Pesticide mixture toxicity assessments differ between single species tests and mesocosm studies

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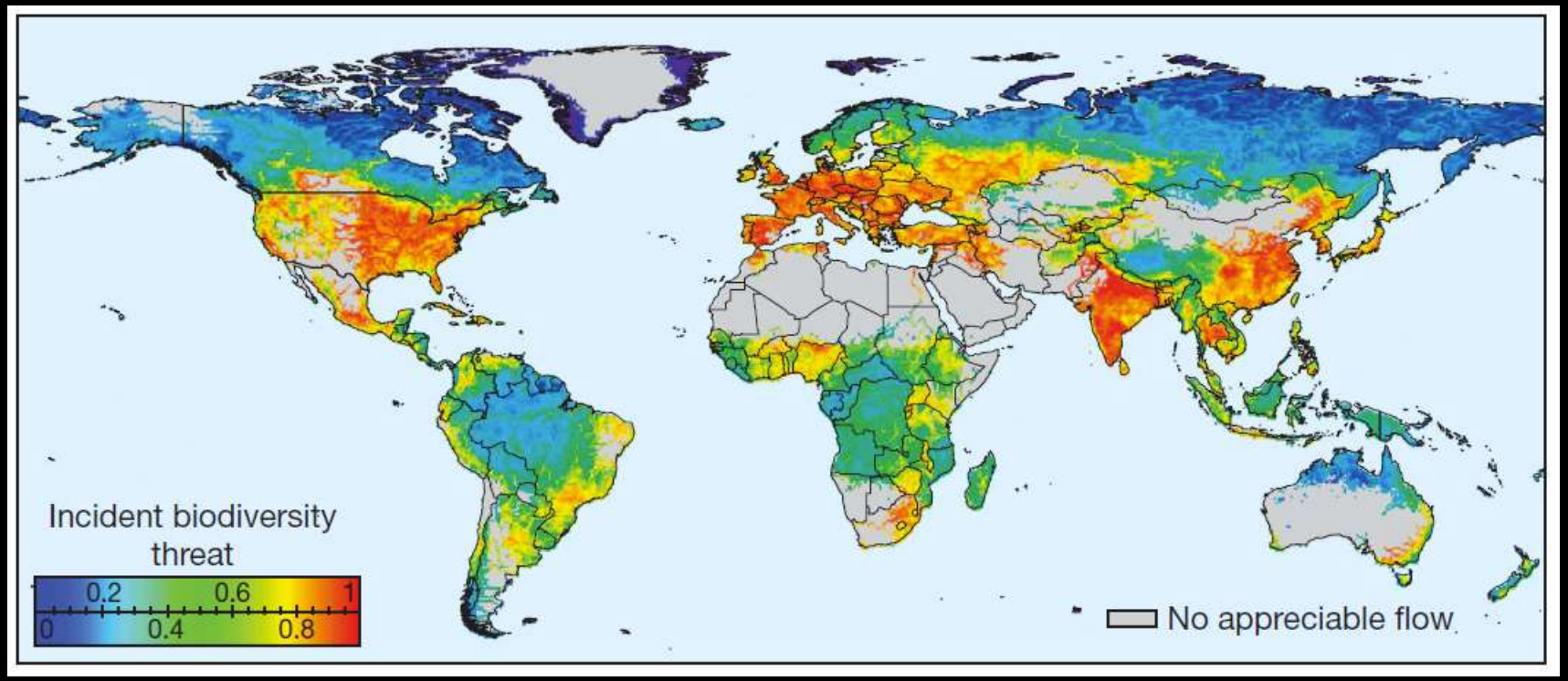
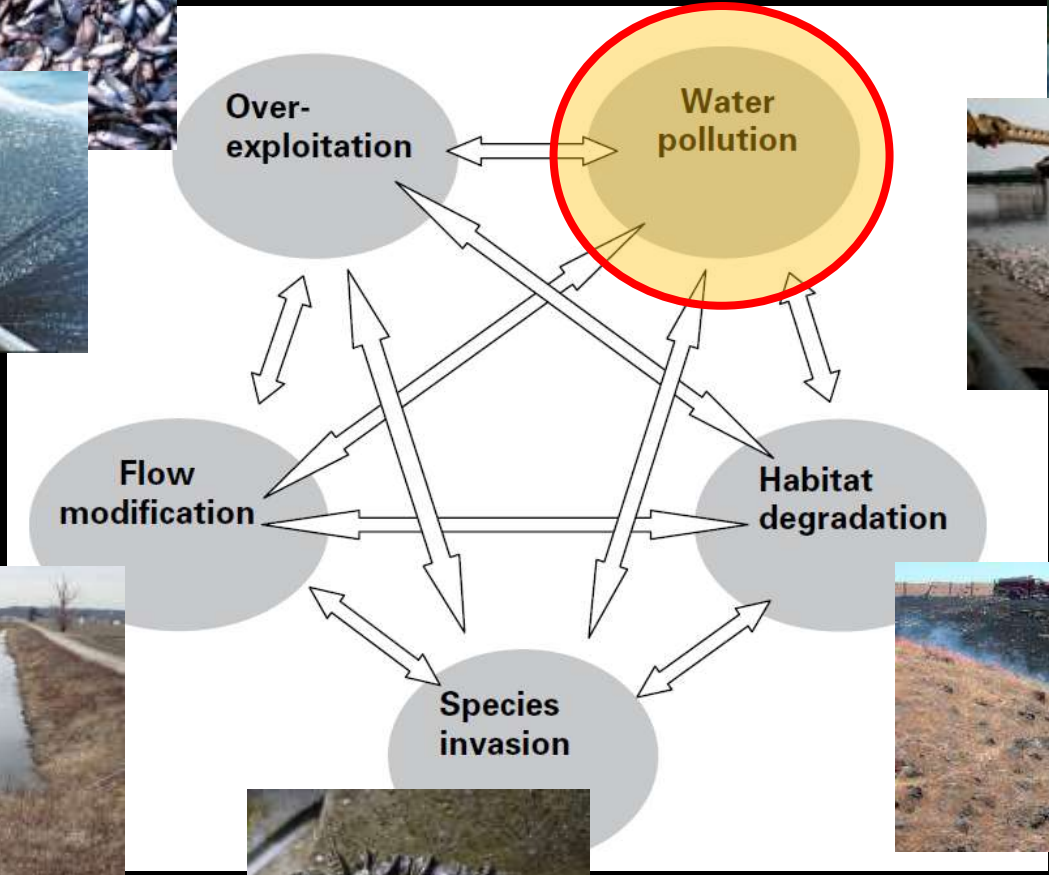
Global geography of incident threat to biodiversity



Aquatic habitats associated with 65% of continental discharge classified as moderately to highly threatened.

Vörösmarty et al., Nature (2010). 467, pp. 555-561

Five major threats to aquatic biodiversity



Dudgeon et al., Biol. Rev. (2006). 81, pp. 163 – 182.

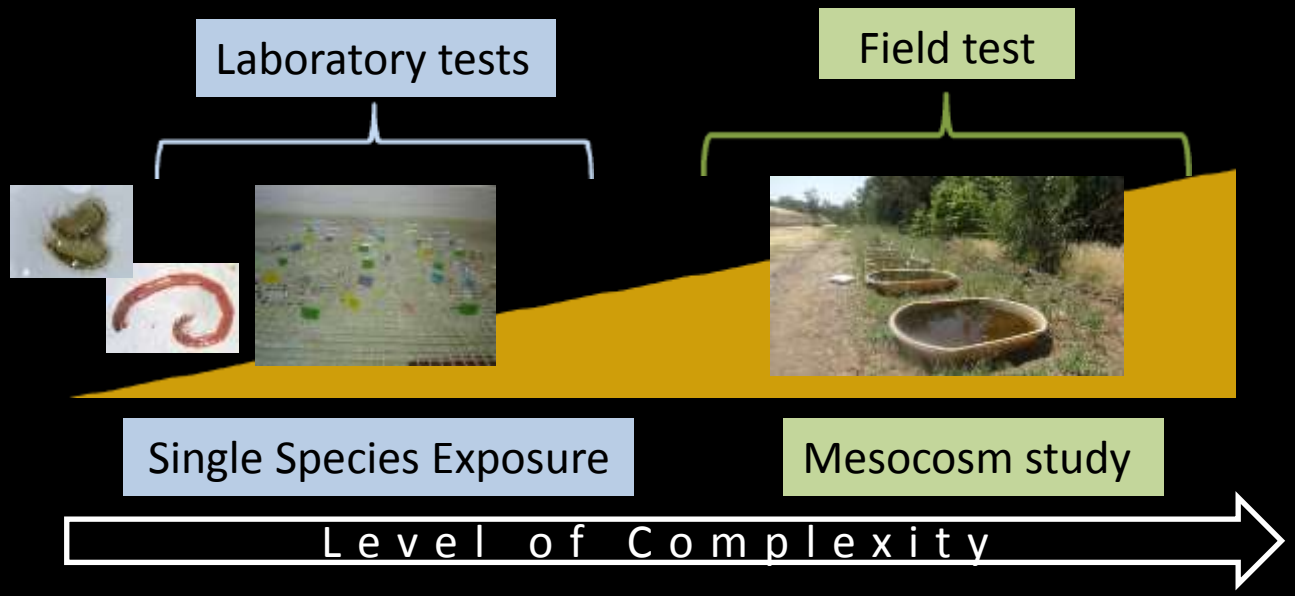
Rationale

Suites of Pesticides = Dominating pollution sources

Invertebrates = crucial components to aquatic ecosystems + among the most threatened species worldwide

New Information on pesticide mixture effects

From short-term laboratory testing towards multiple species in long-term field study using mesocosms

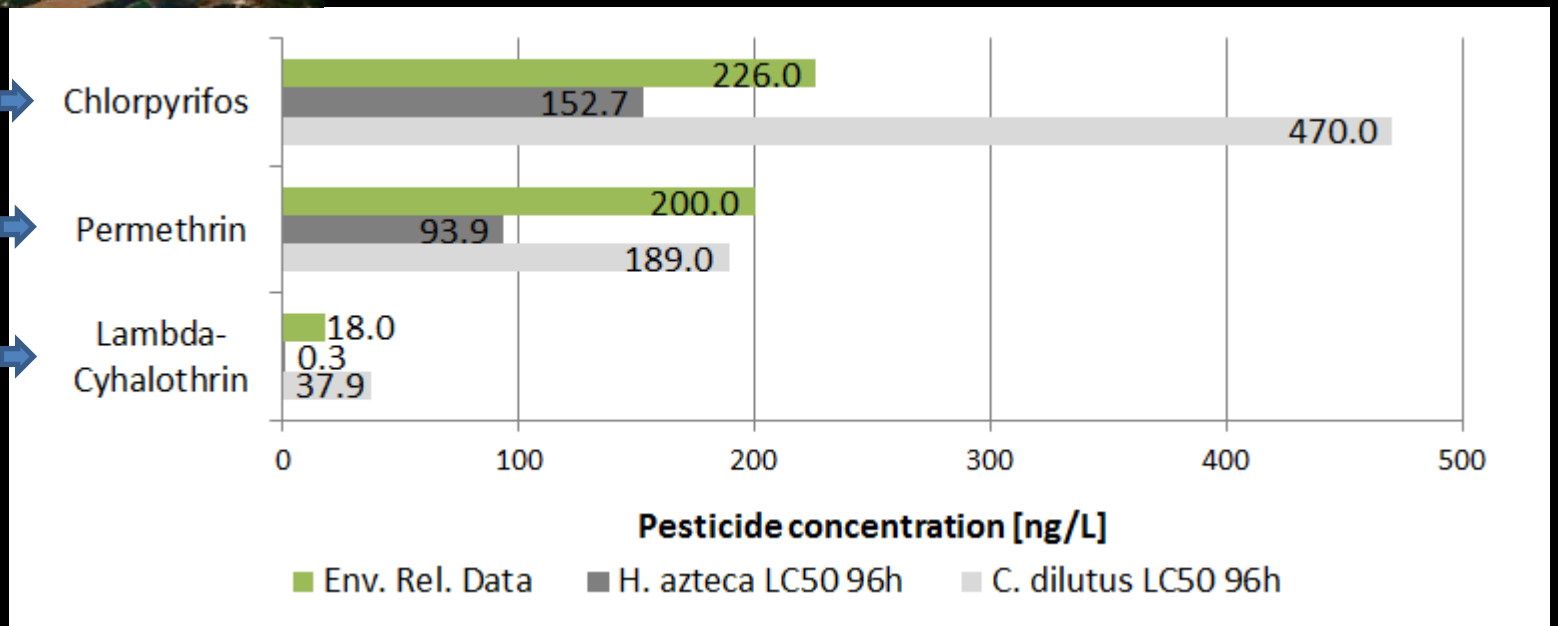


Choice of pesticides

Detected in monitoring projects (Central Valley)

