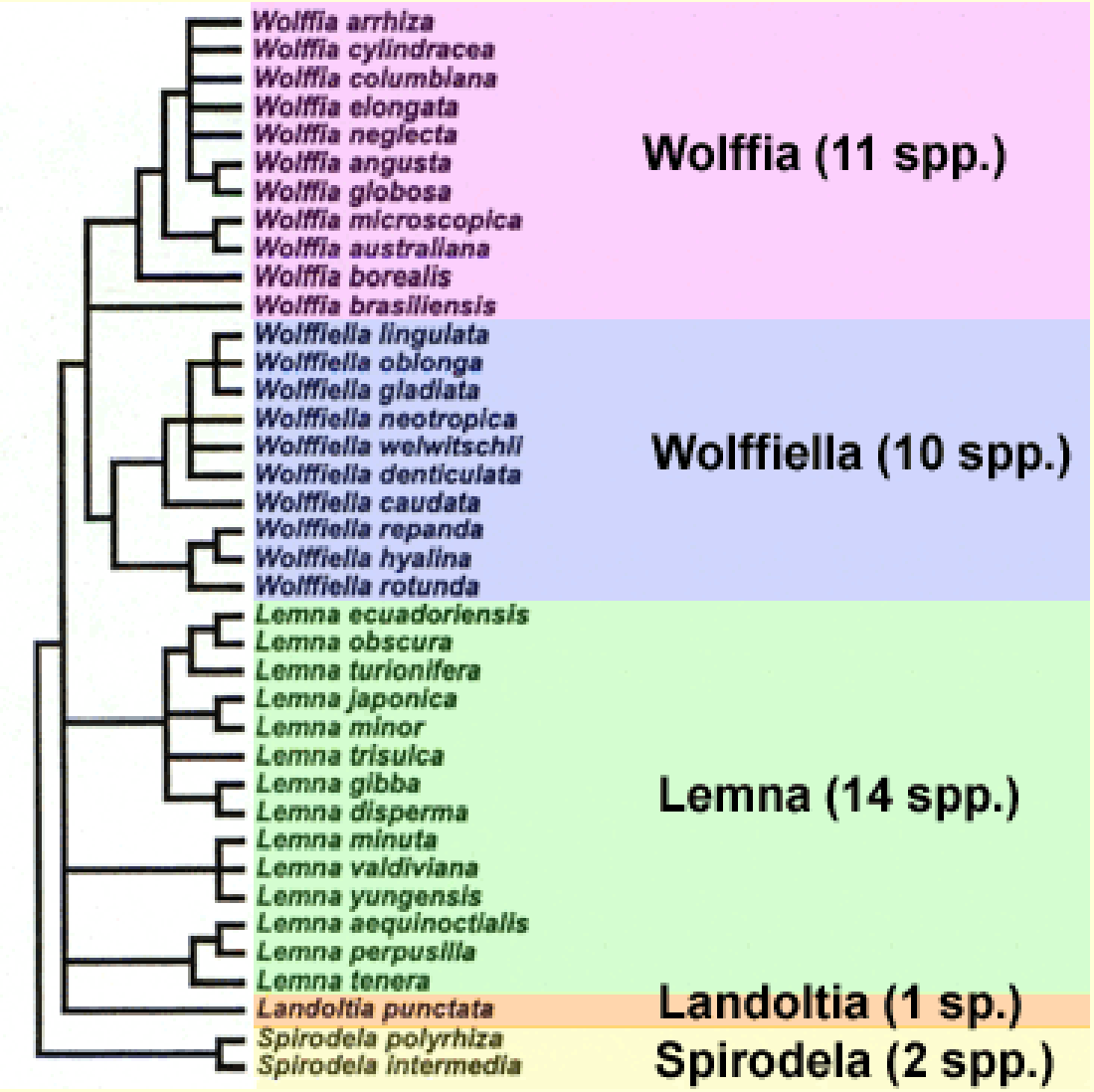
Landoltia punctata

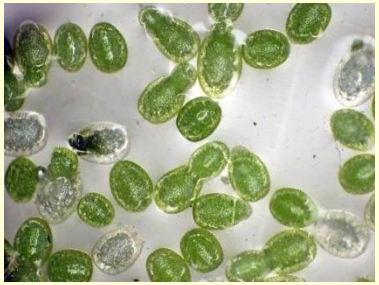


Jansen et al., Physiol Plant 2002

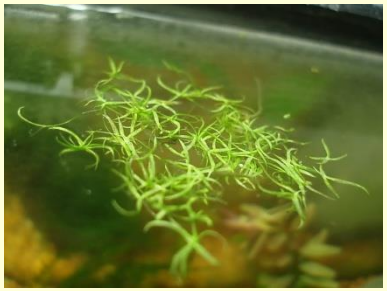
Survival turions

Lemnaceae

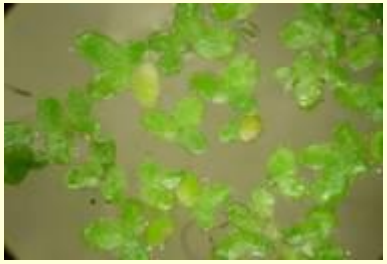




Wolffia globosa



Wolffiella gladiata



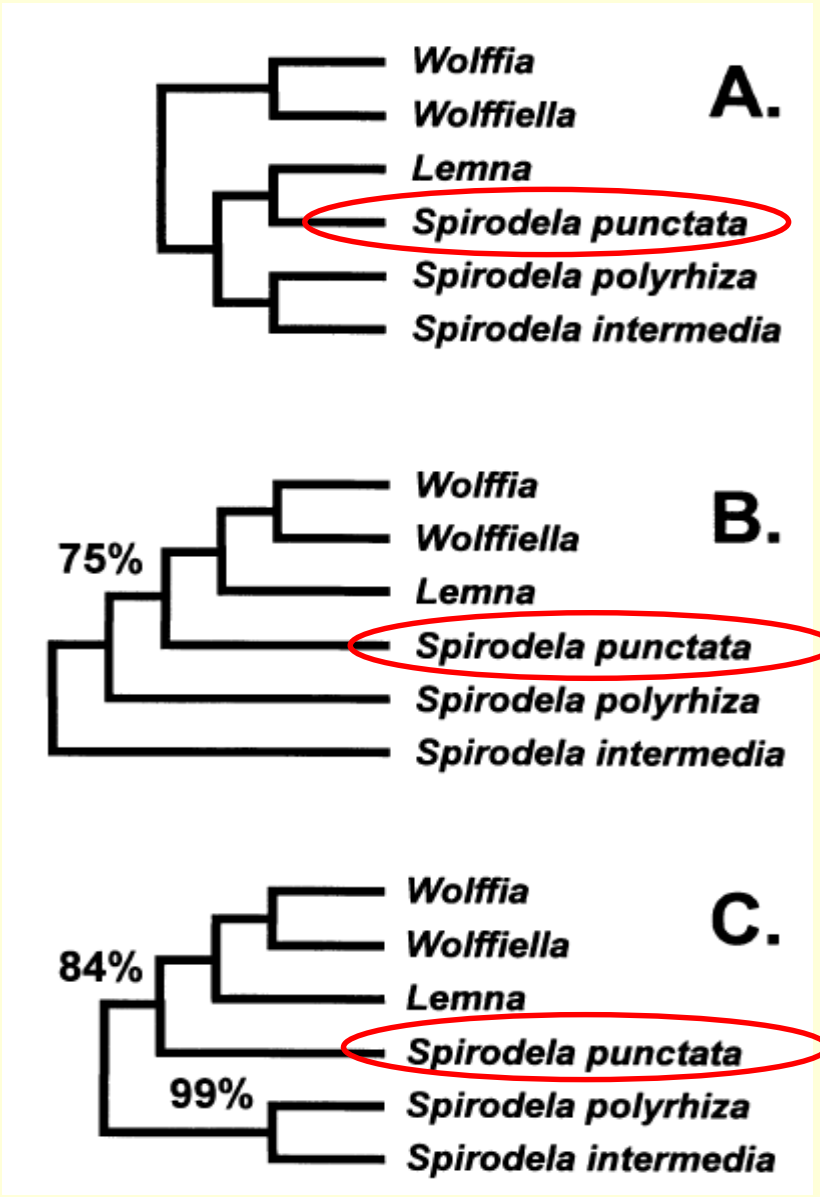
Lemna minor

Spirodela polyrhiza

http://waynesword.palomar.edu

Changing names….!

Lemna oligorhiza



Traditional taxonomic approaches, 1986

Spirodela oligorhiza
Spirodela punctata

Non-molecular, cladistic data,1997

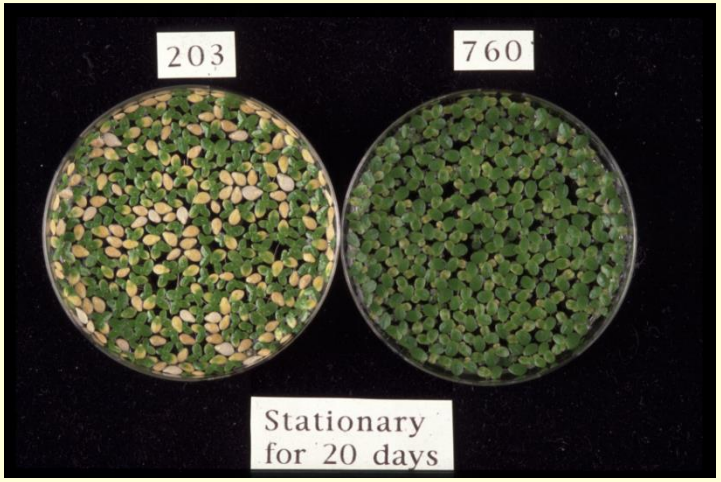
Landoltia punctata

Molecular data (*rbcL*), 1999

Les and Crawford Novon 1999

Advantages Lemnaceae:

- Easy to manipulate
- Fast uptake amino acids, and a whole range of other (in)organic molecules
- Tolerant of range temperatures, nutrient conditions, light levels
- Small
- Clonal cultures
- Axenic cultures are easy to establish and maintain
- Autotropic or heterotropic growth
- Fast growth
- Flat surface



Lemnaceae; what are they?

Lemnaceae; the model species of the 1970 to 1990s

Lemnaceae; the guinea-pigs of the plant kingdom

Lemnaceae; the cleaners of the plant kingdom

Lemnaceae; Invasive aliens?

Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels

Lemnaceae, the great model of the 1970s, 80s and 90s!!!

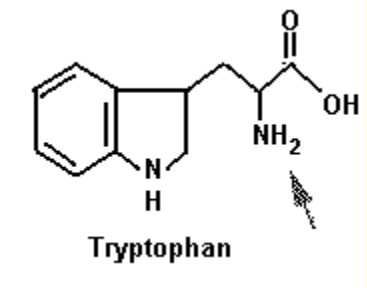
Ageing stars?

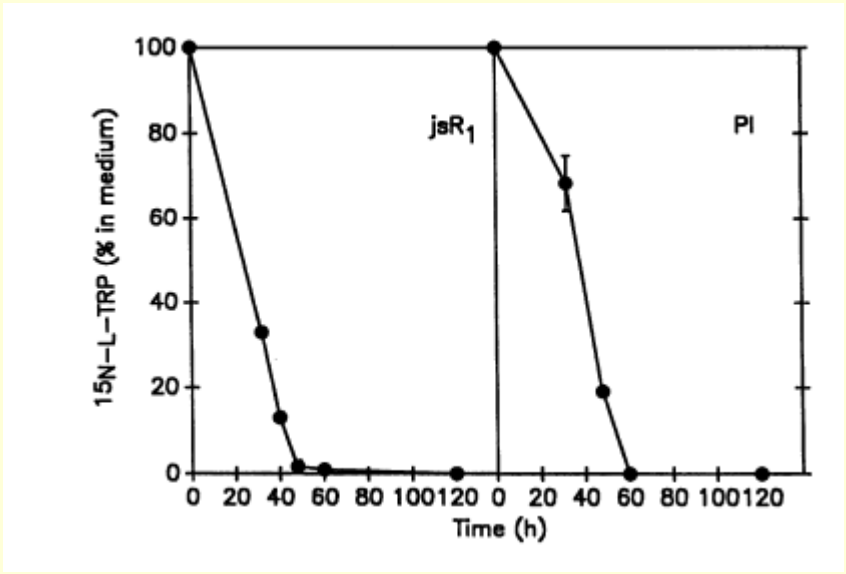
Lemnaceae, the future!

Cool, futuristic kids?

Elucidating metabolic pathways

2 lines of *Lemna gibba*

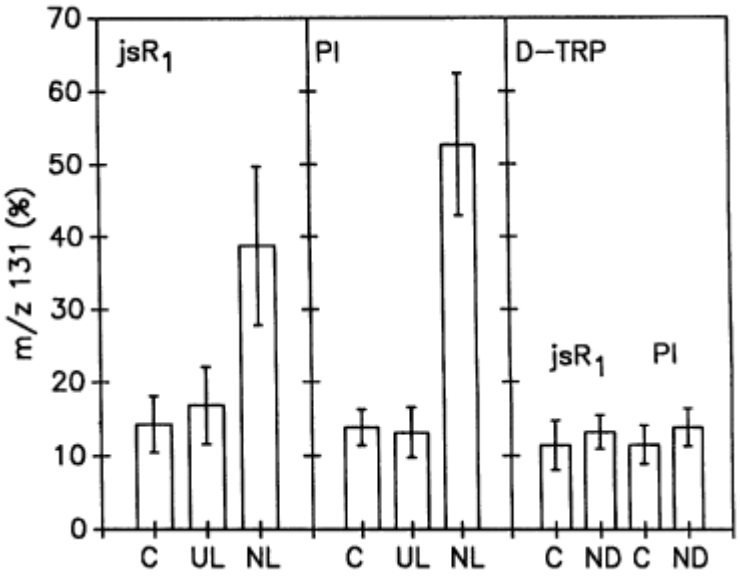




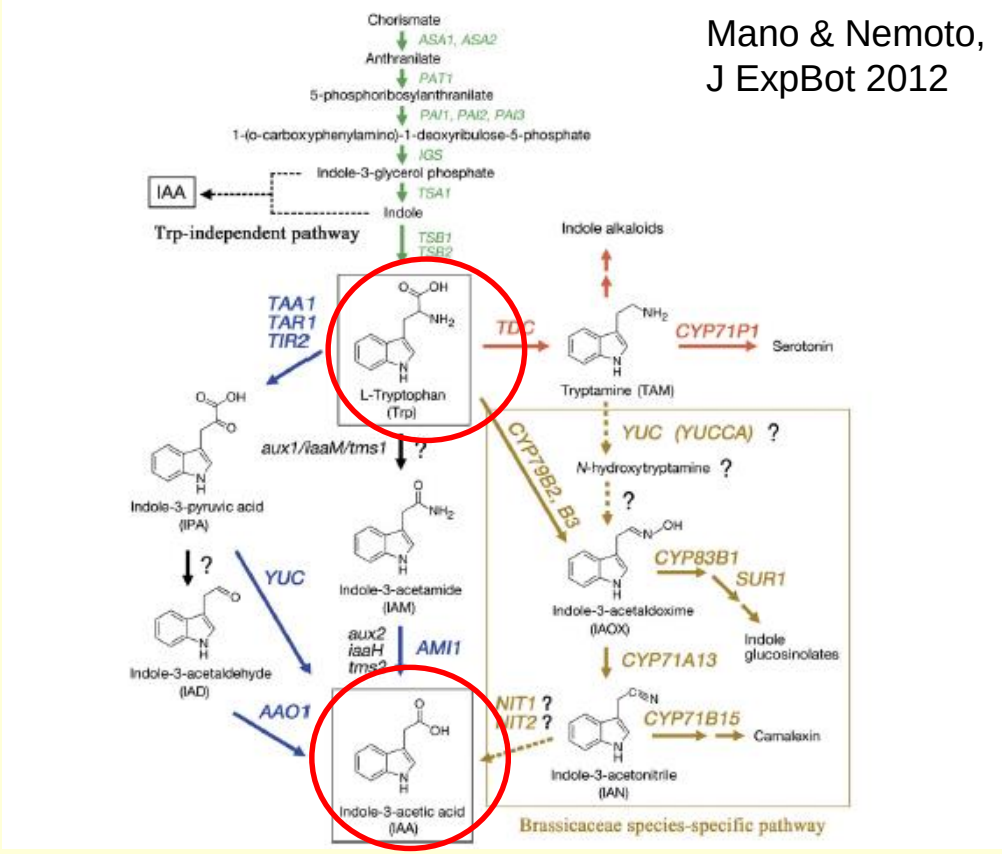
Uptake ^{15}N-labelled tryptophan from medium

