Aquatic Tier-3 effect assessment procedure (microcosm and mesocosm experiments)

Theo C.M. Brock

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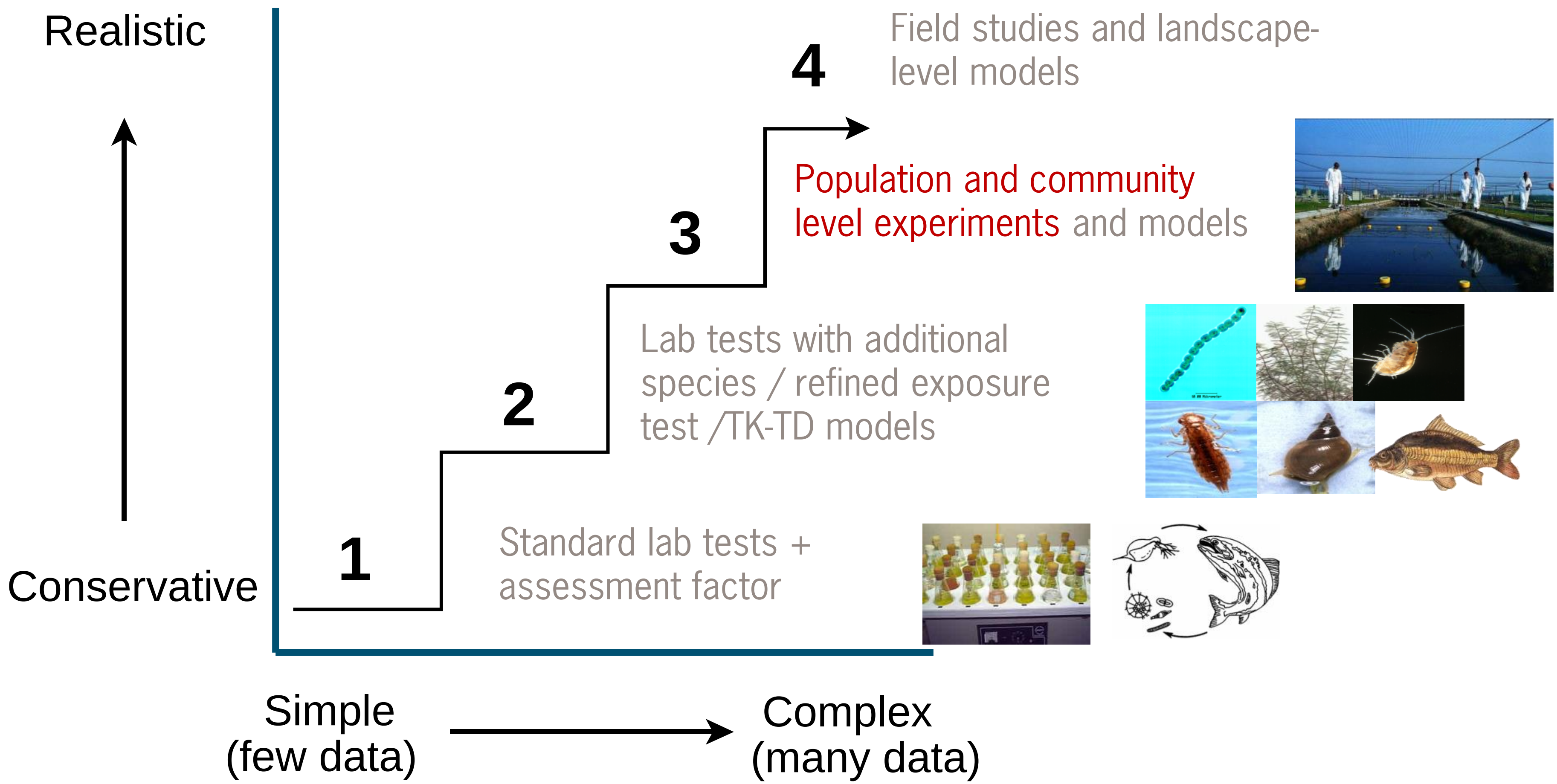
Tiered approach in effect assessment



Model ecosystems (microcosms, mesocosms)

- Constructed artificially with samples from, or portions of, natural ecosystems
- Housed in artificial containers or enclosures
- Reduction in size and complexity when compared with natural ecosystems
- Include an assemblage of organisms representing several trophic levels
- Community should be "adapted" to and in "equilibrium" with its ambient environment

Guidance on interpretation of micro/mesocosm tests: CLASSIC workshop (Giddings et al. 2002); RIVM report (De Jong et al. 2008); EFSA Aquatic Guidance Document (EFSA PPR 2013); Brock et al. 2014

Examples of microcosms and mesocosms

Most experience with lentic freshwater ecosystems

MODEL ECOSYTEMS versus NATURAL ECOSYSTEMS

PROS

- Experimental control and replication
- Their flexibility make them useful to a wide range of experiments
- More easily sampled and quantifid
- Costs may be lower
- Less ethical to perform tests in natural ecosystems

CONS

- No perfect analogs of natural ecosystems
- Lack of large predators
- Wall effects (more periphyton, less turbulence)

Cause-and-effect relations in micro/mesocosms

Direct or primary effects

- Toxicological effects on survival, growth and/or reproduction of organisms
- Fairly well predictable on basis of laboratory toxicity tests and knowledge on field exposure

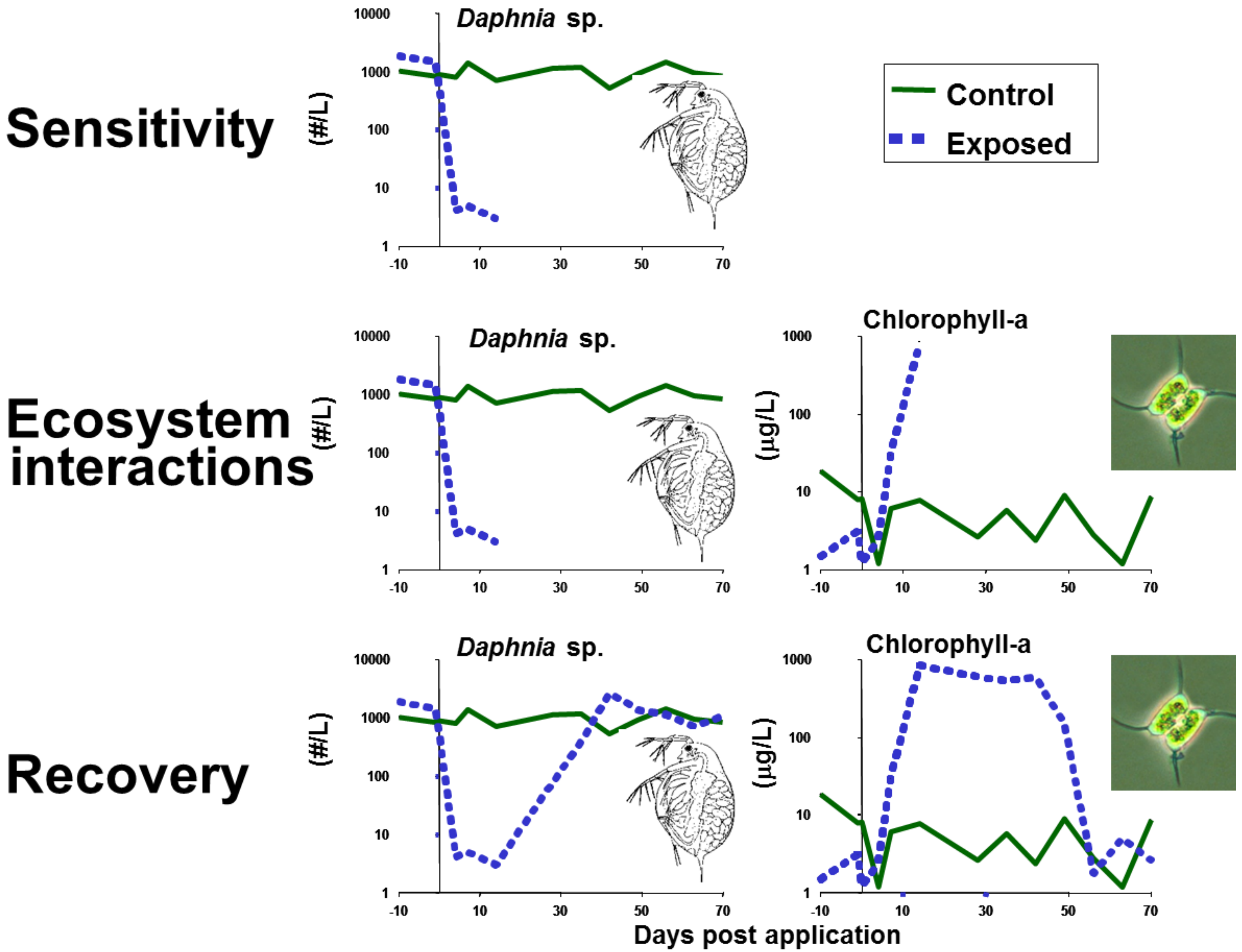
Indirect or secondary effect

- Ecosystem changes that result from a reduction of (toxicant) susceptible species
- The same pesticide, dosed at a similar rate, may cause different secondary effects in different types of ecosystem

Delayed effects and biological recovery

- The duration of the studies may be long enough to observe latency of effects and recovery of affected populations

