Using water quality criteria and surrogate species to protect sensitive life stages of freshwater mussels and other aquatic organisms

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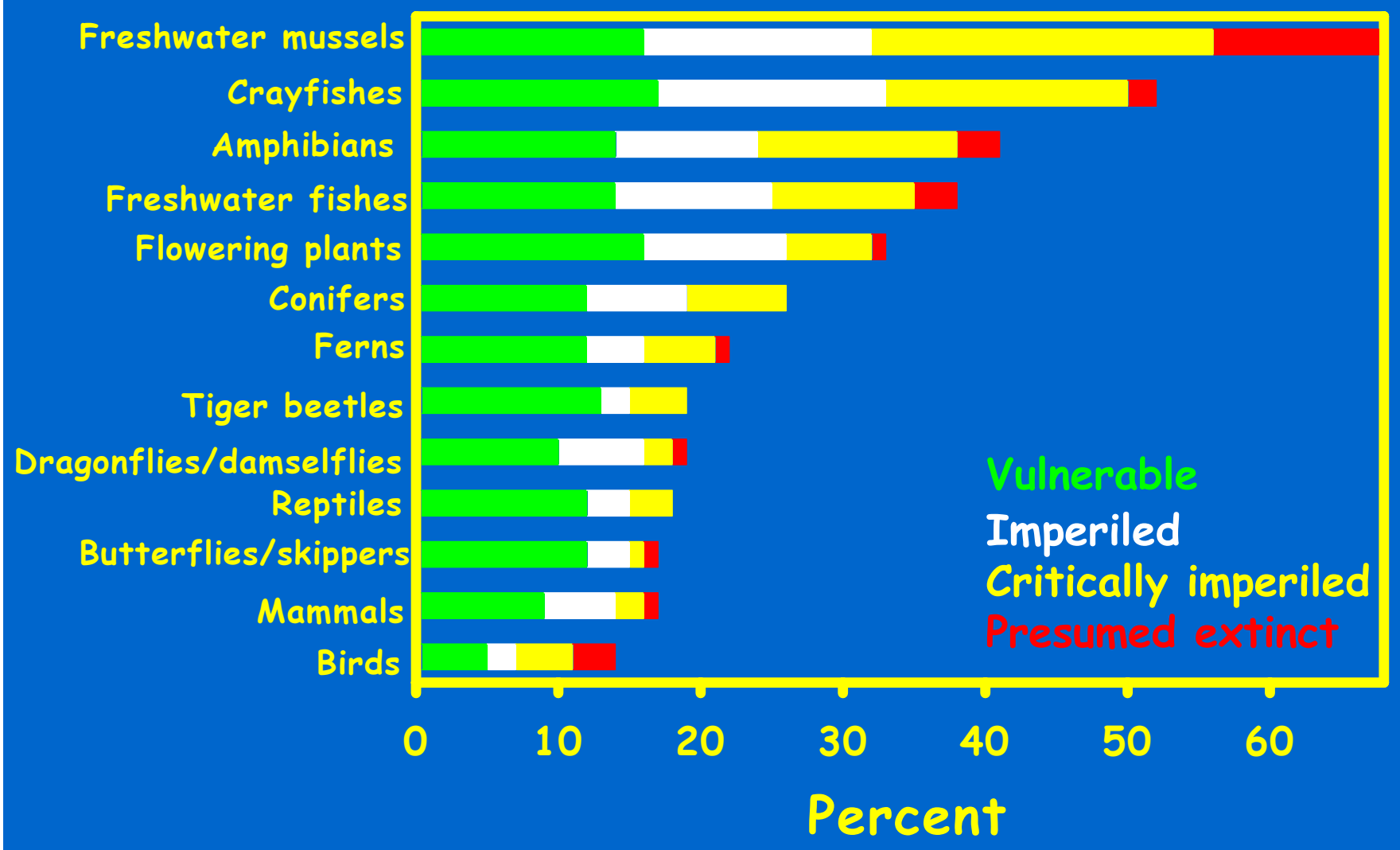
U.S. Department of the Interior
U.S. Geological Survey

The Nature Conservancy



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Master et al. (1998)

EPA-FWS-National Marine Fisheries Service MOA on endangered species (1990s)

- Are existing EPA water quality criteria (WQC) or effluent monitoring procedures adequately protective of endangered aquatic species?
- Are data for surrogate species (e.g., rainbow trout, *Ceriodaphnia dubia*) adequately protective of endangered aquatic species?
- Memorandum of agreement (MOA):
  - National consultation between EPA and Services on existing or new WQC (data gathering, research)
  - Improve consultations procedures for EPA approval of State and Tribal standards and permits

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Overview of talk

- Sensitivity of endangered or threatened (listed) species of fish to contaminants
  - Collaborators: Jim Dwyer, Tom Augspurger (USFWS); Sonny Mayer (USEPA)
- Sensitivity of freshwater sculpin to cadmium, copper, and zinc
  - Collaborators: Chuck Stephan, Dave Mount (USEPA); Chris Mebane (USGS)
- Sensitivity of freshwater mussels to copper, ammonia, and chlorine
  - Collaborators: Jim Dwyer, Tom Augspurger, Andy Roberts, Cindy Kane, Cindy Tibbott (USFWS); Rob Pepin, Cindy Roberts (USEPA); Dick Neves (USGS), Chris Barnhart (Southwest Missouri State University)

Sensitivity of listed species of fish to contaminants

- Acute toxicity tests
  - 5 chemical with different modes of toxic action
  - Up to 20 aquatic species tested
- Effluent toxicity tests
  - 7-d *C. dubia* or fathead minnow testing methods
  - Field-collected effluents or spiked water
- Chronic toxicity tests
  - Copper or pentachlorophenol
  - 2 listed species tested

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Chemical	Use	Mode of Action
Carbaryl	Carbamate insecticide	Inhibitor of cholinesterase activity
Copper sulfate	Mining, industrial, fungicide	Interferes in osmoregulation
4-nonylphenol	Nonylphenol ethoxylate detergents	Narcotic and oxidative stressor
Pentachlorophenol	Wood preservative, molluscicide	Uncoupler of oxidative phosphorylation
Permethrin	Pyrethroid insecticide	Neurotoxin
Ammonium Phosphate	Fertilizer, waste-water treatment	Interferes in respiration
Chlorine (Sodium Hyporchlorite)	Disinfectant	Oxidant; disrupts cell membranes
Diazinon	Insecticide	Inhibitor of cholinesterase activity

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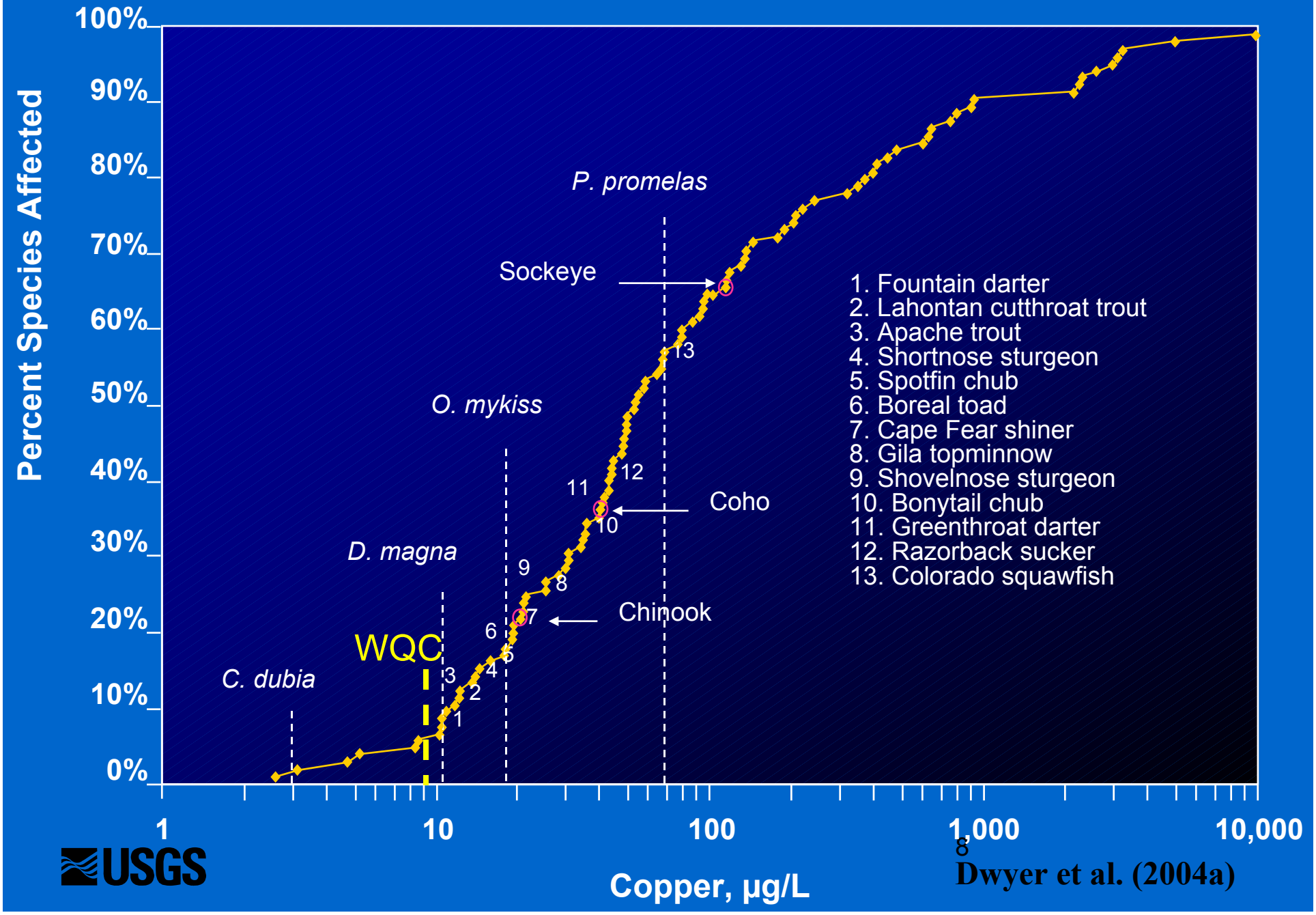
Static-renewal toxicity tests

Acute toxicity of copper to listed species of fish



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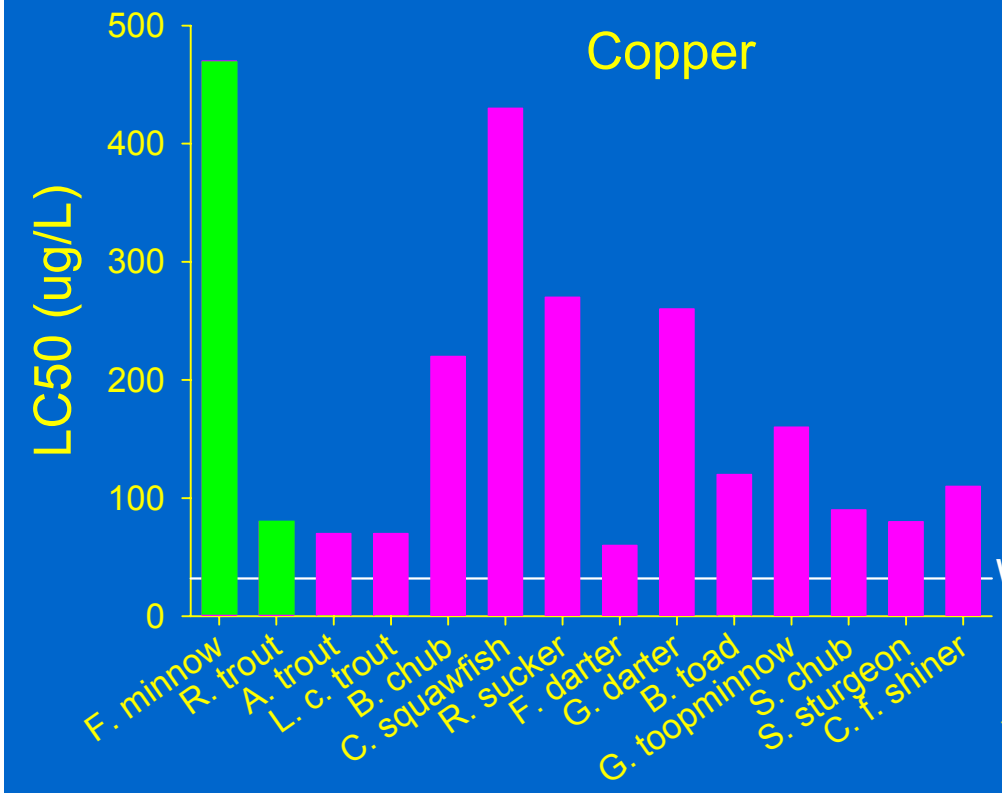
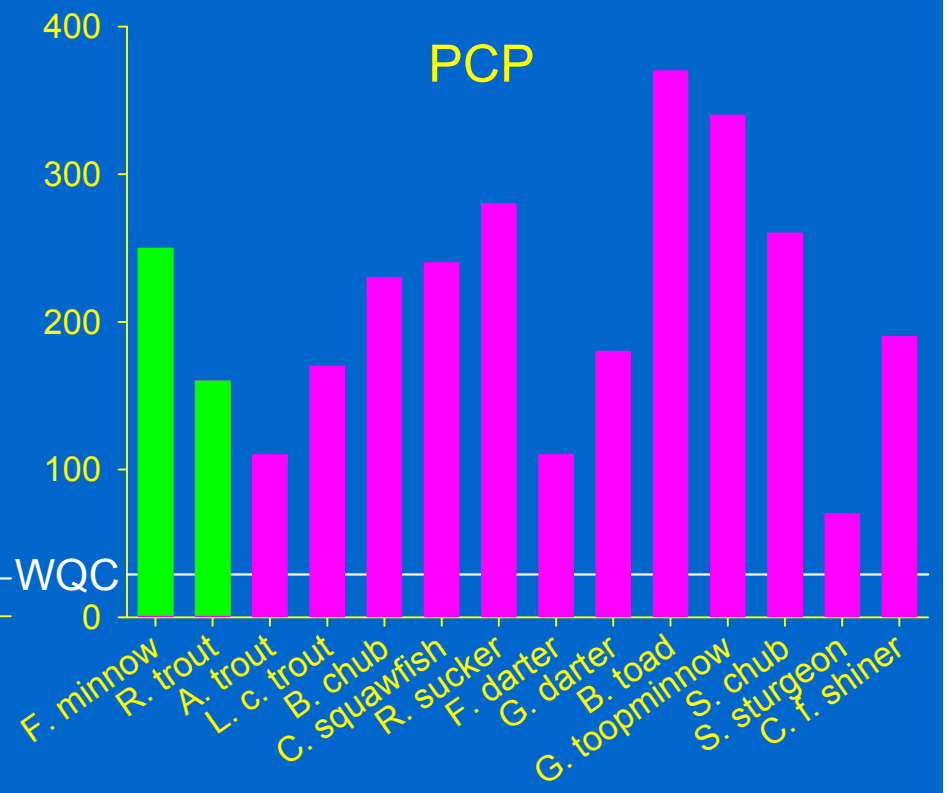
Dwyer et al. (2004a)