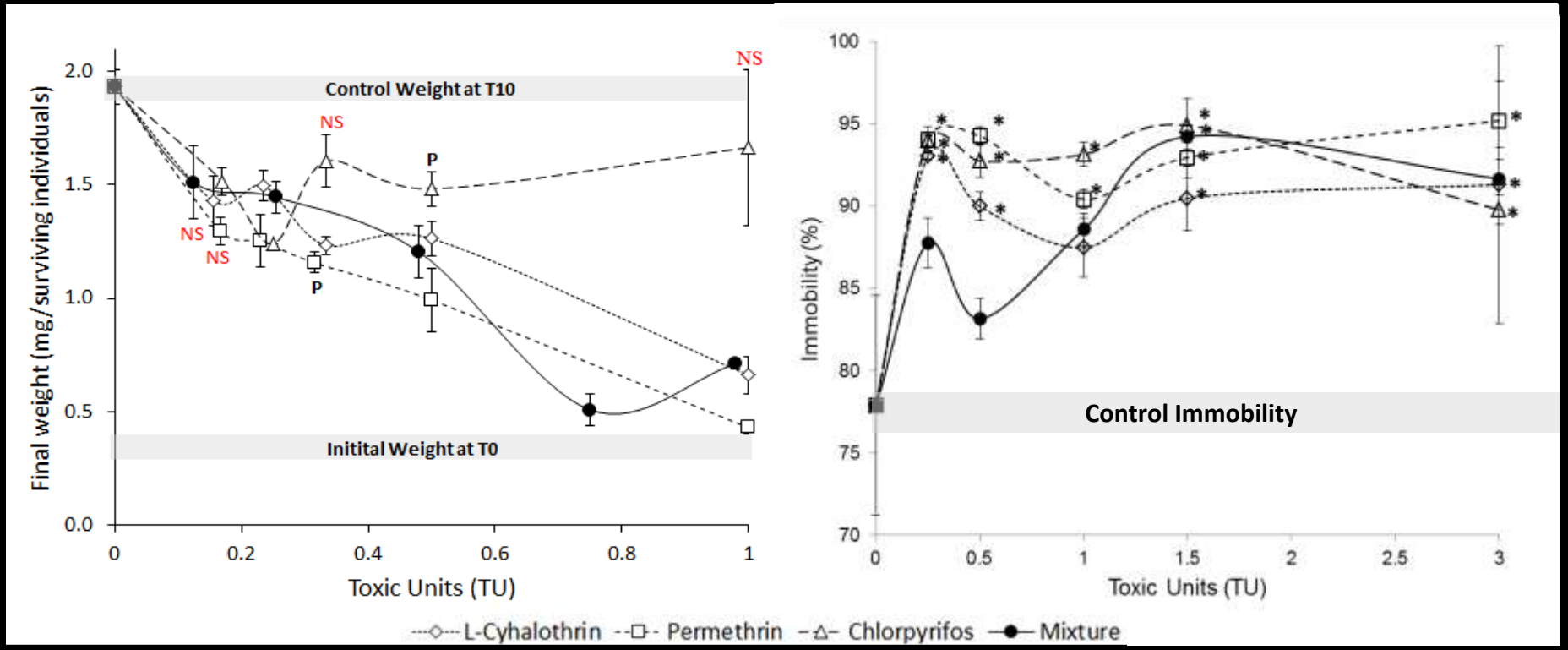
Among most used and most toxic pesticides (DPR database)

Available toxicology data (EPA Ecotox, in-house tests)



10-day weight

Percentage Immobility

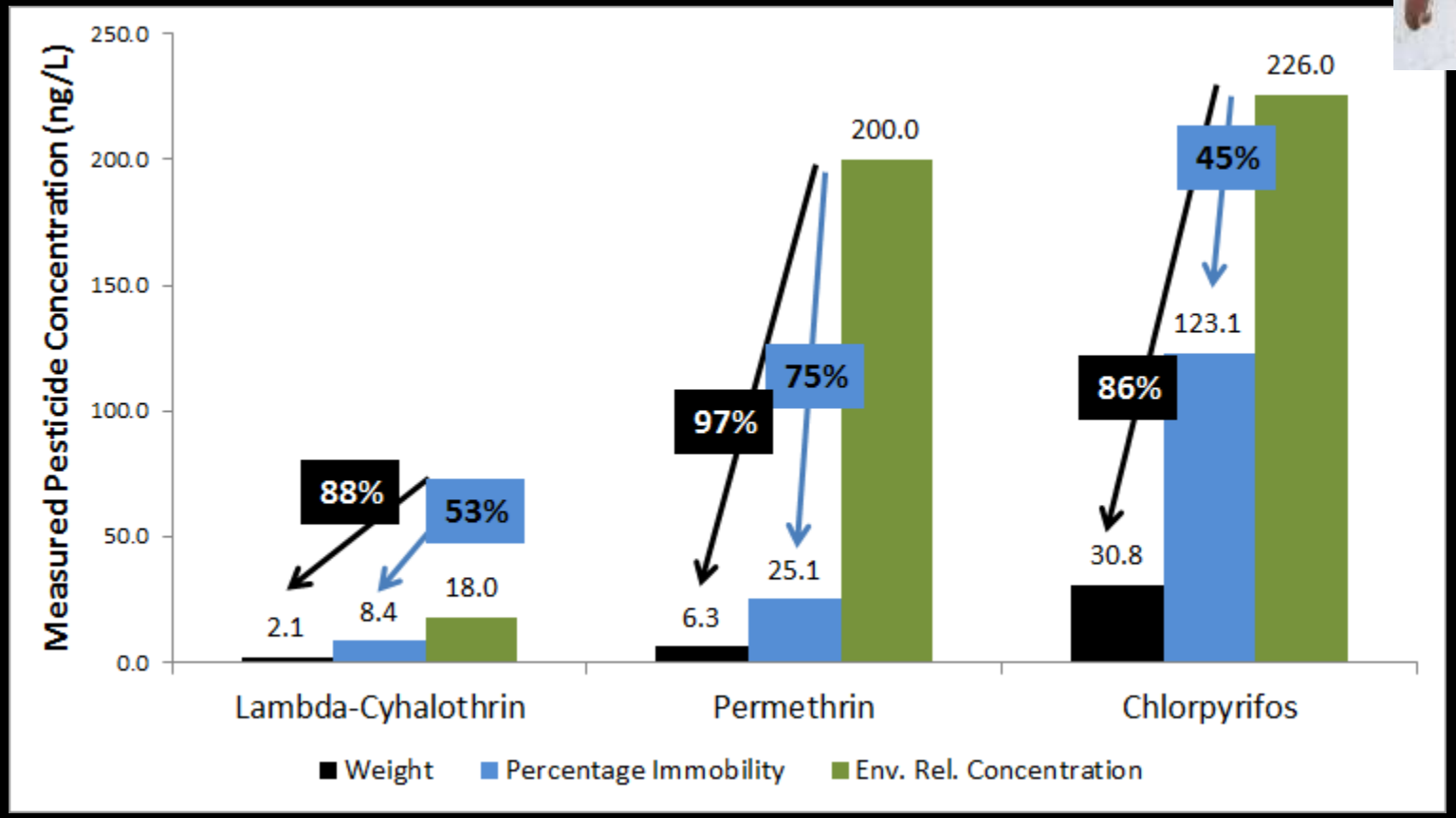


Main Message:
Sublethal responses observed at ≥ 0.125 TU

NS = non-significant to controls, all others = $p < 0.05$; P = single pupae present in treatment.
T0 = Test initiation at day 0, T10 = Test termination at day 10

Hasenbein S, et al., Ecotoxicology

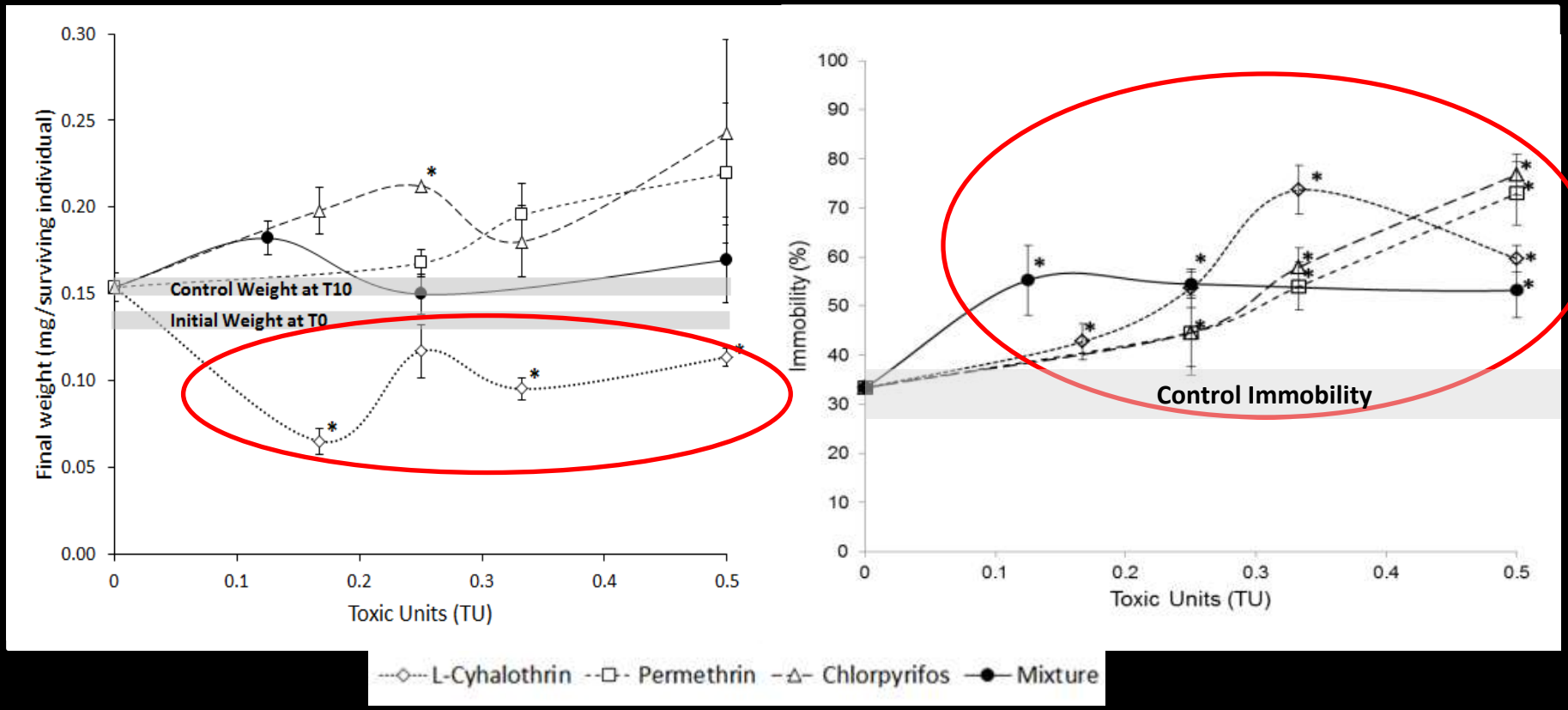
Measured Tertiary Mixture Concentration and its Effect on 10-day Weight and Immobility



