

Life-Stage Sensitivity Data to Optimize Chemical Control Strategies

*DRAFT VERSION, WITH NO FIGURES.
TO BE USED AS PLACEHOLDER ONLY
FINAL VERSION, WITH FIGS, SHALL BE MAILED
ON CD-ROM*

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Companion Presentations

Dr. John Lynn: “Detection of Apoptotic Cells to evaluate Chemical Control Strategies in Early Life Stages” (Poster session)

Chemical Control

Strengths:

- Reliable, versatile, easy to implement
- Cost effective
- Dose-response well characterized

Weaknesses:

- Effects on non-target organisms
- Limited by water quality regulations
- Limited public/agency support
- Environmental persistence
- Expensive

Consequently, chemicals often limited to:

- Confined areas
- Where non-target species of low concern
- Tool of last resort in a limited toolbox

Chemical Control

Target most sensitive life stage:

- Reduce effective dose
 - Reduce environmental risk
 - Reduce cost
 - More regulatory/public support

Study Objectives

- Develop laboratory bioassays for early life stages and adults
- Use two model species
 - Zebra mussel (*Dreissena polymorpha*)
 - Mediterranean mussel (*Mytilus galloprovincialis*)
- Select relevant chemicals
- Identify most sensitive stage(s)

Dreissena polymorpha

- *Background*

- Zebra mussel
- Freshwater
- Central Europe/
Mediterranean
area
- Introduction:
 - Ballast water
released into
Great Lakes
area

- *Testing rationale*

- Recent invasion of
inland Minnesota lake
- Control in small outlet
- Permitted to use
Cutrine[®]-Ultra for algal
control 1 ppm
- Dual use application:
Can permitted
application of Cutrine
eradicate larvae?

Mytilus galloprovincialis

- ***Background***

- Mediterranean mussel
- Marine
- Native: Mediterranean area
- Introduction:
 - Aquaculture
 - Ballast water
 - Ship hull attachment
 - Canals

- ***Testing rationale***

- Invasive to Western U.S.
- Aquaculture
- Selection criteria
 - One of ocean's 10 worst invaders
 - Testing method for native con-specific (*M. edulis*)
 - Can be spawned *in vitro*

Methods: Spawning

Field-collected adults induced to spawn

- *D. polymorpha*: Serotonin (5×10^{-4} M)
- *M. galloprovincialis*: thermal induction (30 °C)

Fertilization

- Composite all viable sperm
- Composite eggs from at least 3 females

Eggs placed in tissue culture dishes

- c. 200 fertilized eggs / well
- 10 mL test solution/well
 - *D. polymorpha*: 12 replicates / treatment
 - *M. galloprovincialis*: 6 replicates / treatment

Do we have a piccie
of the well plates???