Acute toxicity of copper or pentachlorophenol to listed species of fish





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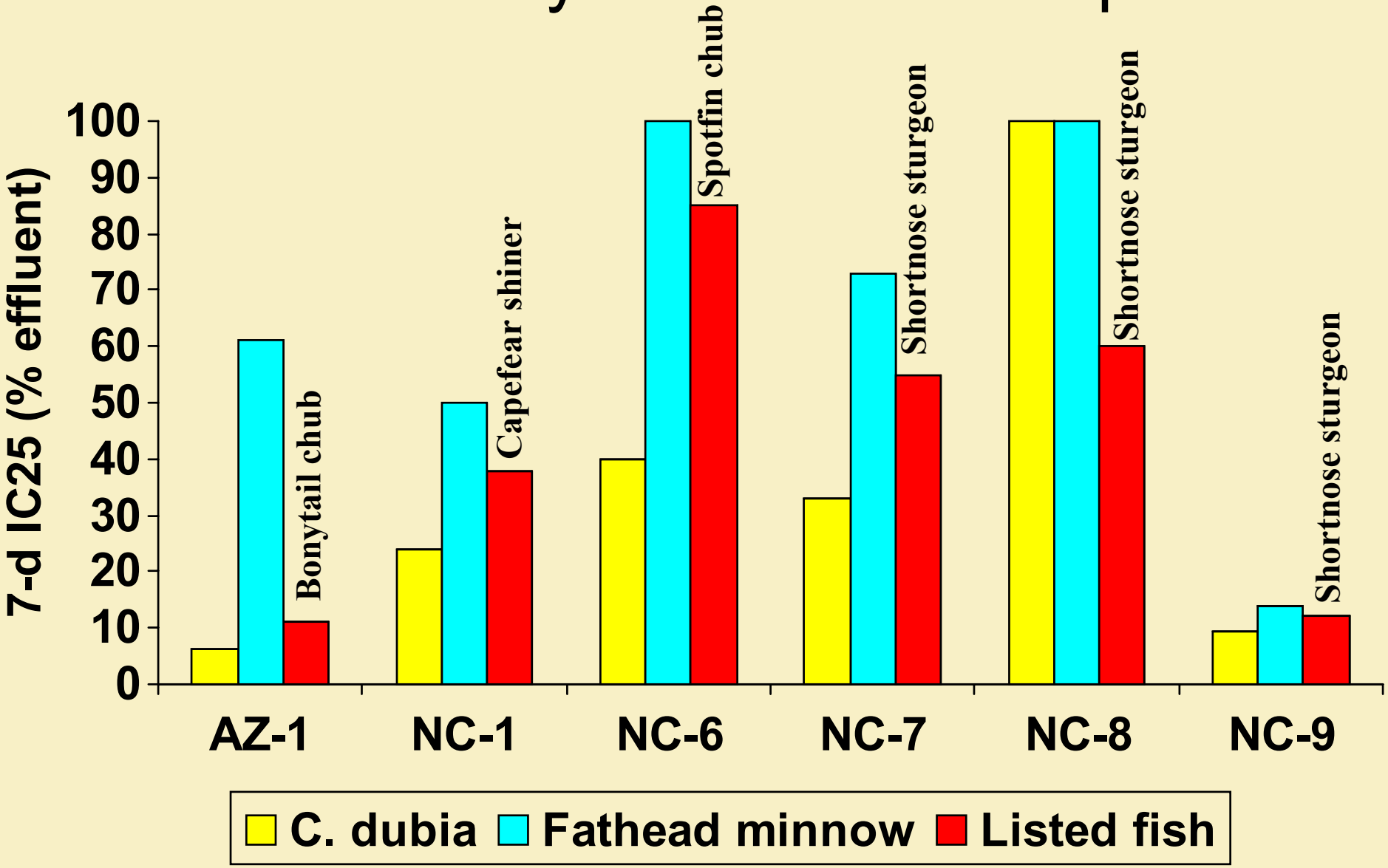
 Dwyer et al. (2004a)

Effluent toxicity tests: Field samples



Dwyer et al. (2004b)

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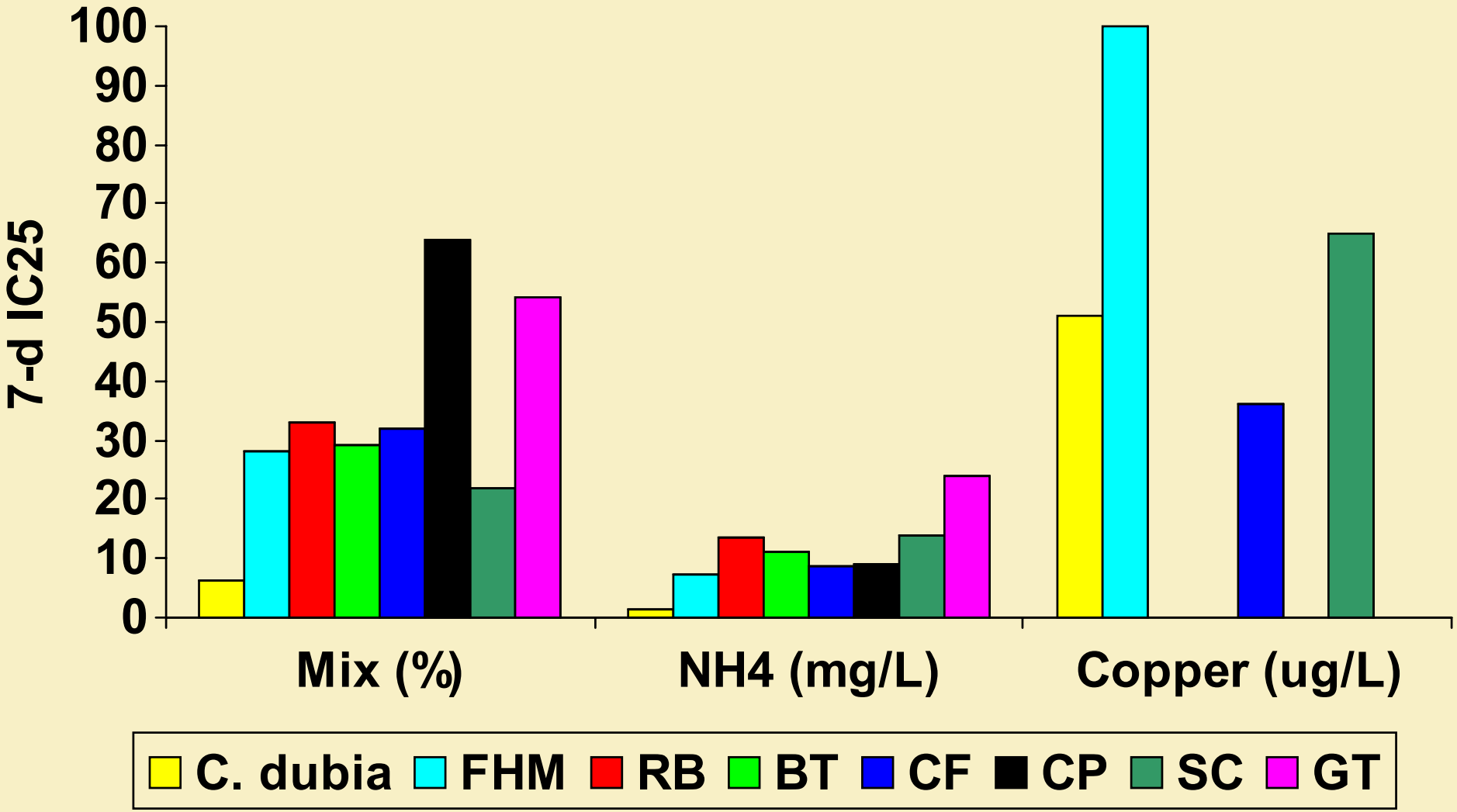
Effluent toxicity tests: Spiked chemicals



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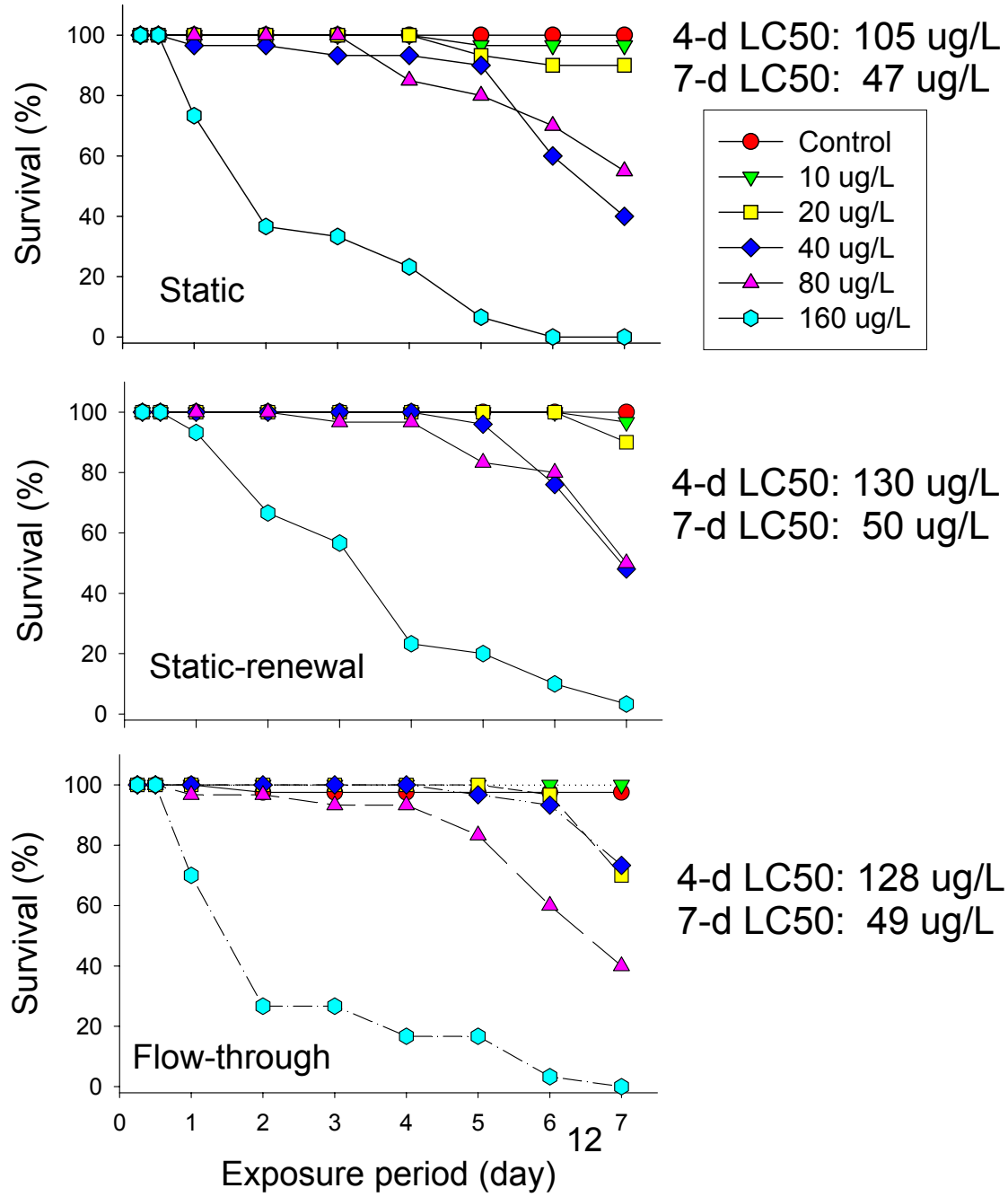
 Dwyer et al. (2004b)

Influence of water replacement on response of shortnose sturgeon to copper in 7-d exposures



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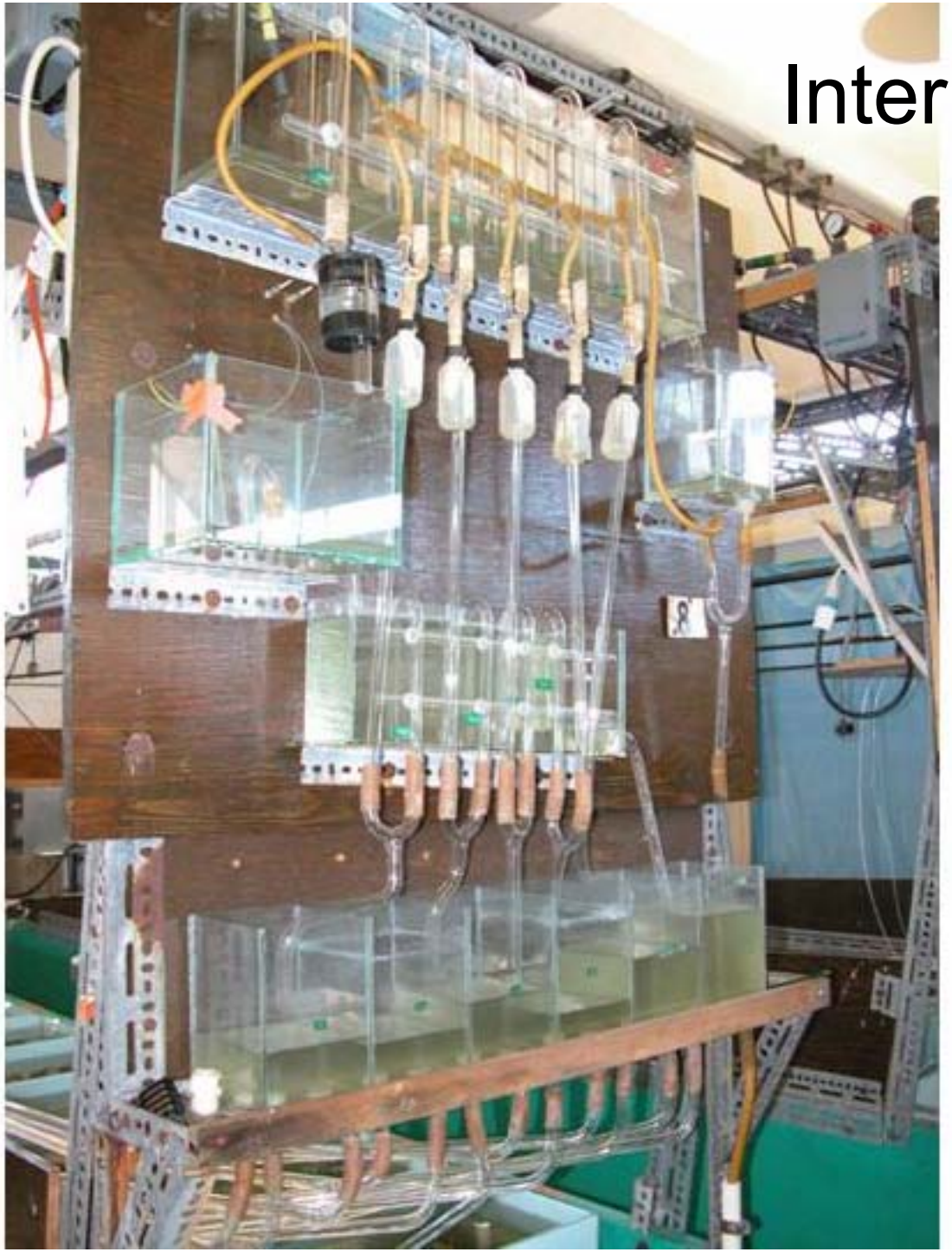
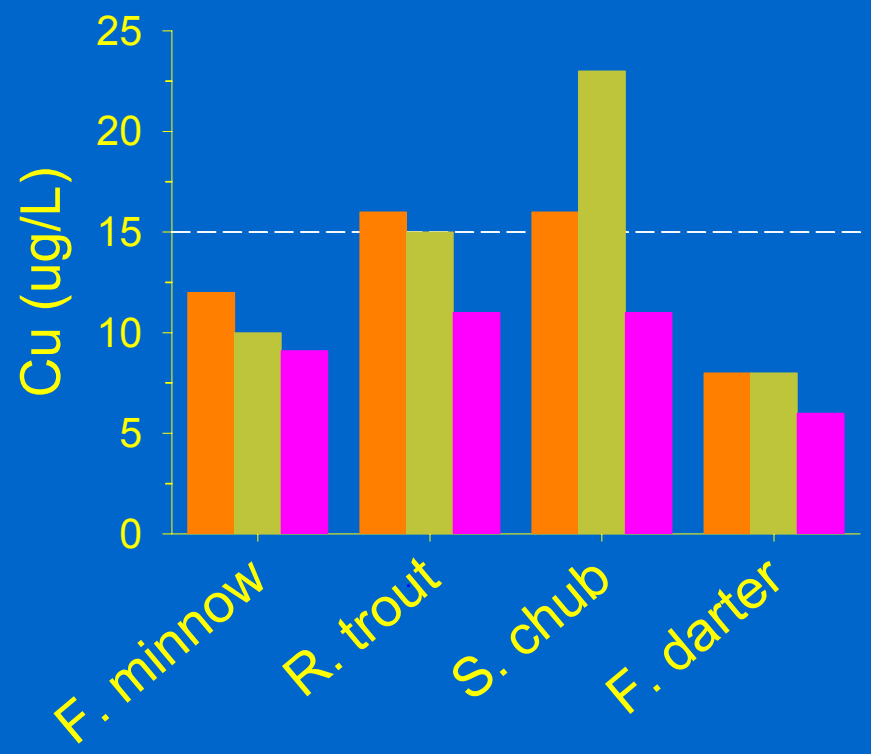
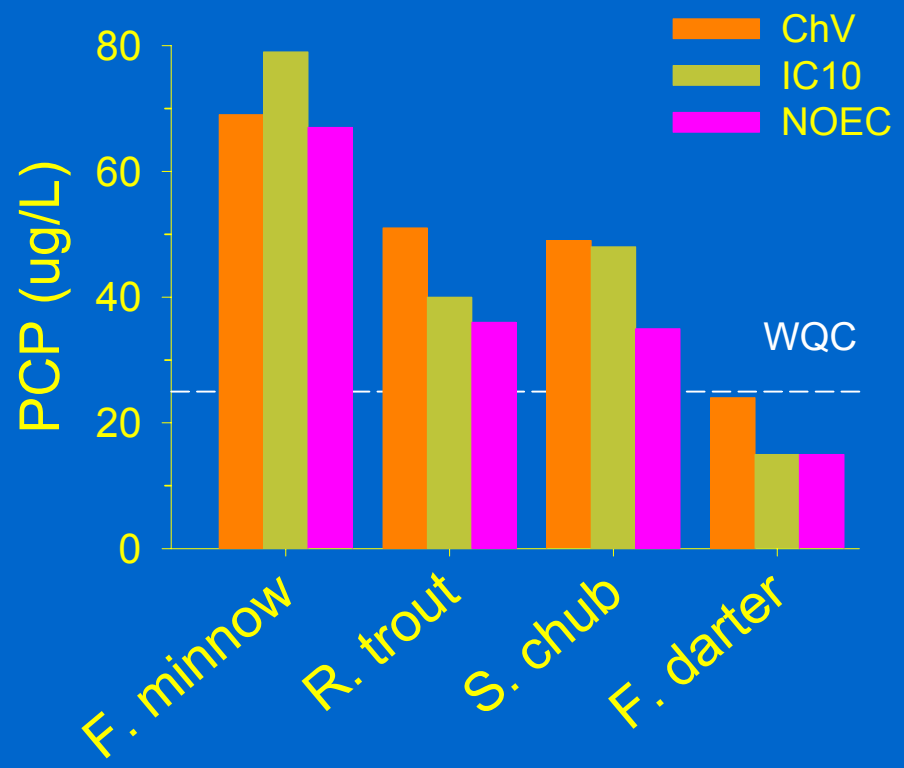
Intermittent diluter