Mixture concentration based on observed significant effects on weight (0.25 TU) and immobility (1 TU)

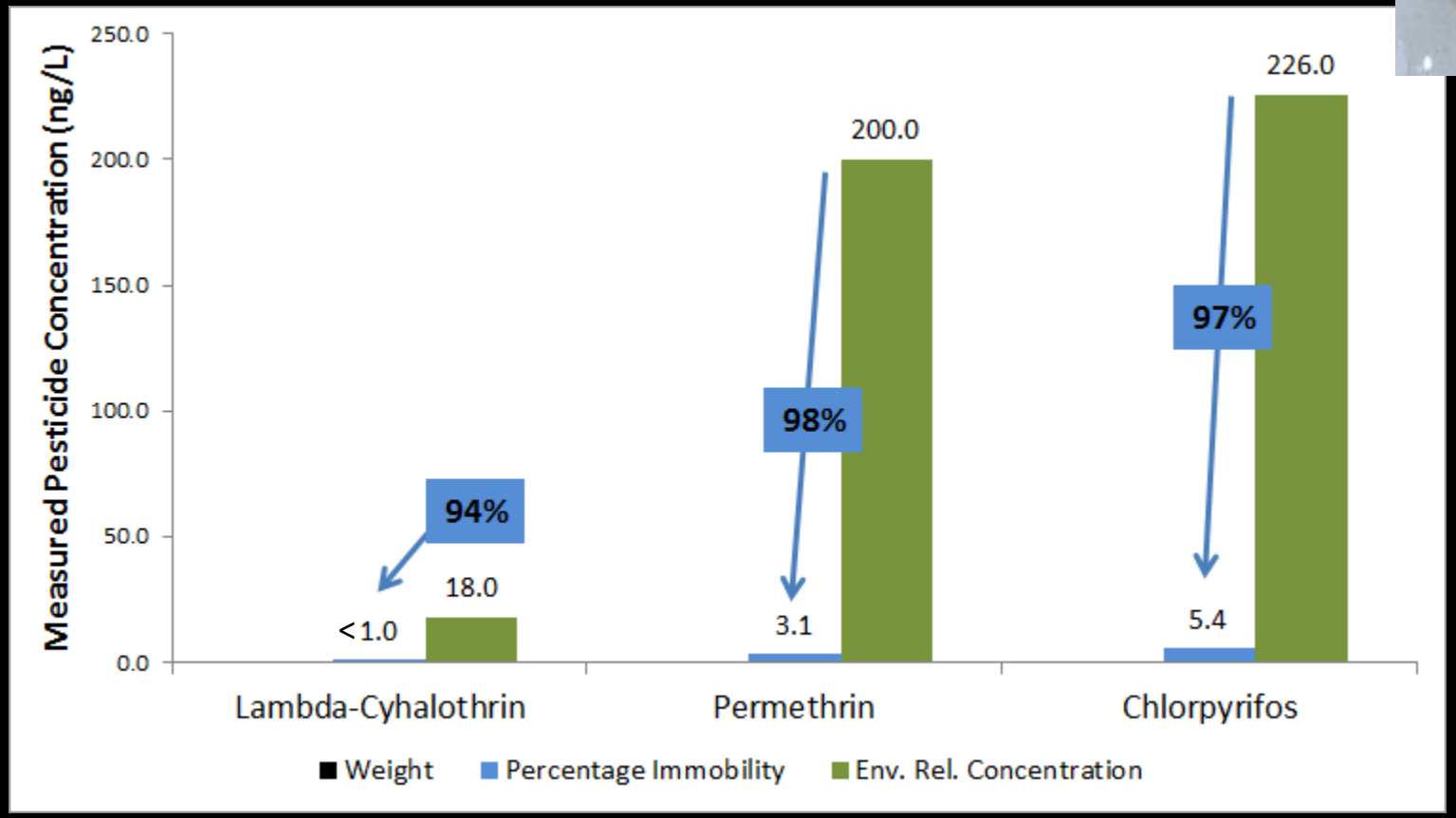
10-day weight

Percentage Immobility



Asterisks = significant compared to controls ($p < 0.05$);
T0 = Test initiation at day 0, T10 = Test termination at day 10

Measured Tertiary Mixture Concentration and its Effect on 10-day Weight and Immobility



Mixture concentration based on observed significant effects on immobility (0.125 TU). Weight: no significant results

Mesocosms

- **Realistic** exposures, yet **controlled**
- Setup: Naturally developed communities, sediment, plants
- Objectives:
  - **Long-term** effects of **multiple** species (macroinvertebrates and zooplankton) and potential **recovery** over 6 months
  - **Dissipation** and **accumulation** of chemicals

→Increased environmental and ecological **relevance**

Pesticide Application

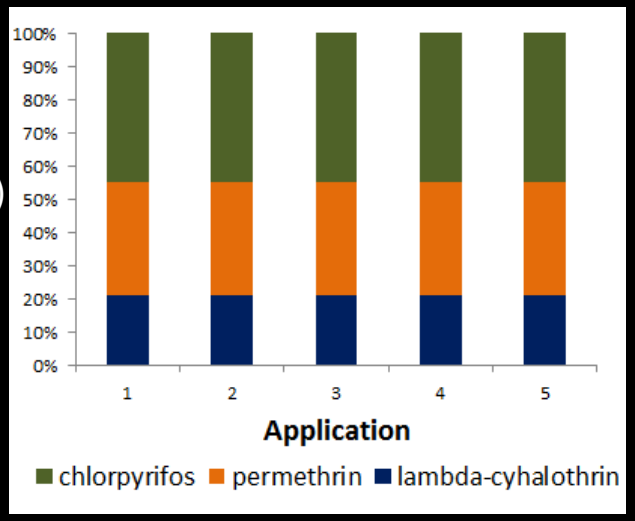
Active Ingredient	Formulation Product
Chlorpyrifos	Lorsban 4-E (44.9% a.i.)
Permethrin	Pounce (25% a.i.)
Lambda-Cyhalothrin	Warrior (11.4% a.i.)

ng/L	LC10		LC25		LC50	
CLF	58.10	161.78	66.95	284.41	77.15	522.97
Perm	48.56	128.52	55.01	192.07	62.30	267.11
LC	0.14	37.78	0.17	43.31	0.21	49.65

LC values determined by using log-logistic modelling approach on 10-day single chemical data. Dark blue-shaded = *H. azteca*

<u>Treatment 1:</u>
Environmentally relevant (CEDEN)

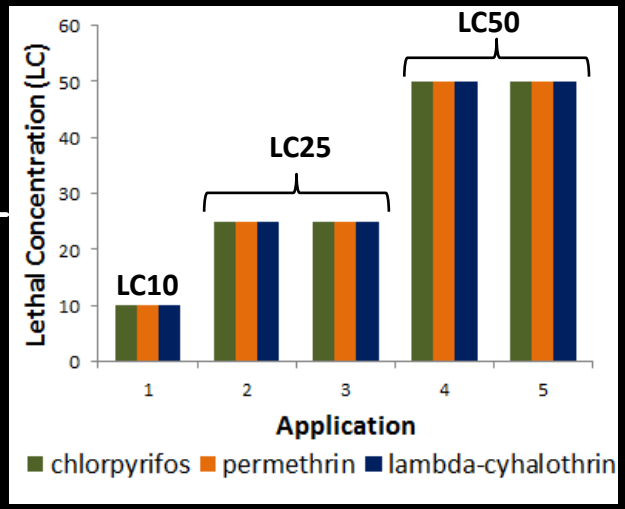
CLF: 7.5 ng/L
Perm: 5.7 ng/L
LC: 3.5 ng/L



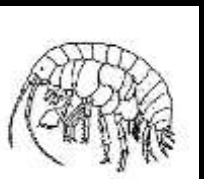
<u>Treatment 2:</u>
LC values *H. azteca**

