Salmon and Trout and Pesticides – Oh My!!

"Impacts of Pesticides on the Biological Requirements of Salmonids"

Photo by Andrew Hendry, NOAA

Jay Davis, U.S. Fish and Wildlife Service

ECS 3119 – Pesticides and Fish & Wildlife Resources

NCTC – Shepherdstown, WV

September 13 – 17, 2010

Pesticides enter salmon picture

...ins impair ability of fish to smell

The suit was filed by the Washington Toxics Coalition, Northwest Coalition for Alterna-tives to Pesticides, Pacific C... Fisherm...

...ated the dis-

agency — in this case the EPA must consult with the fisheries service — to make sure their actions do not harm endangered spe...

"We see that ...

pesticides on salmon. Scholz has recently conducted experiments that show an additive effect when salmon brains are exposed to a mix of insecticides. He has also begun studies looking directly at the impact of pesticides on brain function. The question that needs to be answered, said Scholz, is "what's the link between the real world pesticide levels and the biology of the animal."

In the meantime, the lawsuit will begin winding its way through the courts. Patti Gold-...an, the attorney for the groups who filed the ...it, said she expects it to be several months ...efore there is a ruling.

Yesterday's appointment of Christie Whit-...an as EPA administrator could also affect the ...

Rivers: Scientists find 50 pesticides

Continued from Page One

West Coast basins are threatened with extinction.

The potential listings of the ... under the Endangered S... could bring ...

...ed in the bottom ... the rivers ...

Pesticide promoters endanger Northwest salmon

By ERIKA SCHREDER

The Seattle Post-Intelligencer has done a tremendous public service with its reporting on how pesticides are winding up in Puget Sound streams and threatening salmon runs. ...May 20 opinion piece written by ...the pro-pesticide ...

...creasing number of studies hav... contrary to Hansen's assertions kill salmon directly and may factor in their decline.

Our region has already se... kills due to pesticides. In 19... used in an irrigation canal n... Bear Creek of the Rogue Ri... off thousands of steelhead t... ...almon. When salr...

...esticides are likely to be ...ed supply,

In our state, we can and should make up for EPA's lack of leadership. With the recent listings under the Endangered Species Act, we can no longer ignore the role of pesticides in the diminishing returns of salmon.

...Here's what we need to do: ...should use alternatives ...den care.

Skagit Valley Herald / www.skagitvalleyherald.com

NORTHWEST

Study explores pesticides and salmon

...ble ...ell. ...as ...iter

streams when washed away by rain. Levels of pesticide used in the tests were comparable to levels that the U.S. Geological Survey has routinely found in Western streams, Stein said.

The Seattle-based researchers focused on salmon's antipredatory behavior, but the evidence also suggests pesticides could affect other sur-... Stein said. The fish rely on their ... to find their way

Pesticides wrongly blamed for salmon decline

By HEATHER HANSEN

A recent Post-Intelligencer editorial described pesticides as inherently dangerous to salmon. In March, the City of Seattle ran newspaper ads asking the public to help save salmon by avoiding t... weed killers and pesticides in the...

Whoa, stop the train!

While it is true that some pesti... levels well beyond safety standard... the Environmental Protection Ager... be harmful to salmon, others are reg... for controlling diseases on salmon in hatcheries.

Credible scientists assure me levels of pesticides currently being d... ...ed in regional streams and rivers ...

health and environmental tests, at least 20 of those involving fish, wildlife and ecosystem impacts.

All these tests can ... and cost $20...

More pesticides mean more stream pollution

It shouldn't be surprising that there's a connection between the seasonal sale of pesticides in stores and the annual rise in pesticide contamination in Puget Sound streams.

...rprising is that it's taken ...y officials this ...

The pesticide study by the U.S. Geological Survey, the state Department of Ecology and King County showed concentrations of pesticides rise to lethal levels for aquatic life.

In all, 23 pesticides were detected in 12 streams in King and south Snohomish counties. Five of the pesticides were found at levels above ...concentrations that are safe for ...isms.