Water bath containing exposure chambers
6/14/2001

Chronic toxicity of copper or pentachlorophenol to biomass of listed species of fish





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 Besser et al. (2004)

Sensitivity of freshwater sculpin to cadmium, copper, and zinc

- Declines observed with sculpin in metal-contaminated streams in Idaho where trout are still present
  - Habitat?
  - Sensitivity to metals?
- Methods for spawning field-collected adult sculpin
  - Mottled sculpin (*Cottus bairdi;* 2003)
  - Shorthead sculpin (*Cottus confusus*; 2004 ongoing)
- Acute and chronic toxicity tests with sculpin and rainbow trout (*Oncorhynchus mykiss*) at a hardness of 100 mg/L (as $CaCO_3$)

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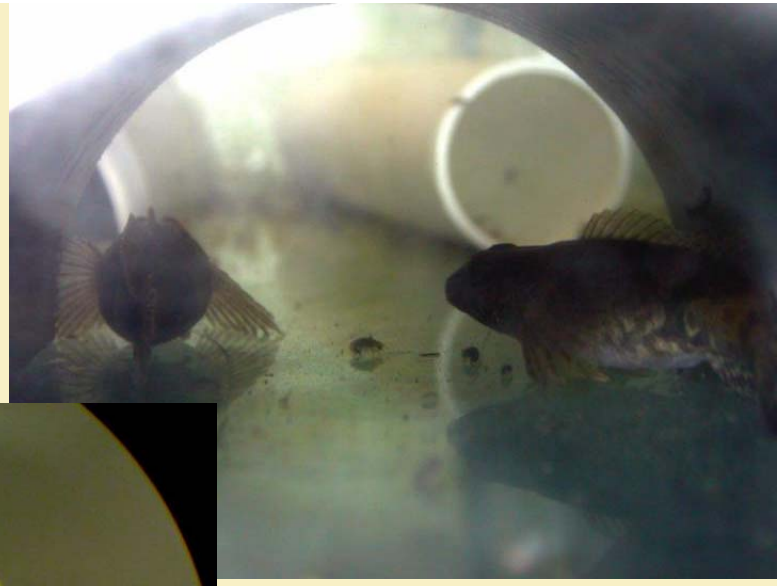
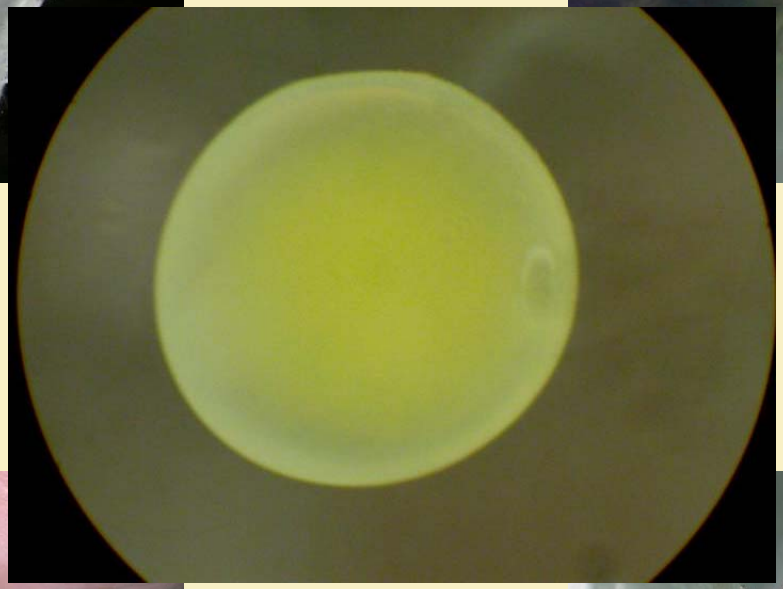
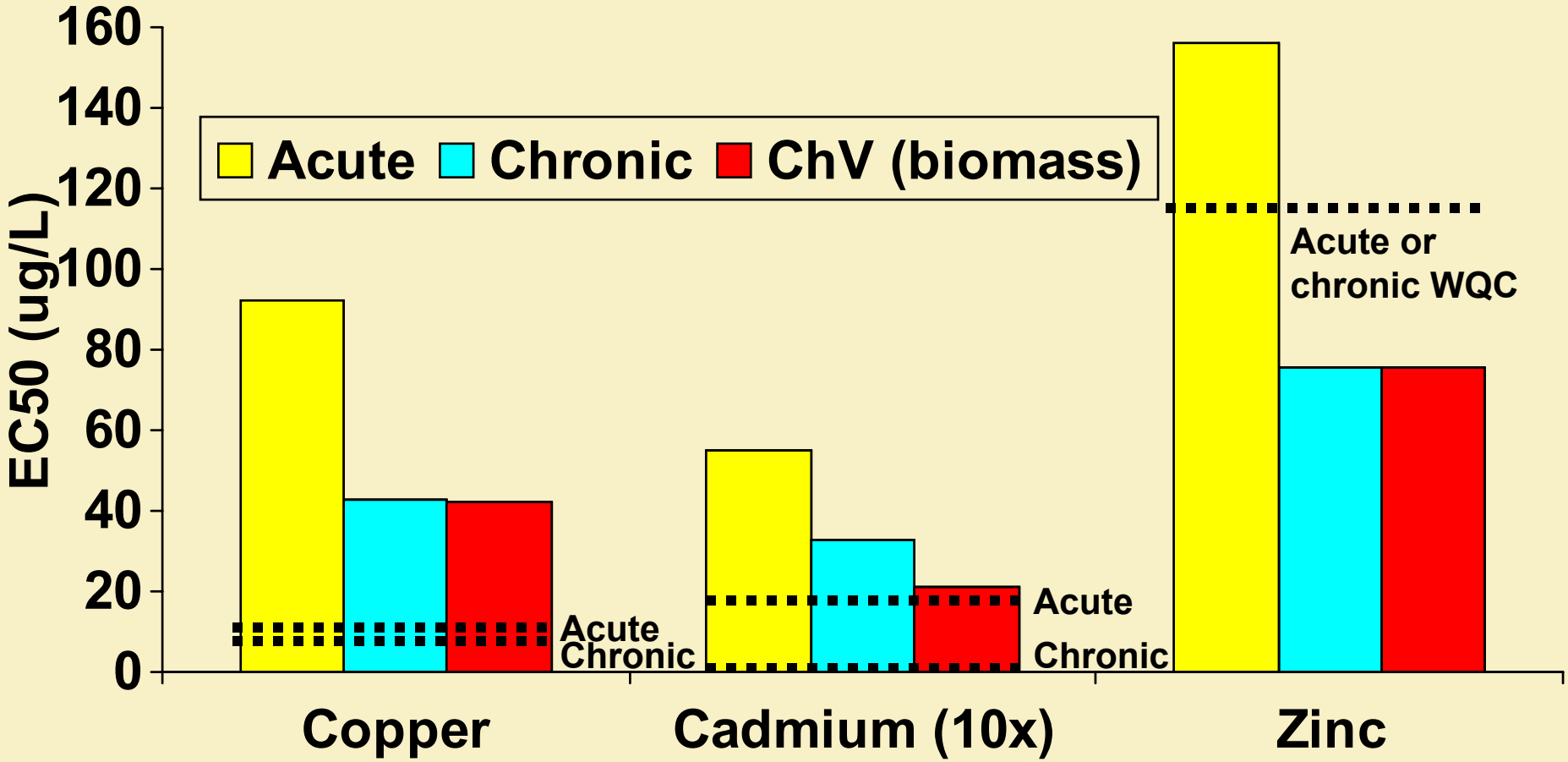


Spawning of sculpin



Toxicity of metals to mottled sculpin



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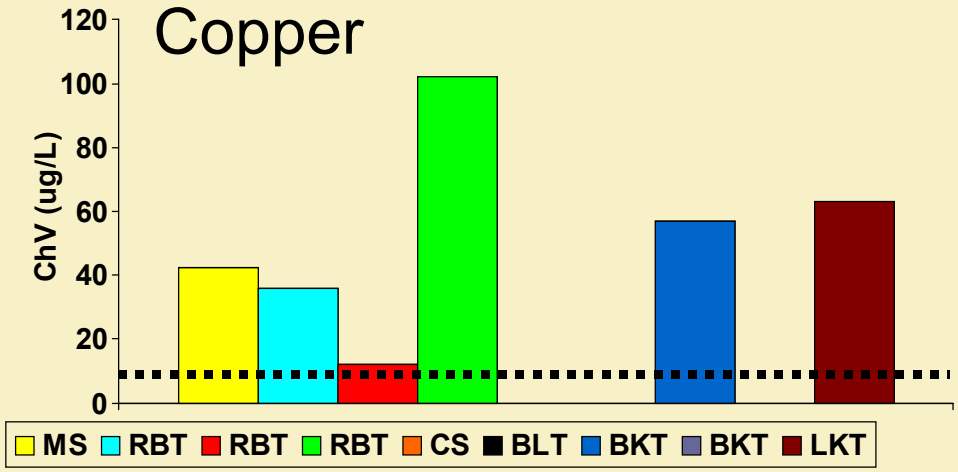
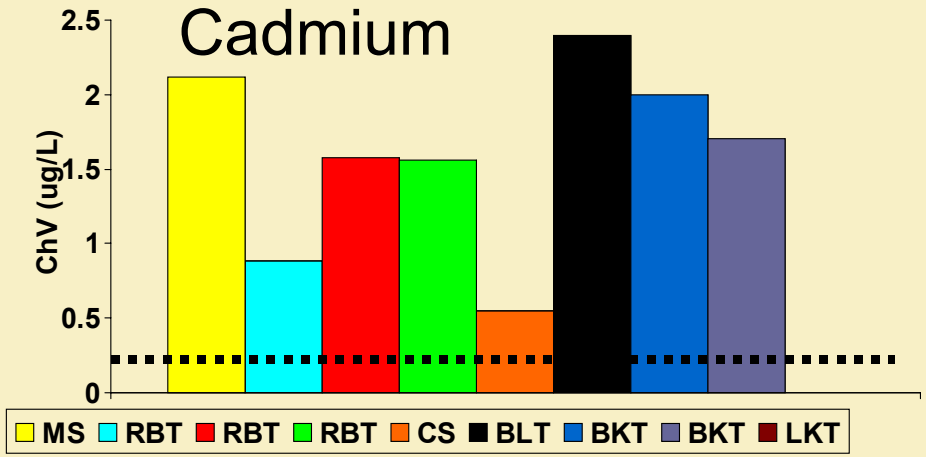
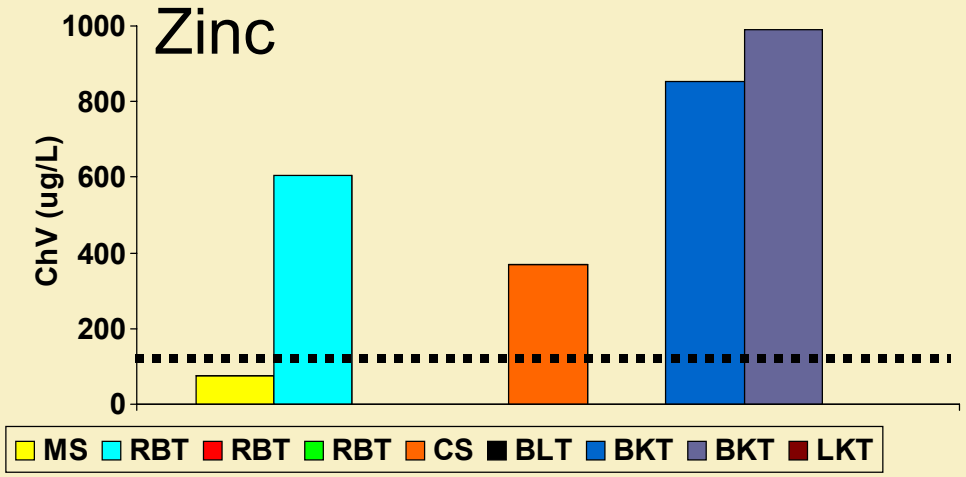
Chronic toxicity of metals to sculpin (present study) or salmonids (softer water)