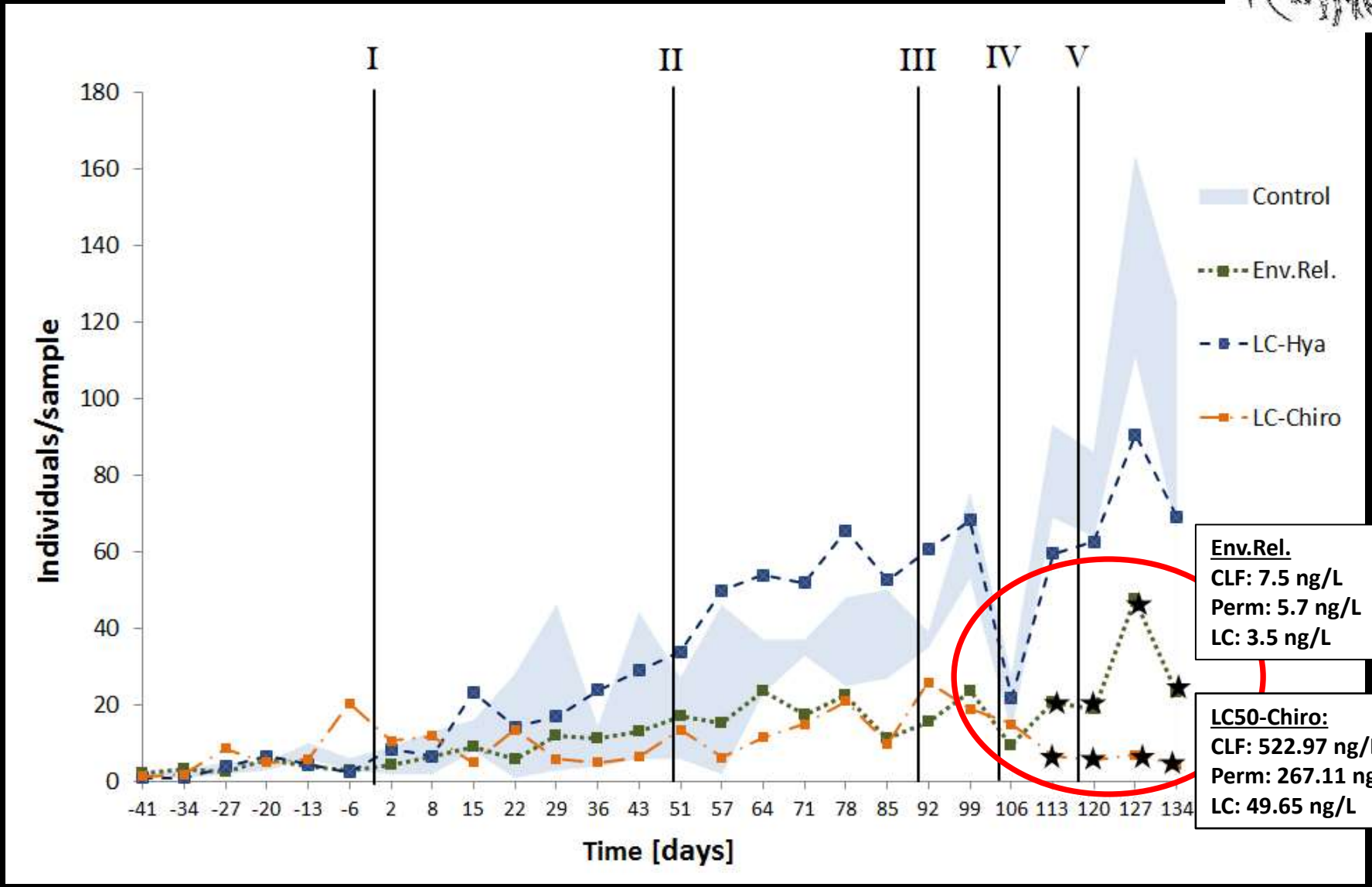
<u>Treatment 3:</u>
LC values *C. dilutus**

*Hasenbein, S. et al, ESPR



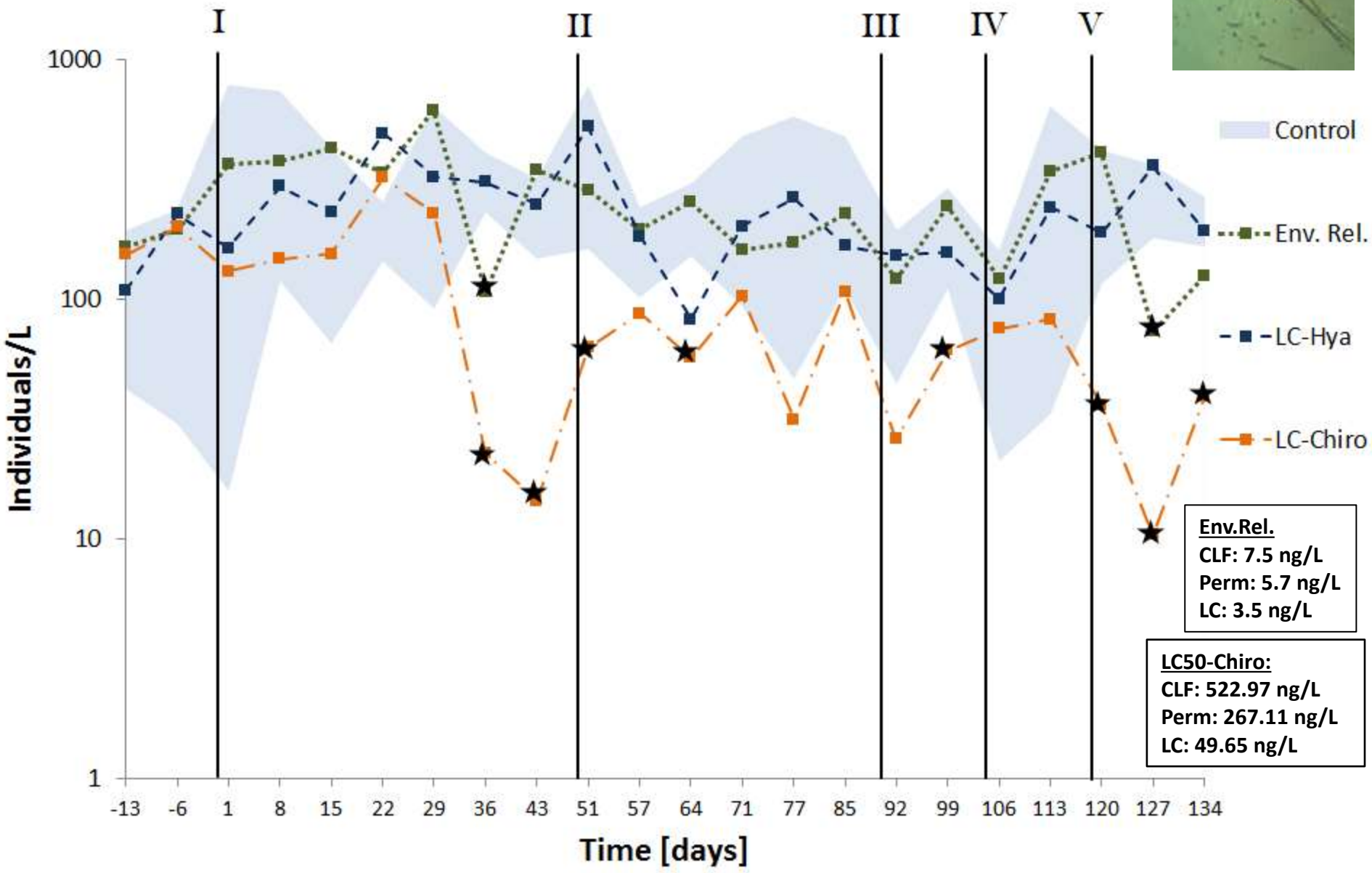
H. azteca



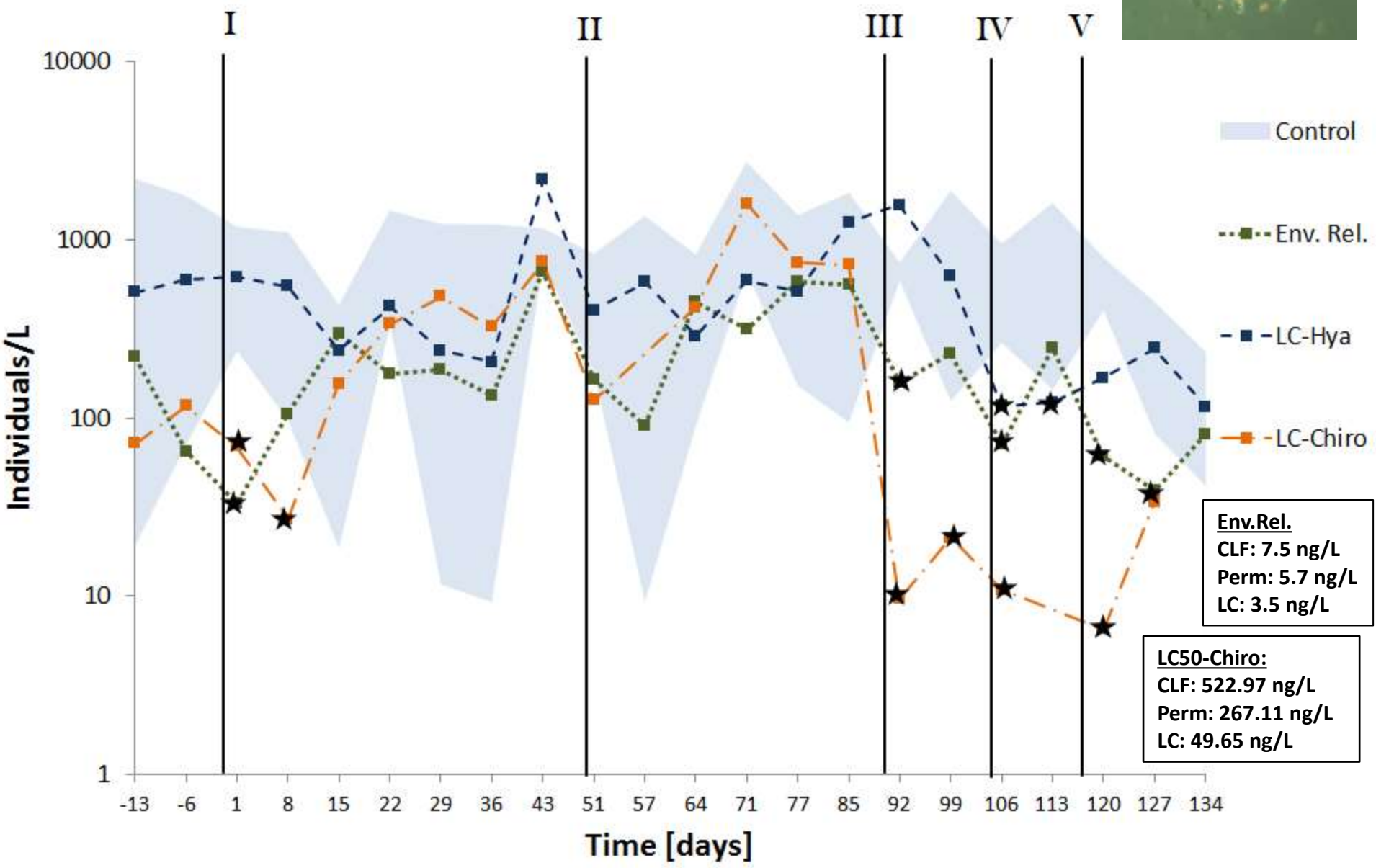


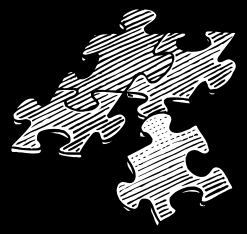
Asterisk = $p < 0.05$

Copepods


I
II
III
IV
V
1000
100
10
1
Individuals/L
-13
-6
1
8
15
22
29
36
43
51
57
64
71
77
85
92
99
106
113
120
127
134
Time [days]
Control
Env. Rel.
LC-Hya
LC-Chiro
Env.Rel.
CLF: 7.5 ng/L
Perm: 5.7 ng/L
LC: 3.5 ng/L
LC50-Chiro:
CLF: 522.97 ng/L
Perm: 267.11 ng/L
LC: 49.65 ng/L

Daphnia magna





What does this mean?