Cause-and-effect relationships in micro/mesocosms



The conduct of micro/mesocosm tests

- Experimental set-up: ANOVA versus Regression

  - 4 replicates * 3 treatments

1	2	3	4	5	6	7	8	9	10	11	12

  - 1 replicates * 12 treatments

1	2	3	4	5	6	7	8	9	10	11	12

Experimental set-up of micro/mesocosm tests

Combine the best of two worlds, replicated regression design

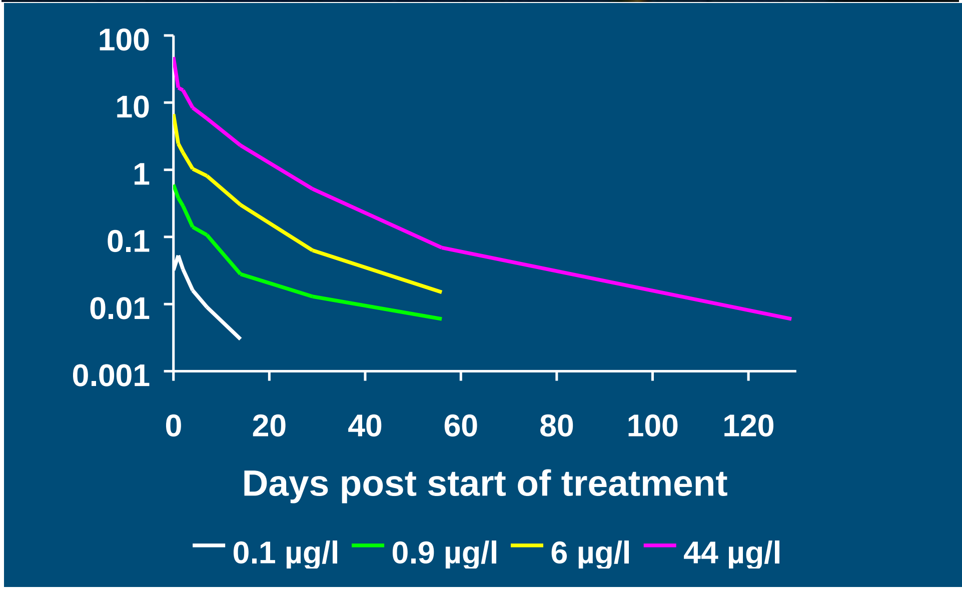
- E.g. the 3 replicates * 5 treatments + 4 controls experimental set-up
- Analysed with Williams test (combination between ANOVA and regression)
- Test assumes an increasing effect with an increasing dose

Chlorpyrifos: mesocosm experiment in the Netherlands

Single application (spray drift)

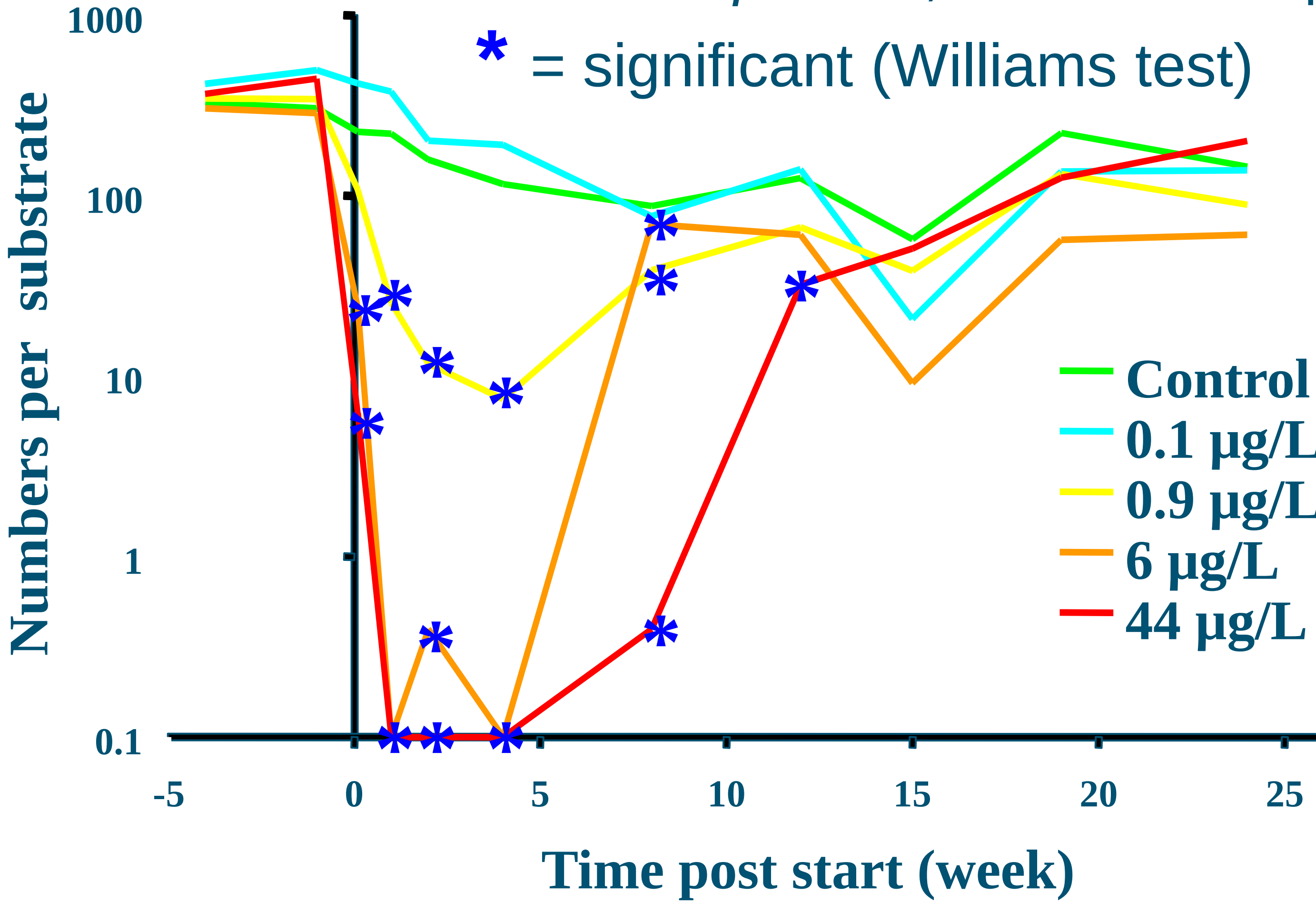
Macrophyte-dominated ditches

• 4 mesocosms	controls
2	0.1 µg/L
2	0.9 µg/L
2	6 µg/L
2	44 µg/L



Univariate statistics sensitive endpoints

Cloeon dipterum; NOEC = 0.1 μg/L



Allows to evaluate effects on more or less dominant populations

Chlorpyrifos example: Evaluation with univariate statistics

Typical outcome univariate NOEC calculations:

Results 19 of the 189 species collected

Taxon	Effect	-4	-1	0.1	1	2	4	8	12	15	19	24
Annelida												
Oligochaeta	+	>	>	>	>	>	0.9	6	>	>	>	0.9
Stylaria lacustris	+	>	>	>	>	>	0.9	0.9	>	>	>	>
Arthropods												
Crustacea												
Simocephalus vetulus	-	>	>	0.9	0.9	0.9	0.9	6	>	>	>	>
Daphnia galeata	-	n.p.	n.p.	n.p.	>	>	0.1	0.1	6	6	>	>
Ostracoda	-	n.p.	L!	6	0.9	6	6	0.9	6	6	6	>
Copepoda (mature stages)	-	>	>	>	L!	6	>	0.9	>	>	>	>
Copepoda (nauplii)	-	>	>	0.9	0.9	0.9	0.9	6	>	n.p.	>	n.p.
Gammarus pulex	-	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.1	L!	0.1	0.1	0.1
Insecta												
Caenis horaria	-	>	>	6	0.9	0.9	0.1	0.9	6	0.9	6	0.9
Caenis luctuosa	-	n.p.	n.p.	6	n.p.	0.9	0.1	0.9	>	>	n.p.	n.p.
Cloeon dipterum	-	>	>	0.9	0.1	0.1	0.1	6	>	>	>	>
Coenagrionidae	-	>	>	>	6	6	6	>	>	>	>	>
Sialis lutaria	-	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.1	n.p.	0.1	0.1	n.p.
Hygrotus versicolor	-	n.p.	n.p.	>	0.9	0.9	n.p.	6	0.9	n.p.	n.p.	n.p.
Mystacides longicornis/nigra		>	>	0.9	0.9	L!	0.1	>	6	6	6	>
Ablabesmyia phatta/monilis		>	>	6	0.9	0.9	0.9	6	6	>	0.9	n.p.
Ceratopogonidae	-	>	>	>	0.9	>	L!	6	6	>	>	>
Chaoborus obscuripes	-	L!	>	0.1	0.9	0.1	0.9	6	6	6	>	6
Chironomus	-	n.p.	n.p.	n.p.	>	n.p.	>	>	6	6	>	>

Chlorpyrifos example: Multivariate analysis

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 18, No. 2, pp. 138–148, 1999

0730-7268/99 $9.00 + .00

PRINCIPAL RESPONSE CURVES: ANALYSIS OF TIME-DEPENDENT MULTIVARIATE RESPONSES OF BIOLOGICAL COMMUNITY TO STRESS

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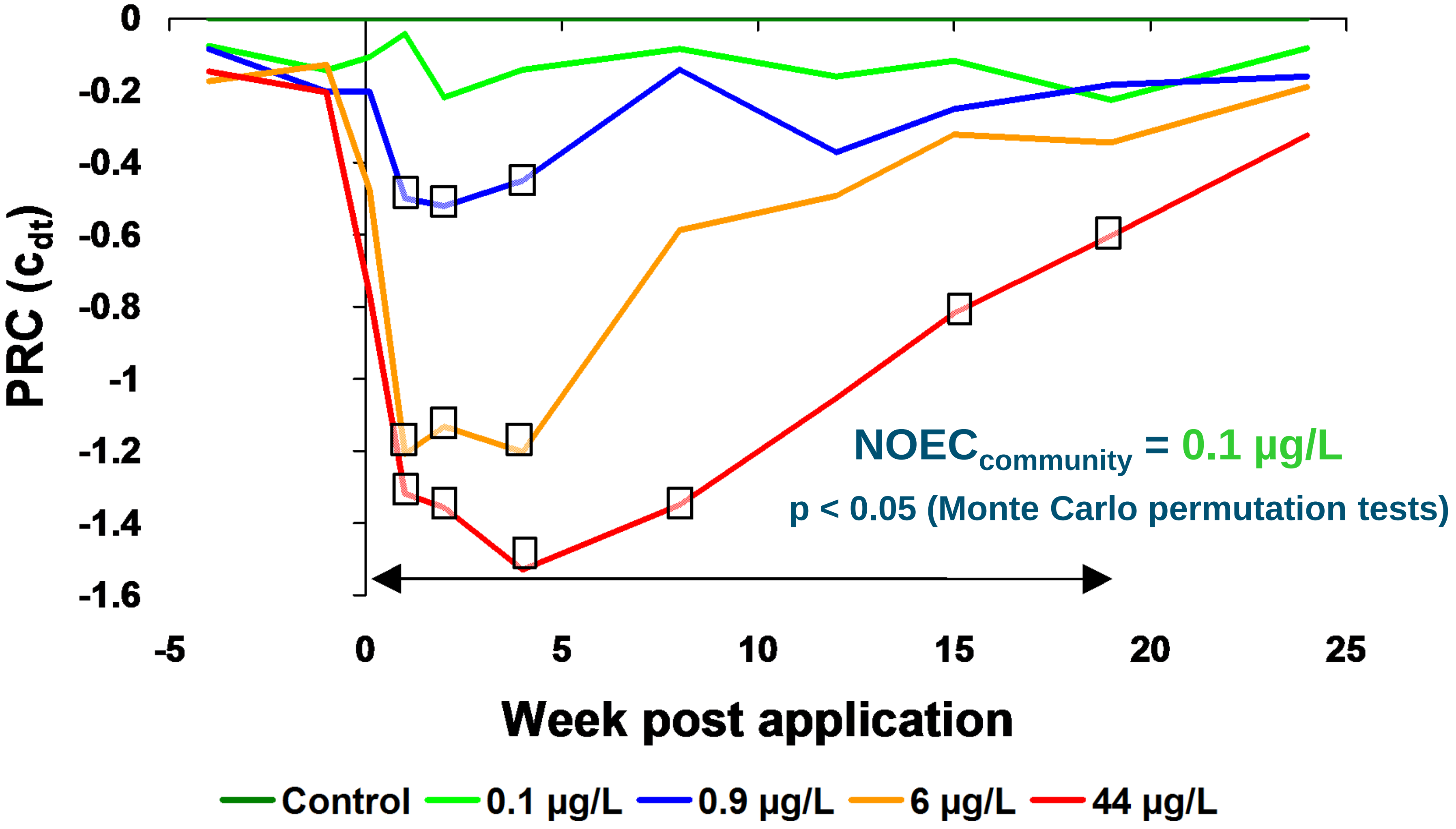
(*Received* 30 *April* 1997; *Accepted* 18 *December* 1997)