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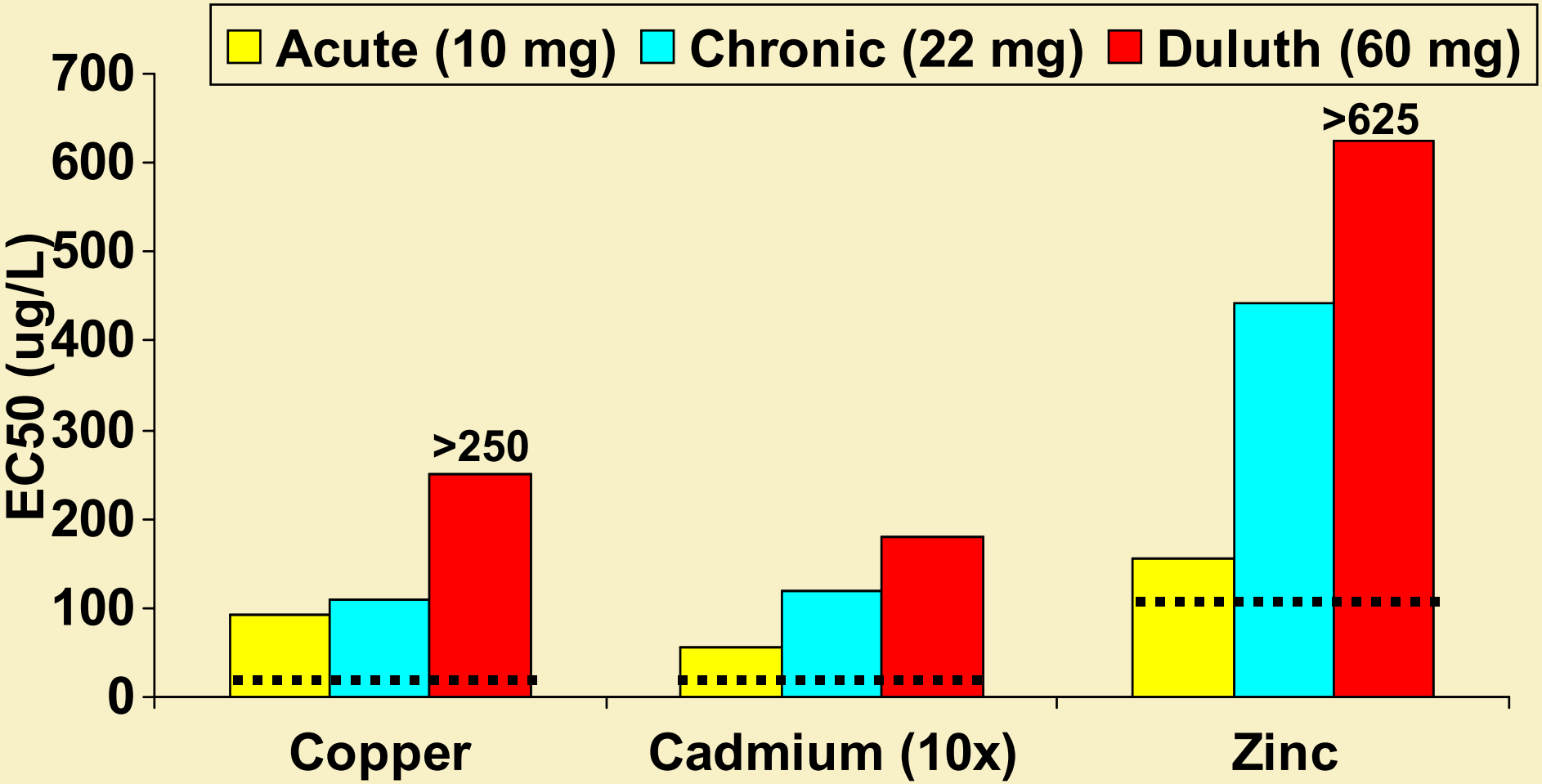
Acute toxicity of metals to different life stages of juvenile mottled sculpin



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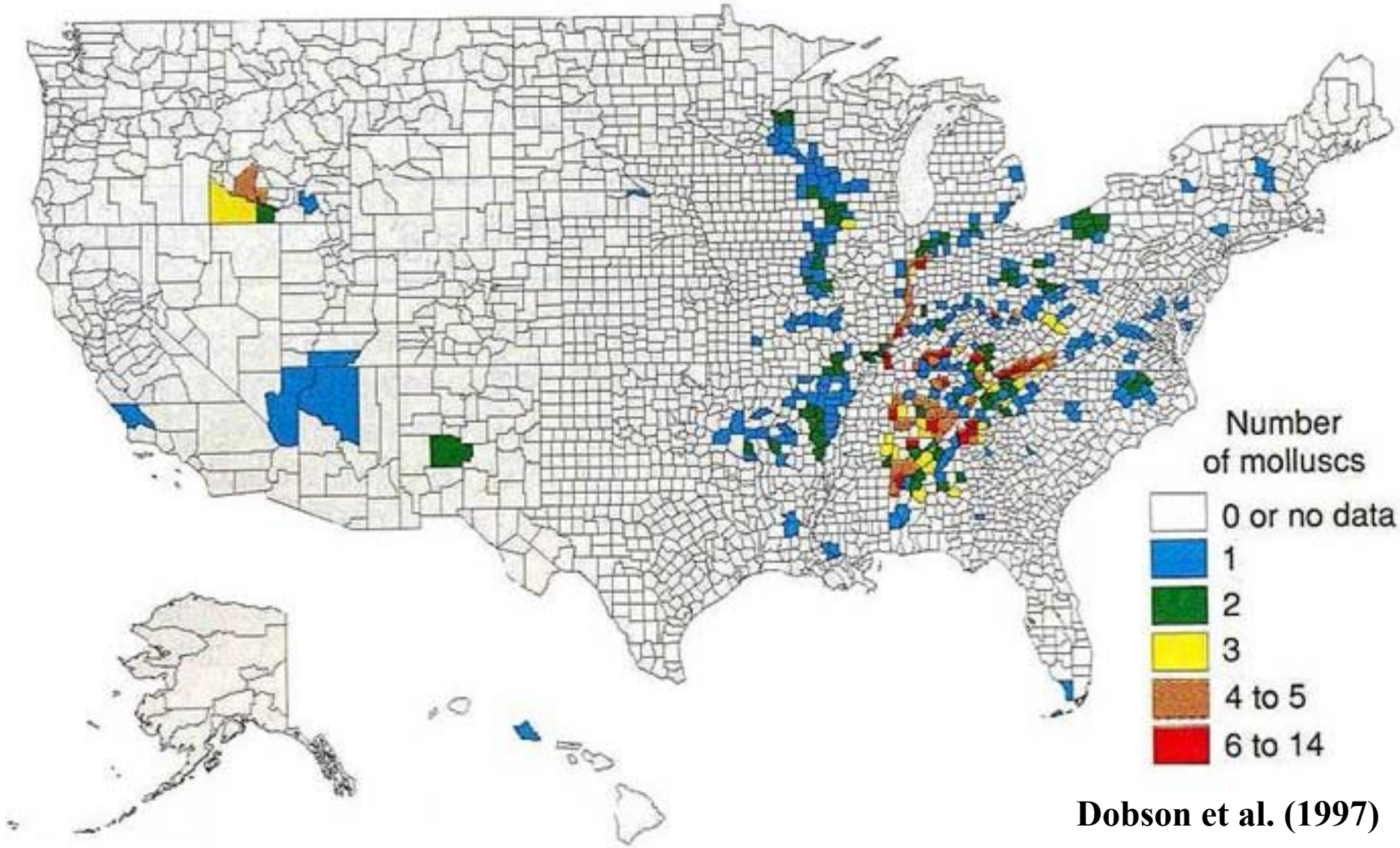
Geographic distribution of endangered mollusks



Dobson et al. (1997)

Potential causes for the decline of mussels in North America

- Greater than 70% of native unionid species in the United States listed as endangered, threatened, or of special concern
  - Habitat alteration
  - Introduction of exotic species
  - Over-utilization
  - Disease
  - Predation
  - Pollution

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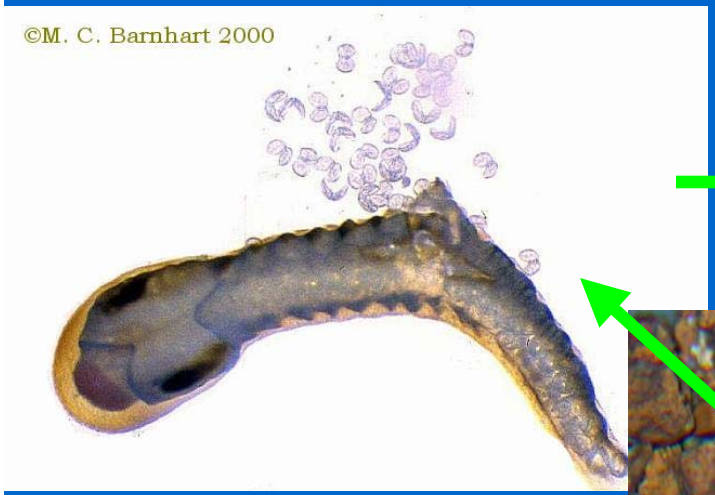
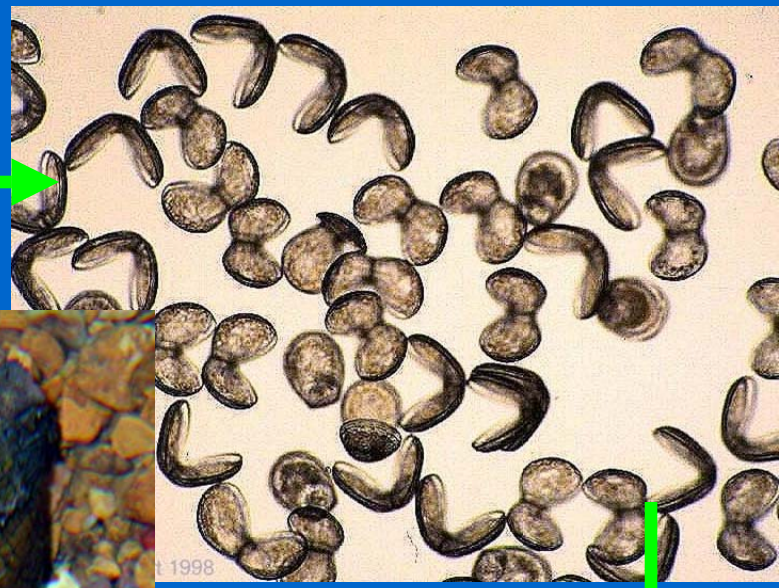
Generic life cycle of a “mollusk”



Ovisac w/glochidia



Glochidia

Juveniles

Glochidia encysted on fish gill

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Sensitivity of mussels to ammonia (mg/L at pH 8)

Rank	Genus	Species Used in GMAV Derivation	SMAV	GMAV
12	*Oncorhynchus*	Golden trout	26.10	21.95
		Cutthroat trout	25.80	
		Pink salmon	42.07	
		Coho salmon	20.26	
		Rainbow trout	11.23	
		Chinook salmon	17.34	
11	*Etheostoma*	Orangethroat darter	17.96	17.96
10	*Notemigonus*	Golden shiner	14.67	14.67
9	*Prosopium*	Mountain whitefish	12.11	12.11
8	***Pyganodon***	**Giant floater**	**8.88**	**8.88**
7	***Utterbackia***	**Paper pondshell**	**7.86**	**7.86**
6	***Actinonaias***	**Pheasantshell**	**7.27**	**7.27**
5	***Lasmigona***	**Green floater**	**6.10**	**6.10**
4	***Villosa***	**Rainbow**	**5.53**	**5.53**
3	***Fusconaia***	**Atlantic pigtoe**	**5.18**	**5.18**
2	***Medionidus***	**Cumberland moccasinshell**	**4.24**	**4.24**
1	***Lampsilis***	**Plain pocketbook**	**9.97**	**3.57**
		Fatmucket	**1.28**	

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Augspurger et al. (2003)

EPA issues regarding toxicity testing with glochidia (aka the "Stephan memos")

- How long should acute tests with glochidia be conducted (i.e., based on the life history of the species)?
- How long can glochidia survive and still be able to attach to a host?
- Are there data that indicate that effect concentrations do not change very much during the last half of a toxicity test?
- Is the use of NaCl (or KCl) to determine the percentage of organisms exhibiting valve closure an appropriate method to judge the acceptability of glochidia used to start a toxicity test? Does the response of glochidia to NaCl (or KCl) relate to the ability of glochidia to attach to a host? Is there an independent way of determining if glochidia are alive or healthy at the start (or end) of a toxicity test?

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EPA issues regarding toxicity testing with glochidia (cont.)

- Should there be a holding time for glochidia after harvesting but before application of a saline solution to determine if glochidia that are initially closed might open?
- Will immature, stressed, or unhealthy glochidia close when exposed to a saline solution? Could glochidia be alive and successfully attach to a host but not close when exposed to a saline solution? Are broken glochidia frequently observed at the start of a test? Would the presence of broken glochidia be indicative of stress during harvesting?
- Should glochidia be rinsed before use in a toxicity test?
- Should glochidia be acclimated to test conditions before the start of a toxicity test?
- What criteria should be used to judge acceptability of a toxicity test conducted with glochidia?

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EPA issues regarding toxicity testing with juvenile mussels (aka the "Stephan memos")

- What life stage should be used to start acute or chronic toxicity tests with juveniles?
- How should death of juveniles be determined at the end of a toxicity test with juveniles?
- What criteria should be used to judge acceptability of a toxicity test conducted with juveniles?

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Overview of mussel project

- **Refine methods** for conducting toxicity tests with glochidia and juveniles of freshwater mussels
- **Evaluate the toxicity of copper, ammonia, and chlorine** to glochidia and juveniles using acute and chronic toxicity tests
- **Develop standard method** through ASTM for conducting toxicity tests with freshwater mussels
- **Aquatic risk assessment** of these chemicals comparing toxicity results to ambient concentrations in important mussel habitats.

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Overview of today's talk on mussels

- Evaluate methods
  - Viability of glochidia
  - Influence of exposure chamber on toxicity
- Compare relative sensitivity of mussels to copper, ammonia, or chlorine based on toxicity tests conducted with the following life stages:
  - Glochidia
  - Newly-released juveniles
  - Two-month-old juveniles
- Compare sensitivity of mussels to USEPA water quality criteria

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Toxicity tests conducted

Species	Glochidia	Newly-released juveniles	2-month-old juveniles
Oyster mussel (listed)	X	X	
Rainbow	X	2 X	X
Mucket	2 X		
Fatmucket	4 X		
Ellipse	X		
Wavy-rayed lampmussel	X	X	
Scaleshell (listed)	X	X	
Pink papershell	X		
Neosho mucket (candidate)	X	2 X	

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Test conditions	Glochidia	Newly-released juveniles	2-month-old juveniles
Test duration	6 h, 1 d, 2 d	2 d, 4 d, 10 d	2 d, 4 d, 10 d
Chemical	Copper, ammonia, chlorine		
Chamber (ml)	200 or 300	30 or 300	30 or 300
Water type	ASTM water (170 mg/L hardness, pH 8.3) at 20°C		
Water renewal	Static renewal or flow through		
Age	<2 h	4 to 6 d	>2 month
Organisms/replicate*	~1000	5	5
Feeding	None		
Endpoint	Shell closure w/NaCl	Survival (foot or shell movement)	
Acceptability (%)	>90		

*glochidia collected from about 3 to 9 females

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Isolation of glochidia from a female mussel using a syringe

Monitoring a test

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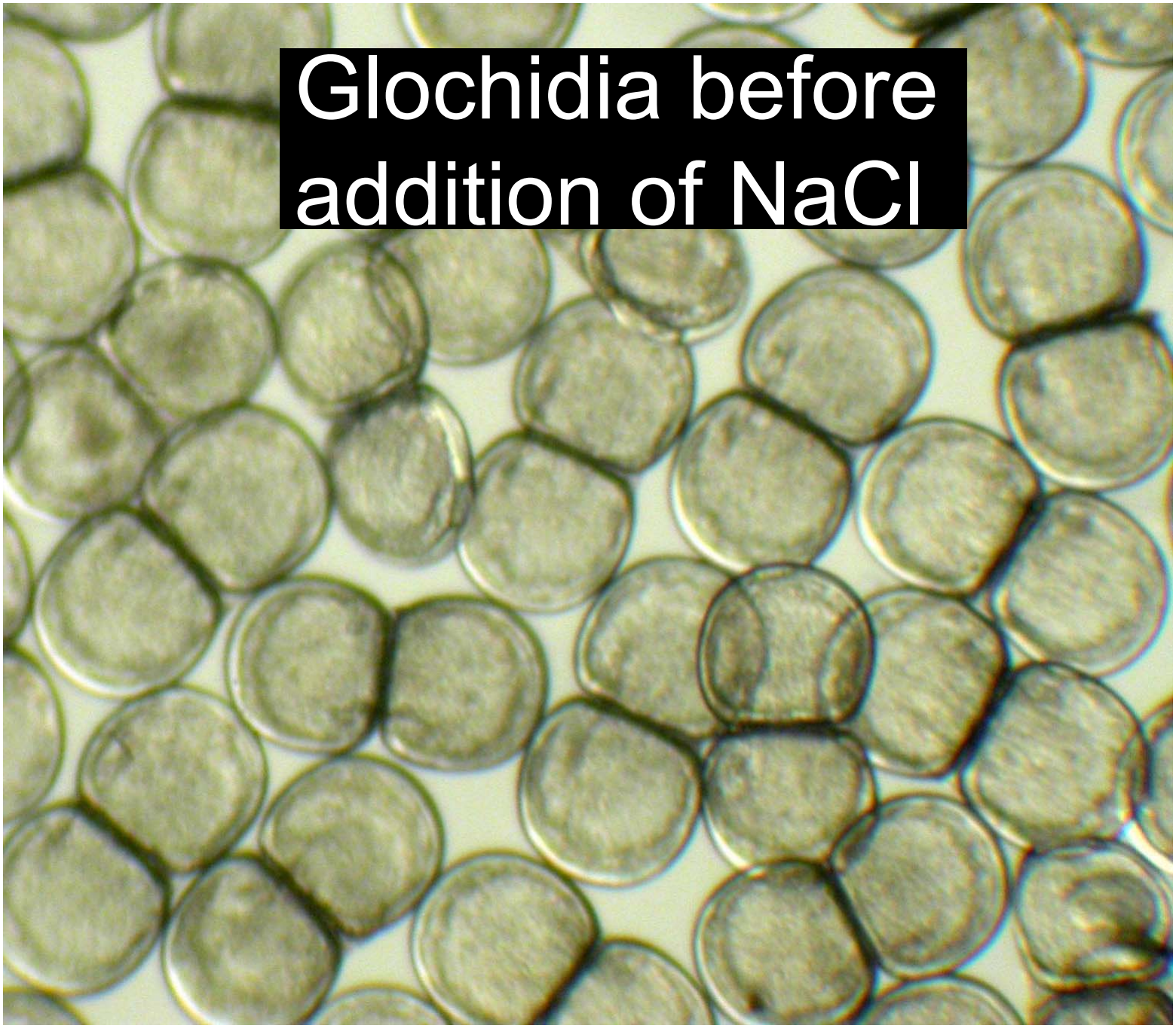
Polystyrene tissue-culture plates and glass chambers used in static exposures