Baldi, Maher, Slovin and Cohen, Plant Physiol 1991

2 lines of *Lemna gibba*



C = no added Trp
UL = unlabeled
NL = labeled L-isomer
ND = labeled D-isomer

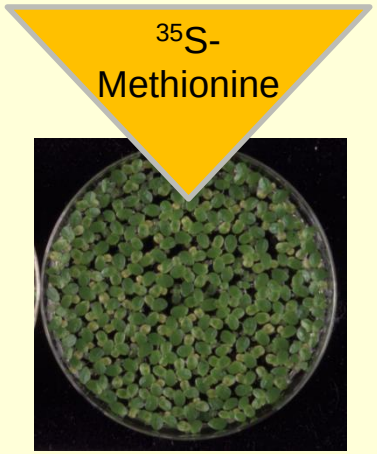


Mano & Nemoto, J ExpBot 2012

^{15}N-labelled free IAA in plant

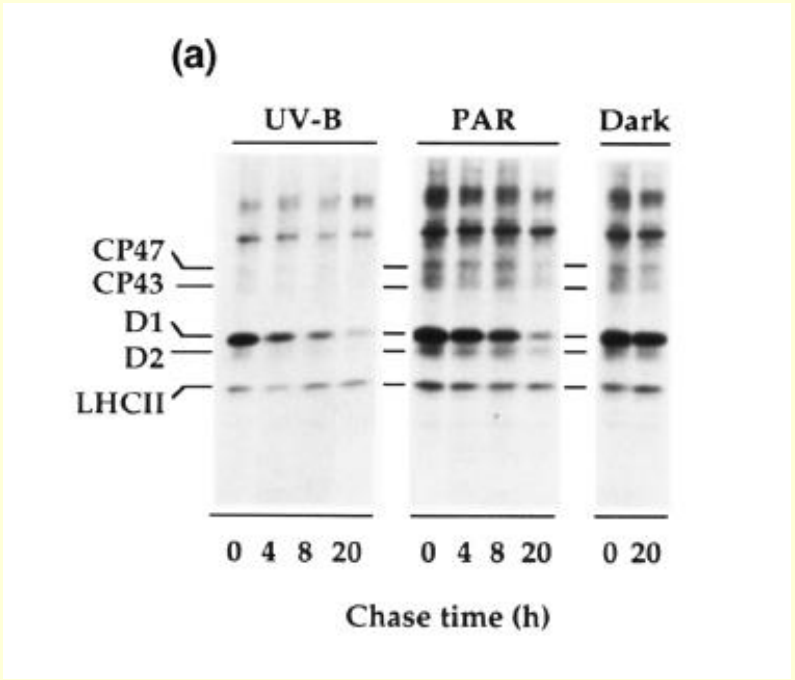
Baldi, Maher, Slovin and Cohen, Plant Physiol 1991

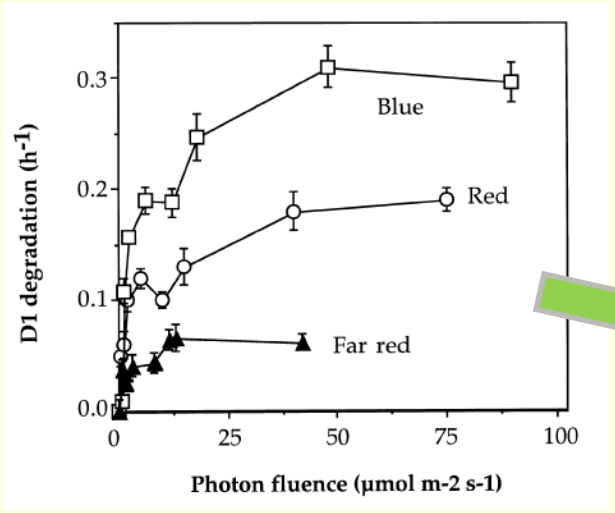
Jansen et al., Plant J 1996

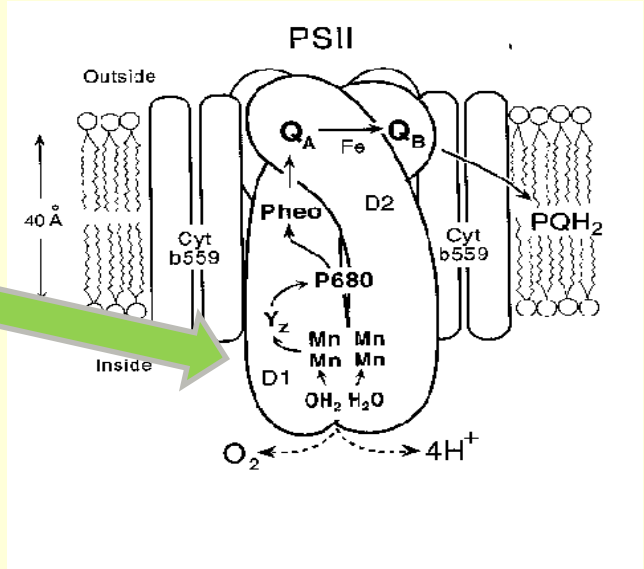


Landoltia punctata

Membrane isolation







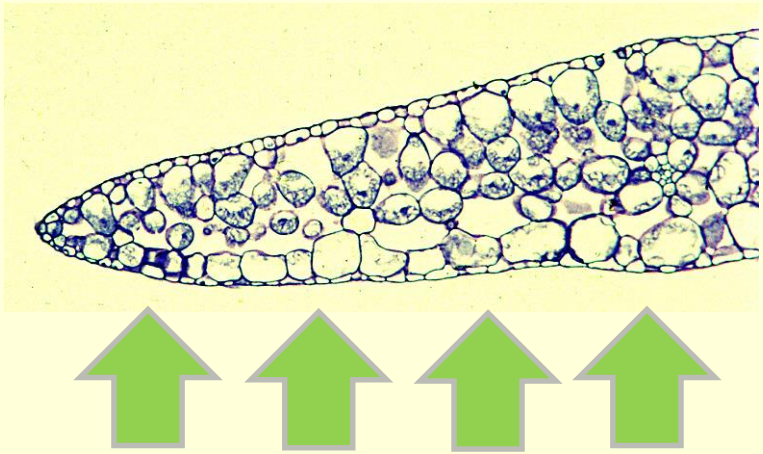
Lemnaceae studies have been instrumental in our understanding of a range of plant metabolic pathways

Why such a good model organism?

Lemna fronds take up water and nutrients **directly** through the lower surface of the frond, and not through the root (Landolt, 1986)

Lemna species can take up minerals, amino acids, sugars, phytohormones, and a broad range of organic compounds

Similarity algae!



Distinct uptake systems for:

- neutral l-α-amino acids,
- basic amino acids,
- purine bases,
- choline,
- ethanolamine,
- tyramine,
- urea,
- Aldohexoses

Specific systems enable utilisation organic compounds in environment

The role of roots is primarily anchorage

Datko & Mudd, *Plant Physiol* (1985)

Lemna minor can grow for long periods in darkness!

TABLE 3.6. Dry matter production in cultures grown at different light intensities of fluorescent light colour 34, without or with 3×10^{-6} M kinetin, on a medium containing 1% sucrose. Multiplication rate G_t is on base of the natural logarithm (section 2.3.2).

Light intensity $\mu W\ cm^{-2}$	Multiplication rate G_t	Dry weight mg/cm^2	Dry weight increase mg/cm^2 day
Without kinetin			
Darkness	0.04	8.2	0.32
1	0.06	5.0	0.30
3	0.08	4.0	0.32
8	0.10	3.0	0.30
30	0.16	2.1	0.34
110	0.20	1.9	0.38
300	0.29	1.9	0.55
1100	0.38	2.5	0.95
2400	0.39	3.0	1.17

Rombach, LUW, 1976

Lemnaceae; what are they?

Lemnaceae; the model of the 1960 and 1970s

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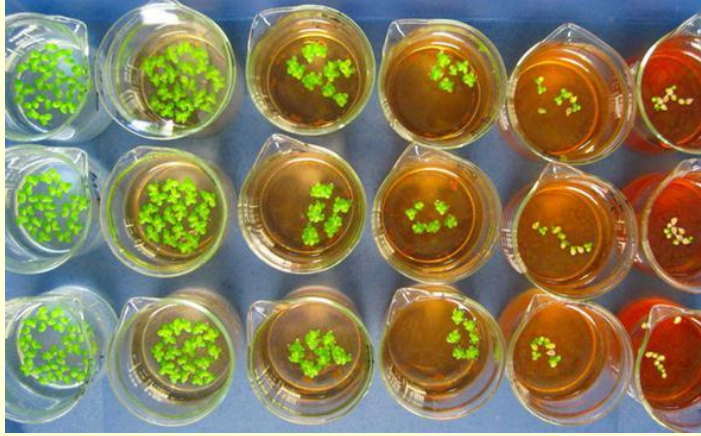
Lemnaceae; the cleaners of the plant kingdom

Lemnaceae; Invasive aliens?

Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels



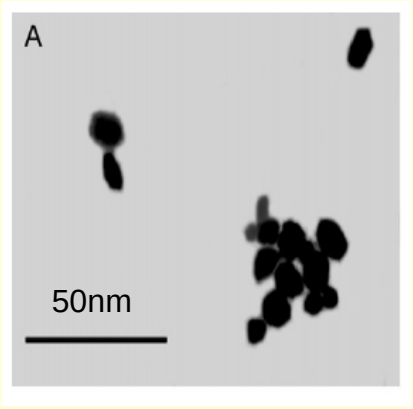
OECD GUIDELINES FOR THE TESTING OF CHEMICALS

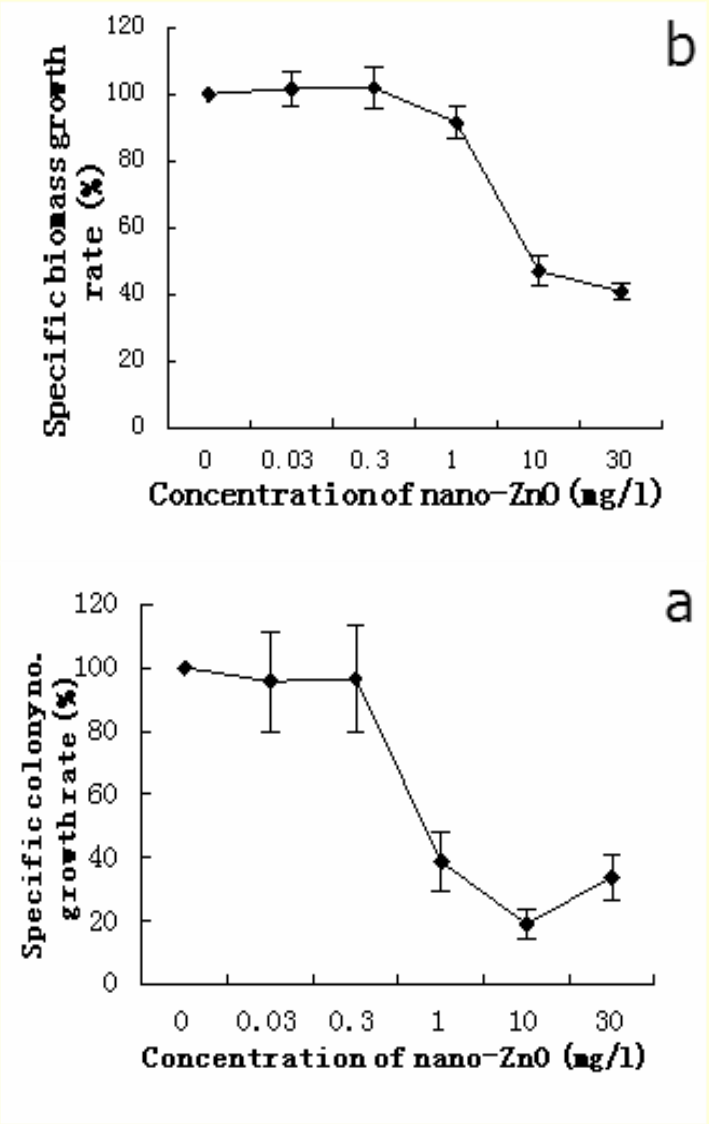
REVISED PROPOSAL FOR A NEW GUIDELINE 221

***Lemna* sp. Growth Inhibition Test**

Lemna sp,; the key model-plant species used for toxicological testing!