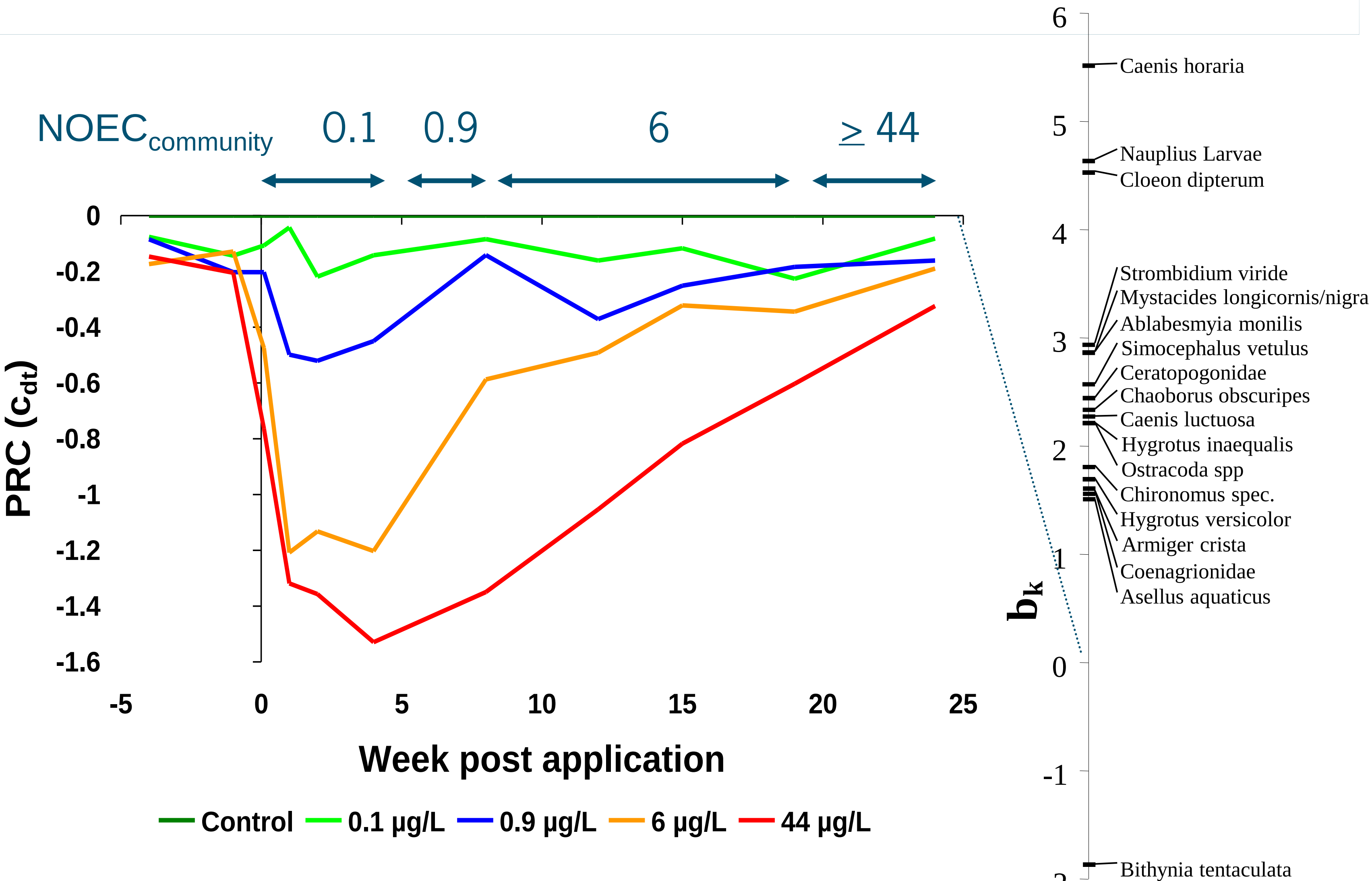
Abstract—In this paper a novel multivariate method is proposed for the analysis of community response data from designed experiments repeatedly sampled in time. The long-term effects of the insecticide chlorpyrifos on the invertebrate community and the dissolved oxygen (DO)–pH–alkalinity–conductivity syndrome, in outdoor experimental ditches, are used as example data. The new method, which we have named the principal response curve method (PRC), is based on redundancy analysis (RDA), adjusted for overall changes in community response over time, as observed in control test systems. This allows the method to focus on the time-dependent treatment effects. The principal component is plotted against time, yielding a principal response curve of the community for each treatment. The PRC method distills the complexity of time-dependent, community-level effects of pollutants into a graphic form that can be appreciated more readily than the results of other currently available multivariate techniques. The PRC method also enables a quantitative interpretation of effects towards the species level.

Keywords—Redundancy analysis Mesocosms Multivariate ordination techniques Pesticides Principal response curves

Chlorpyrifos example: Principal Response Curves



Chlorpyrifos example: Principal Response Curves



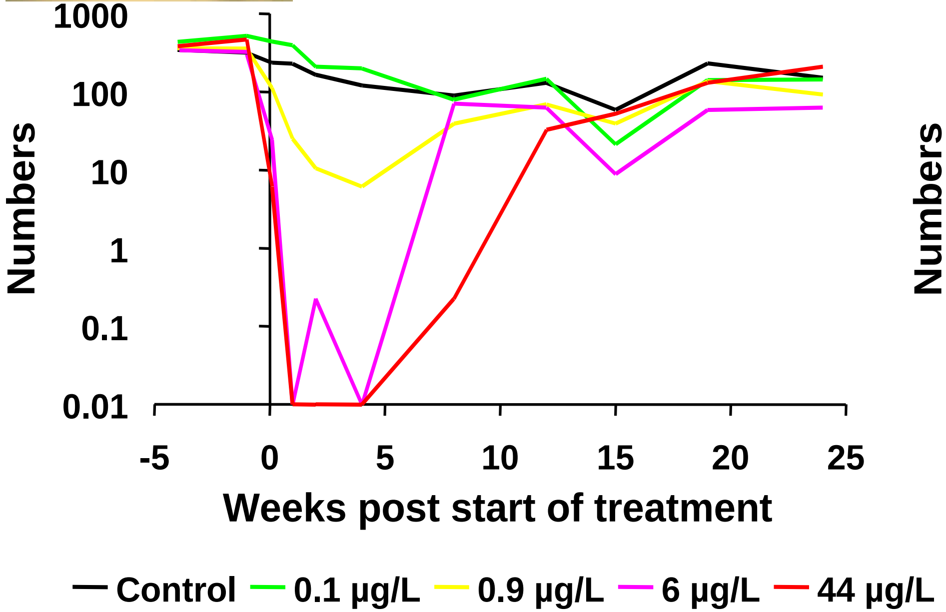
Van den Brink & Ter Braak. 1999

Allows to evaluate community-level effects

Chlorpyrifos exp.: sensitivity and recovery

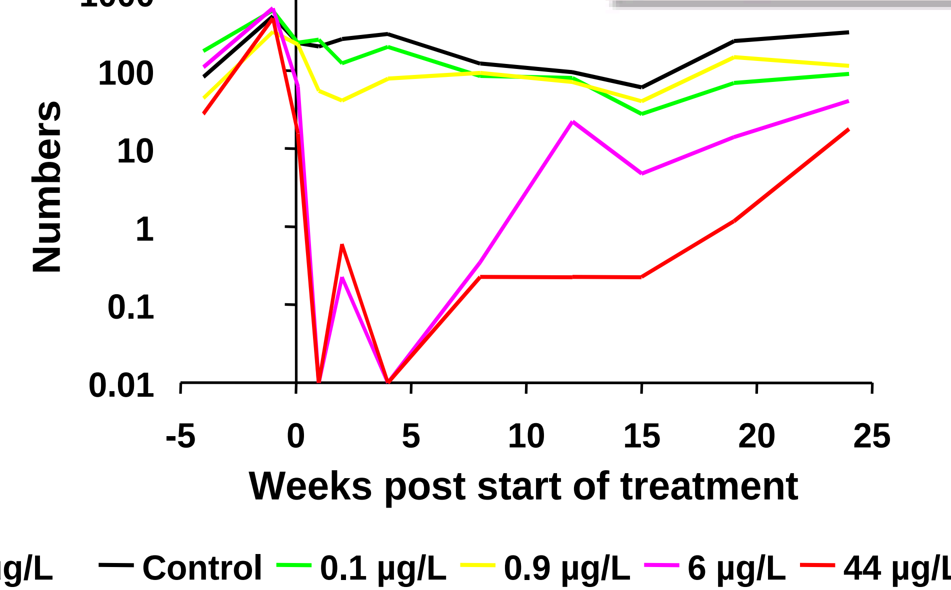
Two mayfly species

Cloeon dipterum
NOEC = 0.1 µg/L



Relatively fast recovery
- appr. 3 generations/year
- egg deposition by terrestrial adults