Renewal of water in chlorine exposures



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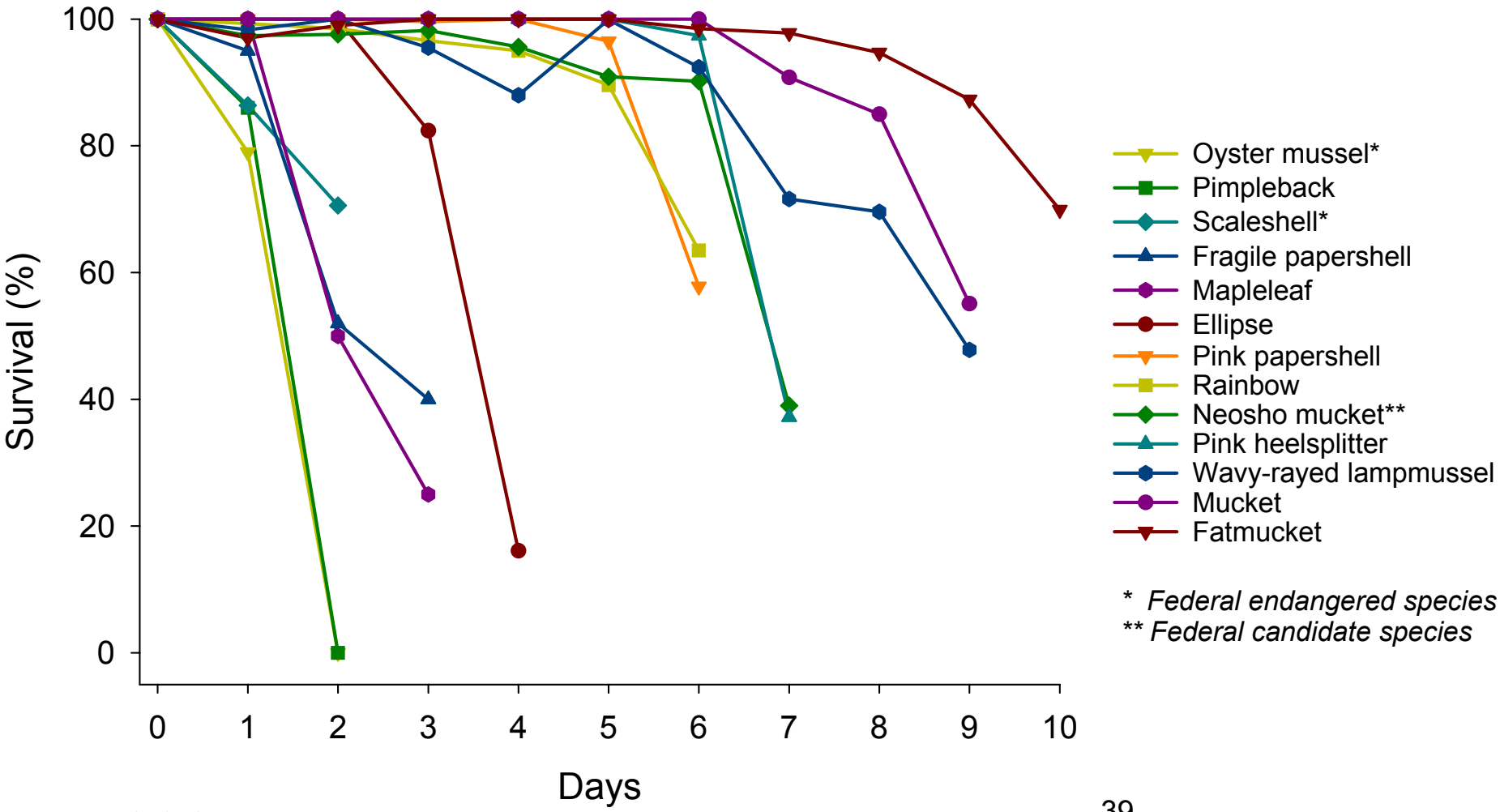
Addition of NaCl to glochidia

Foot movement of newly-released juvenile mussel

Glochidia: Survival time after removal from female mussels



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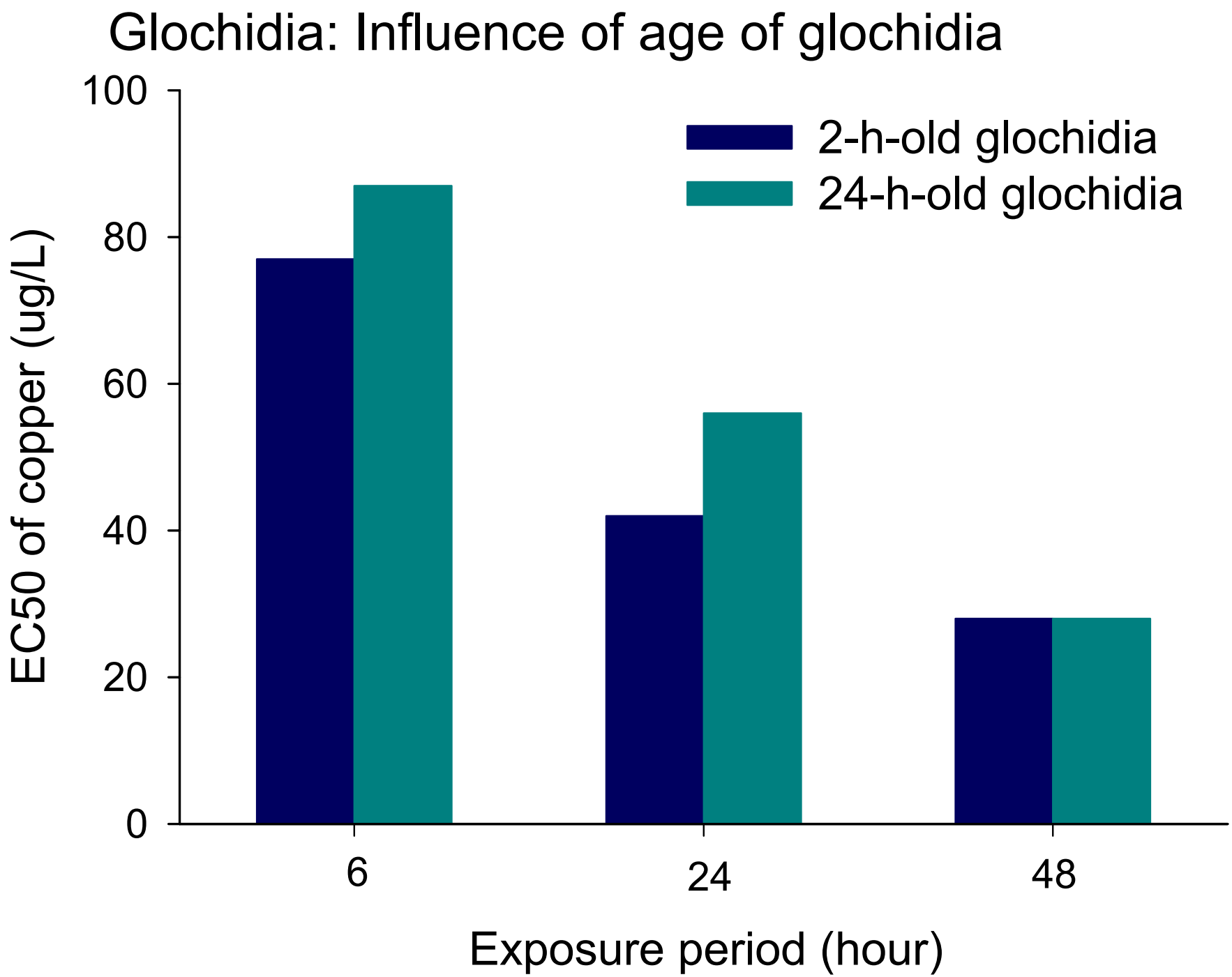
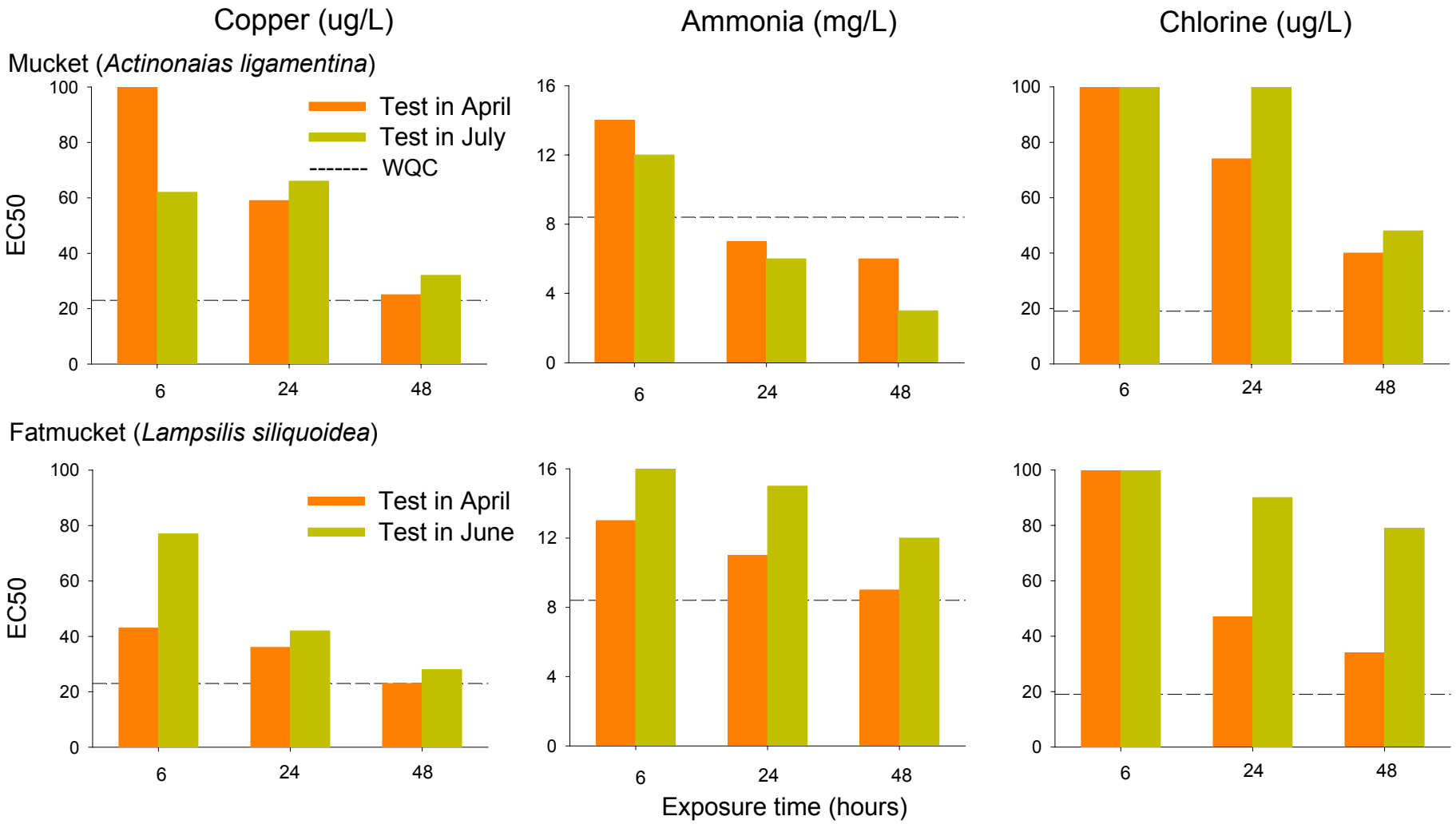
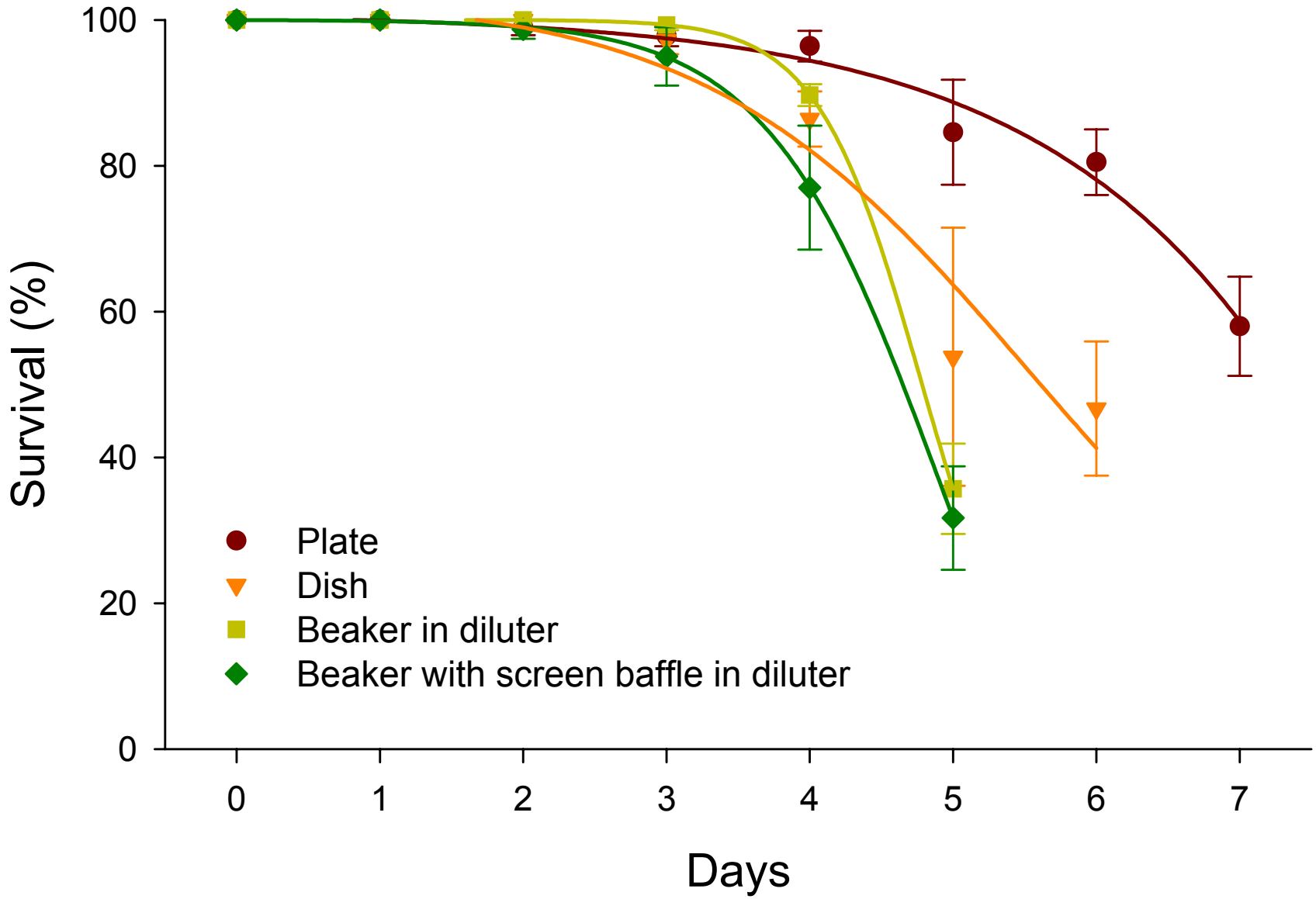