Toxicology ZnO-nanoparticles





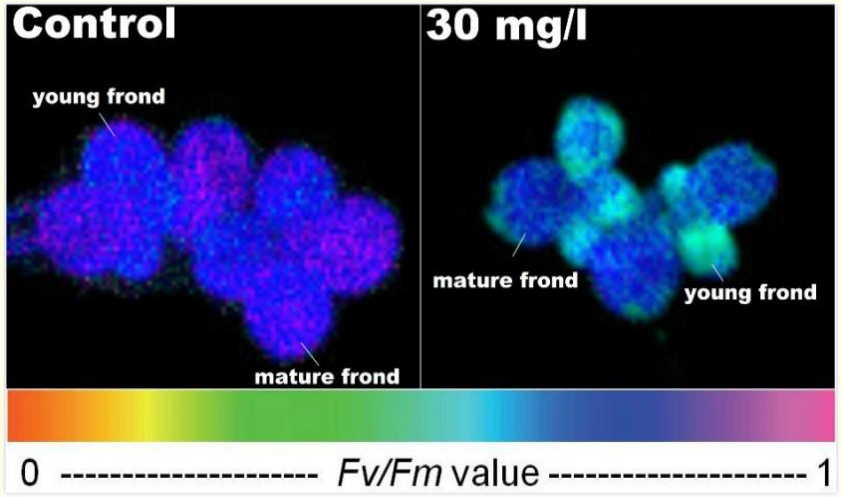
- nominal size: < 100 nm

- average size (TEM): 25.9 +/- 7.8 nm

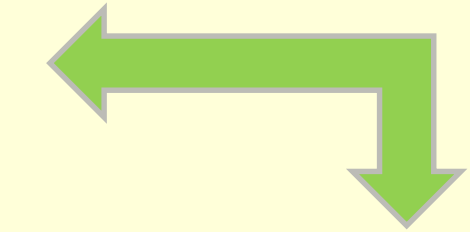
- shape: spherical to square

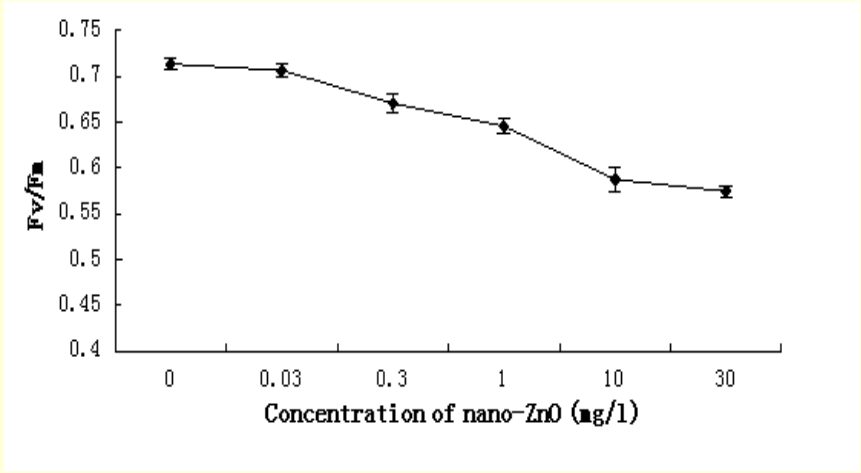
- zeta potential (in duckweed media): -12.13 mV

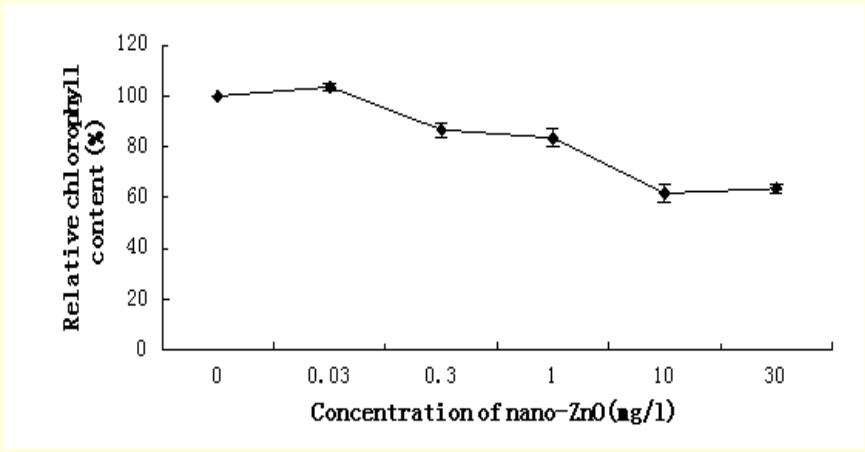
- hydrodynamic diameter at start of experiment (in duckweed medium and at 10 mg/L): 198.6 +/- 39 nm



Maximum photosynthetic efficiency of PSII (Fv/Fm) in *Lemna minor* exposed to 0 mg/l nano-ZnO (Control) and 30 mg/l for 7 days.

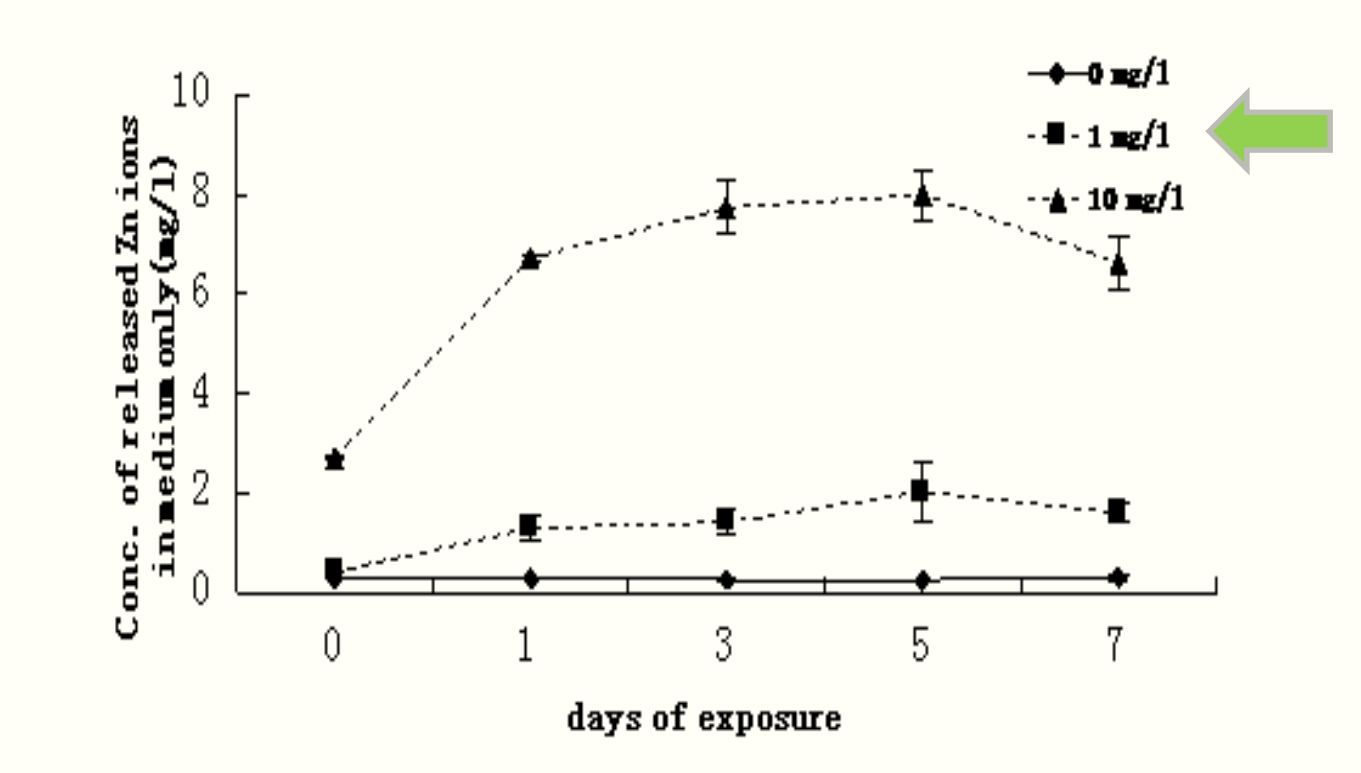






Why are ZnO- nanoparticles toxic?

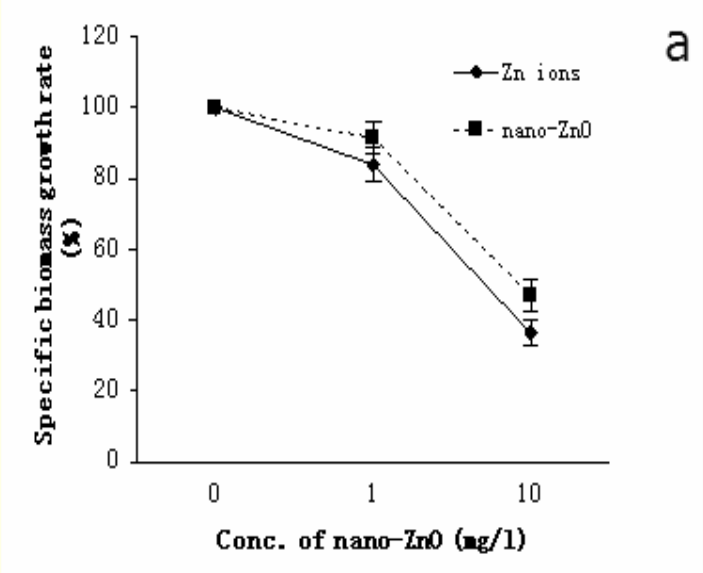
Free Zn^{2+} in the medium

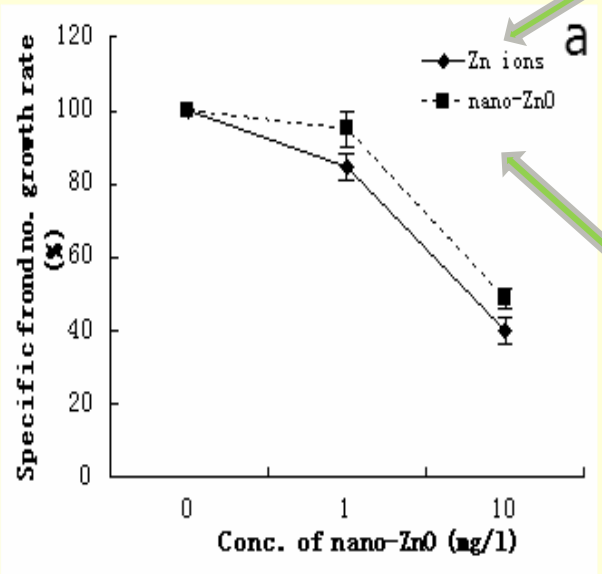


Concentration nanoparticle

At the end of a 1-week experiment, ZnO-nanoparticles completely dissolved

What is toxic? Nano-ZnO or Zn^{2+} ?

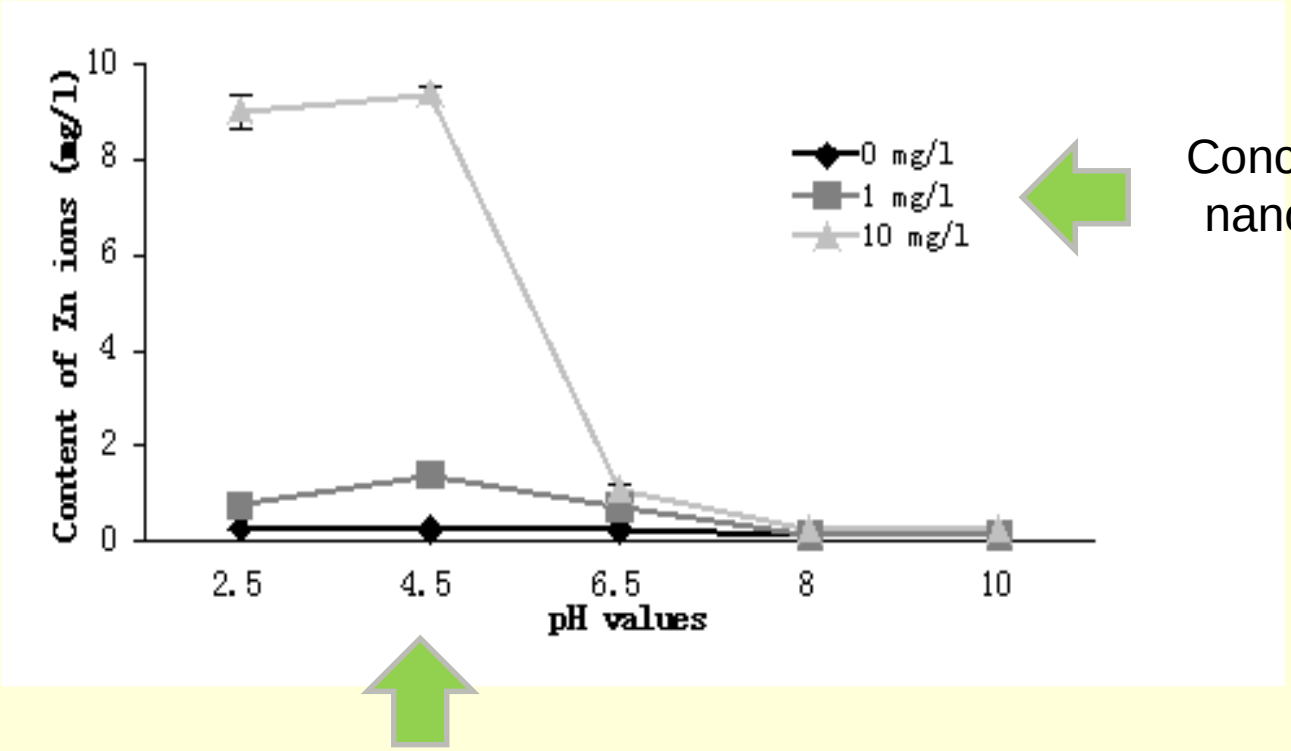




Zn^{2+} equivalent of nano-ZnO

Nano-ZnO

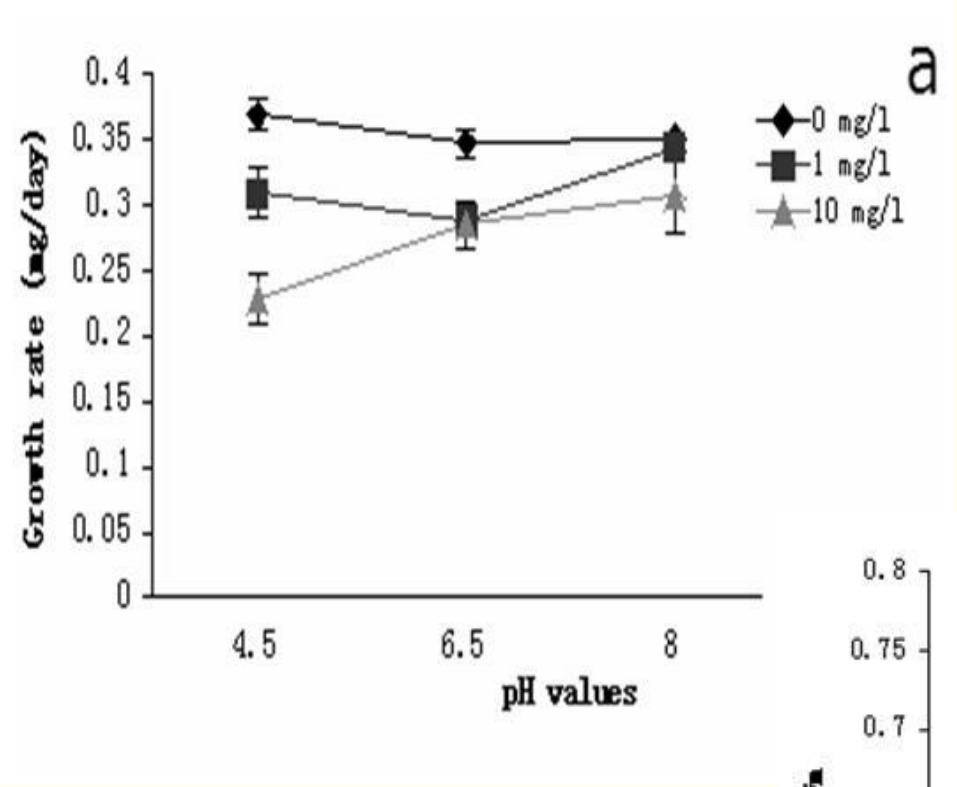
Dissolution nano-ZnO particles is pH dependent