Caenis horaria
NOEC = 0.1 µg/L



Relatively slow recovery
- 1 to 2 generations/year
- egg deposition by terrestrial adults

Rate of recovery is affected by number of generations per year

Chlorpyrifos example: Summary mesocosm experiment

Univariate statistics:

- Consistent NOECs (consecutive sampling dates) most sensitive populations < 0.1 μg/L (slight short term effect)

Multivariate analysis

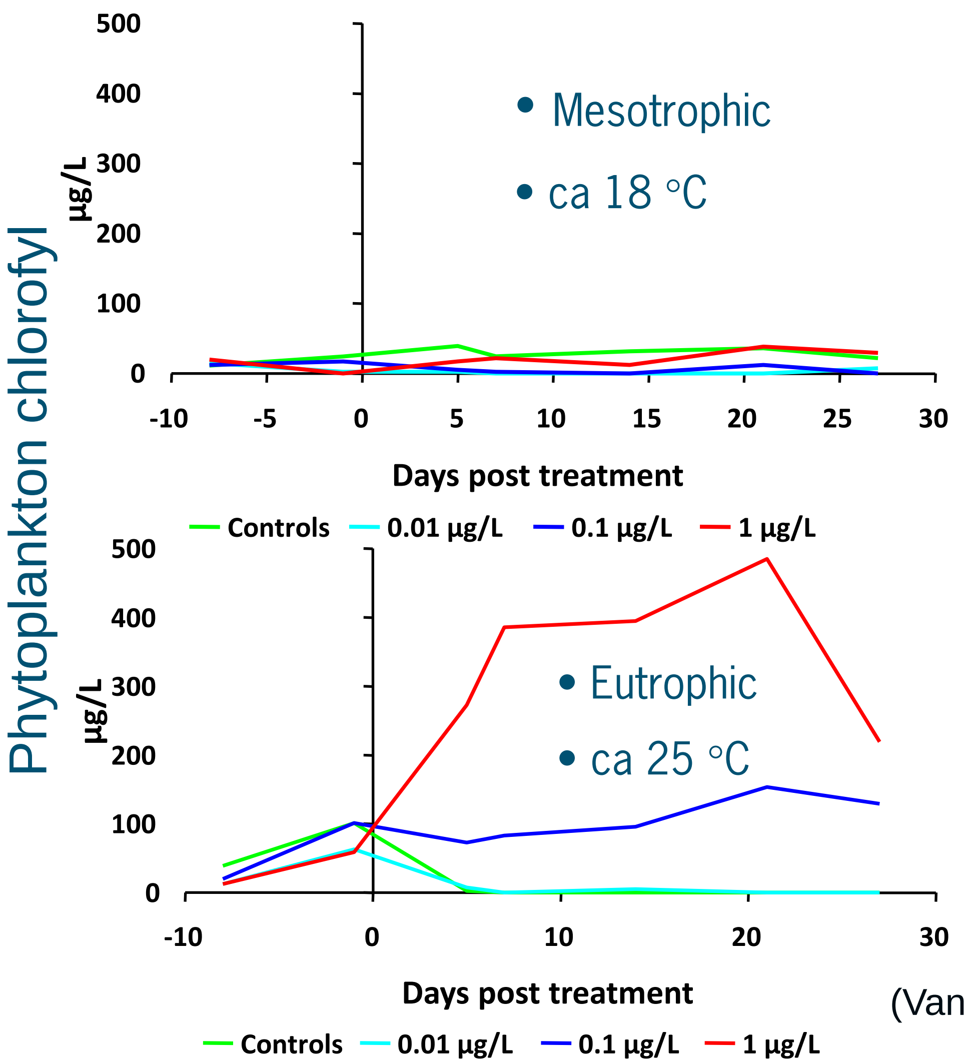
- NOEC invertebrate community = 0.1 μg/L
- At treatment of 0.9 μg/L recovery within 8 weeks

Chlorpyrifos: semi-field experiments, single application exposure

Application regime	Class 1 NOEC	Class 2 LOEC	Class 3A LOEC	Type of test system	Location Reference
6 h pulse	0.1 µg/L	-	(5.0 µg/L)	Experimental streams	Australia Pusey et al. 1994
Single	0.1 µg/L	0.3 µg/L	1.0 µg/L	Outdoor microcosms	USA, Kansas Biever et al. 1994
Single	-	0.1 µg/L	<0.9 µg/L	Experimental ditches	Netherlands Van den Brink et al. 1996
Single	0.1 µg/L	-	1.0 µg/L	Lab microcosms Cool, Mesotr.	Netherlands Van Wijngaarden et al. 2005
Single	0.1 µg/L	-	≤1.0 µg/L	Lab microcosms Warm, Eutrophic	Netherlands Van Wijngaarden et al. 2005
Single	0.1 µg/L	-	1.0 µg/L	Outdoor mesocosms	Spain López-Mancisidor et al. 2005
Single	0.1 µg/L	-	1.0 µg/L	Outdoor microcosms	Thailand Daam et al. 2008
Single	-	-	0.5 µg/L	Pond enclosures	USA, Minnesota Siefert et al. 1989

Threshold levels for effects can be extrapolated with lower uncertainty than responses caused by higher exposures (but representatives of sensitive taxonomic groups need to be present).

Ecosystem interactions and indirect effects are context dependent



1 µg/L chlorpyrifos

- reduction Cladocera
  (direct effect)
- no algal bloom

1 µg/L chlorpyrifos

- reduction Cladocera
  (direct effect)
- pronounced algal bloom
  (indirect effect)

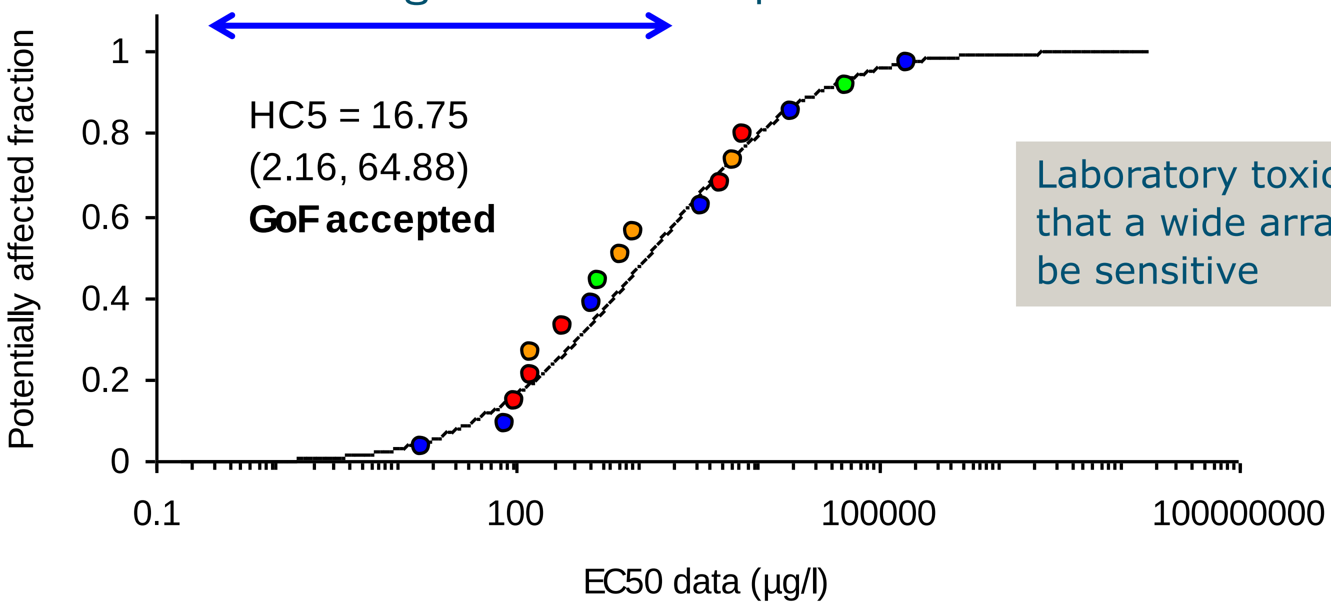
(Van Wijngaarden et al. 2005: *Pest Man Sci* 61:923-935)

Magnitude and duration of direct and indirect effects is context dependent

(In)direct effects fungicide carbendazim

- Lentic macrophyte-dominated microcosms
- 0 – 3.3 – 33 – 100 – 330 – 1000 μg/L
- chronic exposure regime (constant during 4 weeks)