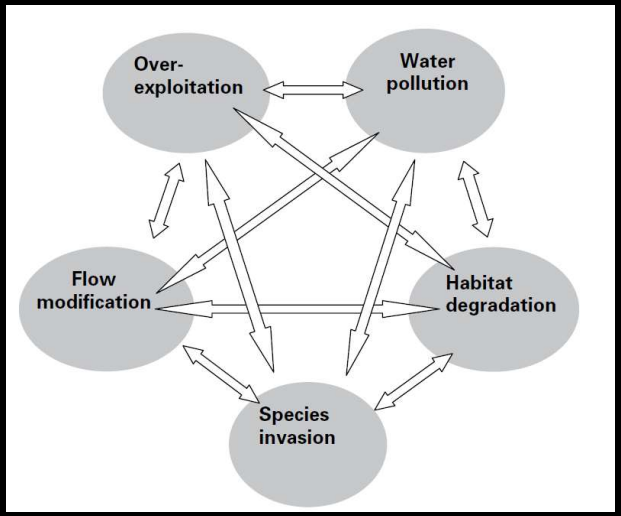
- Lab tests: sublethal effects > 1/8 LC50
- ➢ Focused lab studies with multiple sublethal endpoints → low-level effects (< LOD)

- Meso: multiple species affected: *H. azteca,* **Copepods**, *Daphnia magna* → important "fish food" sources → food web effects

→Contaminants are a HUGE concern

→Not just those three chemicals (herbicide study)

→Many different factors

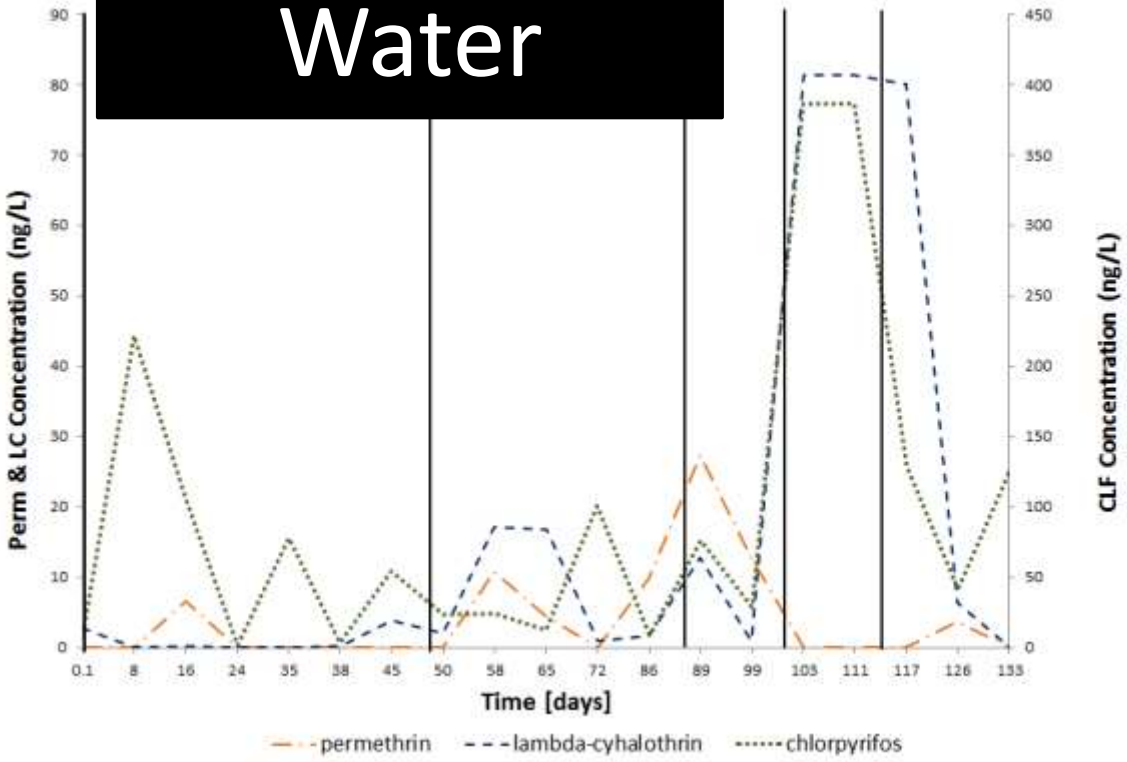


Acknowledgments

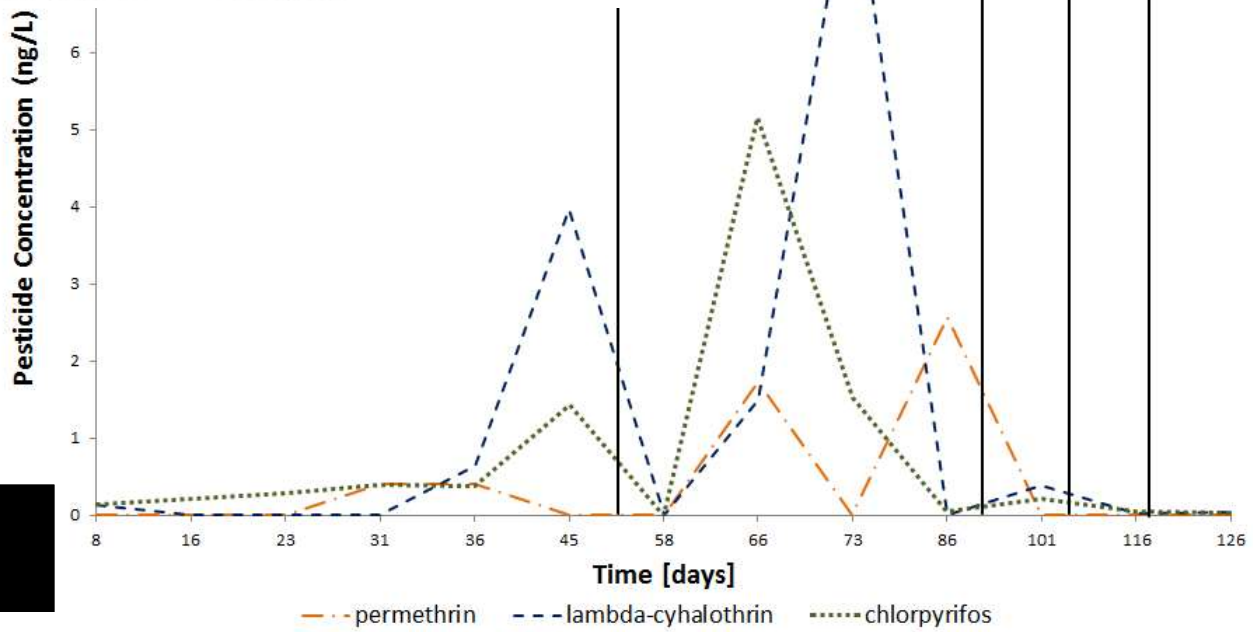
- Putah Creek Riparian Reserve at UC Davis, especially A. Fulks and JP Marie
- Emily Parry & Thomas Young, CEE at UC Davis
- Linda Deanovic & Marie Stillway, AHP at UC Davis
- Many, many volunteers and student assistants!!!