...led containe...

Don't s... wash pest... streams. F... pesticides where th... rains.

And ...rden. ...rs, o... face...

...in which can

EPA must act on pesticide findings

British and U.S. government scientists have found evidence that low levels of pesticides may impair the salmon's se...

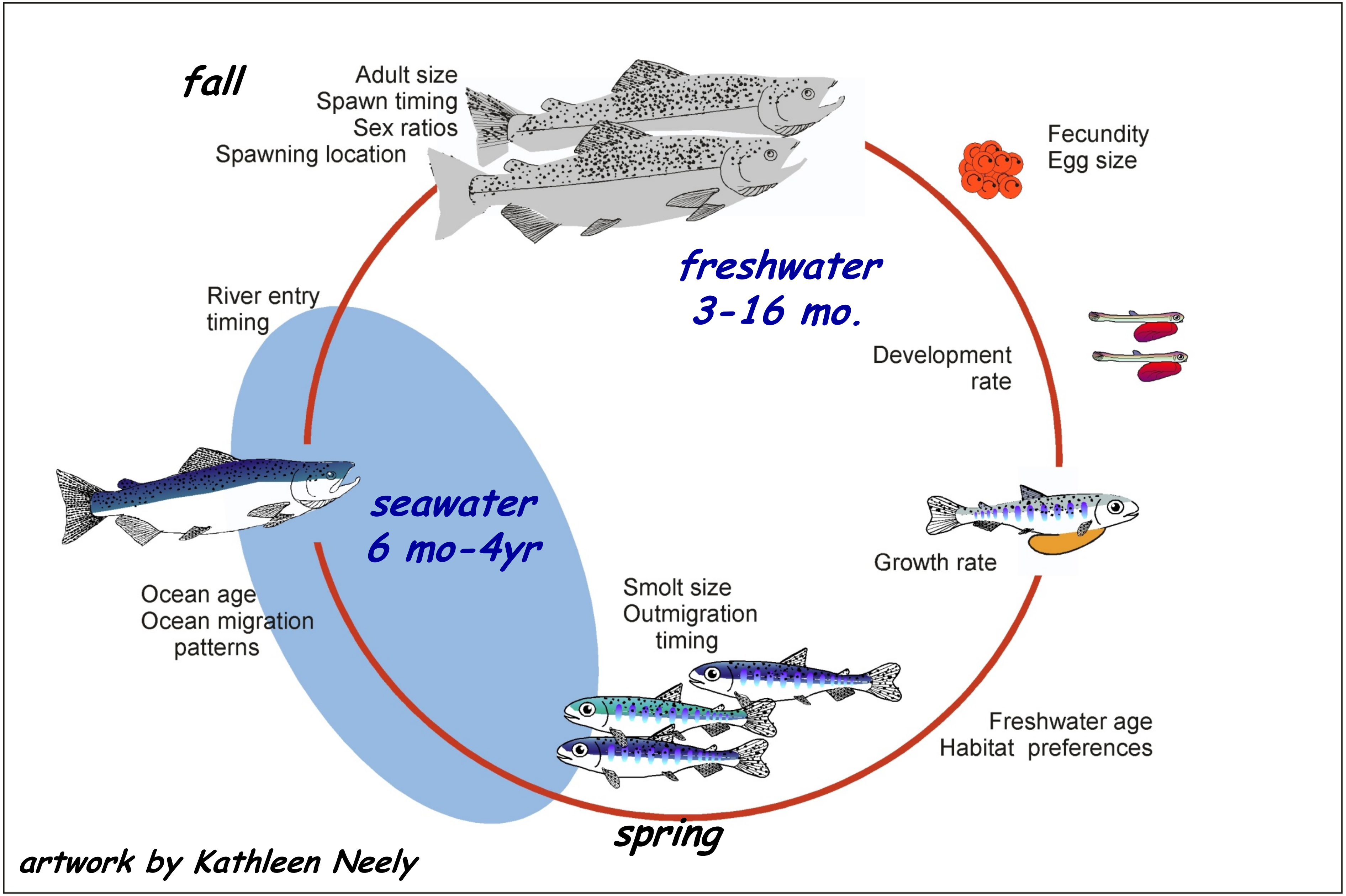
Research suggesting that salmon may be deprived of their sense of smell by a common insecticide has worrisome potential to torpedo efforts to restore the runs.