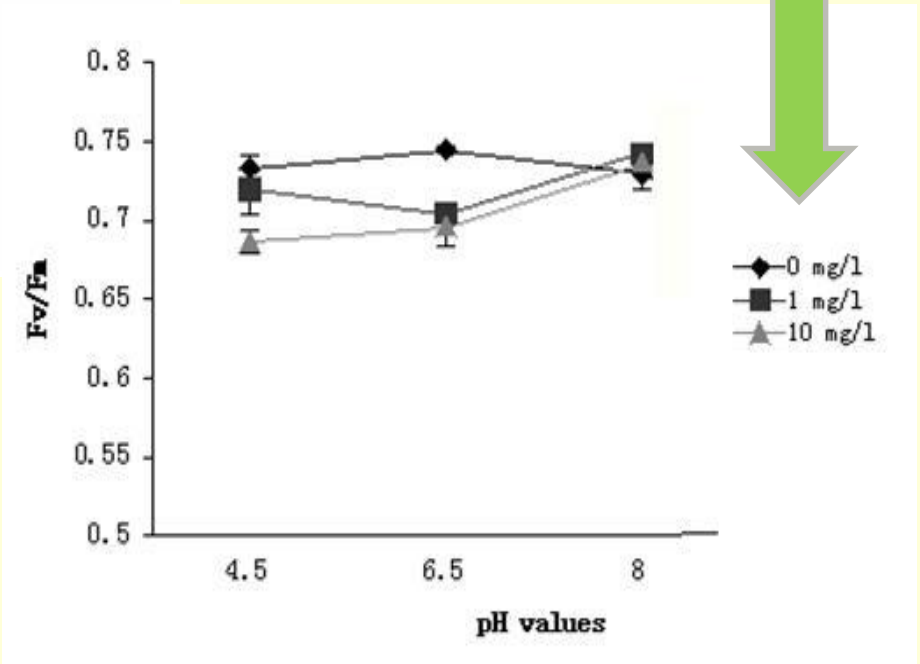
Concentration nanoparticle

Standard duckweed medium

Much decreased nano-ZnO toxicity at pH 8



Concentration ZnO-nanoparticle



A major role for Zn-ions in causing nano-ZnO toxicity at pH 4.5

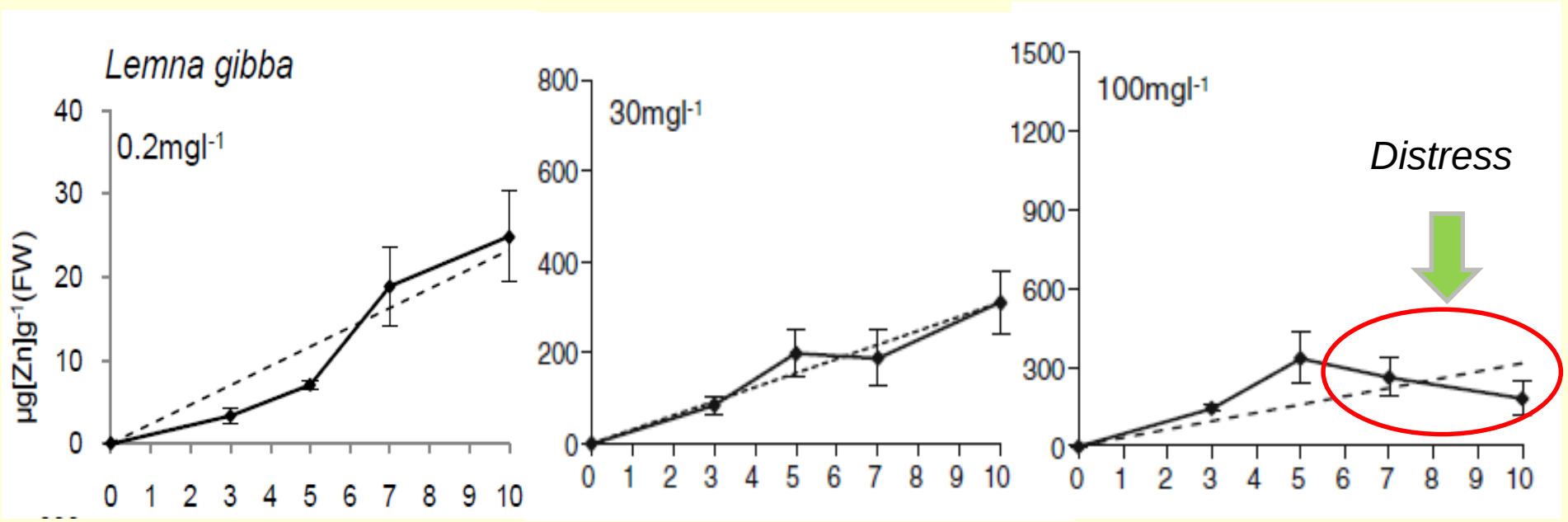
The pH of natural surface waters varies widely, but values in the range between pH 6.5–8.5 are common

Thus, the pH of water bodies is a determinant of the environmental fate and biological impact of nanoparticles,

Irish bogs, pH<5

Limestone with stream, pH>7.5

Zinc uptake by *Lemnaceae*

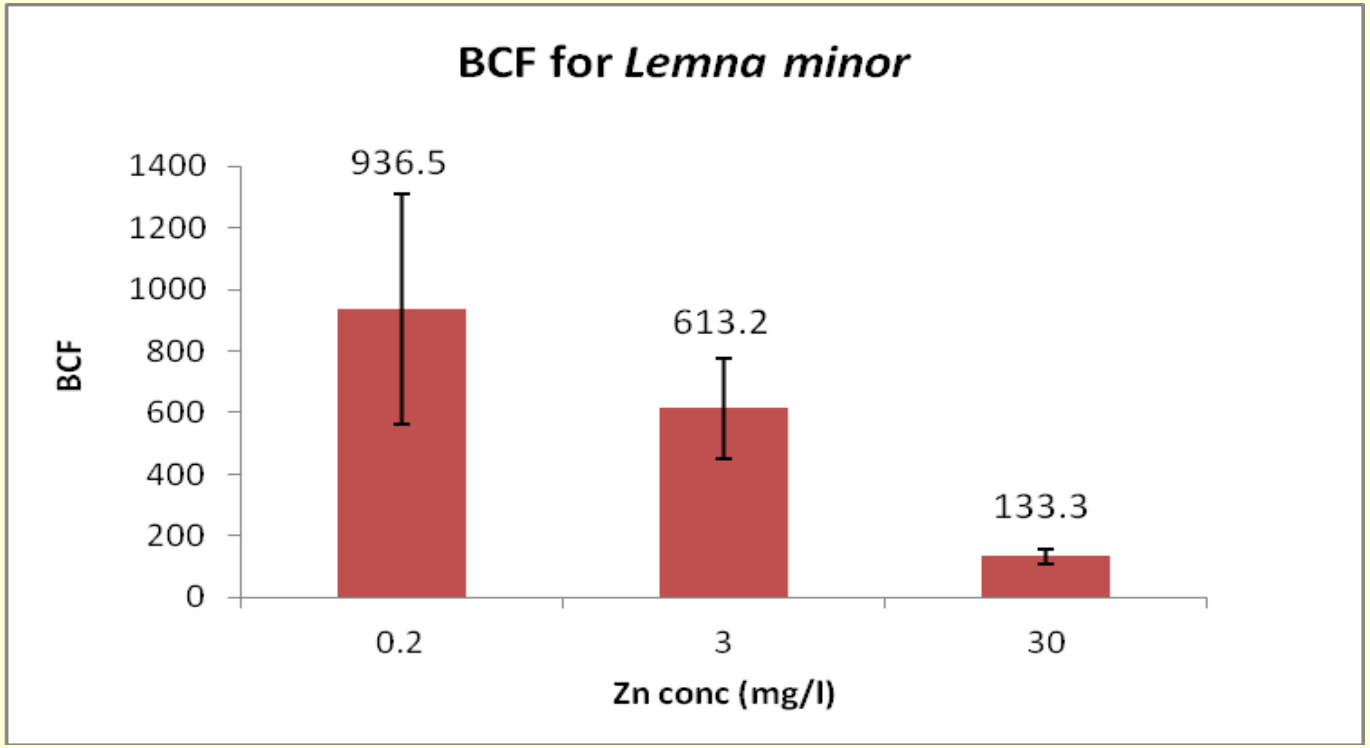


Uptake described by “linear model with first order kinetics”

Lahive et al., 2011

Zinc bioaccumulation by *Lemna minor*

BCF; ratio Zn in plant/Zn in medium



Literature; accumulation factors Zn > 35.000

Cyprinus carpio (Common carp)

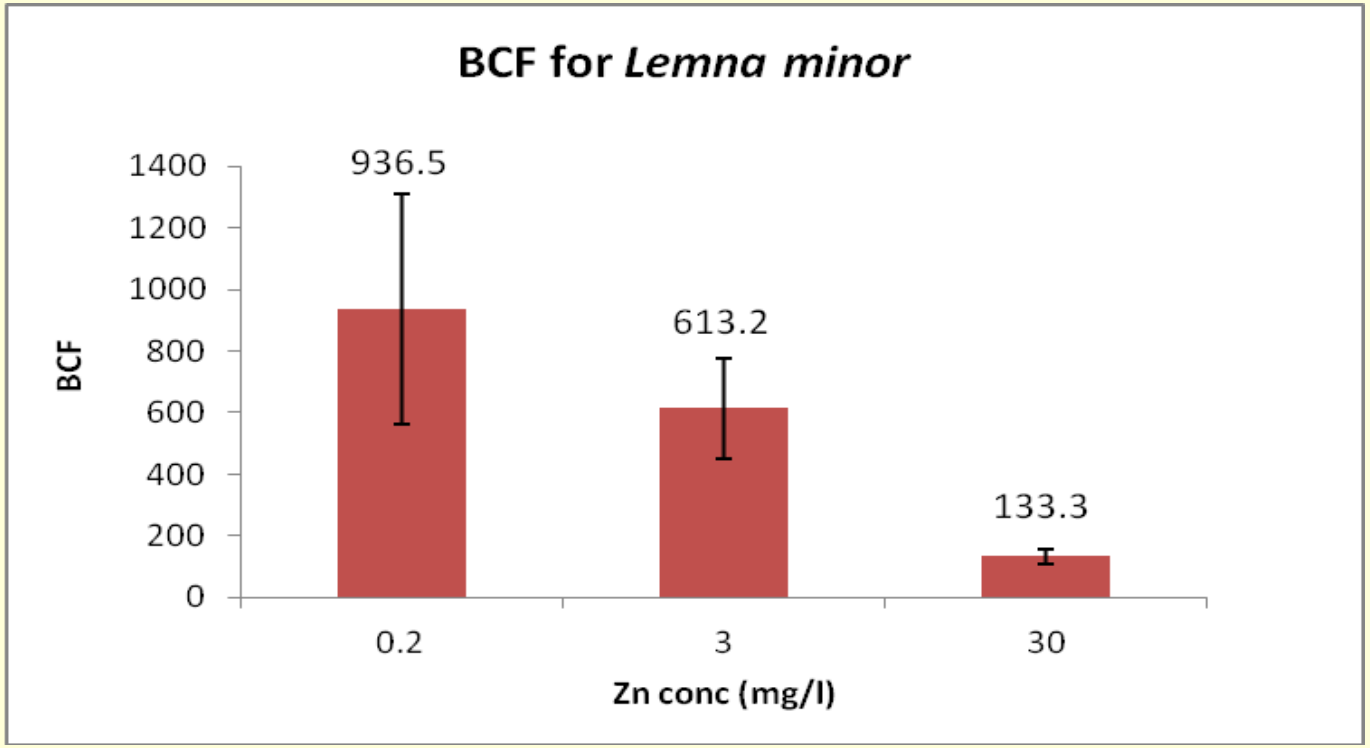
Common carp – zinc defiency leading to:

- Reduced growth
- Cataracts
- High mortality
- Erosion of fins and skin
- Accumulation Fe and Cu in intestine and pancreas

Common carp feeds, amongst others, on Lemnaceae

Zinc bioaccumulation by *Lemna minor*

BCF; ratio Zn in plant/Zn in medium



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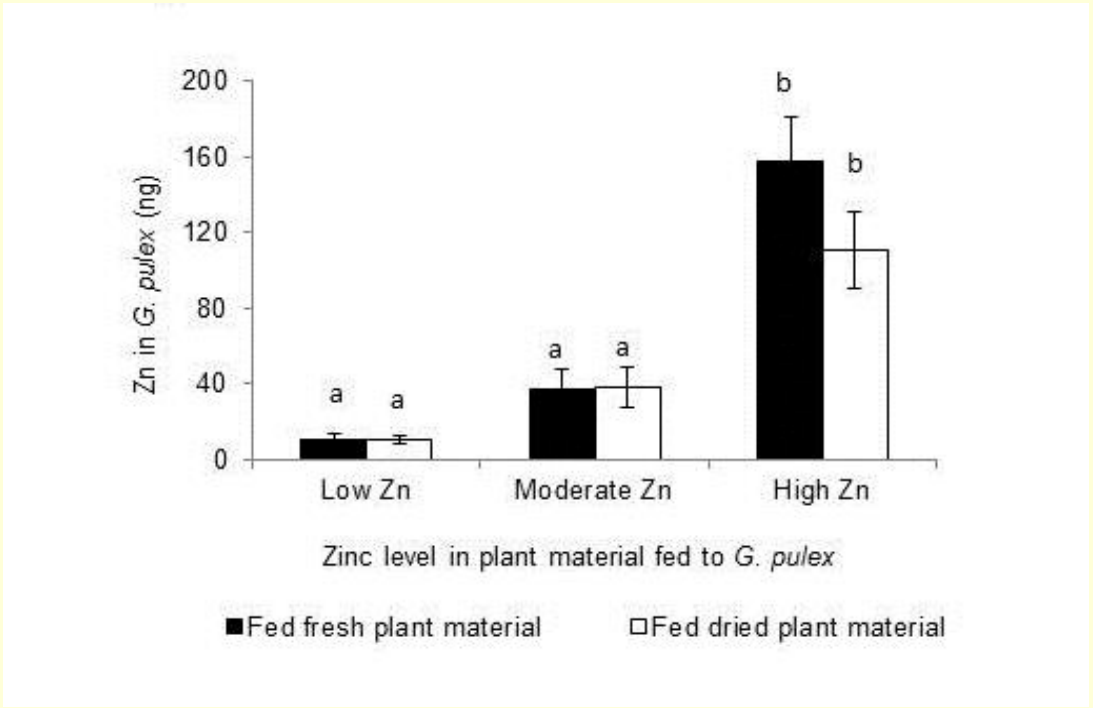
From bioaccumulation to ….
…….food fortification