Concentration range microcosm experiment

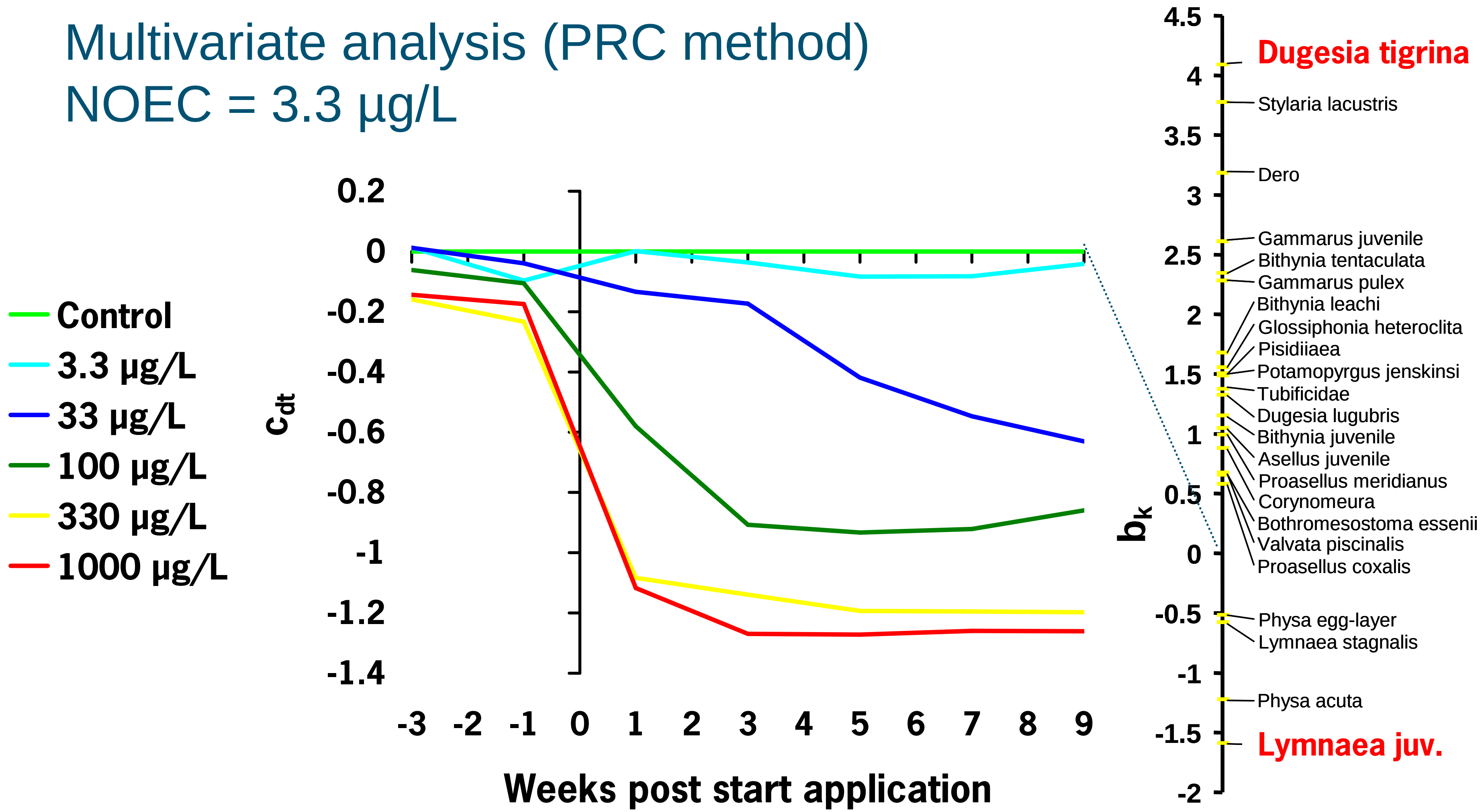


Laboratory toxicity data suggest that a wide array of species may be sensitive

(Van den Brink et al. 2000: *Aquat Toxicol* 48:251-264)

Carbendazim microcosm experiment

Multivariate analysis (PRC method)
NOEC = 3.3 µg/L



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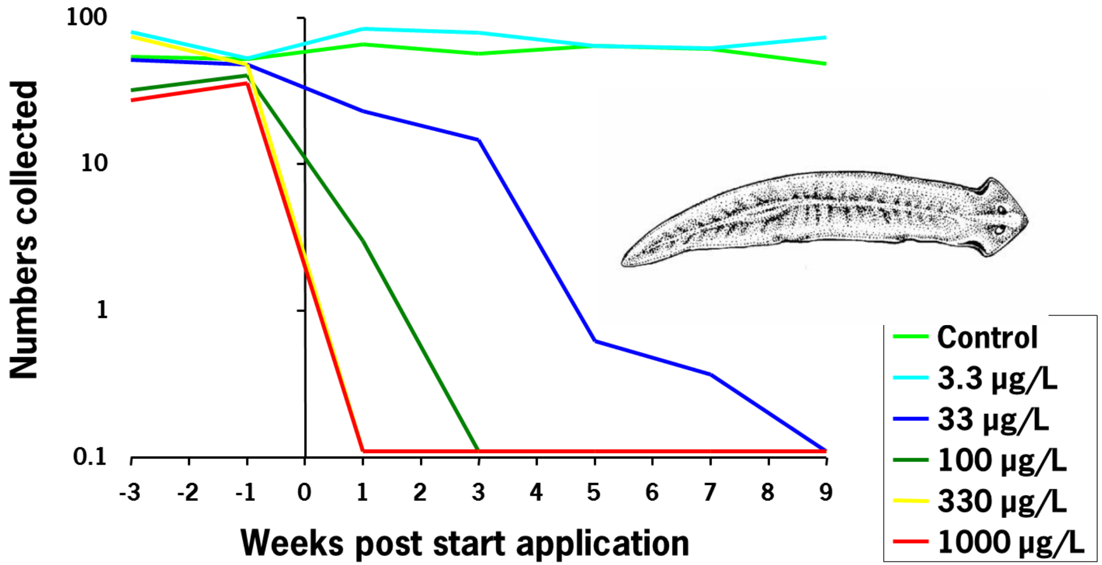
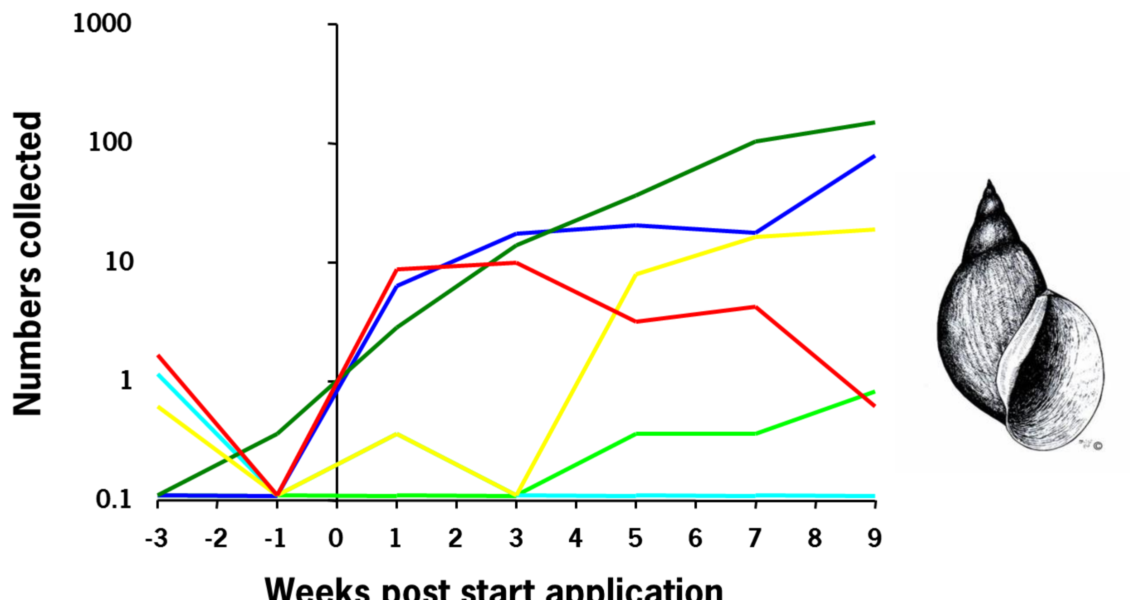
(In)direct effects fungicide carbendazim



Dugesia tigrina
- Flatworm
- Direct effect
- NOEC = 3.3 µg/L
- Concentration – time dependency

Lymnaea stagnalis
- Snail
- Indirect effect
- NOEC = 3.3 µg/L
- Less clear concentration – time dependency



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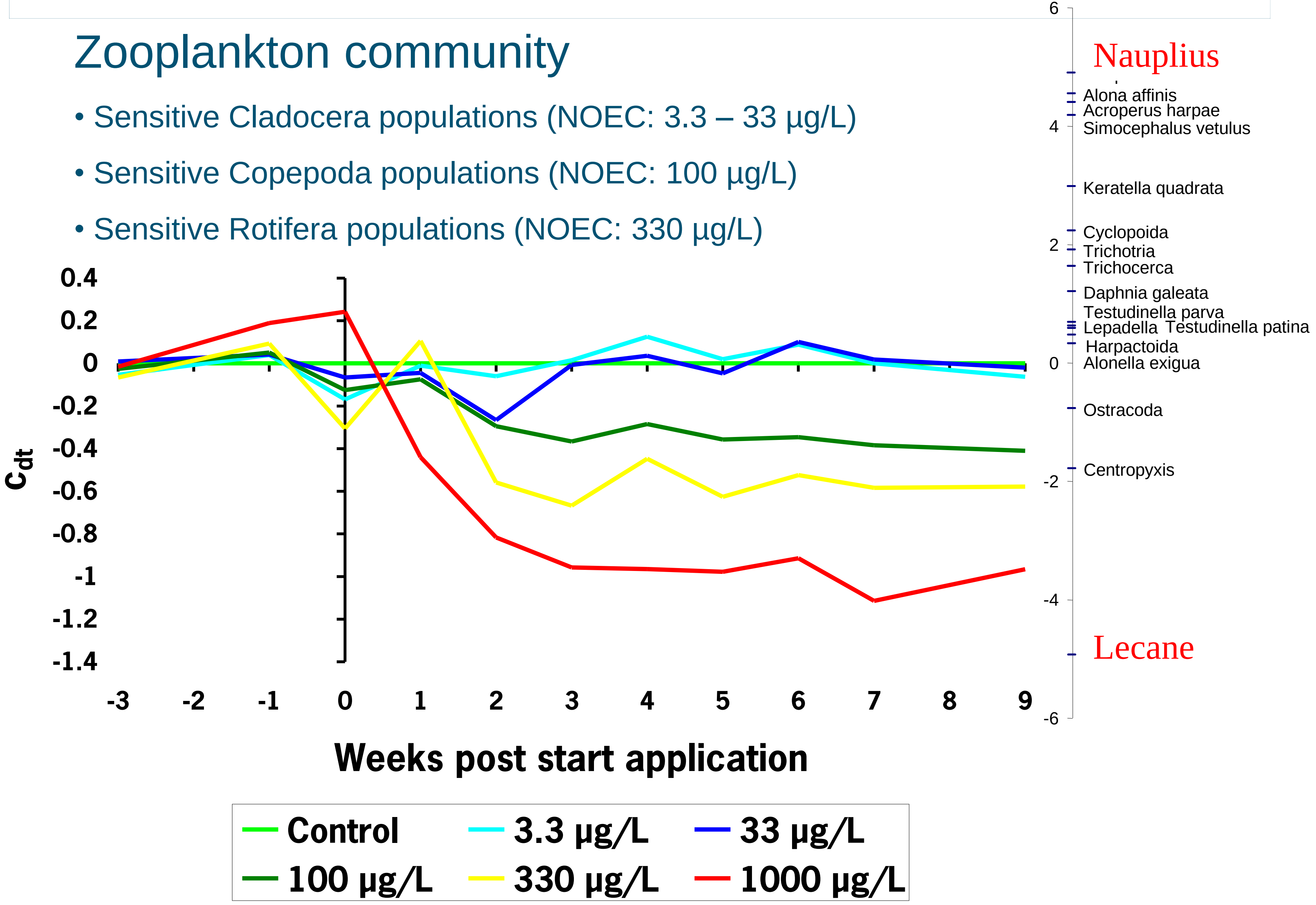
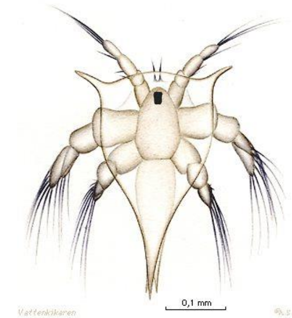
Carbendazim microcosm experiment