This region is spending billions of dollars on salmon restoration. That's why it's important that the Environmental Protection Agency quickl... findings of the National ... scienti...

Another Important Consideration: Sublethal Effects

- Decreased Fertility
- Altered Growth
- Deformities
- Slowed or Less Effective Movement
- Altered Behavior
- Effects on Organ, Tissue, Cell Hormone, or Enzyme Functions
- Genetic Defects

Pacific Salmon Life Cycle



Salmon-centric toxicological endpoints of potential concern

Assessment Endpoints	Assessment Measures
Juvenile growth	Foraging behavior
	Growth rate
	Condition index
Reproduction	Courtship behavior
	Number of eggs produced
	Fertilization success
Early development	Gastrulation
	Organogenesis
	Hatching success
Smoltification	Anion exchange (i.e. gill Na^+/K^+ ATPase activity)
	Blood hormone (i.e. thyroxin)
	Salinity tolerance
Disease-induced mortality	Immunocompetence
	Pathogen prevalence in tissues
	Histopathology
Predation-induced mortality	Predator detection
	Shelter use
	Schooling or shoaling behavior
Migration or distribution	Use of juvenile rearing habitats
	Adult homing behavior
	Selection of spawning sites

3 CASE STUDIES

Act 1 - The sublethal effects of carbaryl on coastal cutthroat trout

Act 2 - The sensory physiology and behavior of juvenile coho exposed to pesticides

Act 3 – Pesticide mixtures and the environmental relevance to fish

Pesticide Gets on Fishes Nerves!!!!!!!

U.S. Fish & Wildlife Service – Western Washington
Fish & Wildlife Office, Lacey WA

NOAA Fisheries-National Marine Fisheries Service
Northwest Fisheries Science Center, Seattle WA

Carbamates and Organophosphates (OPs)