Methods: Chemicals

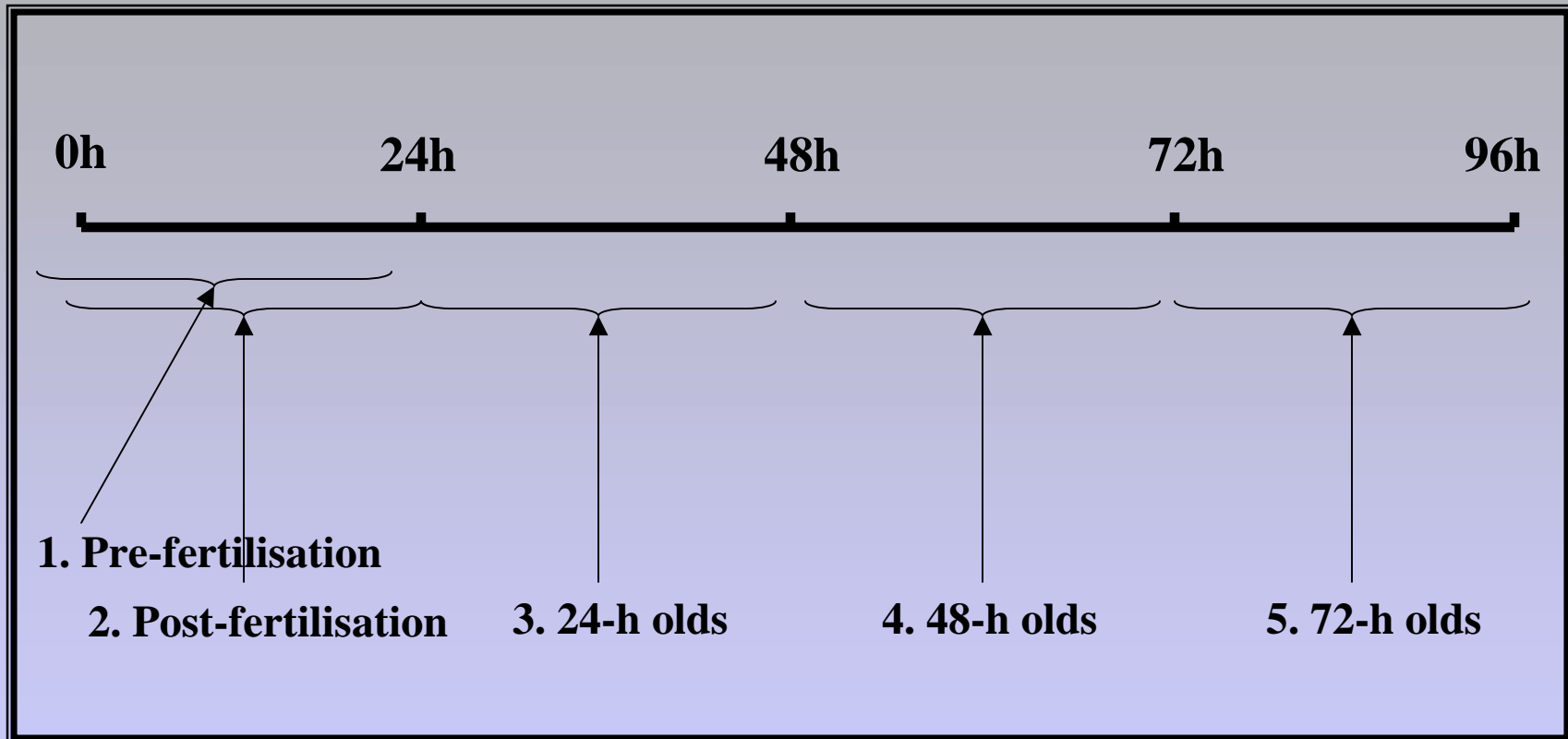
D. polymorpha (hard reconstituted water)

- Citrine-Ultra - chelated copper (9%), emulsified surfactant used to penetrate algal cell walls
 - Larvae nominal concentrations: 1, 2, 5, 10, 20, 50 µg/L
 - Adult nominal concentrations: 10, 100, 500, 1000, 5000 µg/L
- Cupric Sulfate – baseline copper toxicity
 - Larvae nominal concentrations: 1, 2, 5, 10, 20, 50 µg/L

M. galloprovincialis (30 ‰ seawater)

- Cupric Sulfate – known molluscicide
 - Larvae nominal concentrations: 1, 2, 8, 31, 125, 500 µg/L
 - Adult nominal concentrations: 20, 78, 313, 1250, 5000 µg/L
- Chlorine – common molluscicide in closed systems
 - Larvae nominal concentrations: 1, 4, 16, 63, 250, 1000, 4000 µg/L
 - Adult nominal concentrations: 39, 156, 625, 2500, 10000 µg/L
- Bayluscide – effective molluscicide in aquaculture
 - Larvae nominal concentrations: 1, 4, 16, 63, 250, 1000 µg/L
 - Adult nominal concentrations: 4, 16, 63, 250, 1000 µg/L