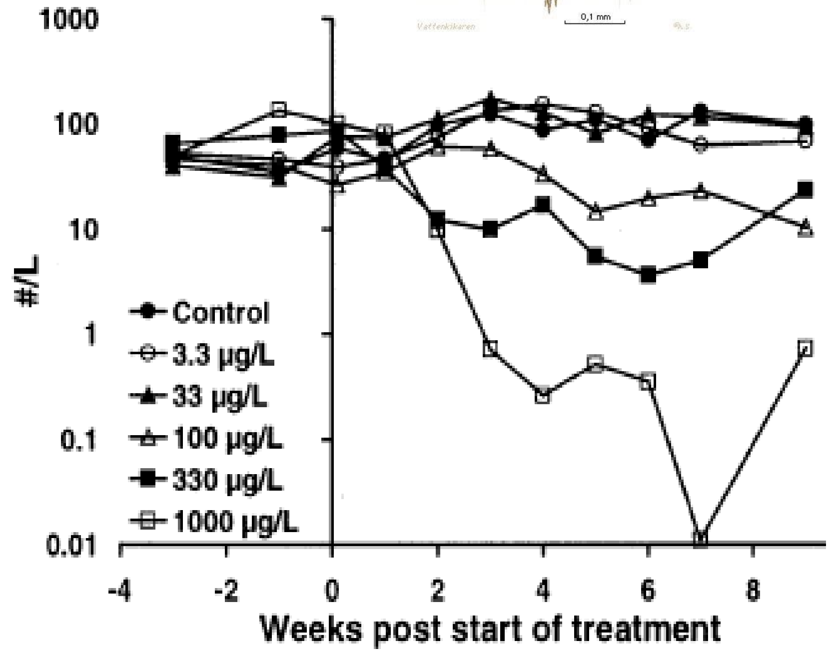
Zooplankton community

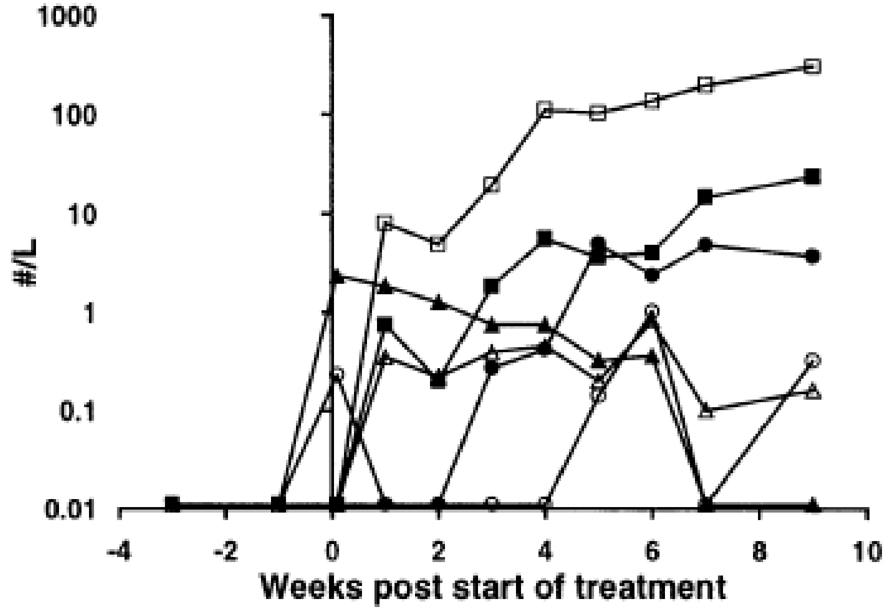
- Sensitive Cladocera populations (NOEC: 3.3 – 33 µg/L)
- Sensitive Copepoda populations (NOEC: 100 µg/L)
- Sensitive Rotifera populations (NOEC: 330 µg/L)



(In)direct effects fungicide carbendazim







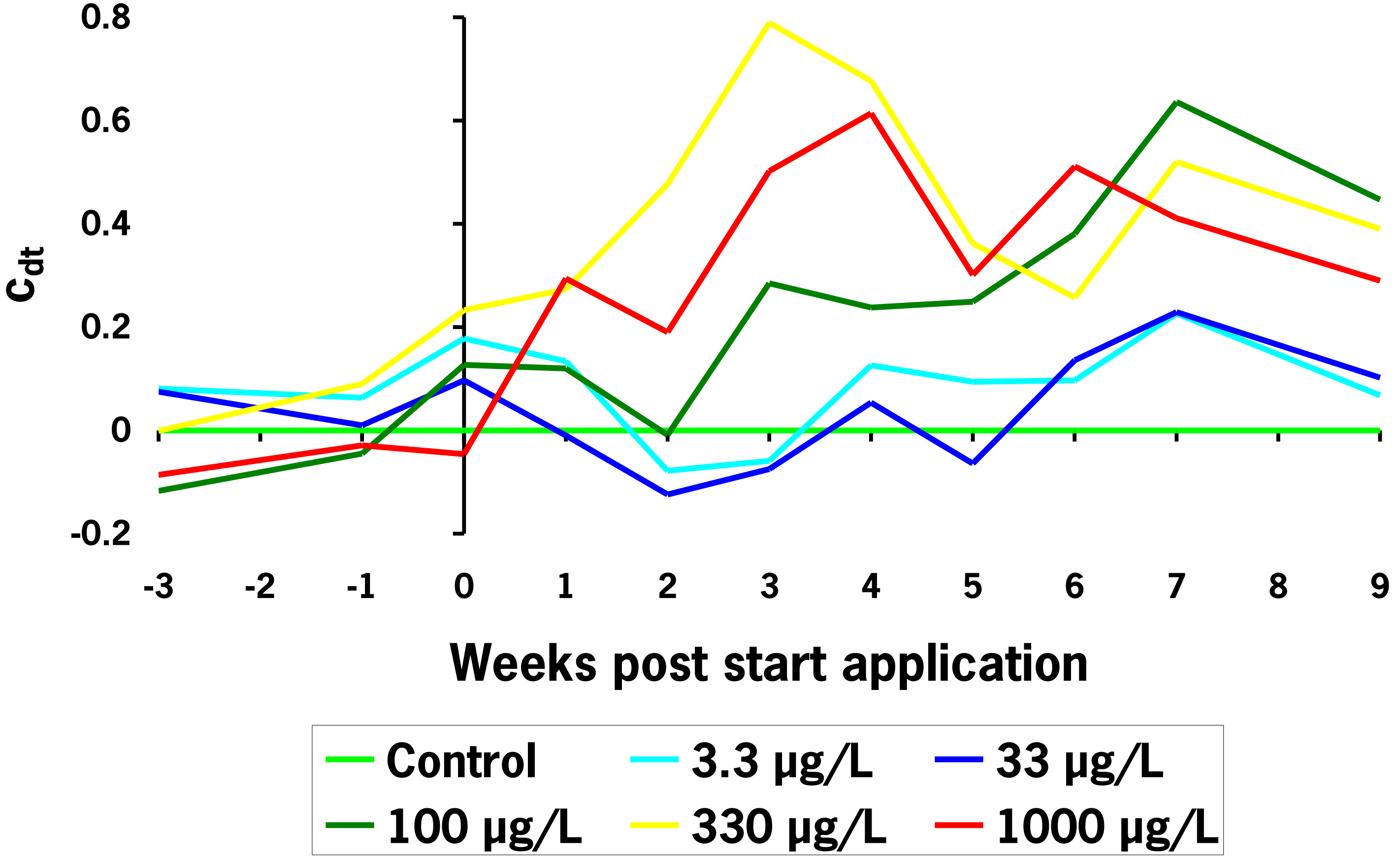
Nauplius (NOEC = 33 µg/L) Direct effect

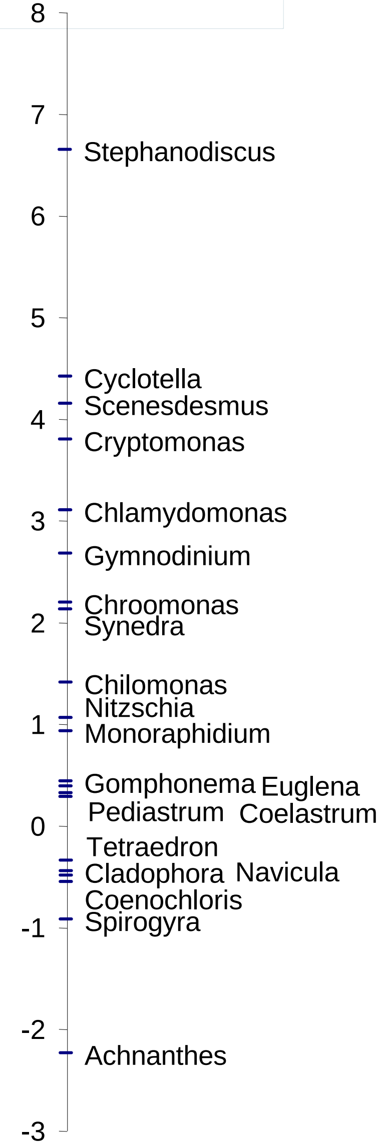
Lecane sp (NOEC = 330 µg/L) Indirect effect