Chemical Fate – Treatment 1

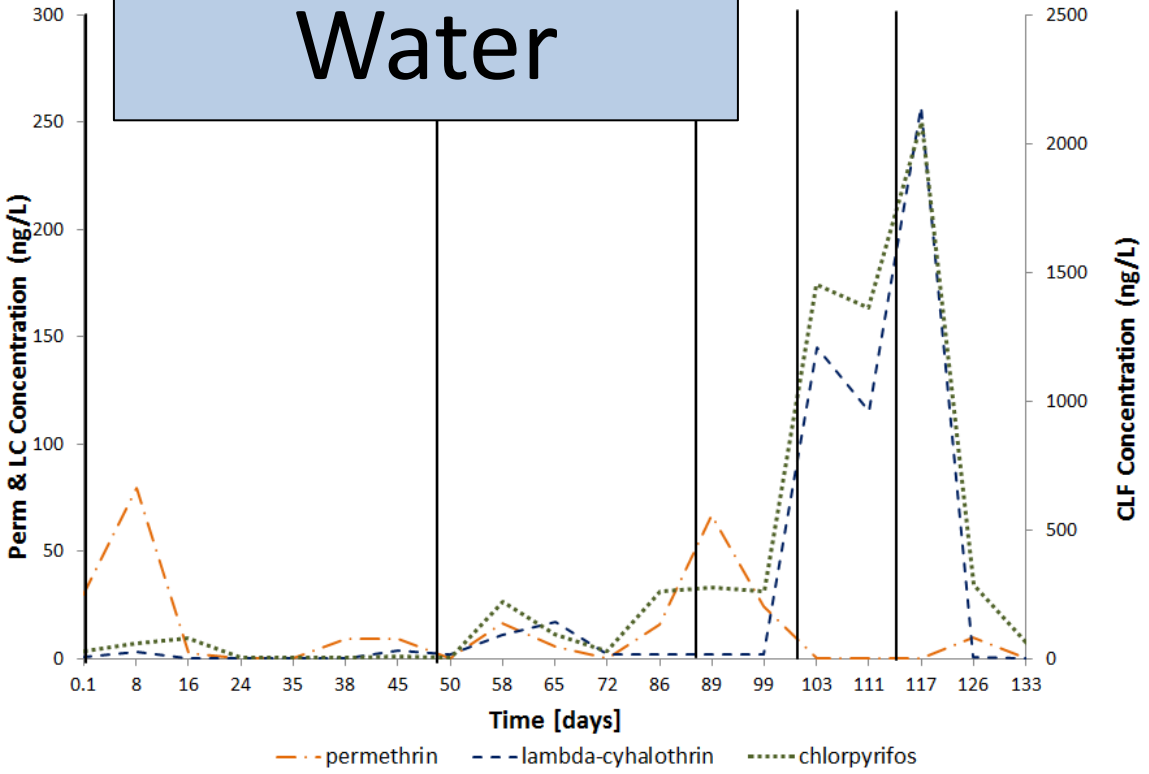
Water



Sediment

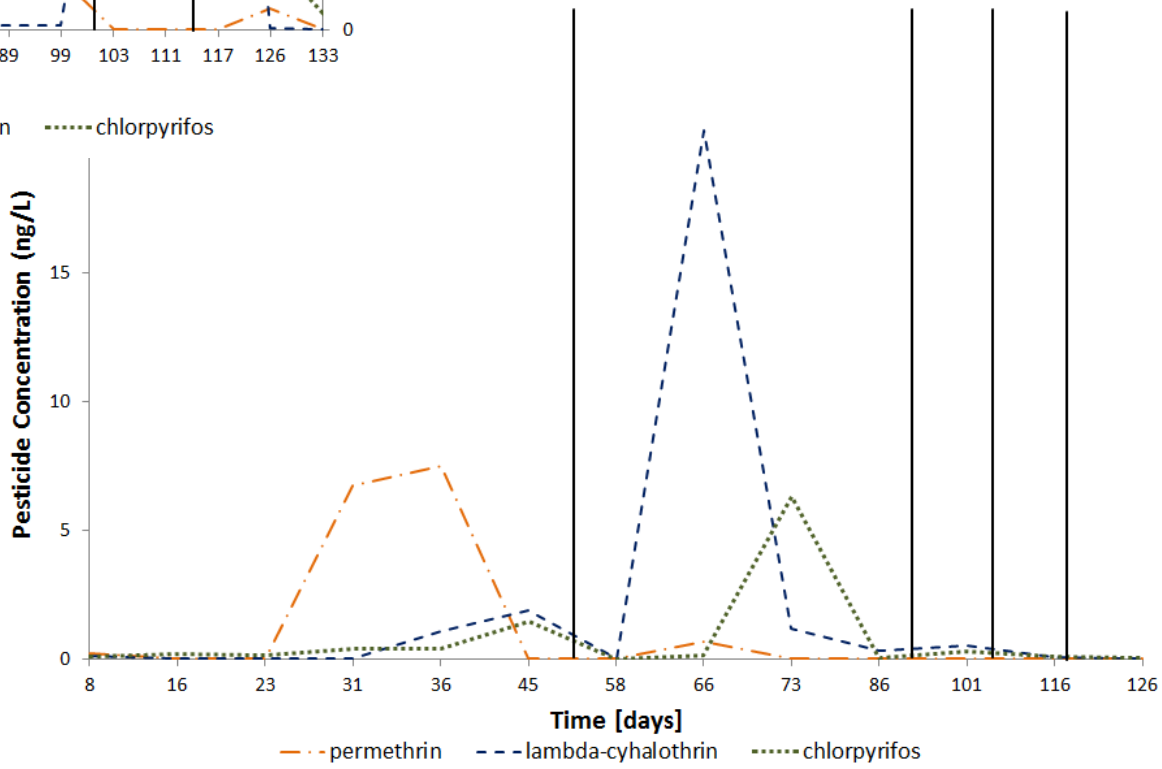


Water

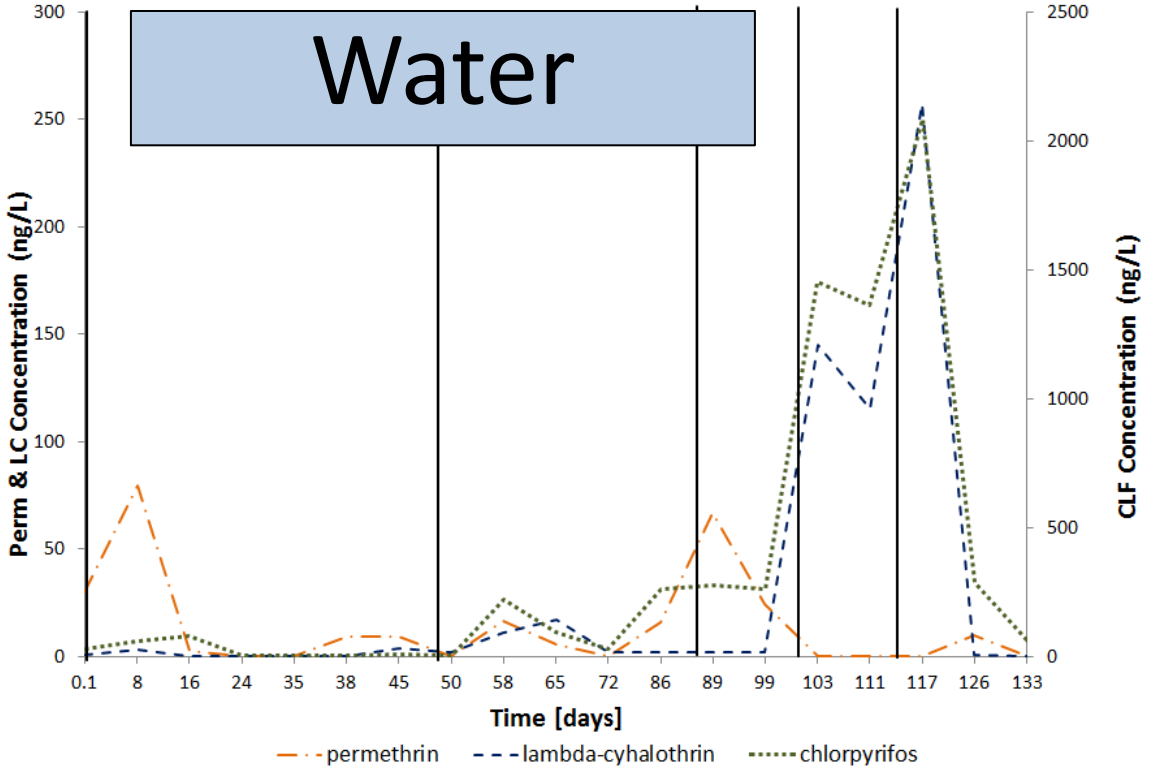


Chemical Fate – Treatment 2

Sediment

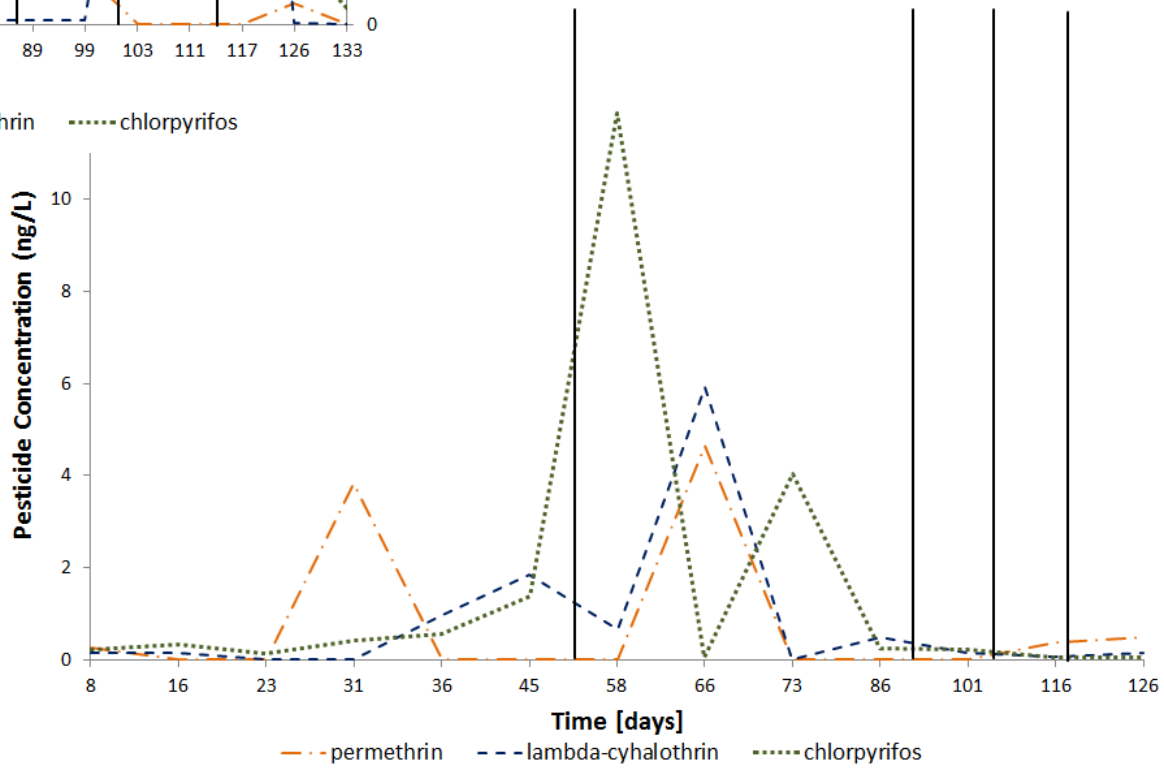


Water



Chemical Fate – Treatment 3

Sediment



Laboratory Exposure tests

Toxic Unit Approach:

$$3TU = 1LC_{50}\ (A) + 1LC_{50}\ (B) + 1LC_{50}\ (C)$$
$$1TU = 1/3LC_{50}\ (A) + 1/3LC_{50}\ (B) + 1/3LC_{50}\ (C)$$

10 day exposure

Endpoints:

Mortality (daily)

Swimming behavior

Dried Weight (60°C)

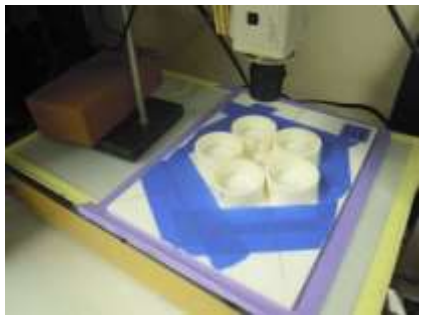
} At test termination

Endobenthic:
Silica sand

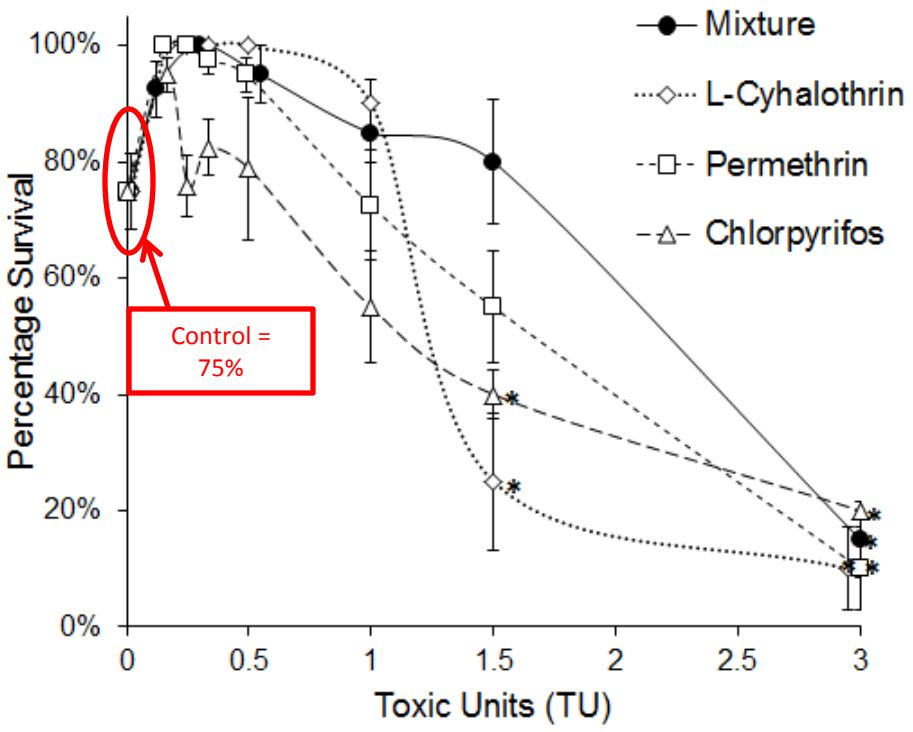
Epibenthic:
Nitex screen

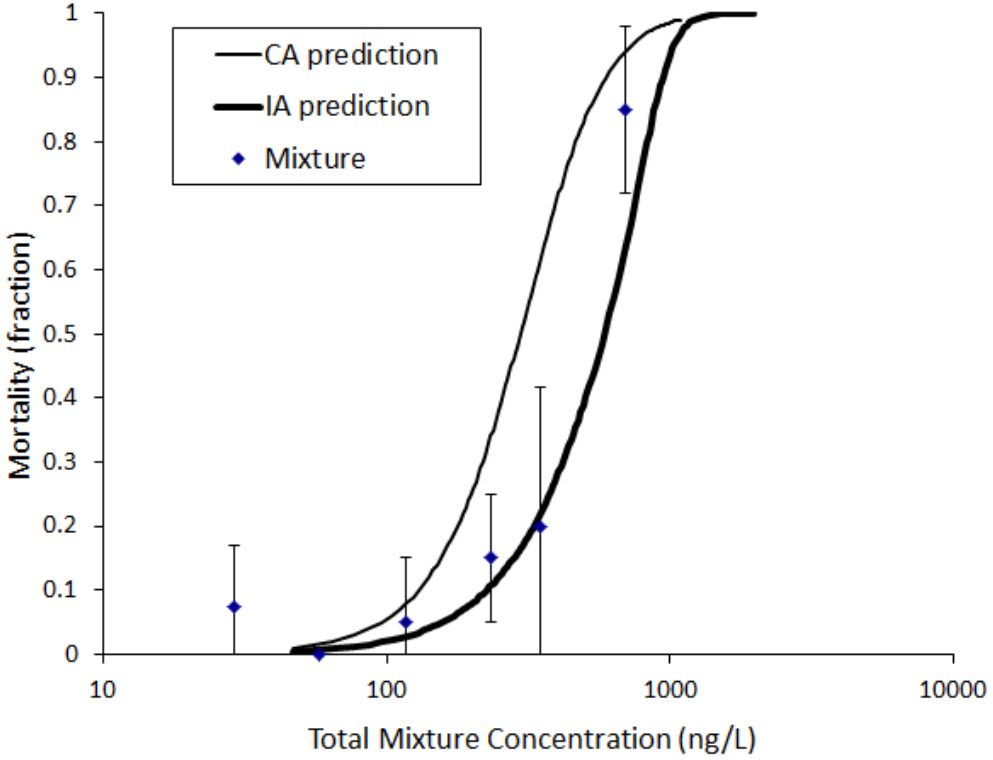
Non-target species of pesticide runoff

Represent taxonomic Orders = potentially vulnerable component of the food web in aquatic ecosystems of North America