Methods: Early life stage exposures



Methods: Adult exposures

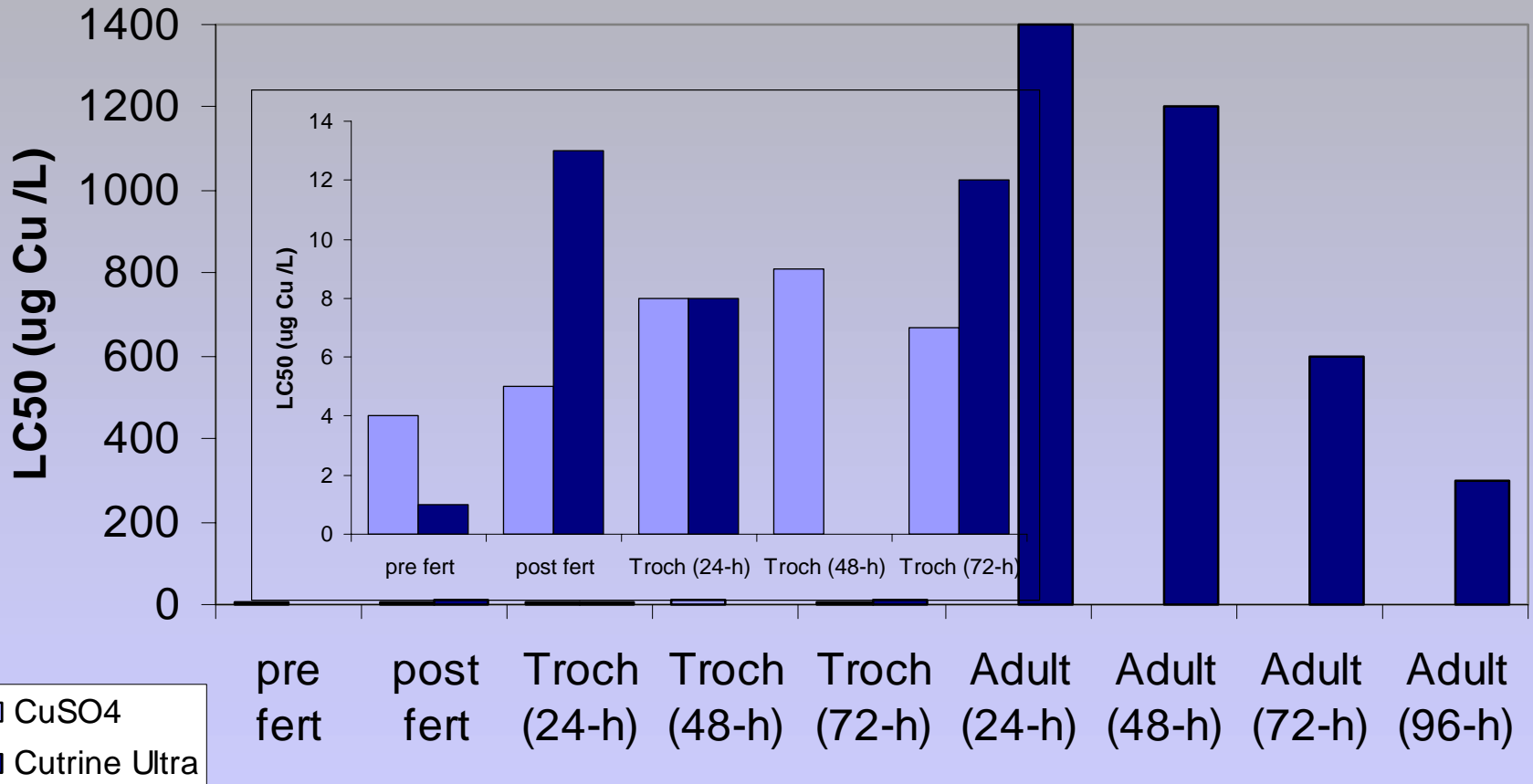
Adult mussels exposed for

- 24 h