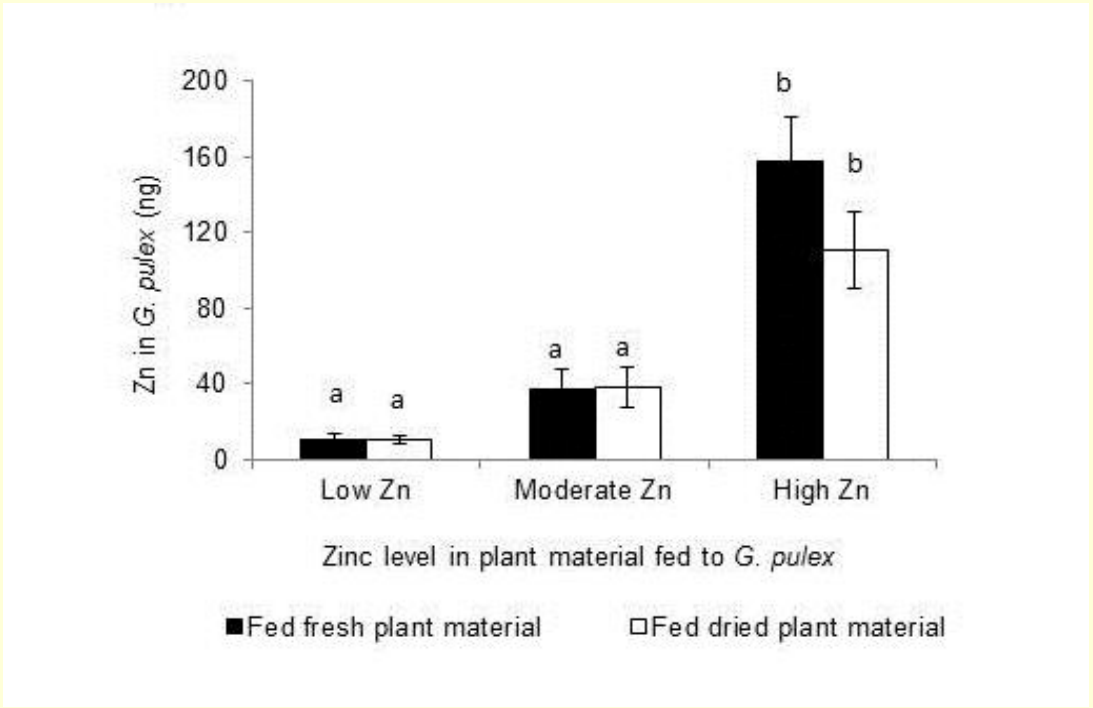


Zinc in *Gammarus pulex* fed 2-days on *Lemna minor* and after 24h depuration

From bioaccumulation to ….
…….food fortification

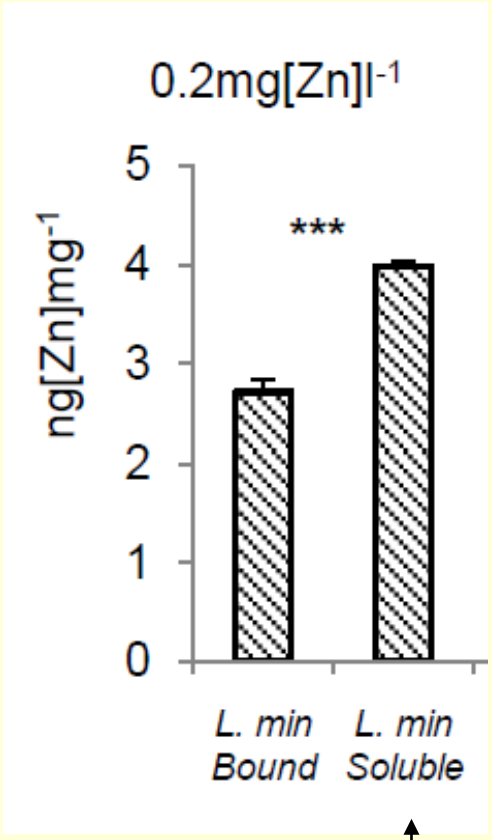
25-fold increase Zn in plant

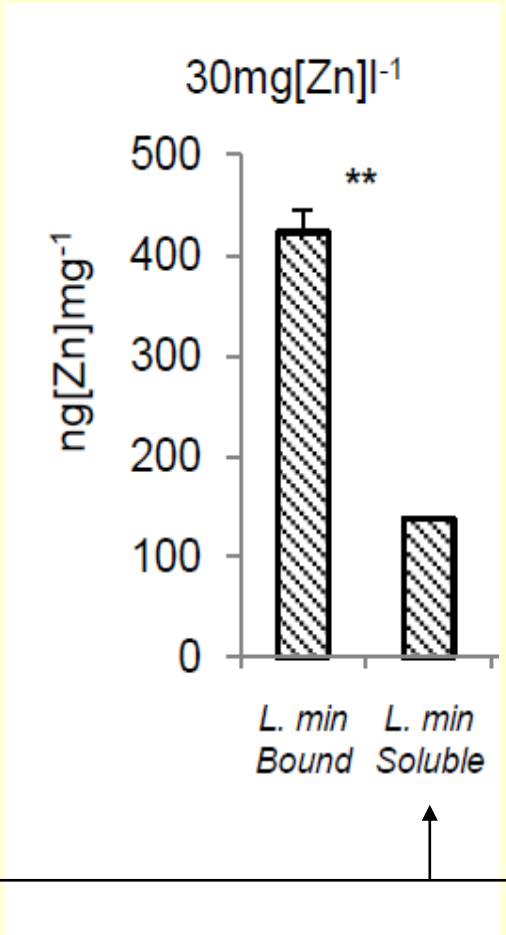
16-fold increase Zn in Gammarus



Zinc in *Gammarus pulex* fed 2-days on *Lemna minor* and after 24h depuration

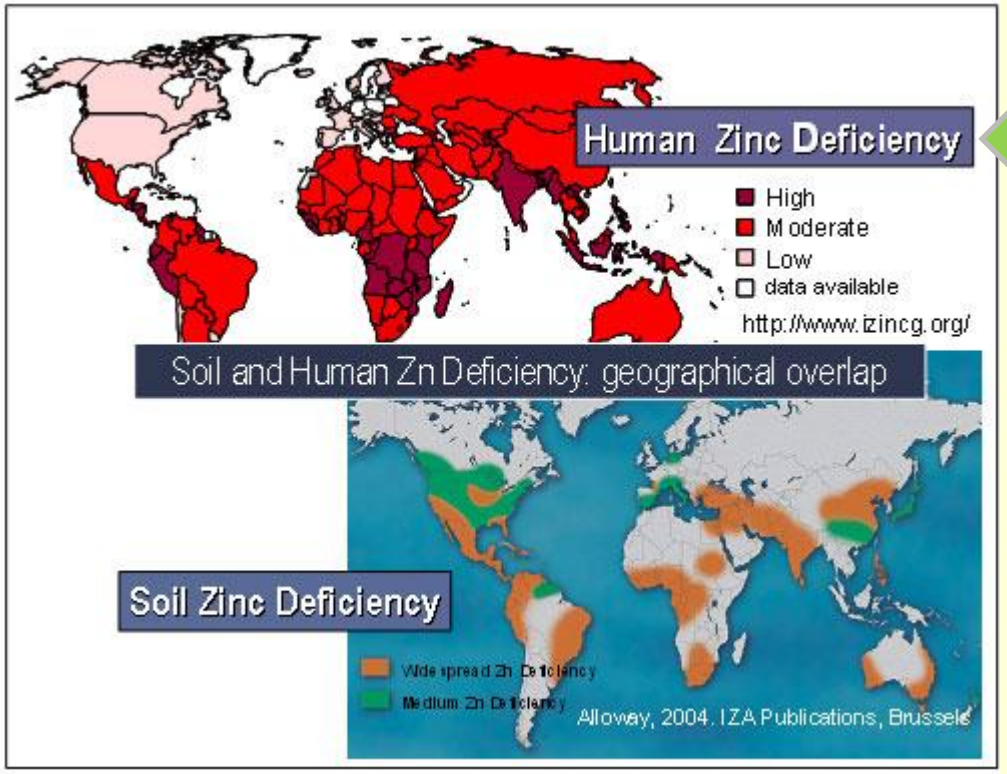
Zinc bioavailability





Available for trophic transfer?

Metals, plants and food quality



Close geographical linkage between soil zinc deficiency and human zinc deficiency

Issue for some 25% of world population

Frozen Lemnaceaea

Lemnaceae as a model system

Toxicity testing

Food/feed fortification

Lemnaceae; what are they?

Lemnaceae; the model of the 1960 and 1970s

Lemnaceae; the guinea-pigs of the plant kingdom

Lemnaceae; the cleaners of the plant kingdom

Lemnaceae; Invasive aliens?

Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels

Lemnaceae in the cleaning business!

Devils Lake – North Dakota – 10.000 inhab.

Tertiary wastewater treatment (N/P removal)

Plant nutrient uptake
Anaerobic microbial processes

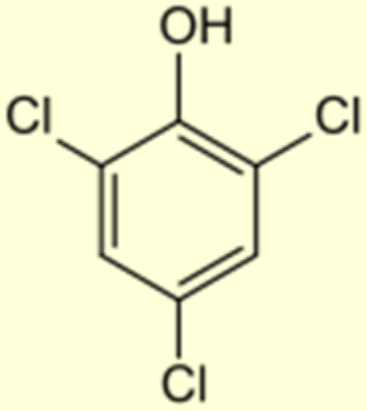
Marriage between biology & engineering

http://www.ci.devils-lake.nd.us/departments/sewer-department.html

Phytoremediation - TCP

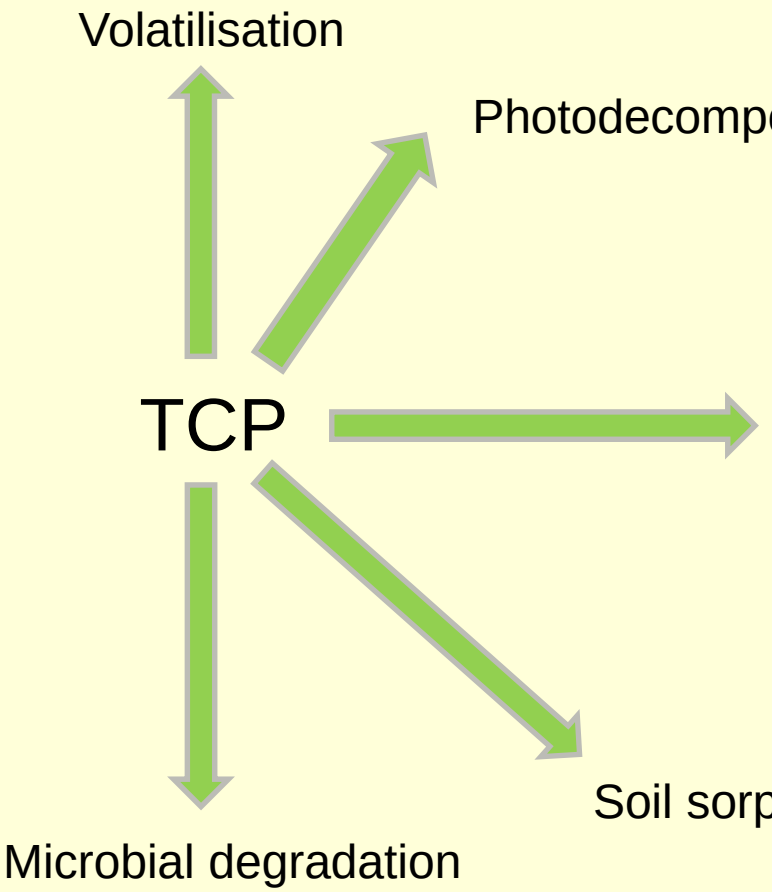
Chlorophenols

- Used as broad-spectrum biocides; residues & breakdown products ubiquitous in the environment
  - Sawmills (wood impregnation agents)
  - Precursors / degradation products chemicals
  - Landfill sites
- Priority pollutant
- Genotoxic, mutagenic, carcinogenic



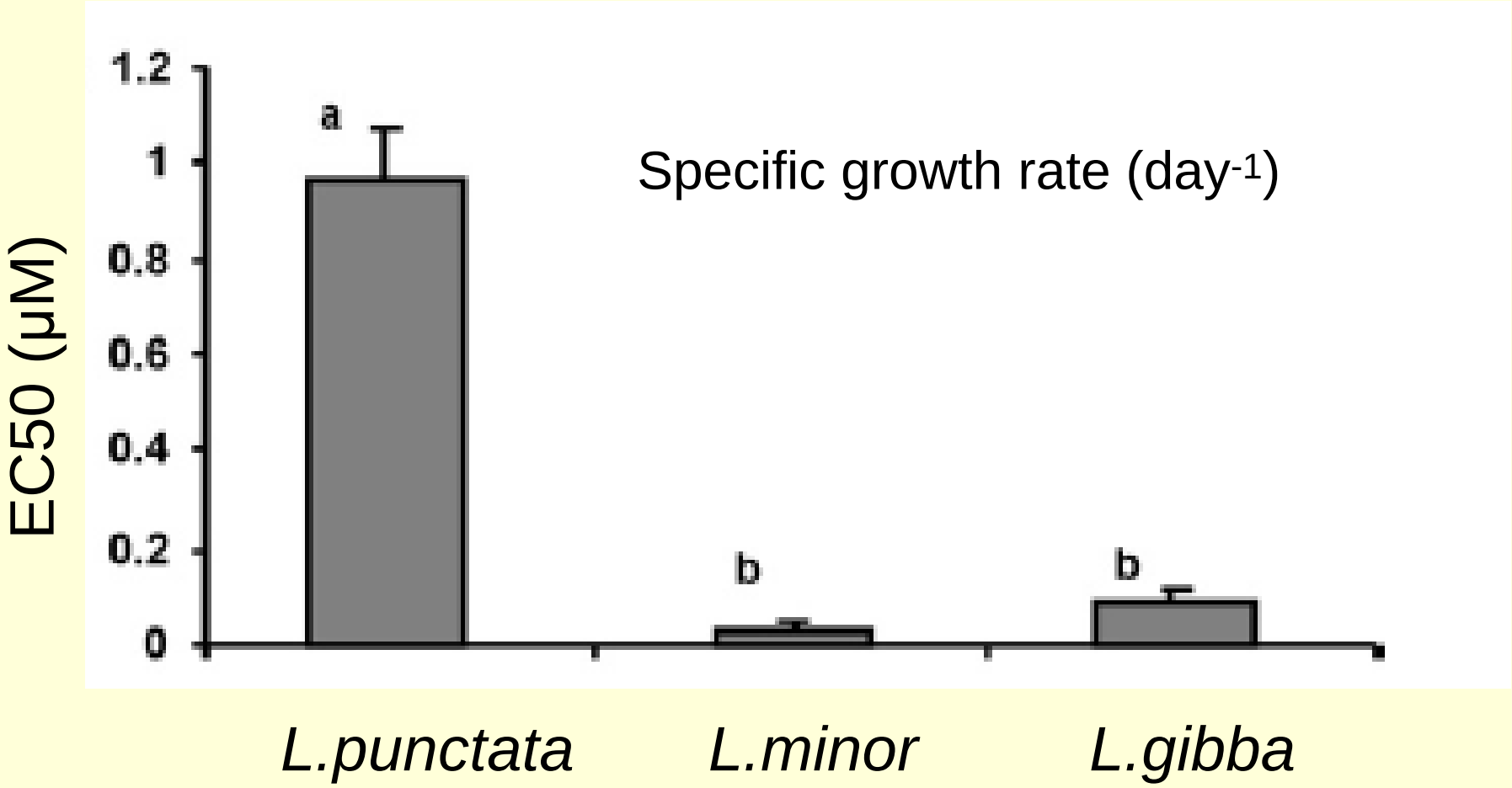
2,4,6, trichlorophenol

Chlorophenols associated with timber treatment plants



- Plant uptake
- Phytotoxicity
- Conjugation by Glutathione S-transferases (GSTs)