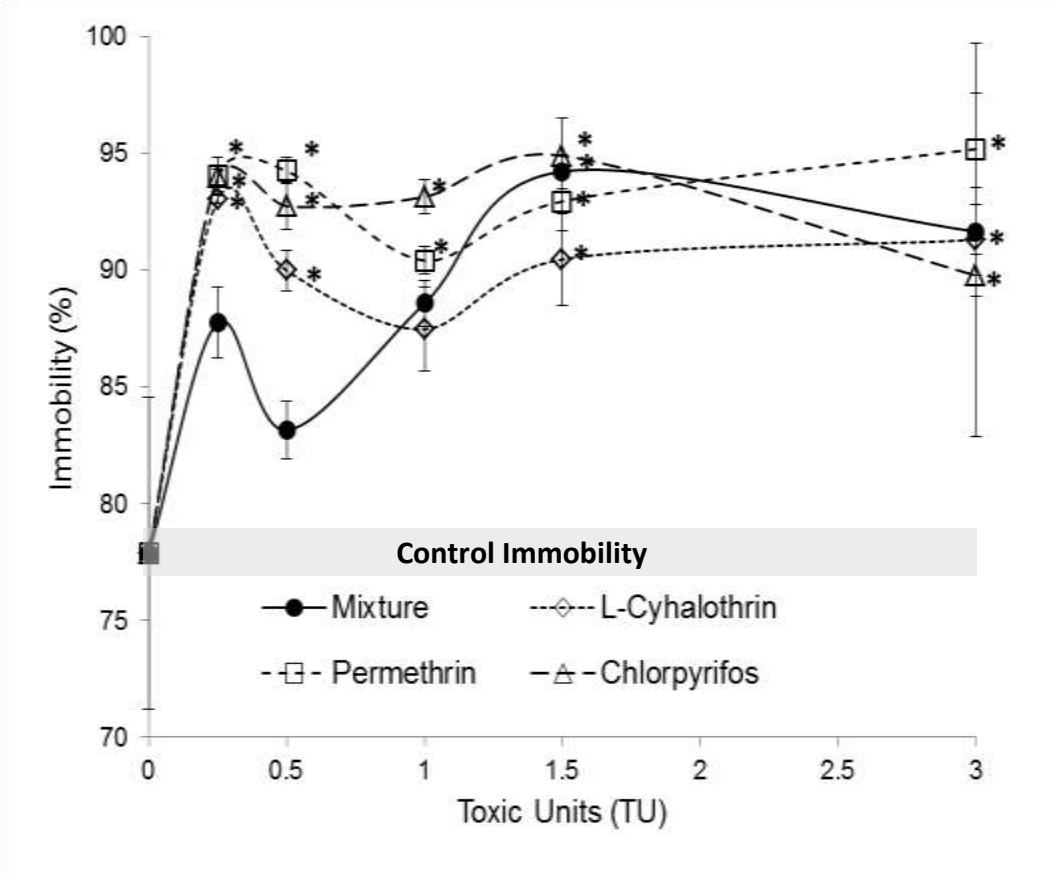
Survival *C. dilutus*





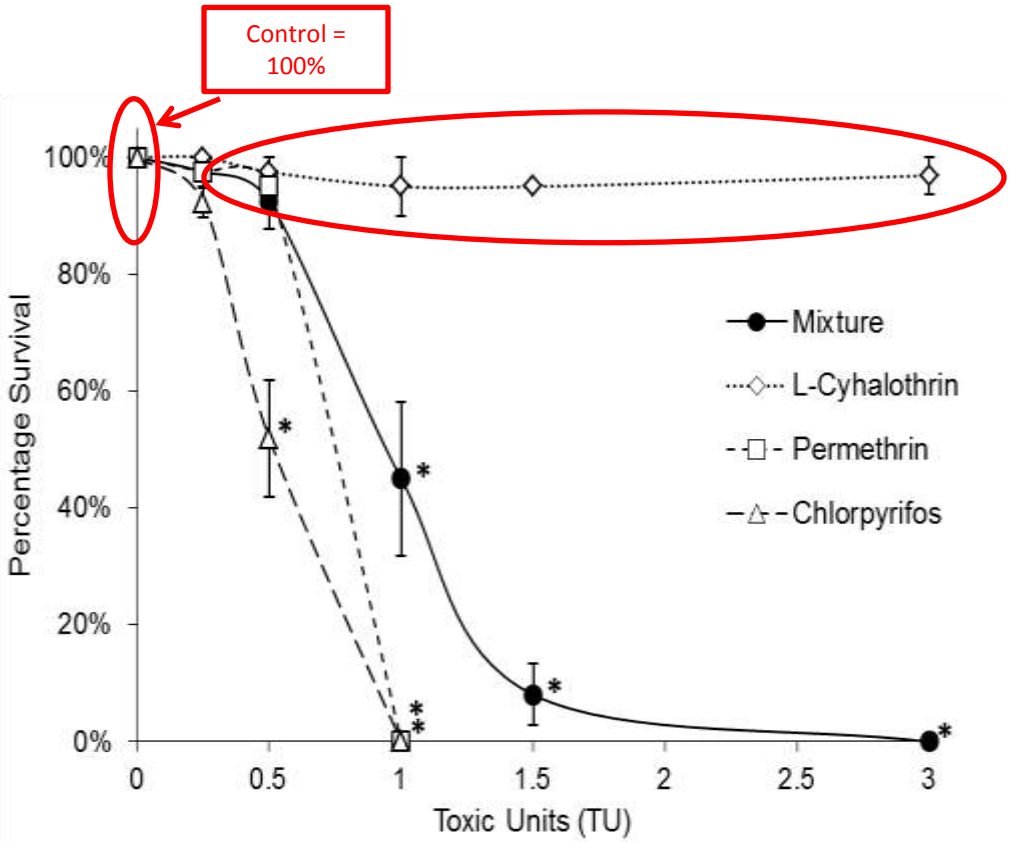
* = $p < 0.05$

Percentage Immobility *C. dilutus*



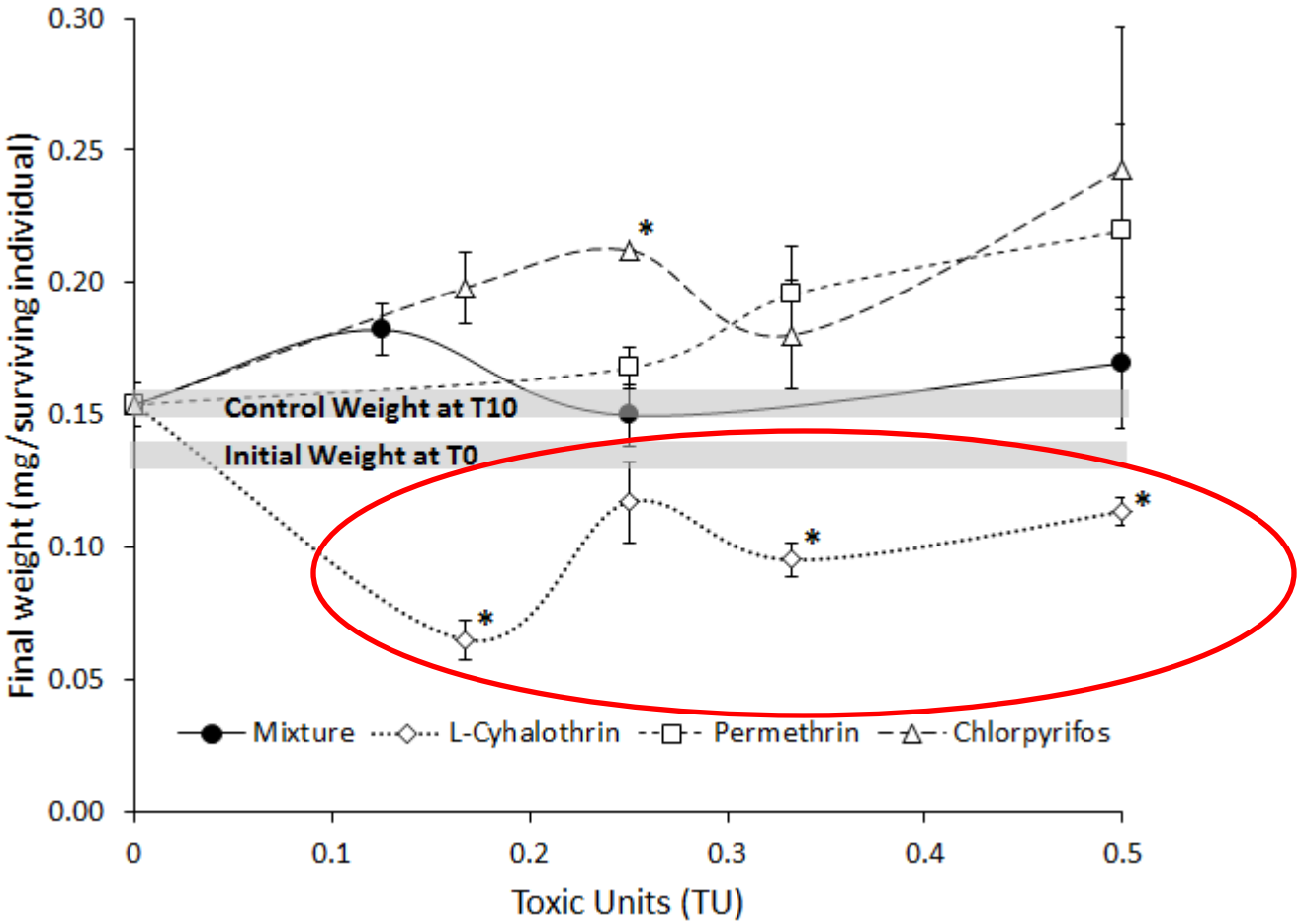
* = $p < 0.05$

Survival *H. azteca*



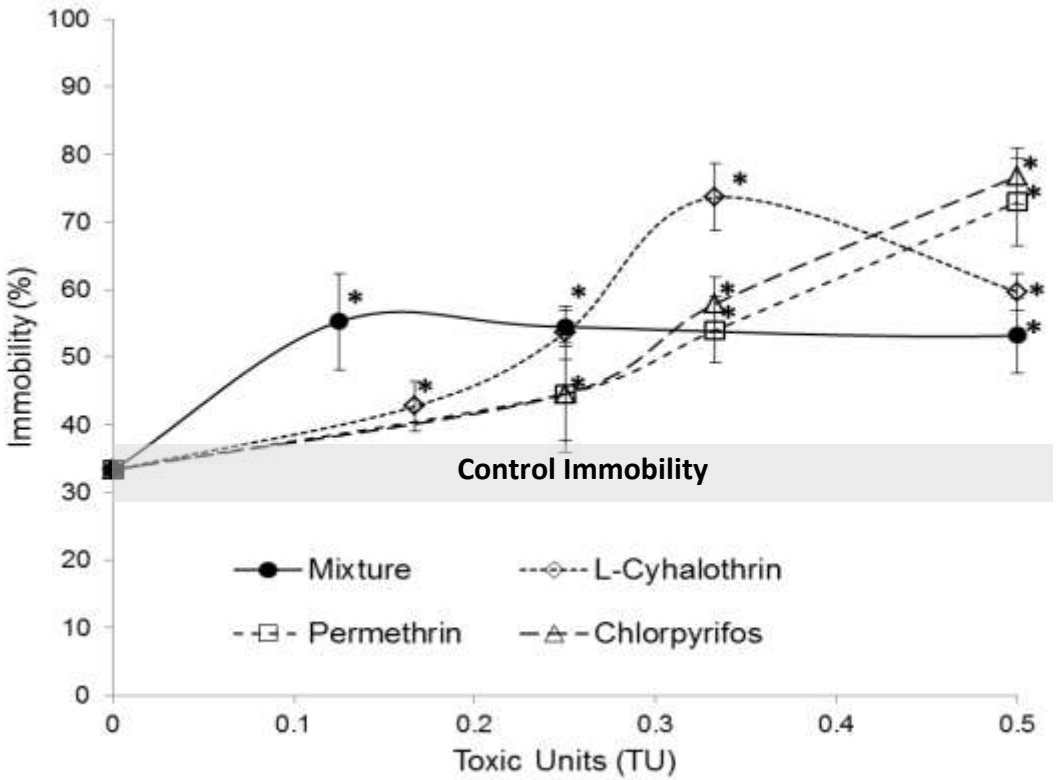
* = p < 0.05

10-day weight *H. azteca*



* = p < 0.05, T0 = Test initiation at day 0, T10 = Test termination at day 10

Percentage Immobility *H. azteca*



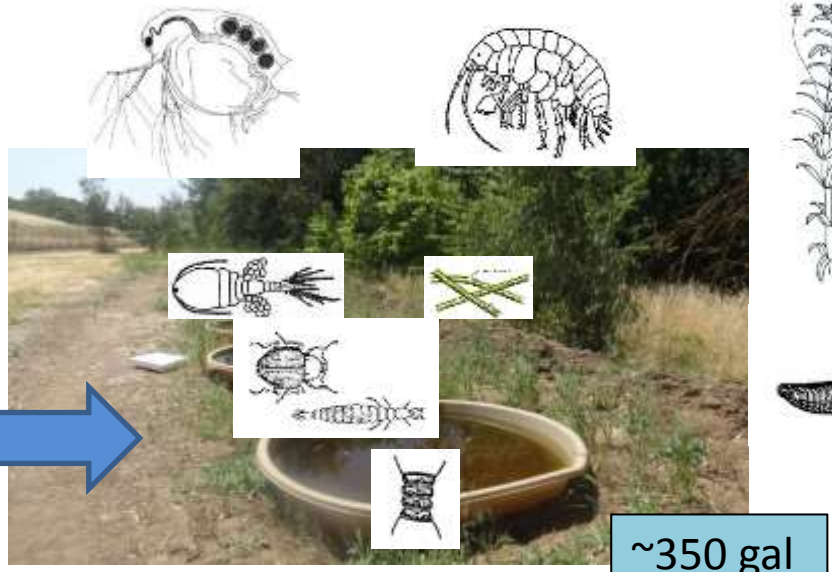
* = $p < 0.05$

Conclusion Lab Studies

- Survival response of *C. dilutus* fits best to Independent Action Model
- Sublethal endpoints for both species significantly affected at concentrations of around 0.125 TU (environmentally relevant) → Ecologically effects on population and reproduction
- No mortality for lambda-cyhalothrin in *H. azteca,* still sublethal effects → various endpoints required