- 48 h
- 72 h

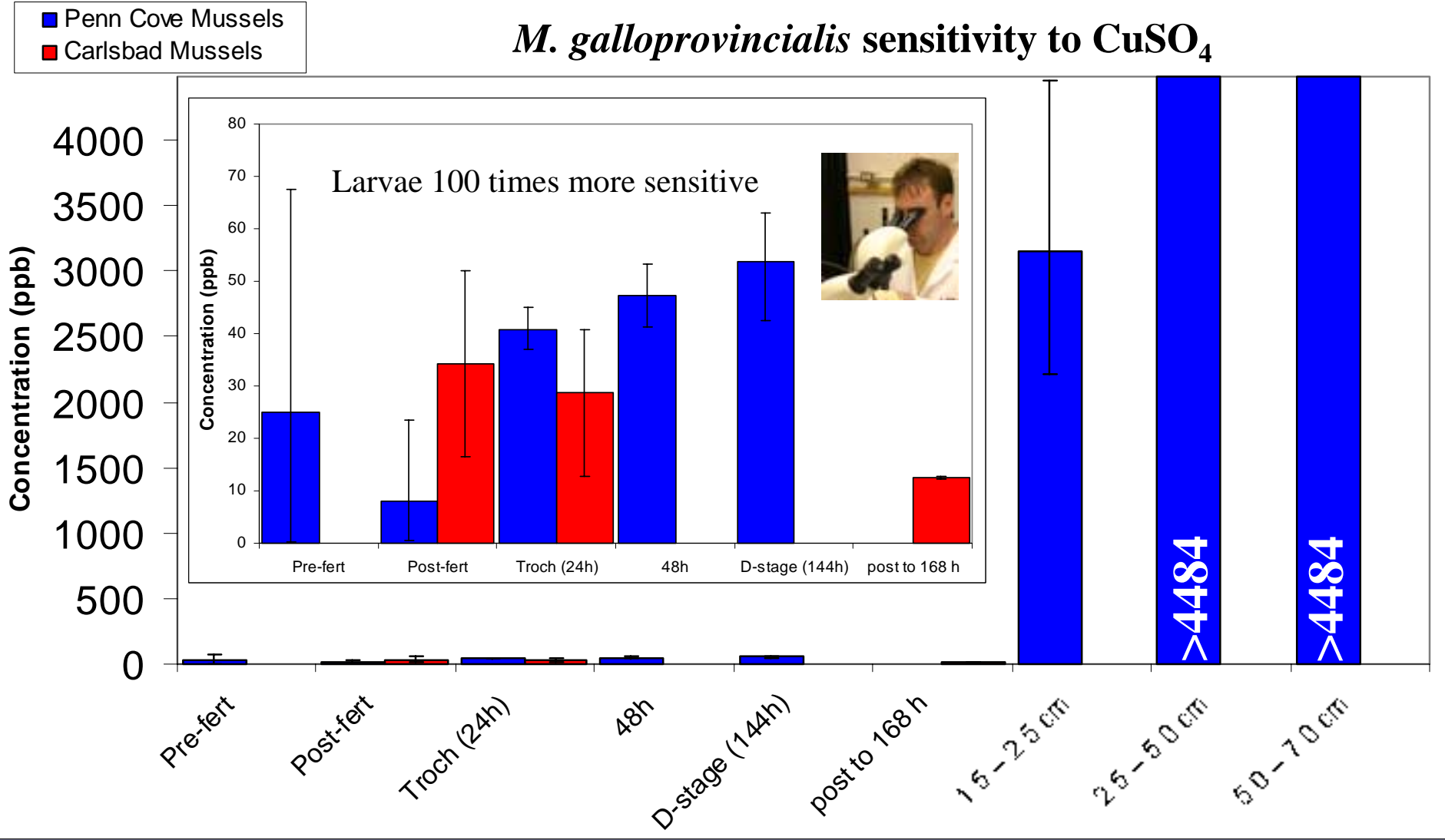
In addition, tested M. galloprovincialis juveniles

- Info on testing protocol?