Carbendazim microcosm experiment

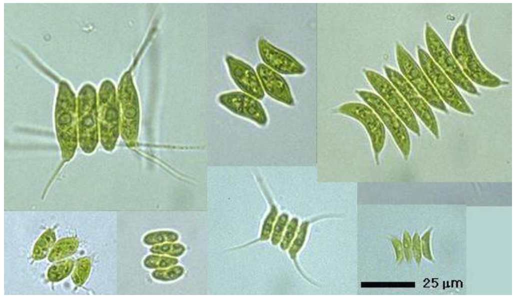
Phytoplankton community

NOEC = 100 µg/L

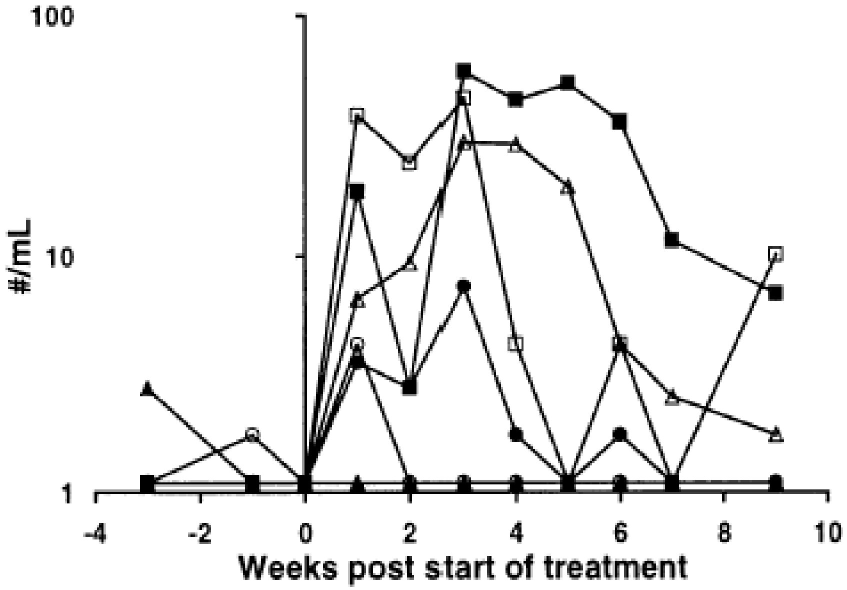




(In)direct effects carbendazim on *Scenedesmus* sp.