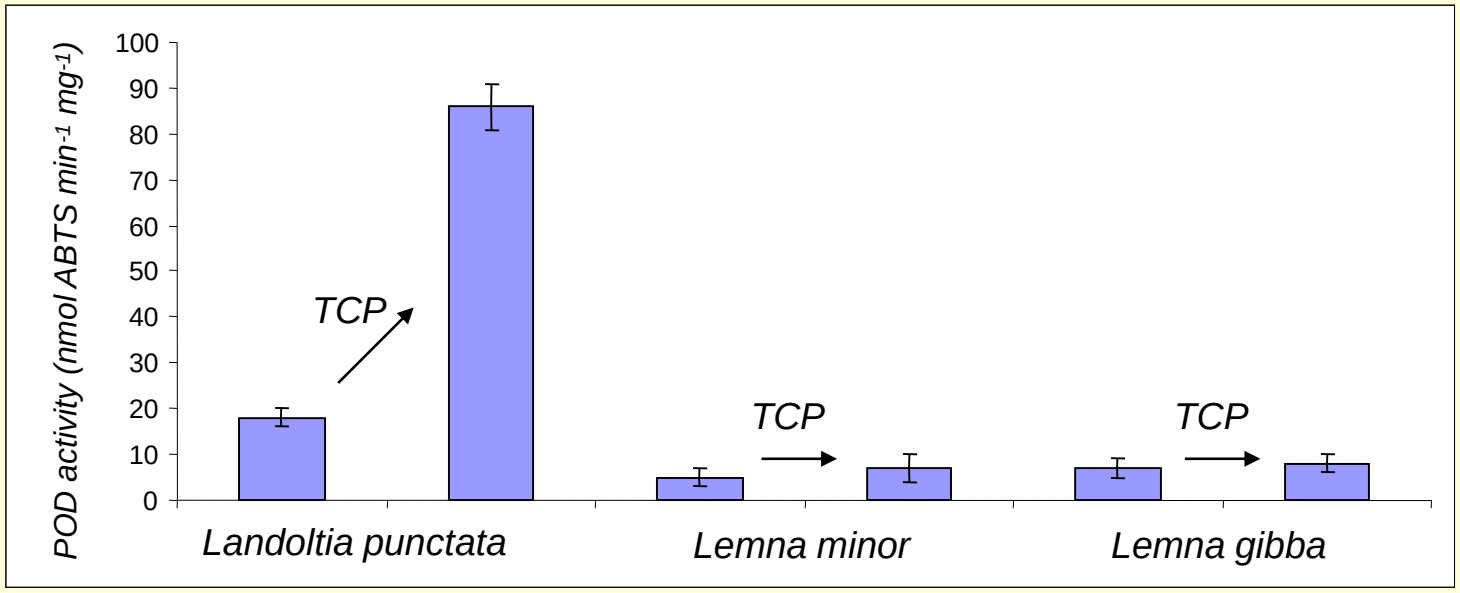
TCP is phytotoxic



Peroxidase activity "in medium" *L. punctata*

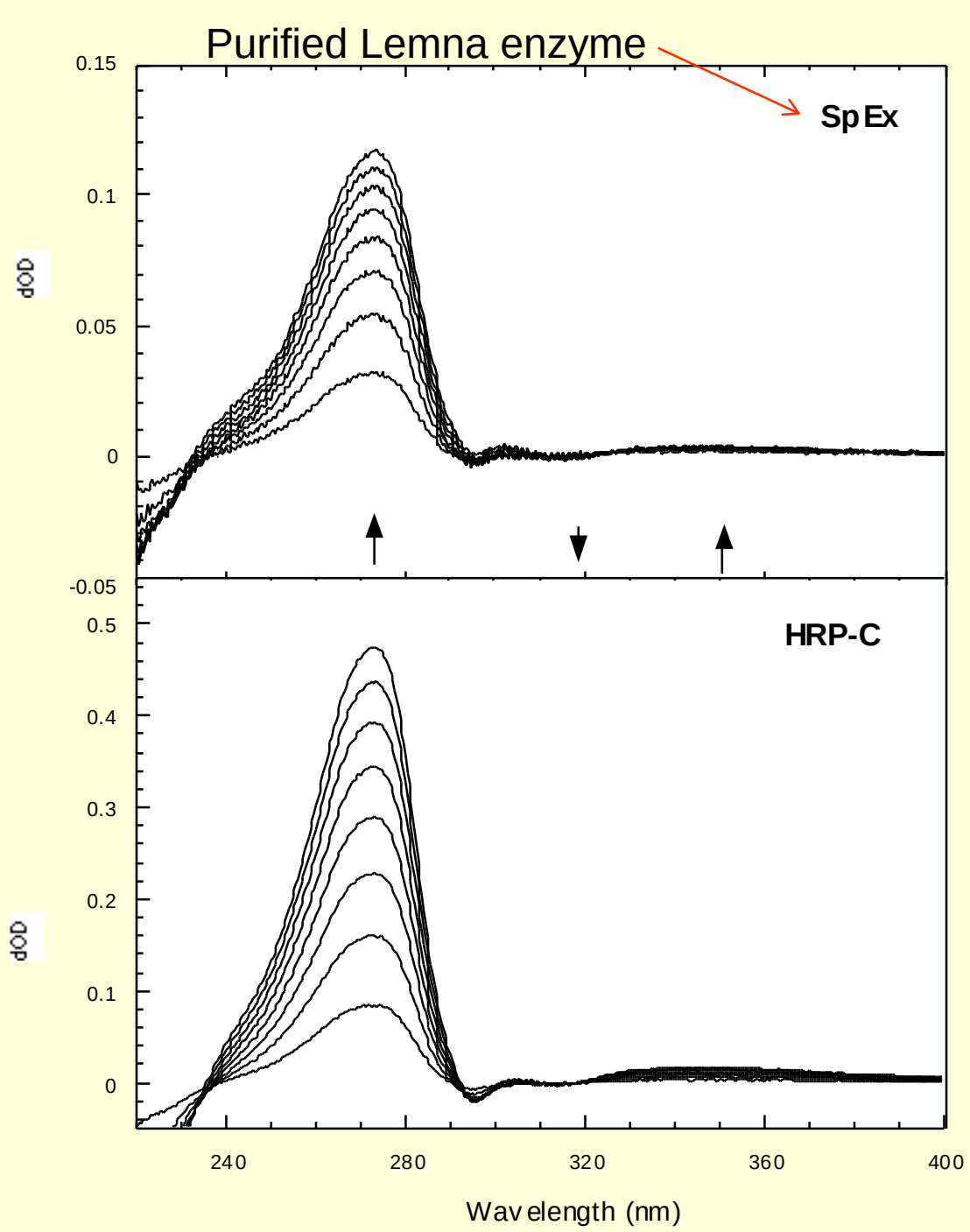
(stressor induced up-regulation of intracellular POX is common)

(no upregulation extracellular POX by heavy metals, herbicides, elicitors)



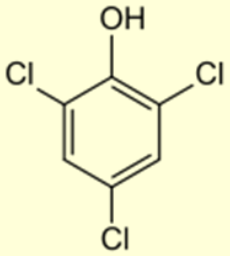
Response to TCP concentration that gives ca. 15% growth inhibition

Severe TCP stress results in decreased extracellular POX activity

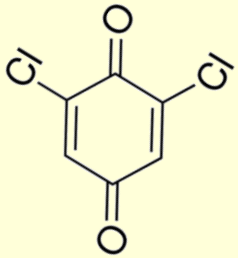


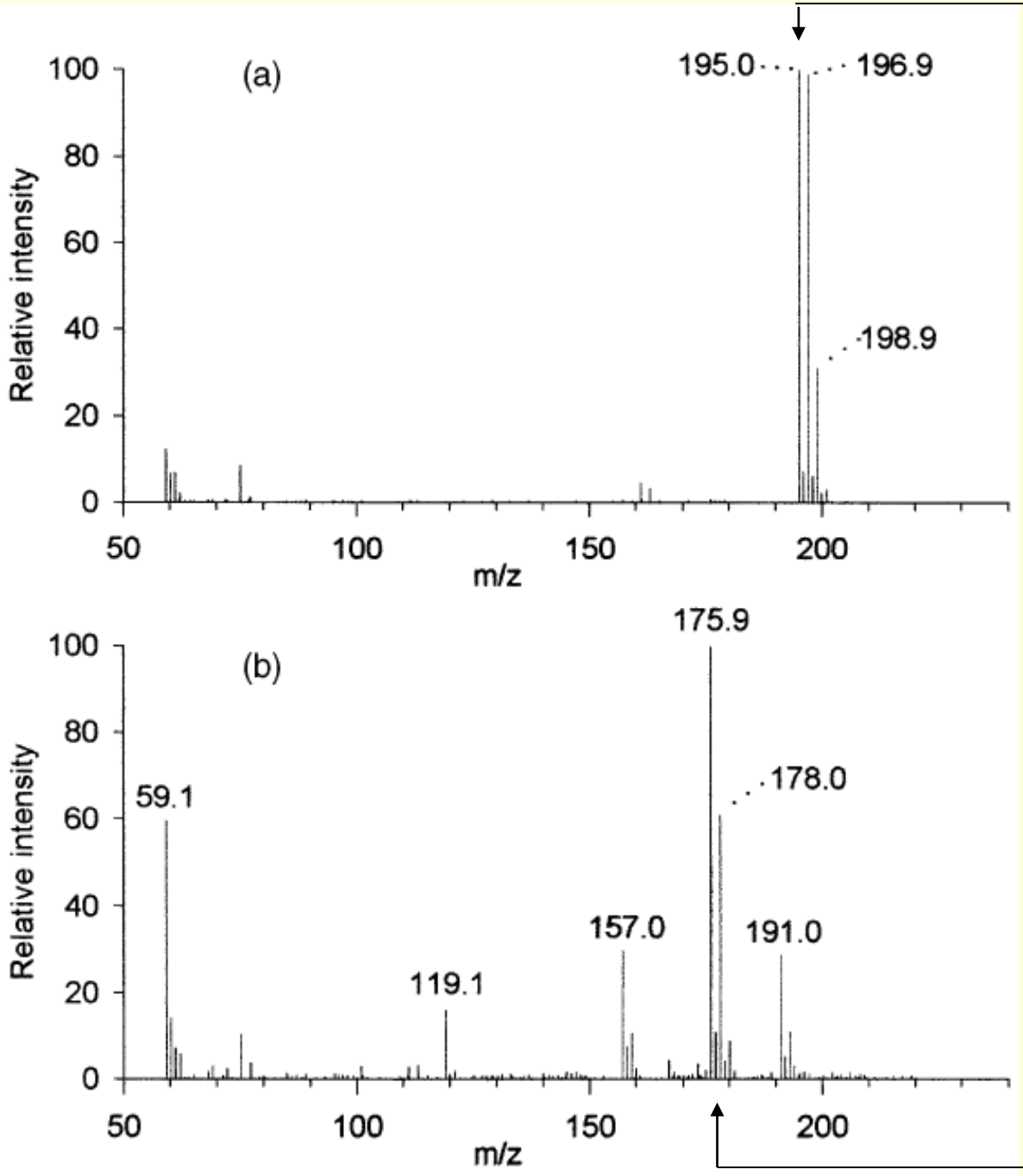
UV/VIS spectrophotometry showing oxidative dechlorination TCP

Enzyme + H_2O_2 + 2,4,6, TCP



Oxidation to 2,6 dichloro-1,4 benzoquinone





m/z 195 TCP
(+2, +4, +6 for ^{37}Cl)

TCP - no enzyme

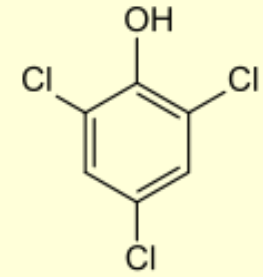
LC-MS

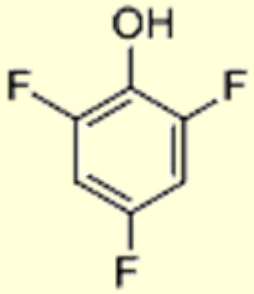
TCP - peroxidase

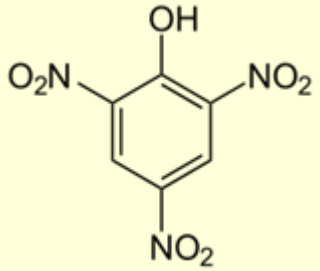
Isomer dichloro benzoquinone

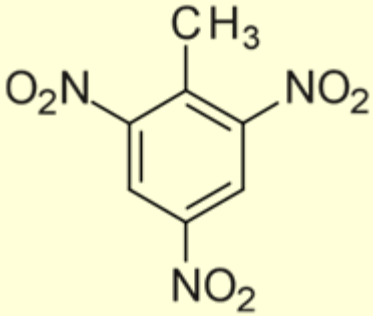
Substrates for class III peroxidases

(TCP, TBP and TFP all induce extracellular POX)

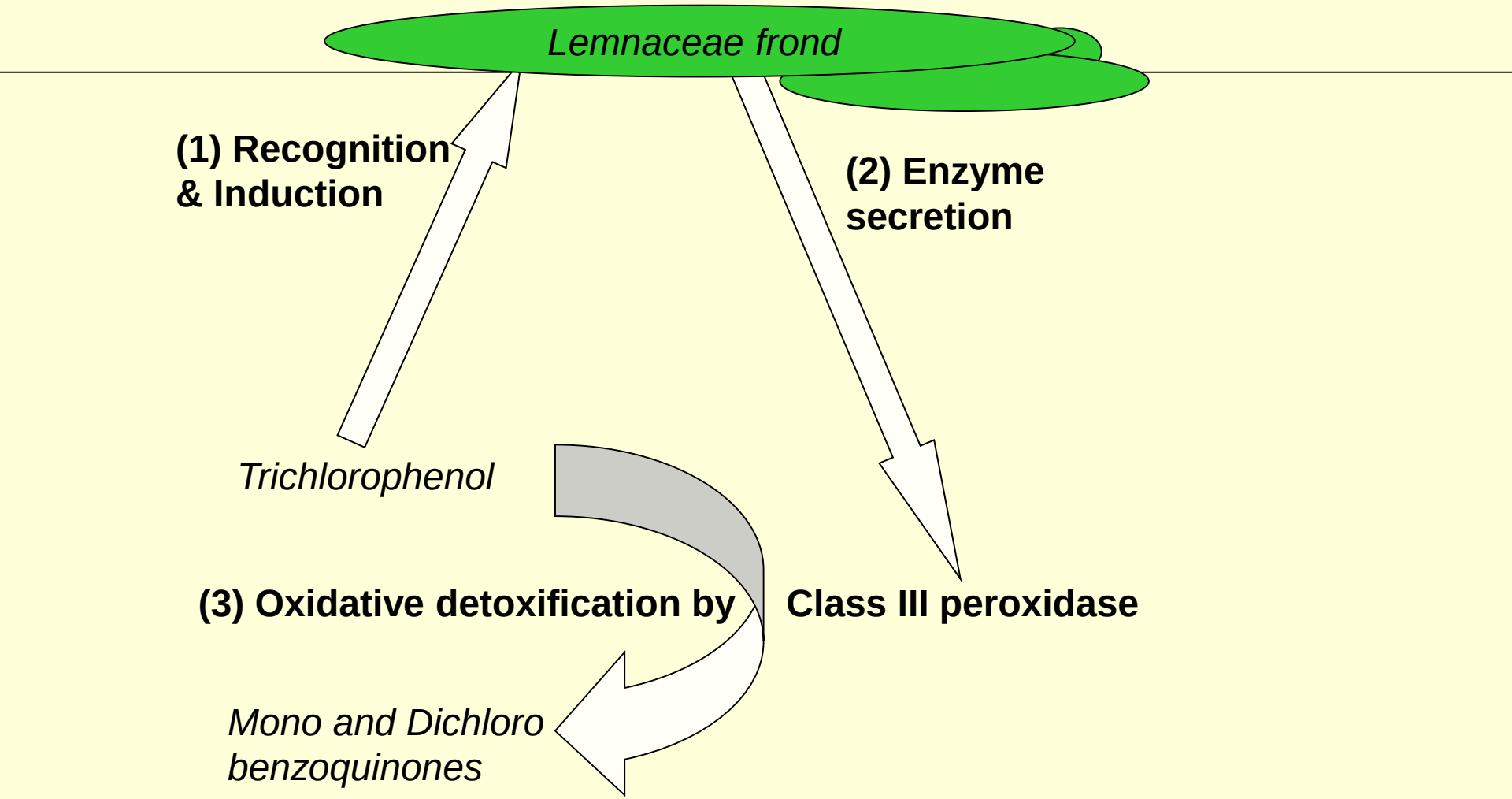








Three components of *Lemnaceae* response system



Lemnaceae; what are they?