Glochidia: Influence of age of glochidia
2-h-old glochidia
24-h-old glochidia
EC50 of copper (ug/L)
100
80
60
40
20
0
6
24
48
Exposure period (hour)

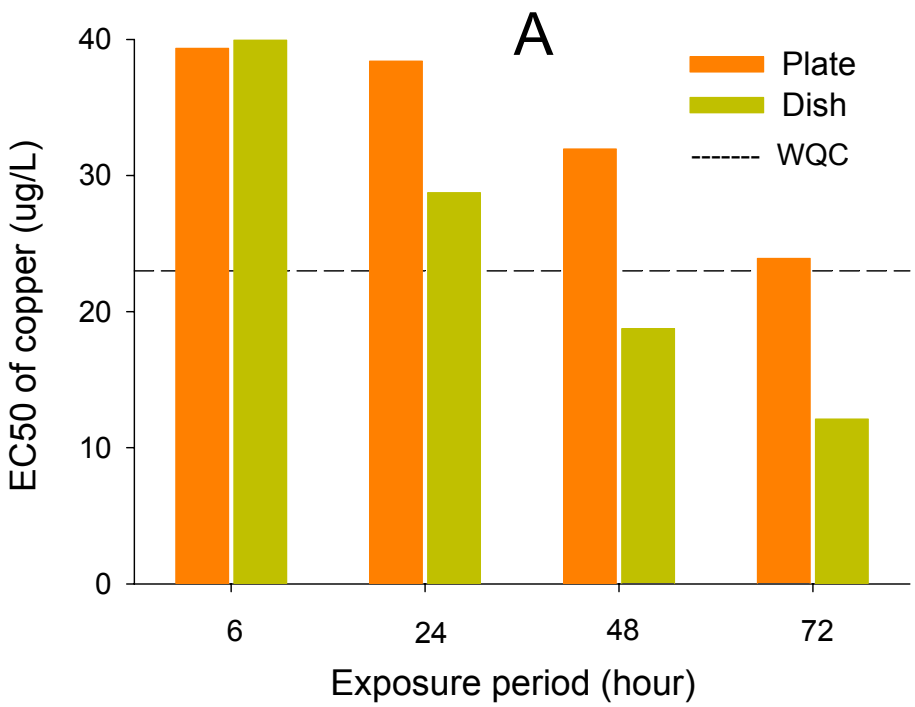
Glochidia: Repeated sampling of the same females

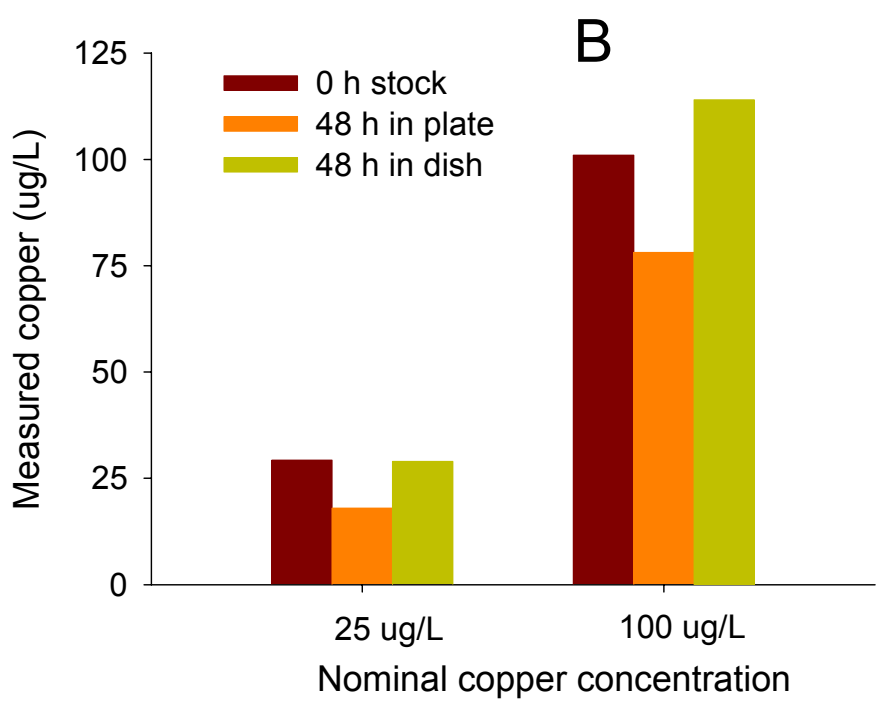


Glochidia: Influence of chamber type on survival of fatmucket glochidia



Glochidia: Influence of type of exposure chamber on toxicity





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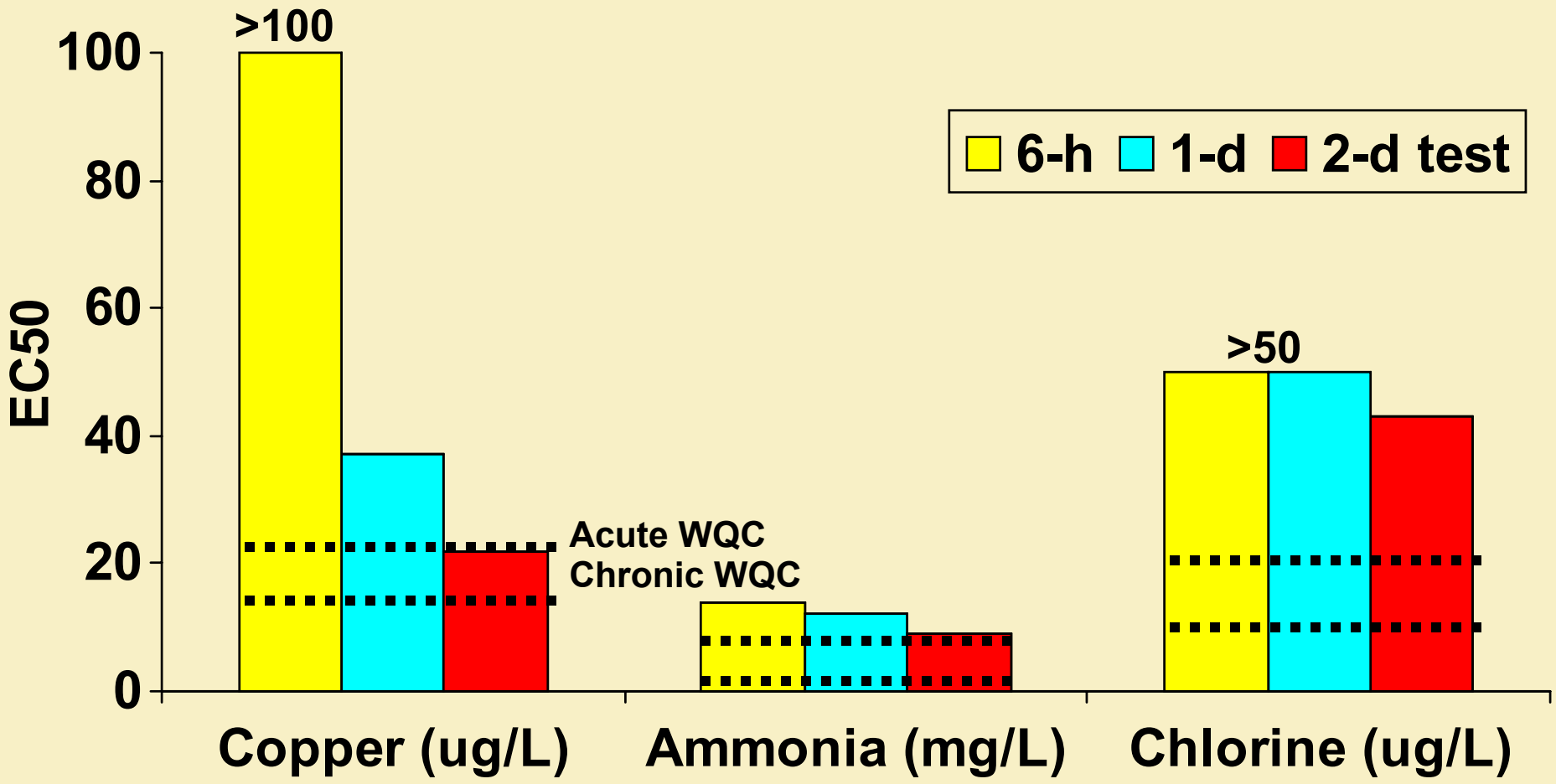
Glochidia: Rainbow



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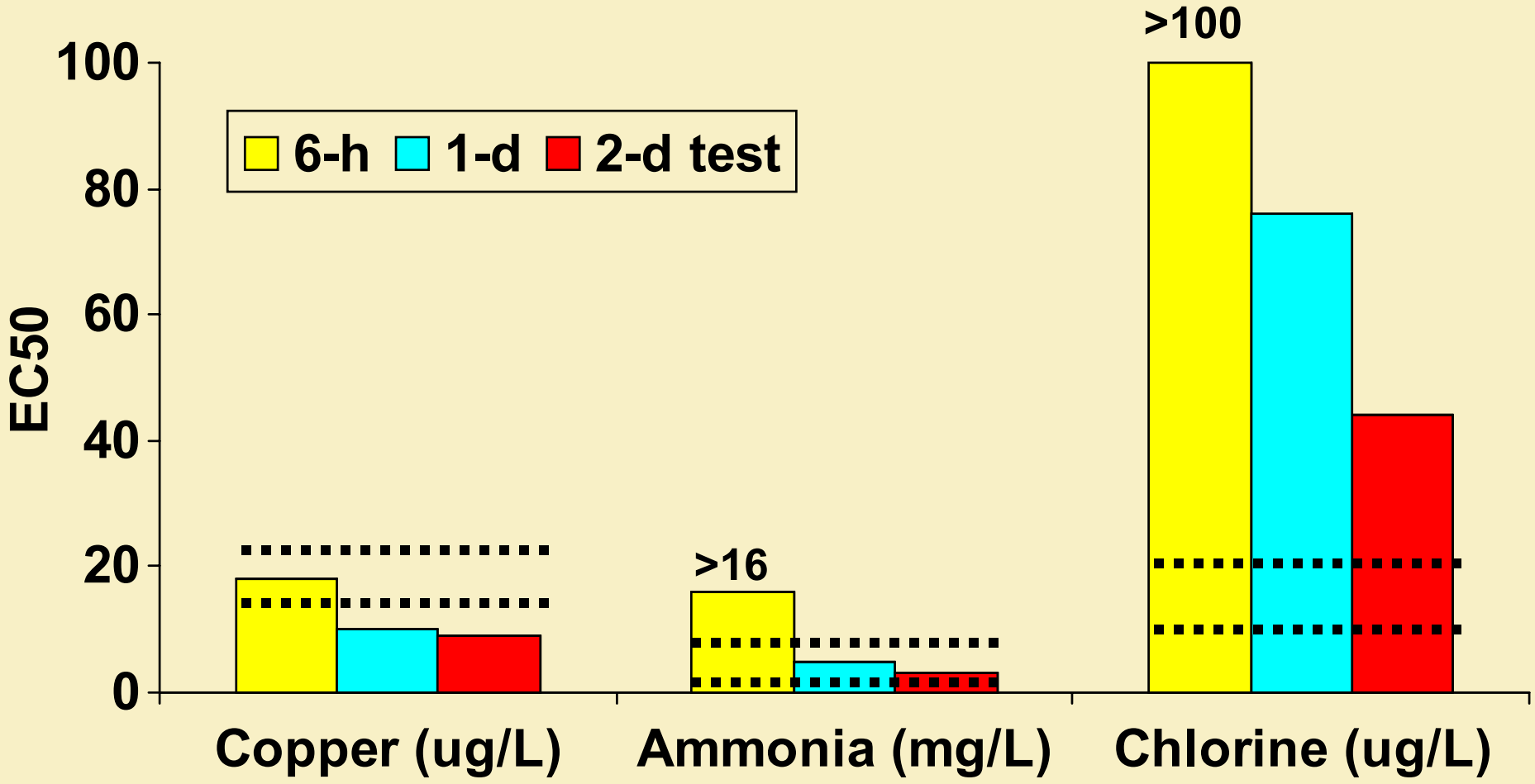
Glochidia: Ellipse



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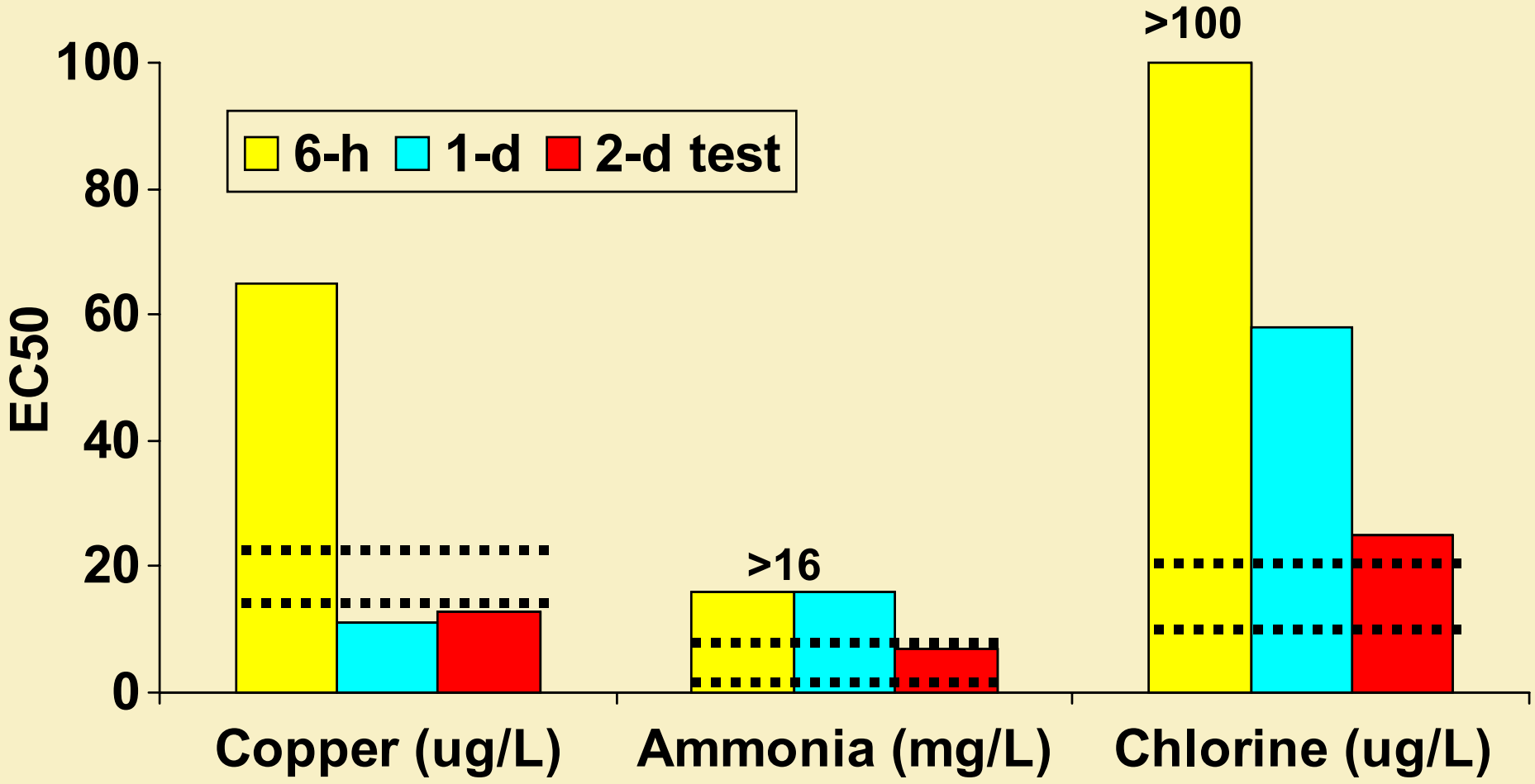
Glochidia: Pink papershell