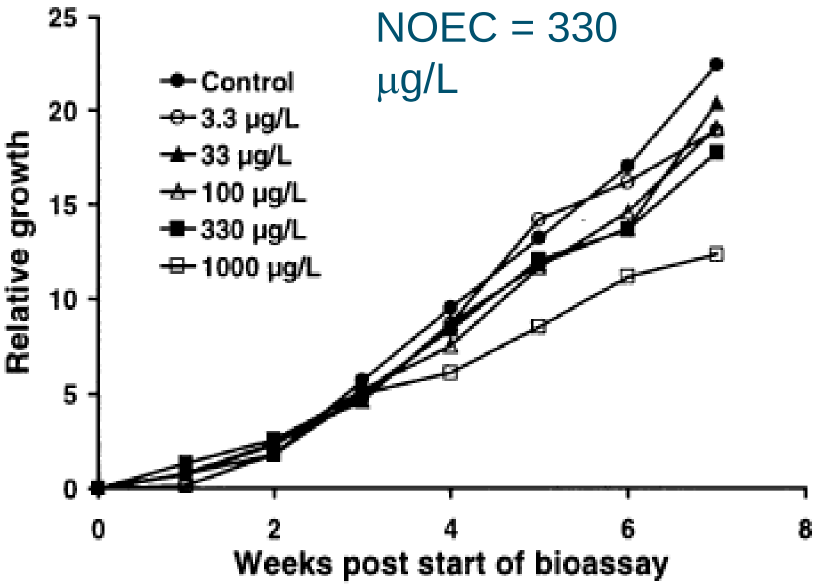


Free-living in microcosm



Bioassay

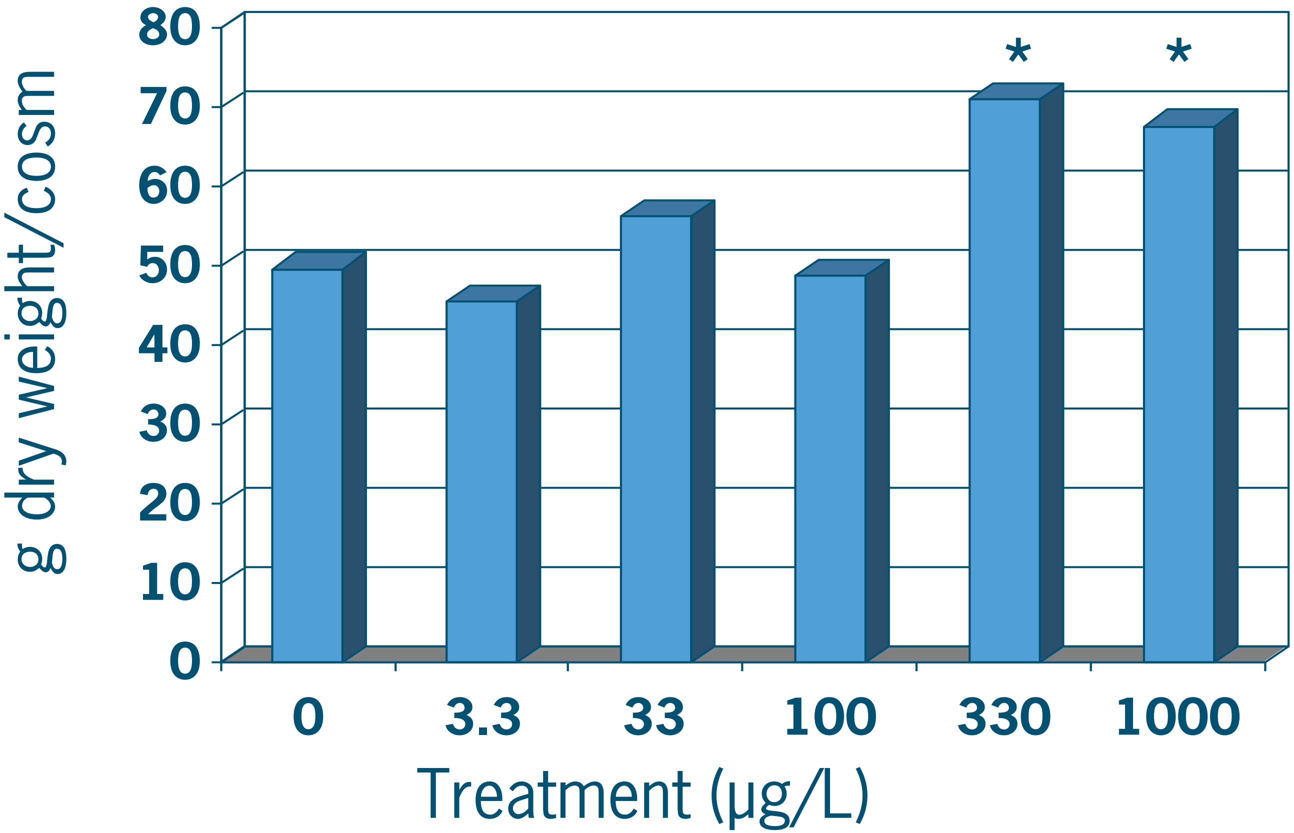
NOEC = 330 µg/L



Influence decline of sensitive grazers may be larger than stress by toxicant

Carbendazim microcosm experiment

Dry weight biomass of *Elodea nuttallii* at end of experiment



* P < 0.05

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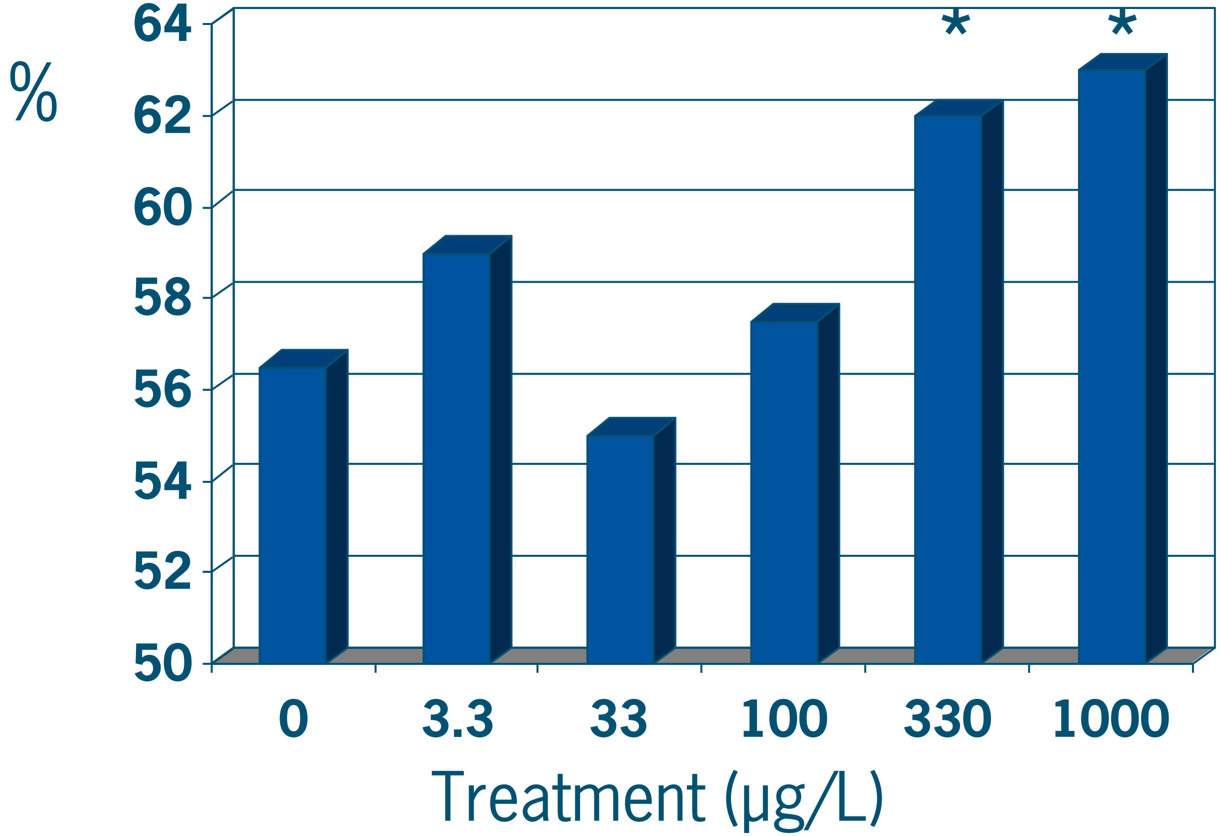
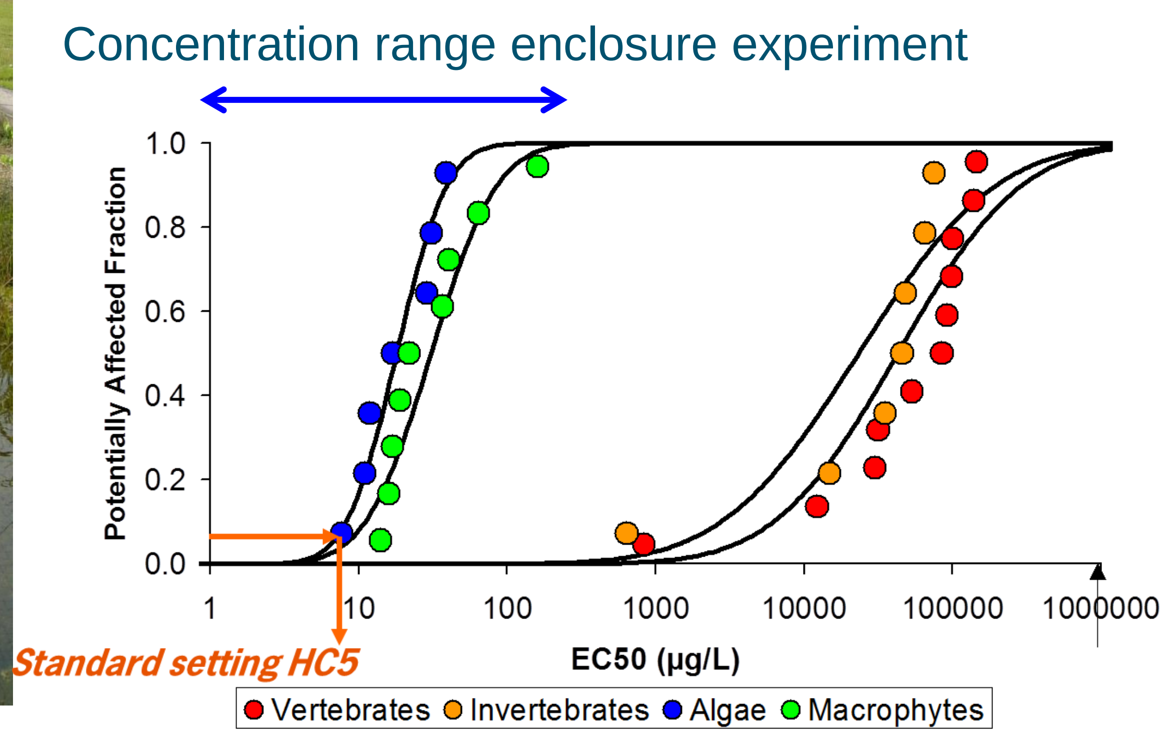
Carbendazim microcosm experiment

Residual dry weight of decomposing *Populus* leaves in small mesh litter bags after 4 weeks (exposure period)



(In)direct effects herbicide metribuzin

SSD acute laboratory toxicity data



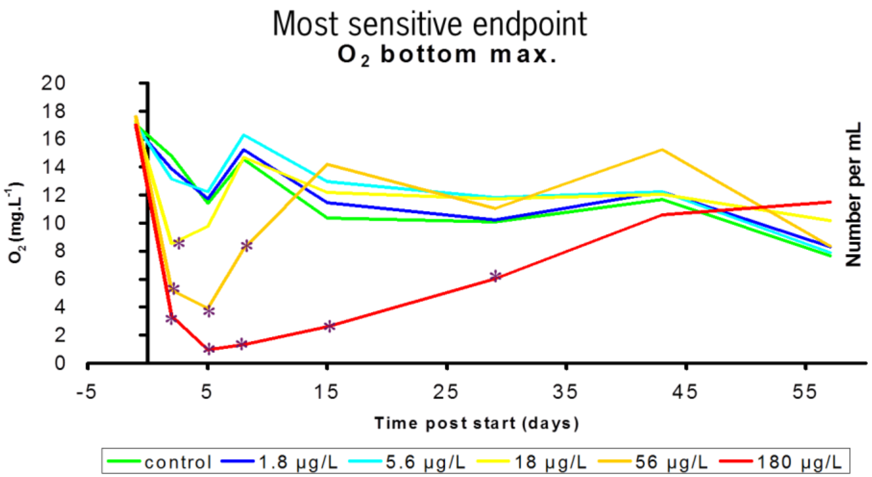
- Field enclosure experiment
- single application metribuzin
- 0 – 1.8 – 5.6 – 18 – 56 – 180 μg/L
- dissipation DT50 = 6.5-9.4 day

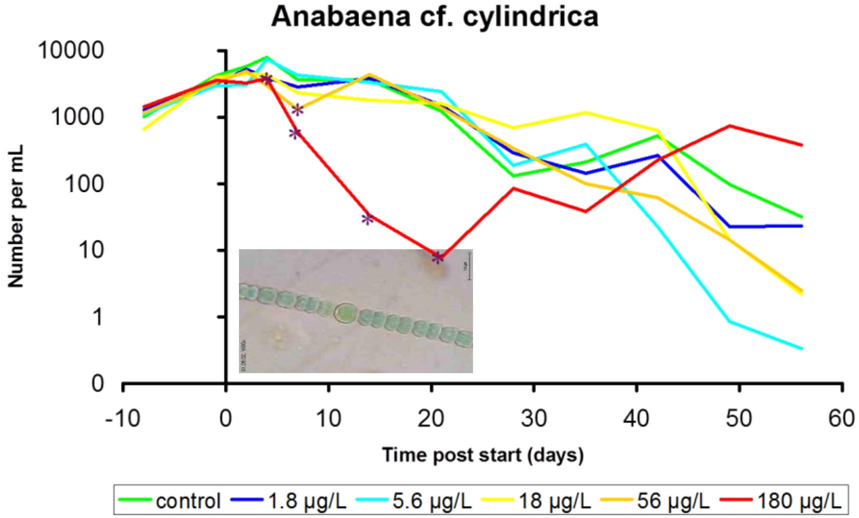
Laboratory toxicity data suggest that primary producers will suffer direct toxic effects at the exposure concentrations tested

(Brock et al. 2004: *Environ Poll* 130:403-426)

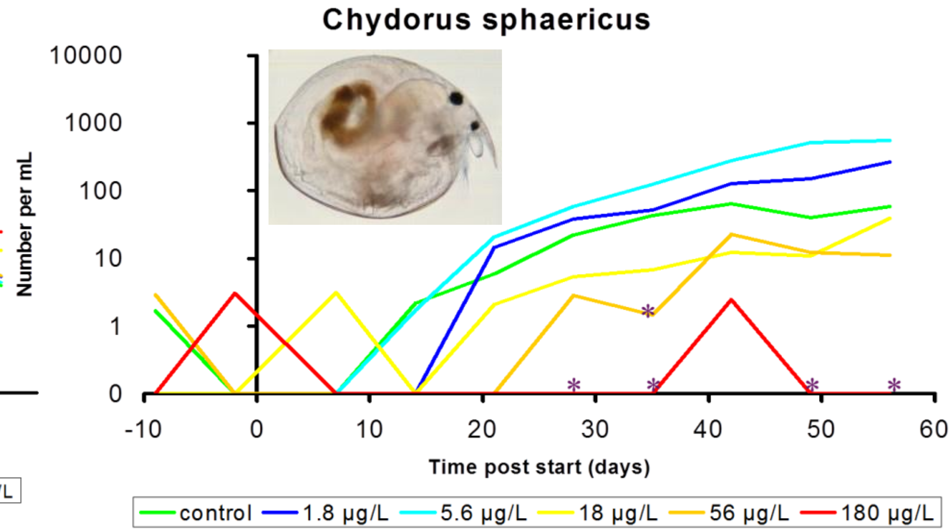
Indirect effects may persist longer than direct effects

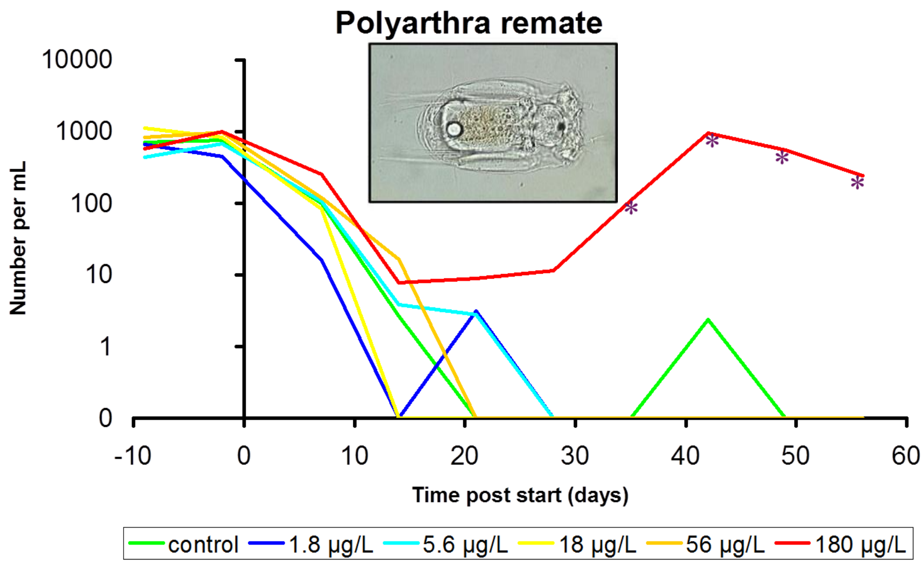
Direct effects





Indirect effects





What type of ecological risk can be assessed in micro/mesocosms?

- Concentration-response relationships of dominant populations
  - Univariate statistics
- Concentration-response relationships of community
  - Multivariate techniques
- Relatively large species with complex life cycles are underrepresented in microcosms
  - Diversity of macrophytes is low
  - No large predators (e.g. fish)
- Isolated micro/mesocosms
  - recovery potential of r-selected species

Thank you for your attention
Questions ?

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