- Swimming behavior: Most sensitive endpoint. Growth variable endpoint across the two species

System Setup

Buried half-way
Natural Sediment/Sand Mixture



~350 gal

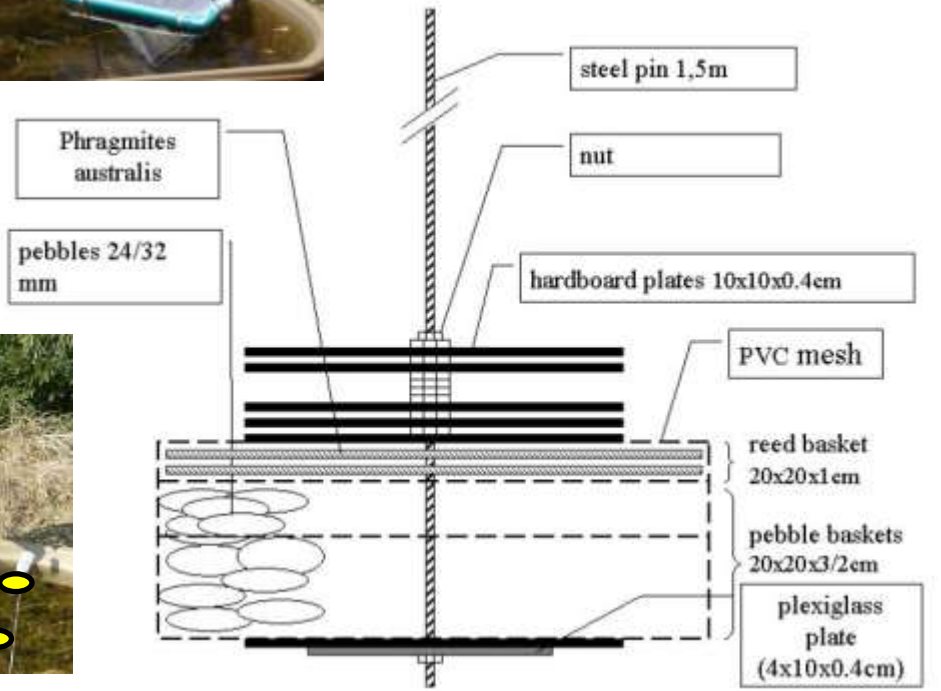
Sampling

- Weekly - from 6 weeks before 1st application on June, 19th until Oct, 31st (day 133 p.a.) → 6 months in total

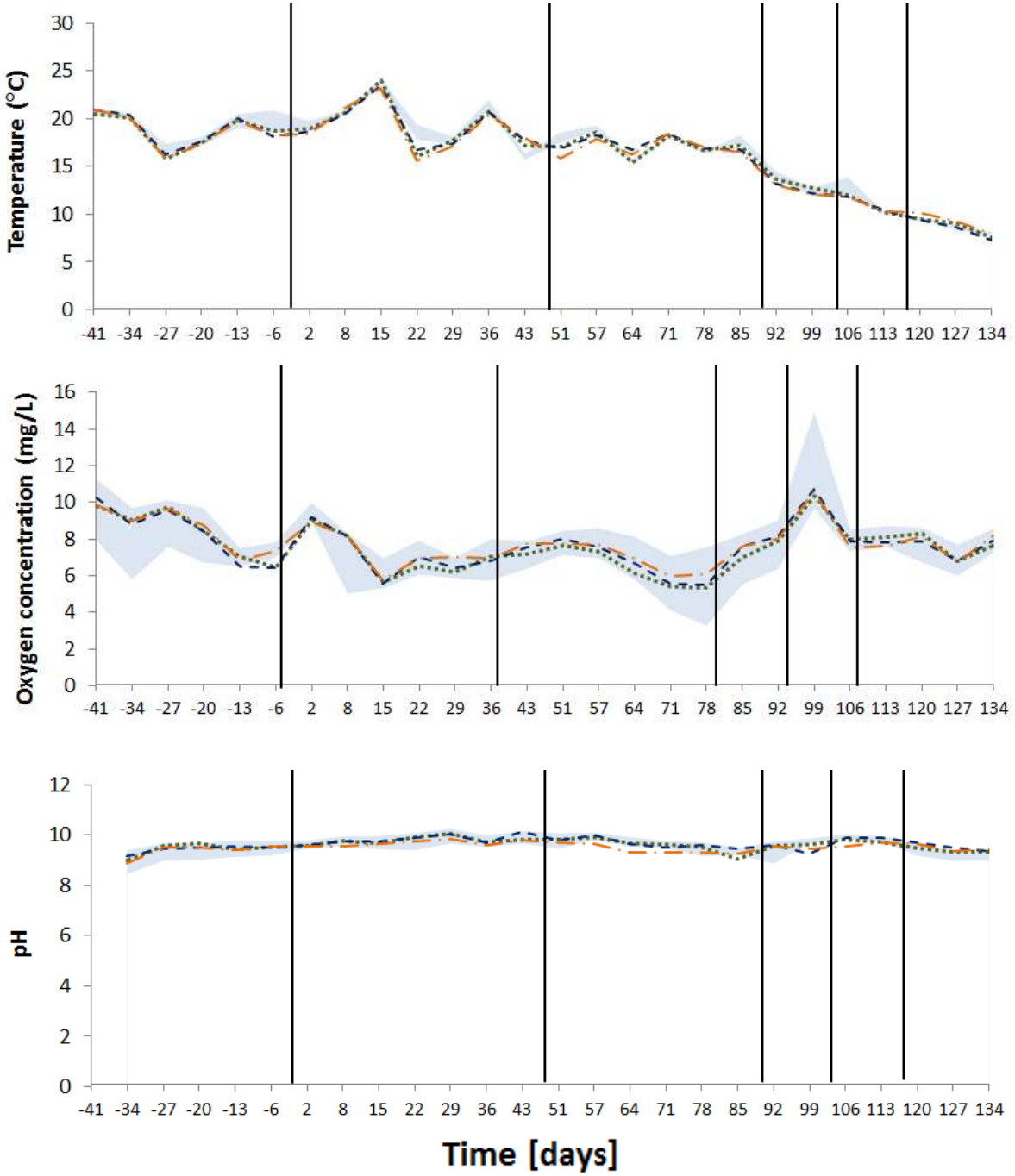
- Physical chemical Parameters:
  - DO, pH, T, EC, SC

- Organism Response:
  - ❑ **Macroinvertebrates**
  - ❑ **Zooplankton**

- Pesticide Fate:
  Water and Sediment



Physical-chemical parameters



Outlook

- Include Phytoplankton, nutrients (bottom-up approach)
- Different sets of contaminants → herbicides, fungicides, pharmaceuticals ….
- Mesocosm study:
  - Fall/spring application,
  - Focus on certain key species (cages)