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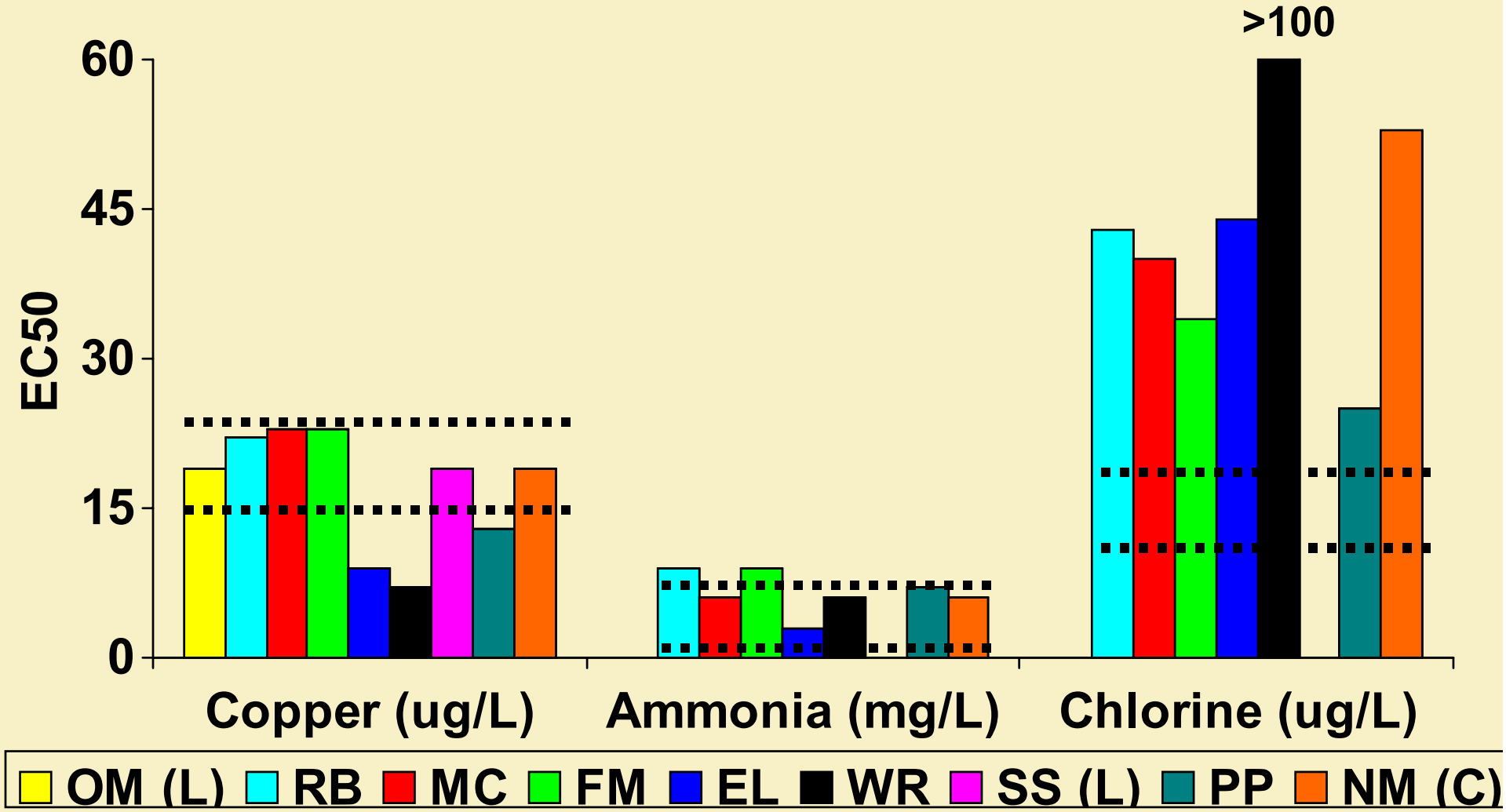
Glochidia: 2-d tests



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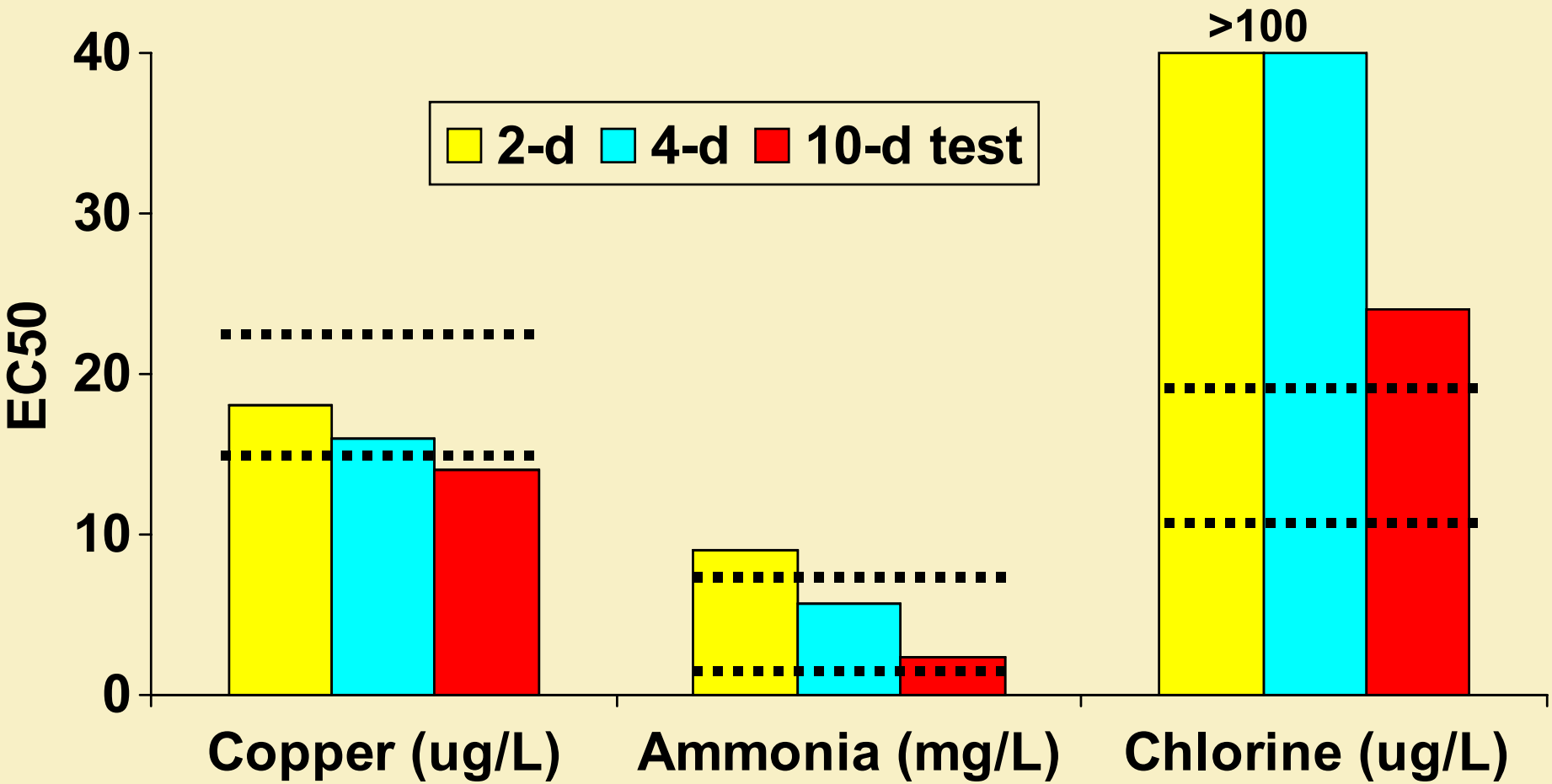
Newly-released juveniles: Oyster mussel



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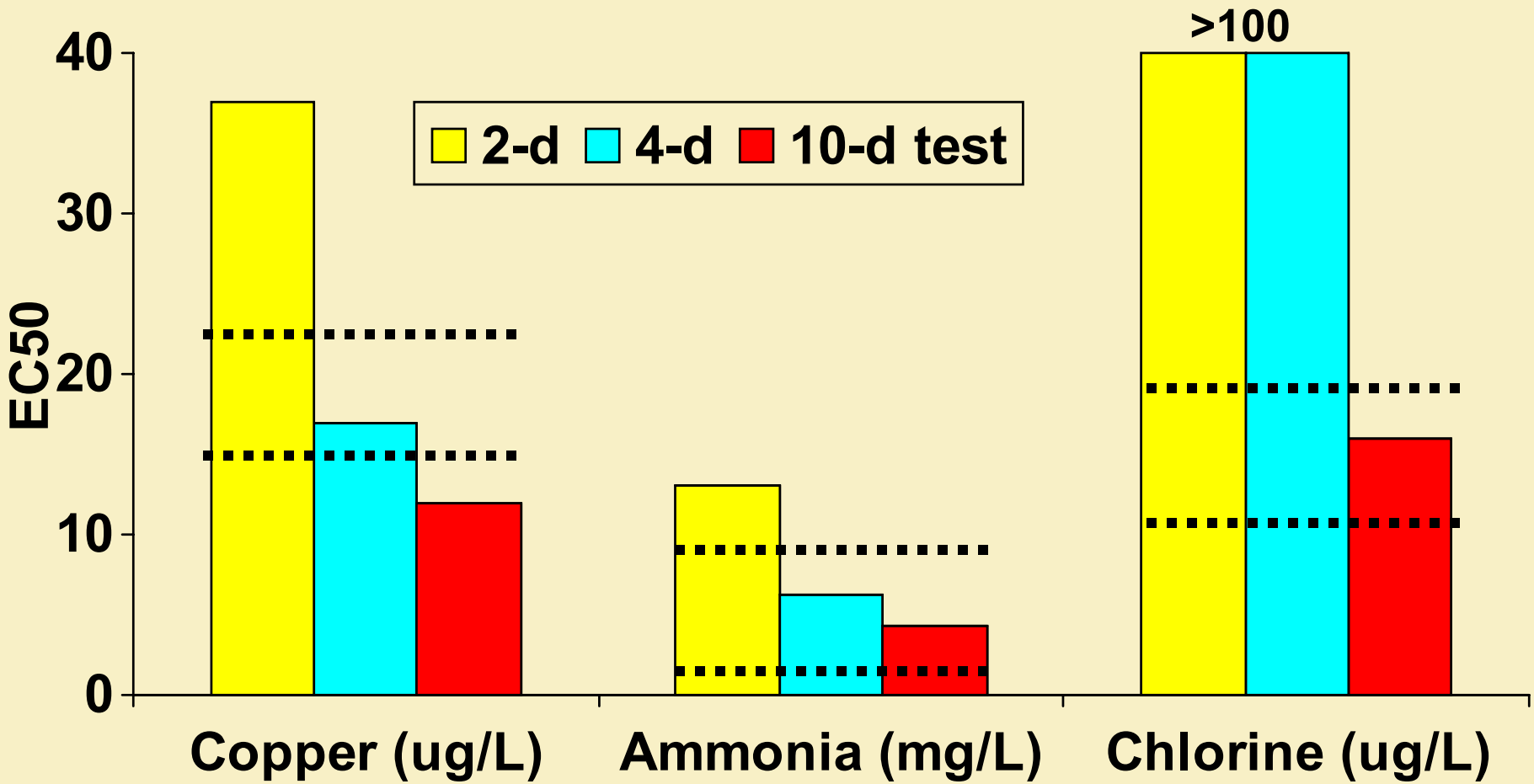
Newly-released juveniles: Rainbow



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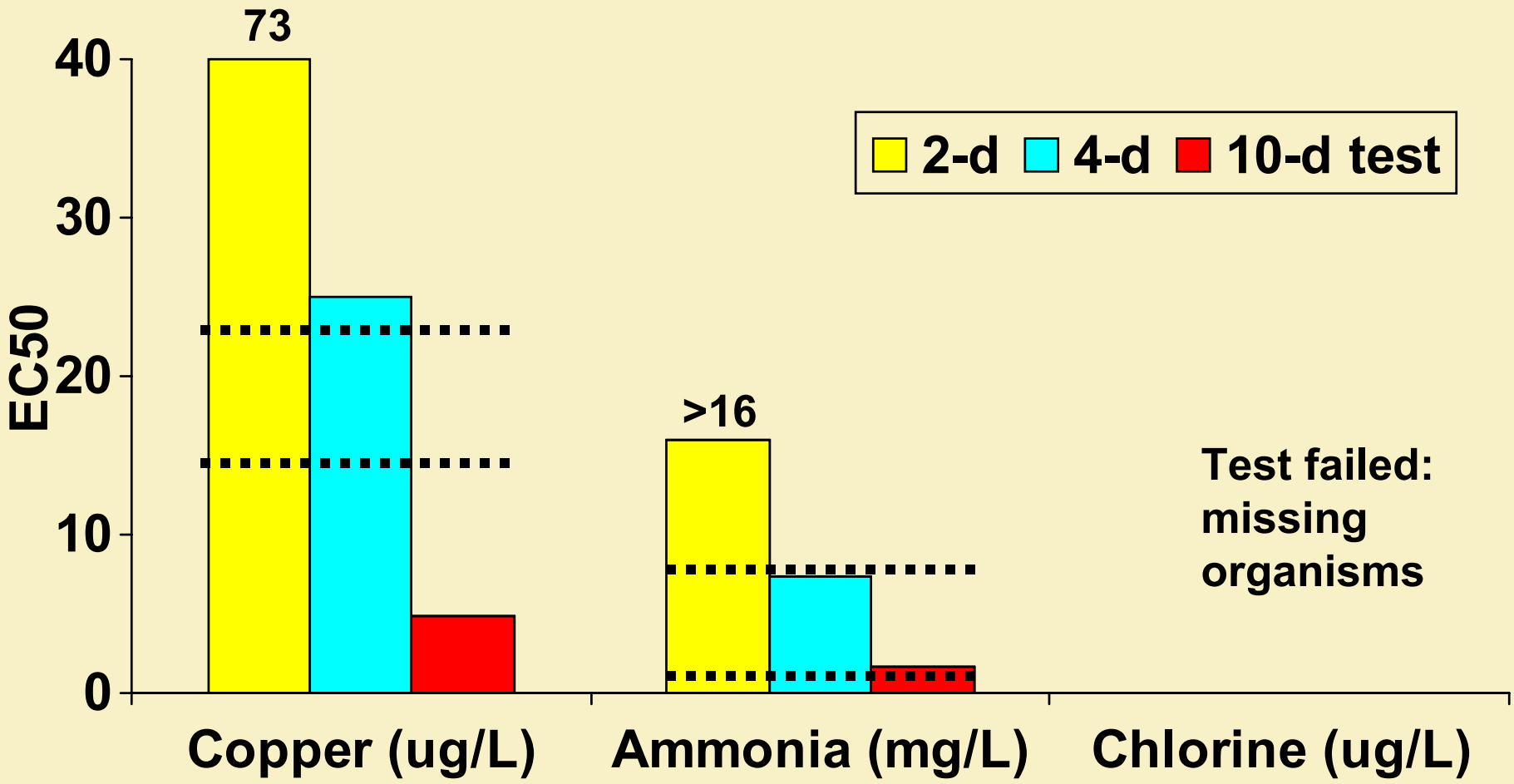
Newly-released juveniles: Wavy-rayed lampmussel