Lemnaceae; the model of the 1970 through to 1990s

Lemnaceae; the guinea-pigs of the plant kingdom

Lemnaceae; the cleaners of the plant kingdom

Lemnaceae; Invasive aliens?

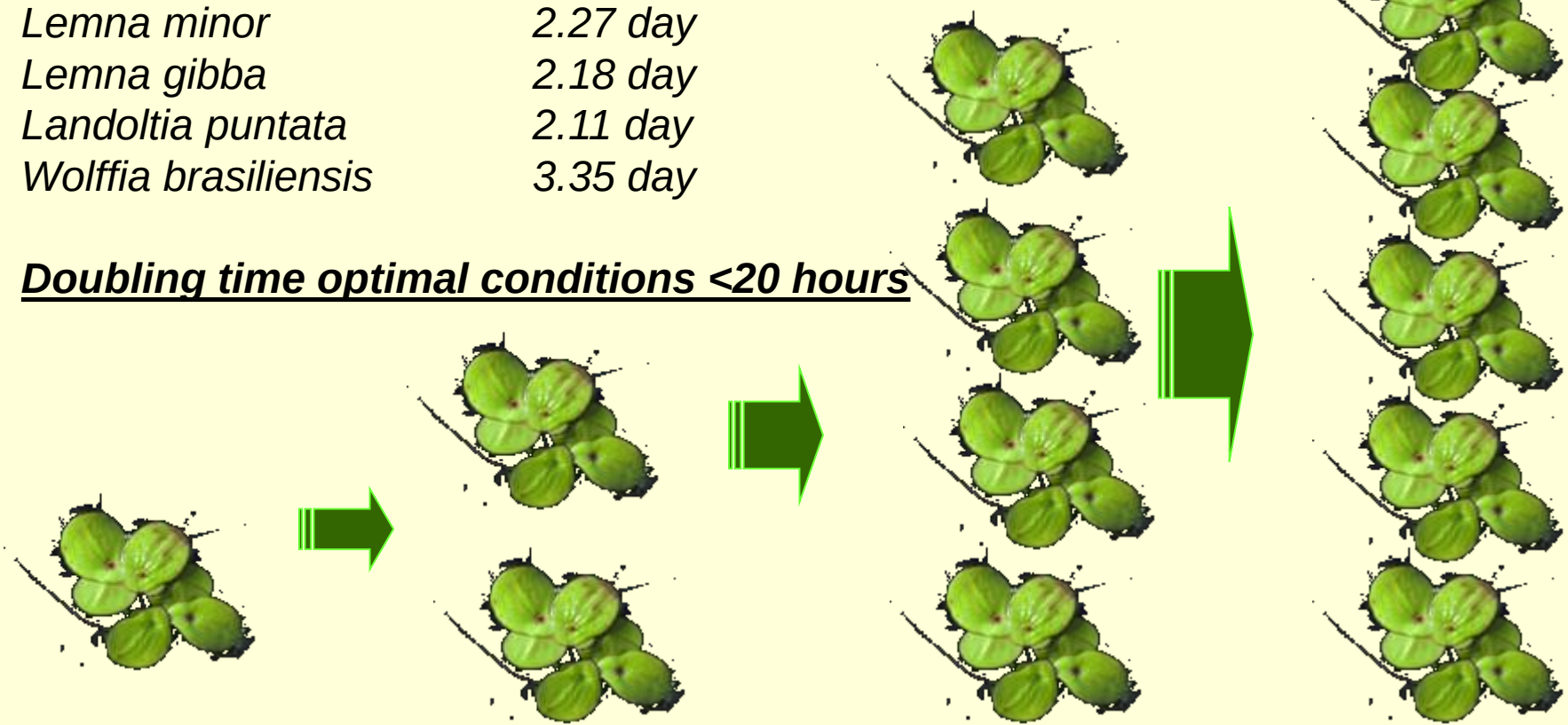
Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels

Small plant – fast grower!

Doubling time in growthroom at UCC

Lemna minor	*2.27 day*
Lemna gibba	*2.18 day*
Landoltia puntata	*2.11 day*
Wolffia brasiliensis	*3.35 day*

Doubling time optimal conditions <20 hours



http://www.mobot.org/jwcross/duckweed/duckweed.htm

Fast growth; problem *or* opportunity ?

Pennsylvania

http://www.voltairenet.org/The-Promise-of-Restitution-of

Lake Maracaibo - Venezuela

http://imageshack.us/

Cork

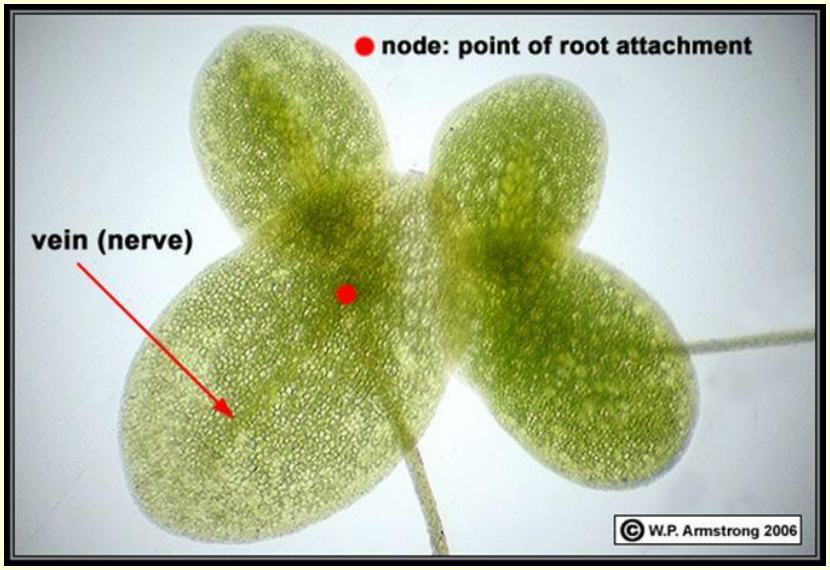
Fast growth; undesirable, invasive, alien species

Distribution *Lemna minuta*

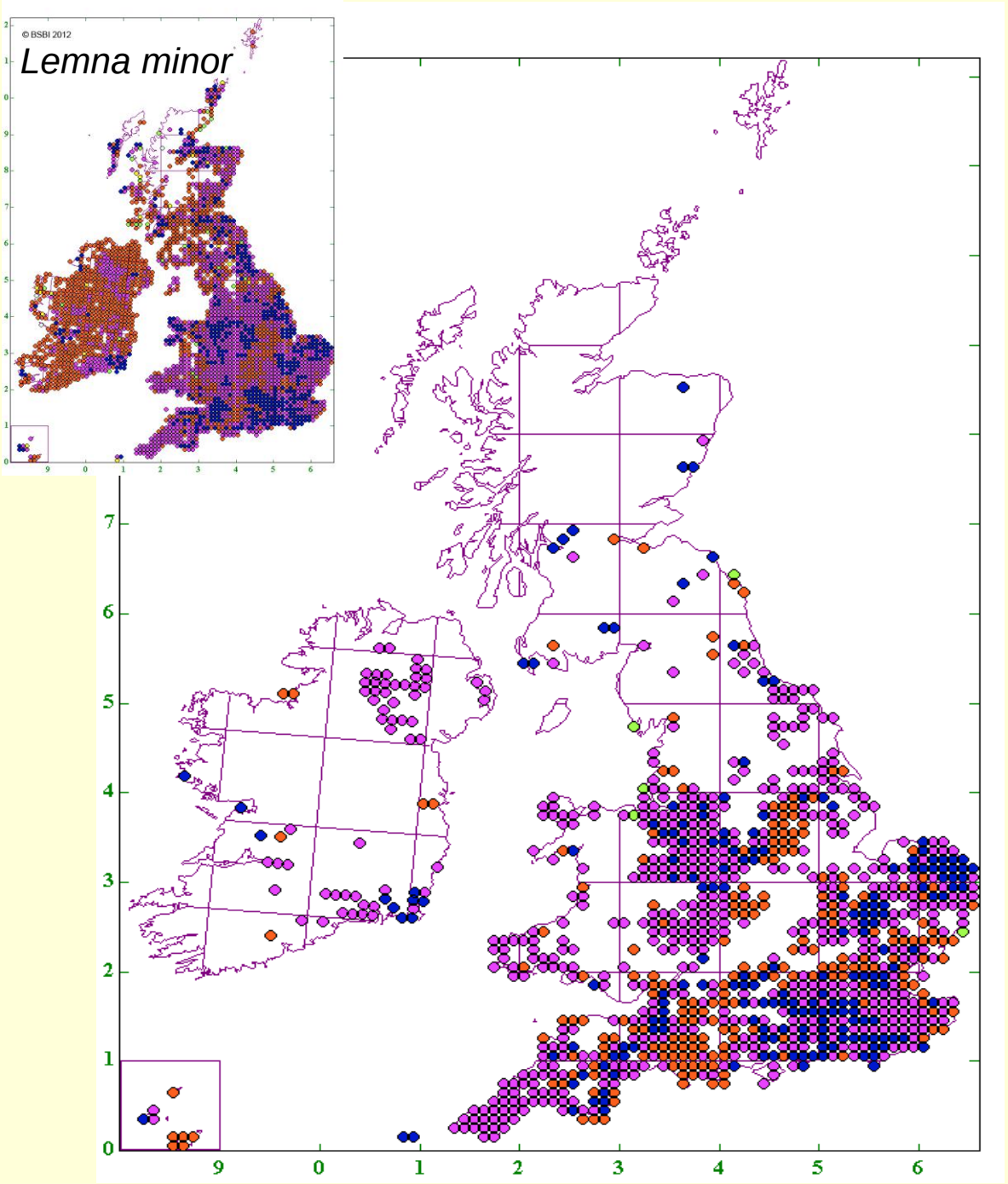
Landolt and Kandeler, 1987

Native in all (parts?) America's

Invasive, alien species in Europe and parts of Asia



waynesword.palomar.edu



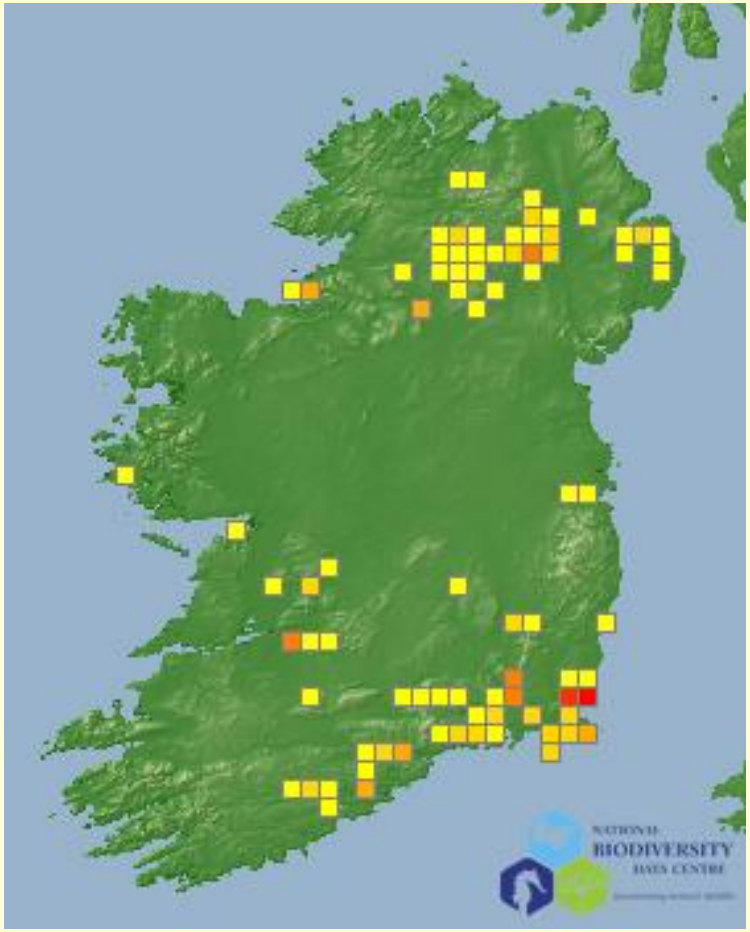
Lemna minuta in the UK

Competes with *L. minor*

First discovered 1977

Green; pre-1986
Red; 1987-1999
Purple; 2000-2009
Blue; since 2010

BSBI Maps Scheme



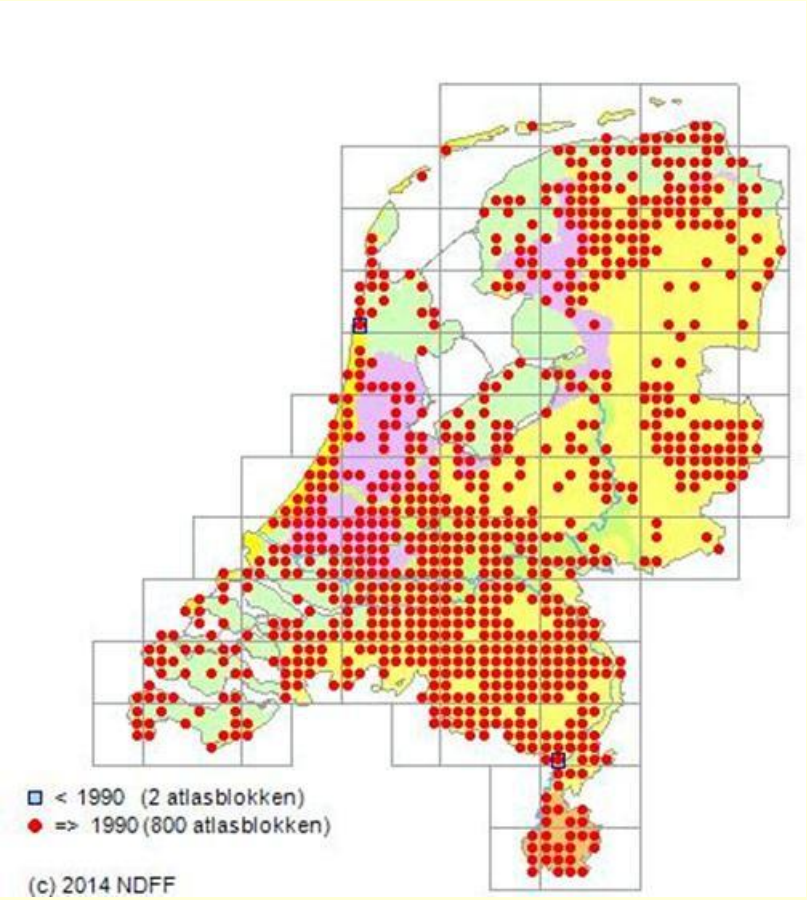
Lemna minuta in Ireland

Competes with *L. minor*

First discovered 1993

Yellow – Red ; Gradient of increasing density

National Biodiversity Data Centre



Lemna minuta in the Netherlands

Competes with *L. minor*

First discovered 1989???