Results: Zebra mussels, copper



M. galloprovincialis sensitivity to CuSO₄

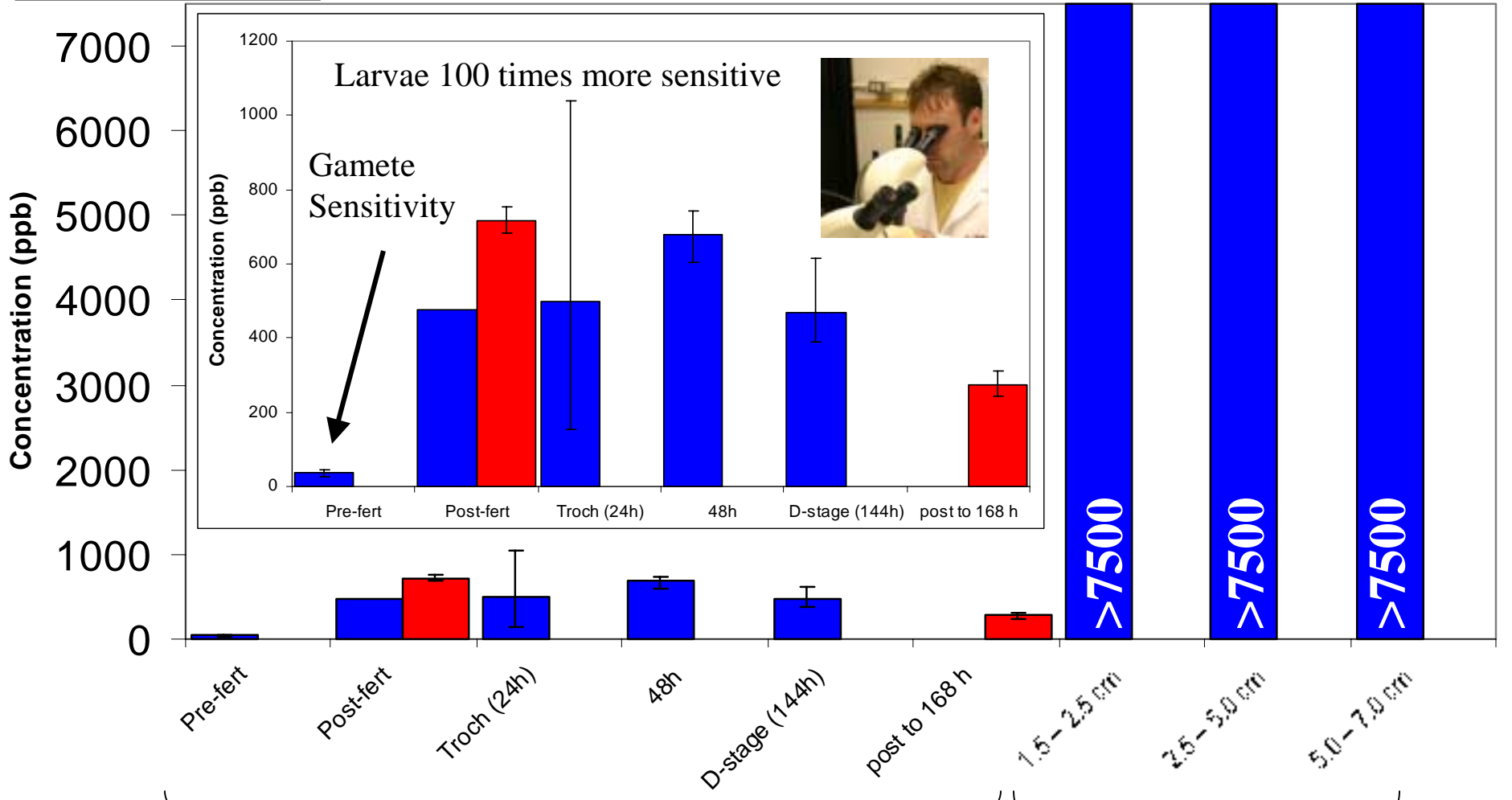


Early life stages

Adults (96-hr)