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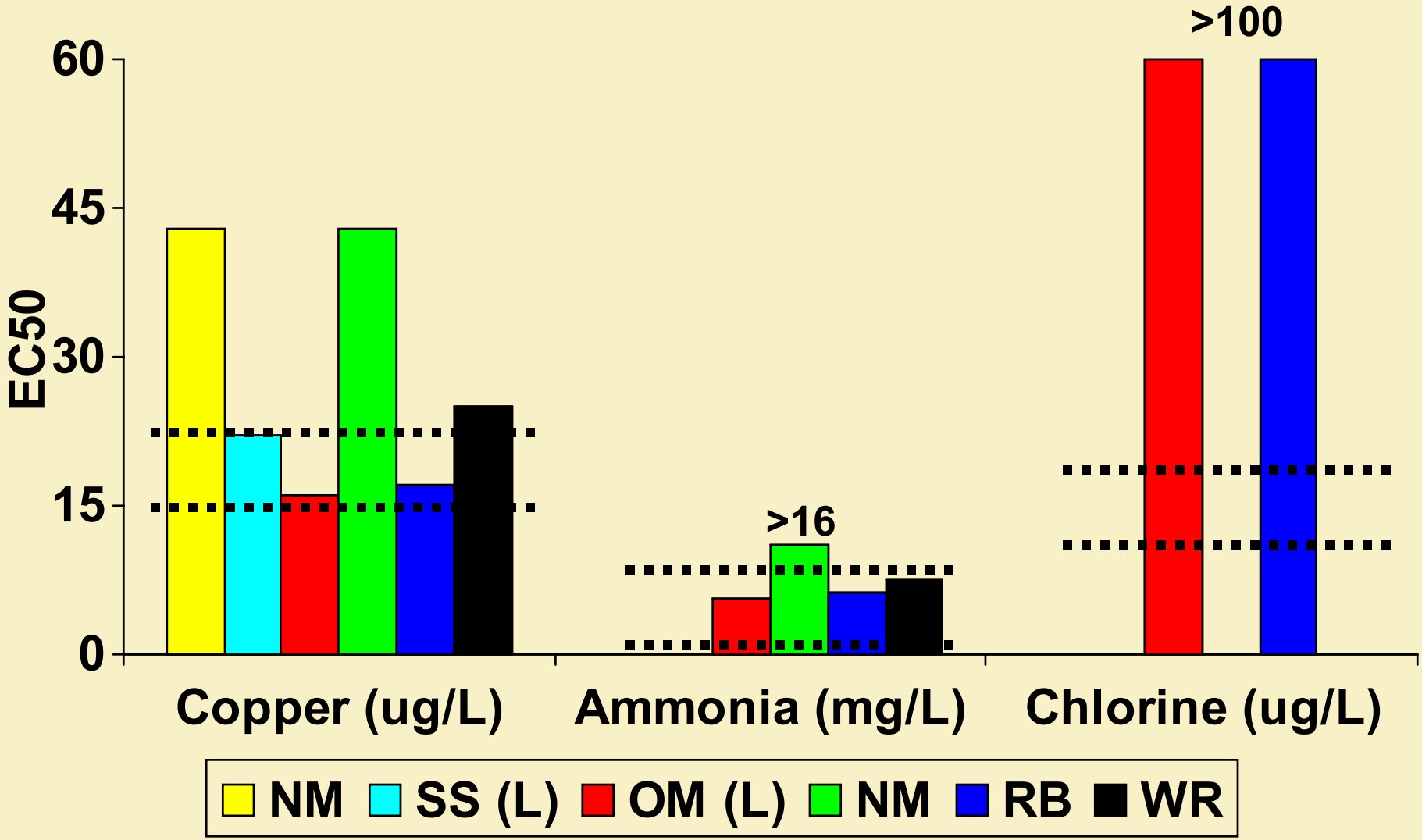
Newly-released juveniles: 4-d tests



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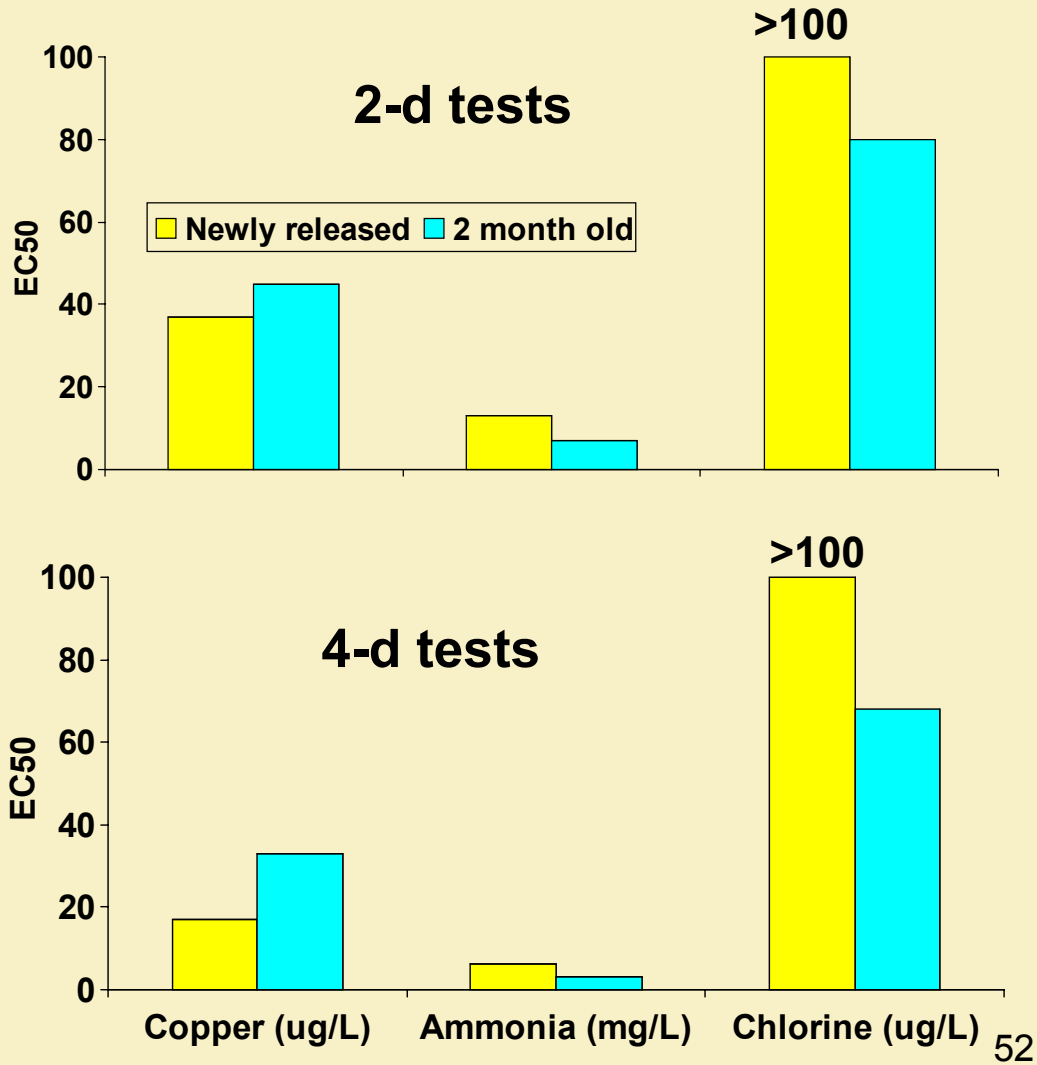
Newly-released vs. 2-month juveniles: Rainbow 2- or 4-d tests



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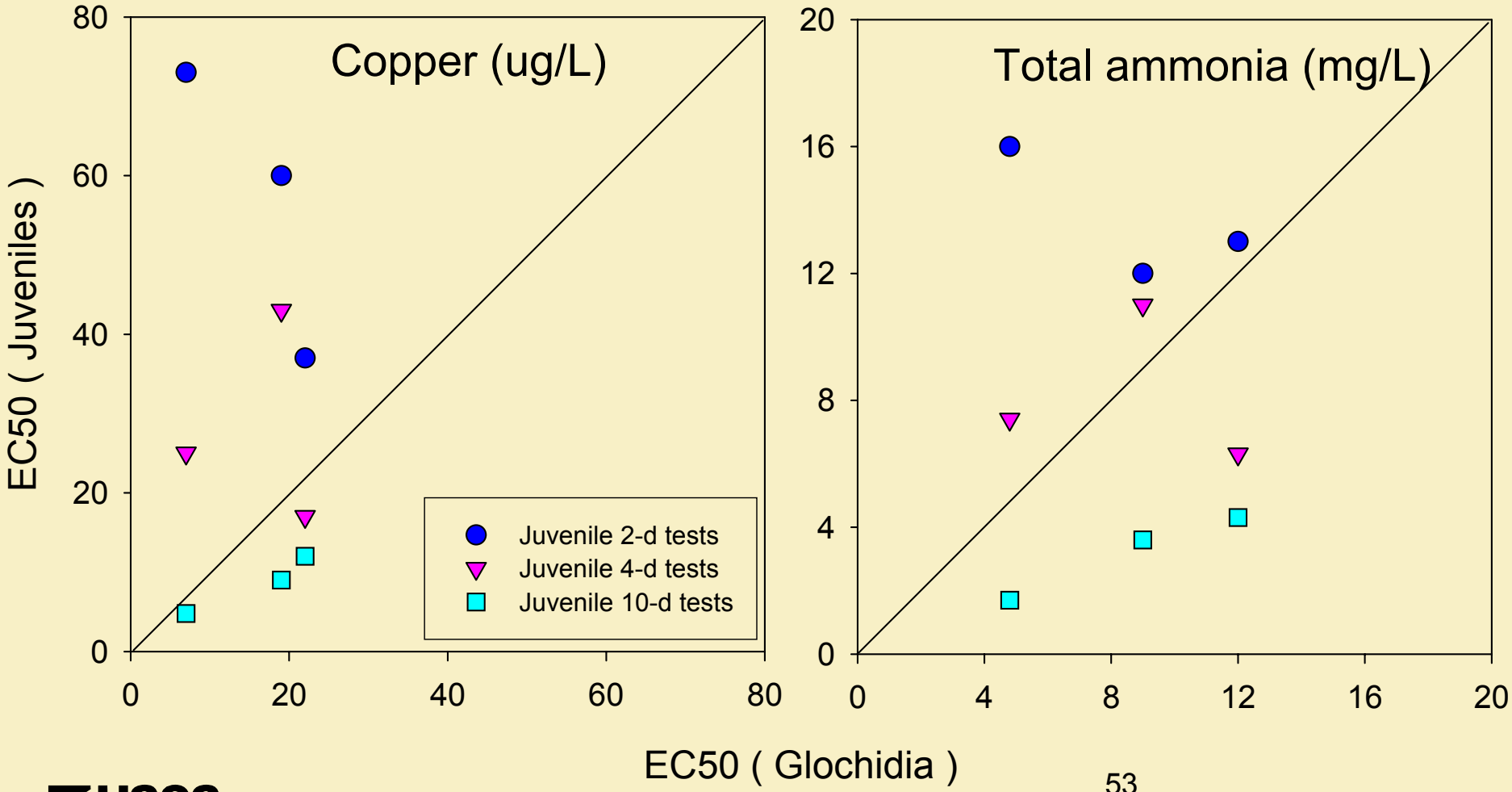
EC50s for glochidia (2-d tests) vs. newly-released juveniles (2-, 4-, or 10-d tests)



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Conclusions for mussel studies

- Toxicity of copper, ammonia, and chlorine increases with increasing exposure time.
- 2 days is a reasonable duration for toxicity tests with glochidia, although shorter exposures may be needed based on the life history and survival time of glochidia.
- EC50s for 2-d glochidia tests are lower than EC50s for 2-d juvenile tests and are higher than EC50s for 4- to 10-d juvenile tests (e.g., difficult to predict toxicity observed with juveniles with results of glochidia tests).

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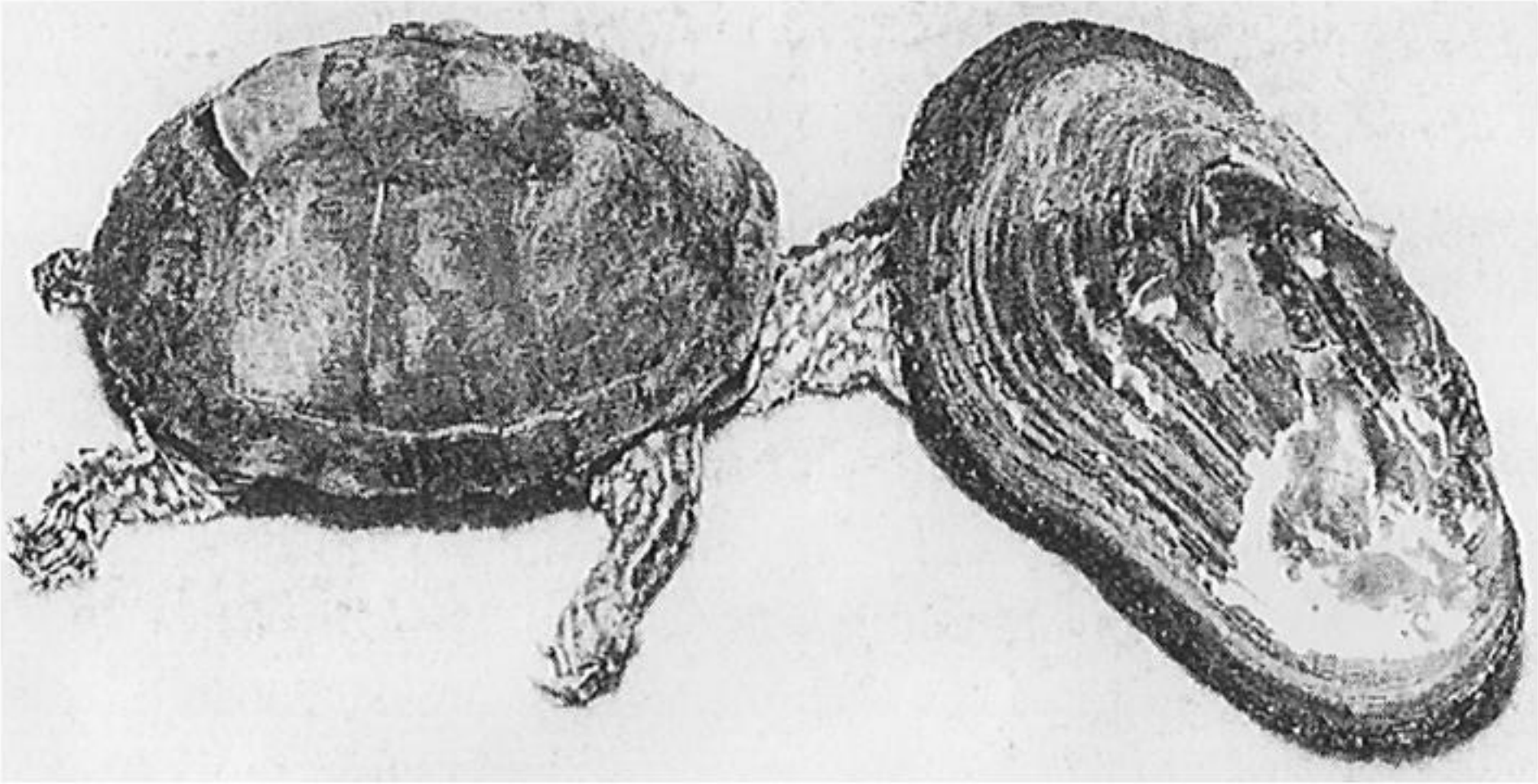
Conclusions for mussels (cont.)

- 2-month-old juvenile rainbows exhibit similar sensitivity to newly-released rainbow juveniles.
- EC50s for copper and ammonia frequently at or below acute EPA water quality criteria for glochidia or juveniles.
- Protecting most sensitive mussels would be protective of listed mussels (scaleshell and oyster mussel)
- Future studies: Long-term toxicity testing with juveniles (e.g., 28-d tests).

USGS

Questions?