Blue; Before 1990

Red: Since 1990

FLORON Verspreidingsatlas Planten

Species present in France (first European record 1965?), Austria, Belgium, Denmark, France, Germany, Greece, Hungary, Ireland, Netherlands, Poland, Spain, Sweden, Switserland, Ukraine, UK.

Also present in India, Japan and Australia.

IUCN, the World Conservation Union, states that the impacts of **alien invasive species** are immense, insidious, and usually irreversible.

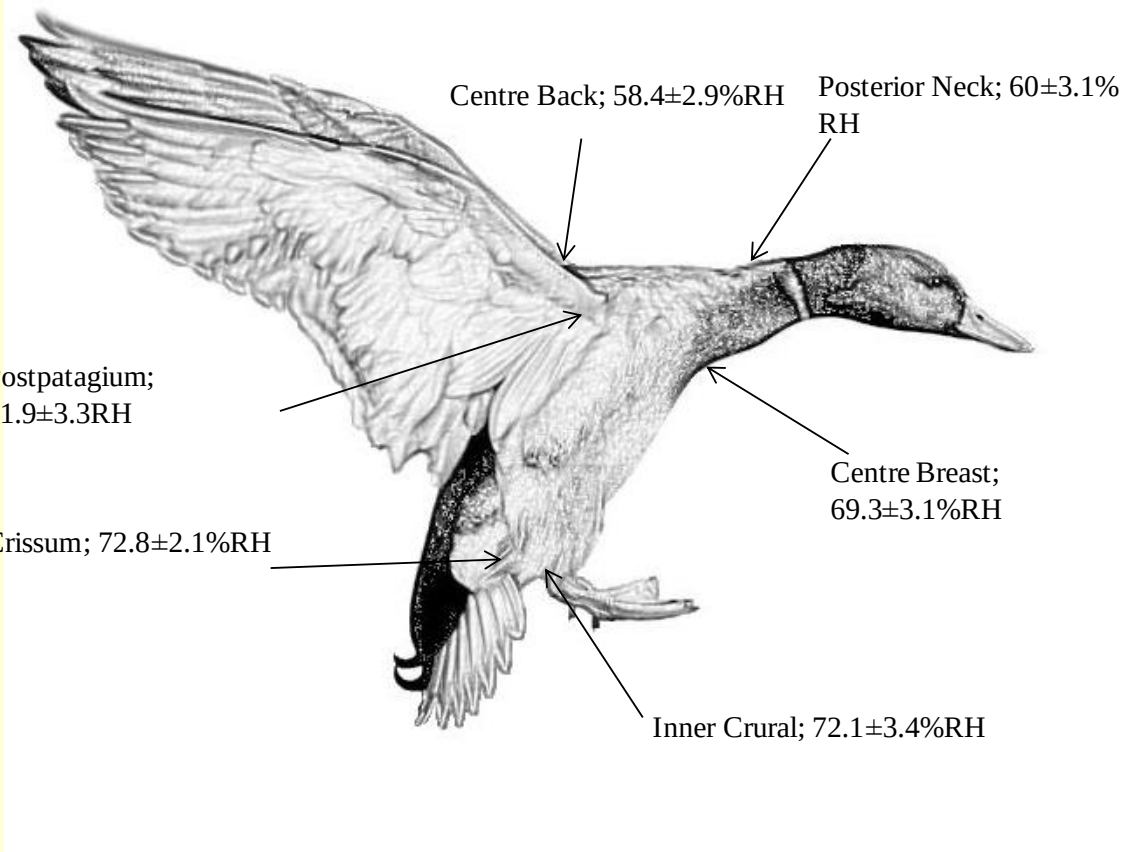
Impacts on;

- biodiversity
- human health
- economies

Lagarosiphon major in the Corrib; impacts on water extraction, boating, diving, and fishing

How does *Lemna minuta* travel?

Epizoochory



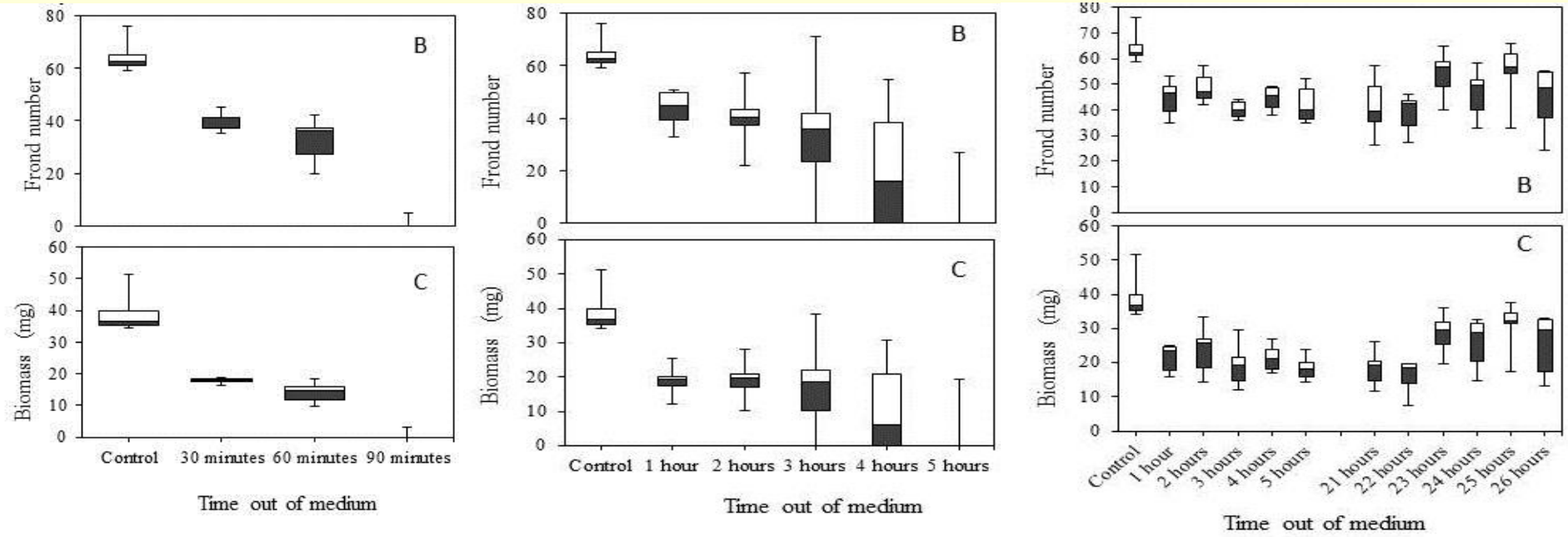
Coughlan, Kelly, Jansen, 2014

Desiccation tolerance is the key limitation to the “colonization capability” of Lemnaceae species

RH 44%

RH 58%

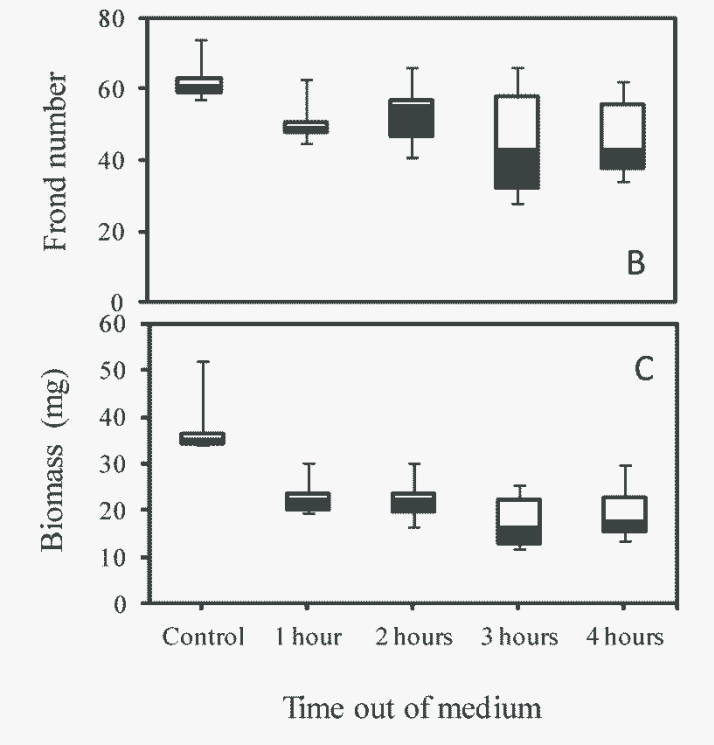
RH 95%



Survival of *Lemna minuta* outside aquatic medium

At a moderate RH (58%) still substantial survival after 2 hours out the water

What about the drying impact of wind?



New colonies (B) and biomass (C) produced by drought stressed *L. minuta*.

Plants were drought stressed between the feathers of the inner crural area of the leg.

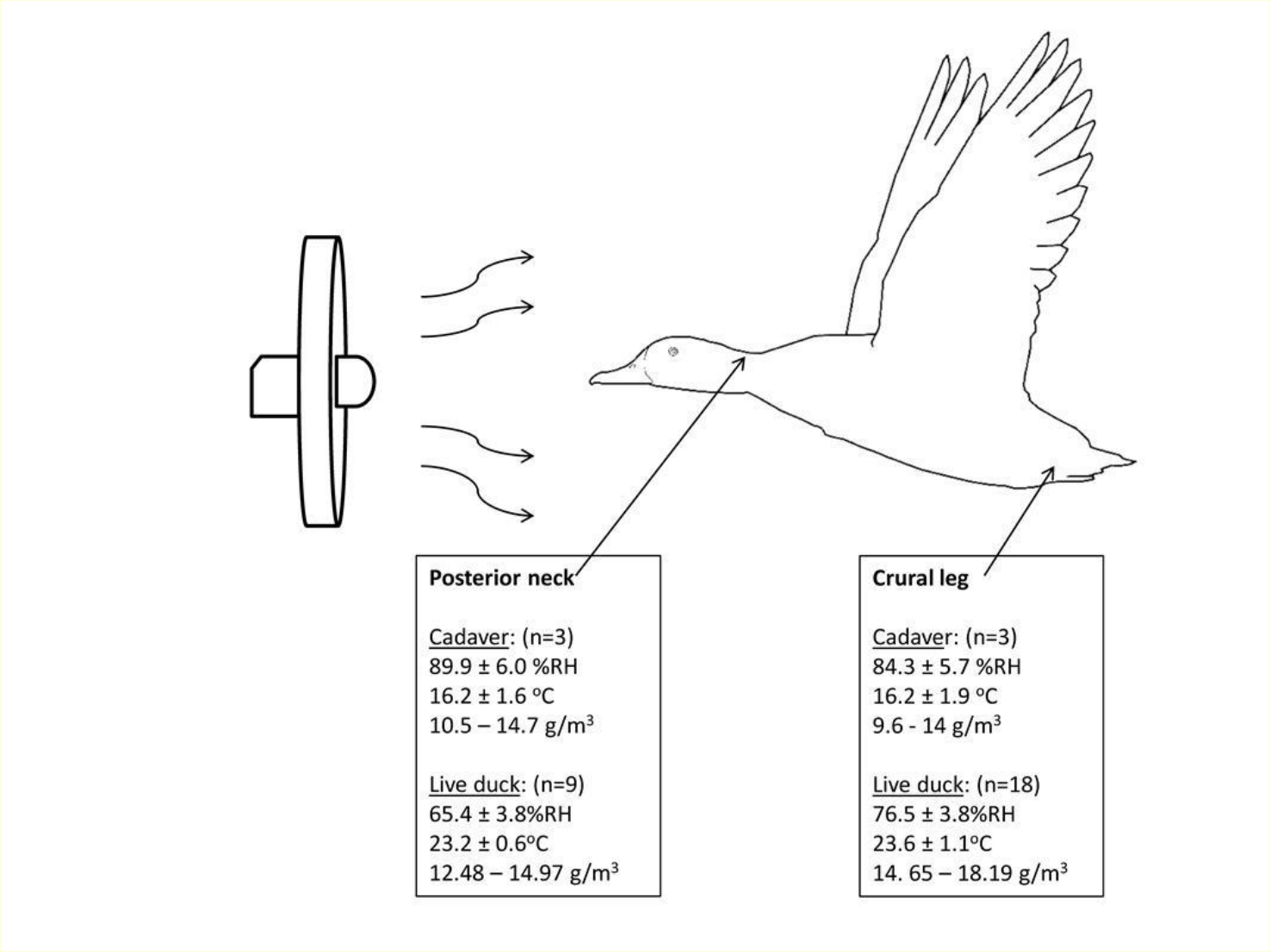
Relative humidity of 84.3 ± 5.7 %, temperature of 16.2 ± 1.9 ˚C, and a vapour density ranging between 9.6 and 14.0 g/m^3.

Prolonged (2-4 hours) viability of *L. minuta* fronds inserted between the feathers of a mallard duck

Pieces of a puzzle:

- Evidence of entanglement and retention *L. minuta* between feathers
- Mallard ducks travel at up to 65km/h, i.e. within 2-4 hours they can travel from;
  - Ireland to England,
  - England to Europe
  - Brno to Prague
- Mallards from northern areas such as Iceland, Russia, Baltic States, northern Poland and Germany, display seasonal migrations to France, Ireland and Britain
- Role birds in dispersal first suggested by Charles Darwin in 1859

The drying impact of wind versus RH and T?



What is next?

- Real flights of different durations
- Use of homing-pigeons with climate and GPS sensors
- Evidence retention and survival
- Modelling and mapping

Lemnaceae; what are they?

Lemnaceae; the model of the 1960 and 1970s

Lemnaceae; the guinea-pigs of the plant kingdom

Lemnaceae; the cleaners of the plant kingdom

Lemnaceae; Invasive aliens?

Lemnaceae; fashionable, high tech, sustainable foods, feeds and fuels?

Lemnaceae; problem *or* opportunity ?

Lemnaceae in traditional /sustainable agriculture (FAO 2011)

http://www.fao.org/ag/againfo/resources/documents/DW/Dw2.htm

Lemnaceae; problem *or* opportunity?

- Yield up to 4 ton FW / hectare / day
- Technology for growth, harvesting and processing
- Bio-crude (renewable fuel)
- Protein (43% DW) feed aquaculture
- Essential amino acids (Lys, Leu, Ile, Phe, Thr, Val)

PetroAlgae Inc Florida Lemna production

http://www.petroalgae.com/

Lemnaceae; “fashionable, new” opportunity ?

Cheese Pie Crisp

- 1 cup Lemna gibba
- 2 medium-large chopped onions fried
- 30 grams butter
- 2 crushed garlic cloves
- 0.5 cup flour
- 1 cup chopped mushrooms
- 50 grams grated cheddar cheese
- 1 tablespoon powdered vegetable soup
- pepper, paprika, nutmeg to taste.

The onions were fried in 1 teaspoon of oil until light brown.
Lemna gibba L. cv. Galilee was added to the mix just before baking.
Prepare puff pastry, spread or roll flat and place in baking pan.
Spoon cheese mixture into pastry shell and bake in a preheated oven at 200° C. for 20 minutes.

Lemnaceae bioreactor?

- Food fortification?
- Phytoremediation?
- Secretion desirable products (insulin, vaccines)
- Biolex; production therapeutic glycosylated proteins, including monoclonal antibodies and interferon (IFN-alpha2b)

http://www.lemnagene.com/

…….*Lemnaceae* and the Green Economy…..?

Molecular transformation technologies

Understanding Physiological potential

DOE-JGI genome sequence

Engineering solutions

Green technology

Protein/biofuel

Phytoremediation
Food fortification

Pharmaceuticals