M. galloprovincialis sensitivity to Chlorine

- Penn Cove Mussels
- Carlsbad Mussels

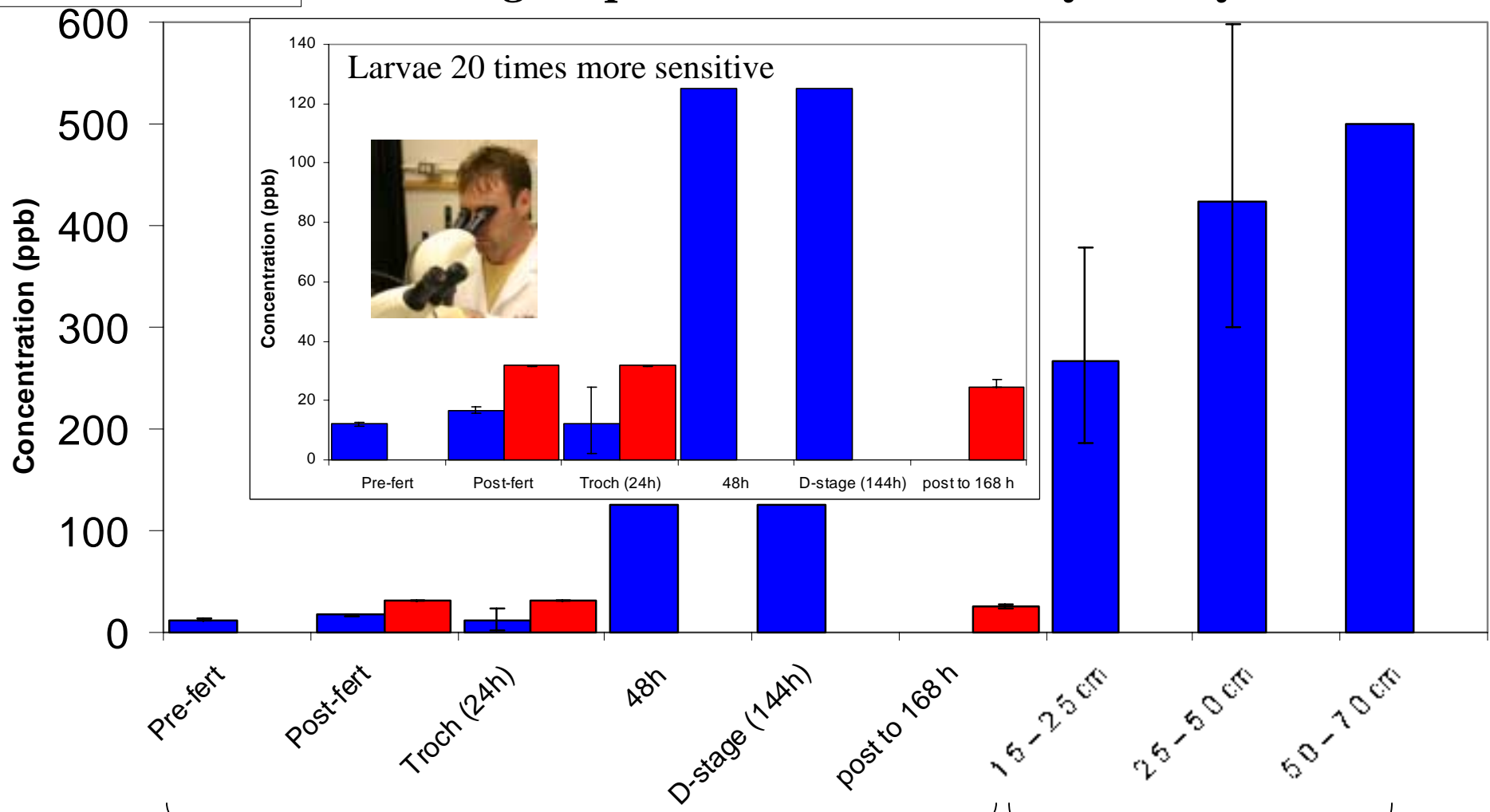


Early life stages

Adults (96-hr)

M. galloprovincialis sensitivity to Bayluscide

- Penn Cove Mussels
- Carlsbad Mussels



Early life stages

Adults (96-hr)

Summary: Life stage sensitivity comparison

Mussel	Chemical	LC ₉₉ (µg /L)			Reduction in chemical needed for 99% control of larvae
		Larvae (all stages)	Adult (24-h)	Adult (96-h)	
<i>Dreissena</i>	Cutrine	14 – 21	Control not obtainable	1,800	99%
<i>Mytilus</i>	Copper	99 – 433	Control not obtainable	157,400	99%
	Cl ₂	2,454 – 3,381	Control not obtainable	Control not obtainable	100%
	Bayluscide	202	Control not obtainable	1,602	87%

Can we change ‘control not available’ to > X ug/L?

Conclusions: Zebra mussel

- *Trochophores 90 – 100 times more sensitive to copper than adults*
- *Adults actively avoided both forms of copper*
 - Very low mortality and valve closure after 24-hours
 - Increasing mortality to 96-hours of exposure
- *Cutrine-Ultra and CuSO_4 similar in toxicity to larvae*
 - Copper accounted for the majority of toxicity
- *Cutrine-Ultra may have been more toxic than copper alone in gamete assays*
 - Surfactant possible spermicide?

Conclusions: Mediterranean mussel

- *Early life-stages of mussels 20 – 100 times more sensitive than adults*
- *Gametes and trochophores most sensitive*
- *D-stage larvae may be more tolerant than trochophores to some chemicals*
- *Gametes especially sensitive to chlorine*
- *Adults actively avoid chlorine, copper, not Bayluscide*
- *Pulse exposures of chlorine ineffective for adult control*

Conclusions and Applications

- **Gametes/larvae orders of magnitude more sensitive than adults**
- **Less chemical means lower cost and undesirable environmental impact**
- **Note that strategy targets population reproduction, not the existing adults**
- **May have utility as part of an integrated management strategy**
- **Efficacy might be modeled using population models**