- **Phase out older pesticides for the next generation**

- **Inhibit AChE – i.e. disrupt neurological function.**

<u>Examples</u>

Carbaryl

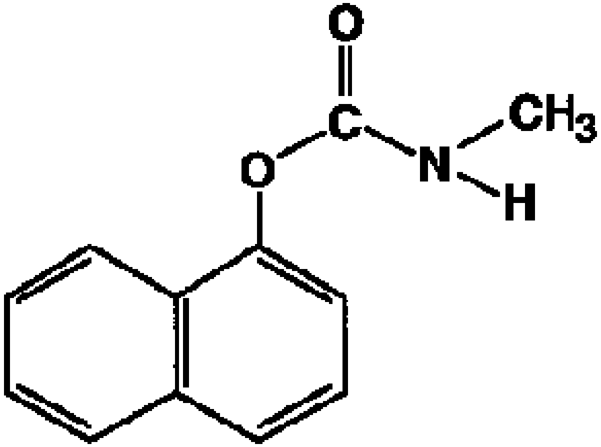
Carbofuran

Aldicarb

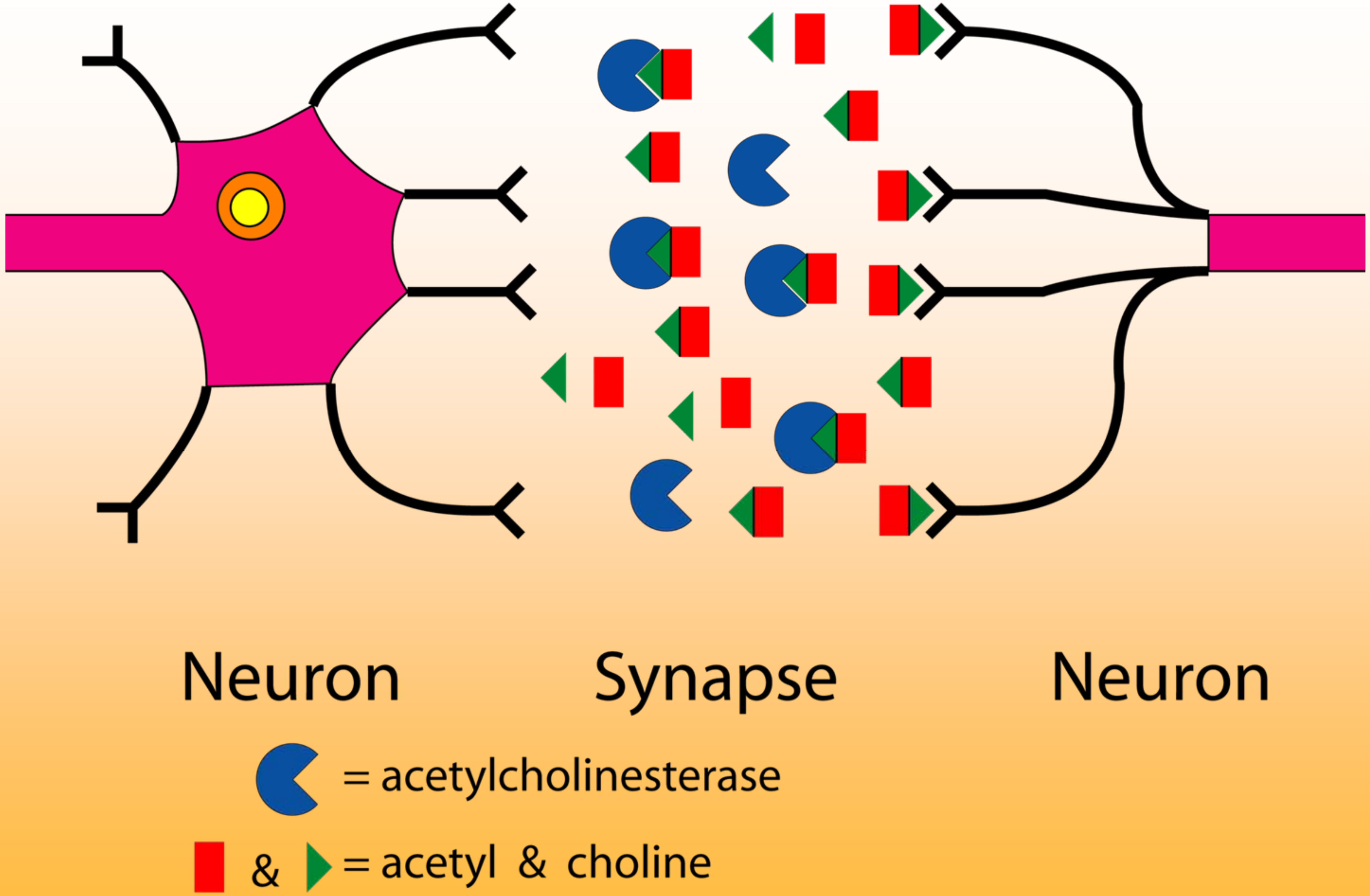
Diazinon

Azinphos-methyl

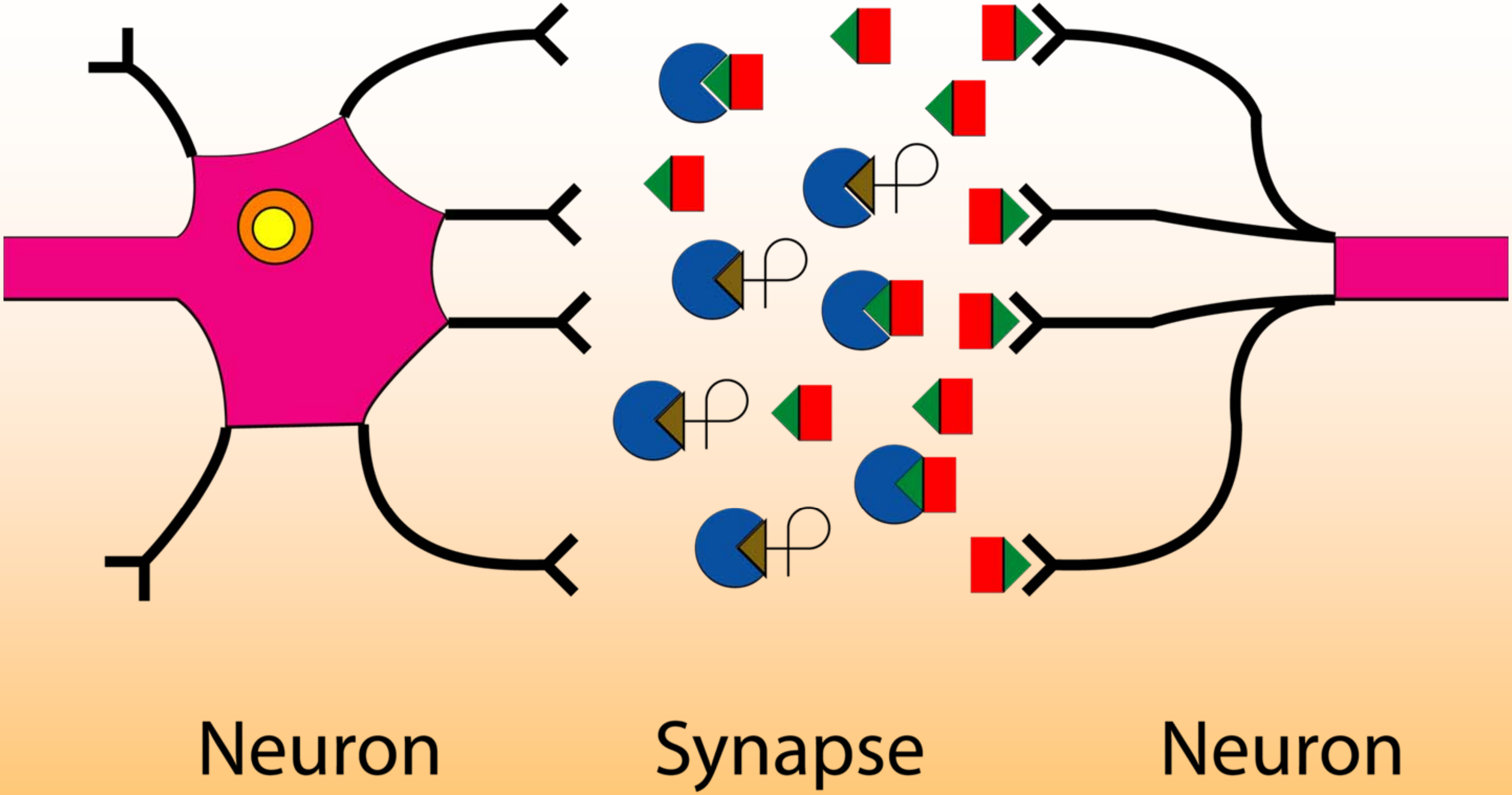
Parathion

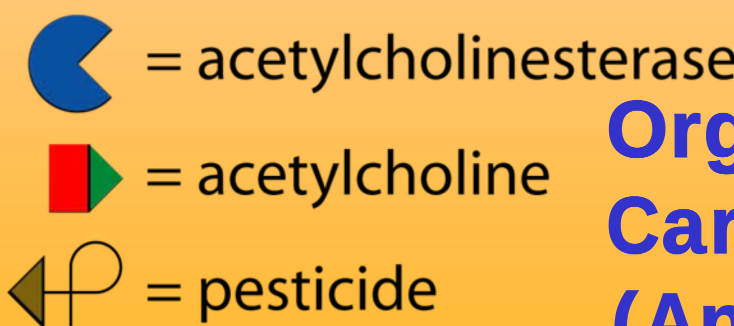


Carbaryl



Overview of the nervous system

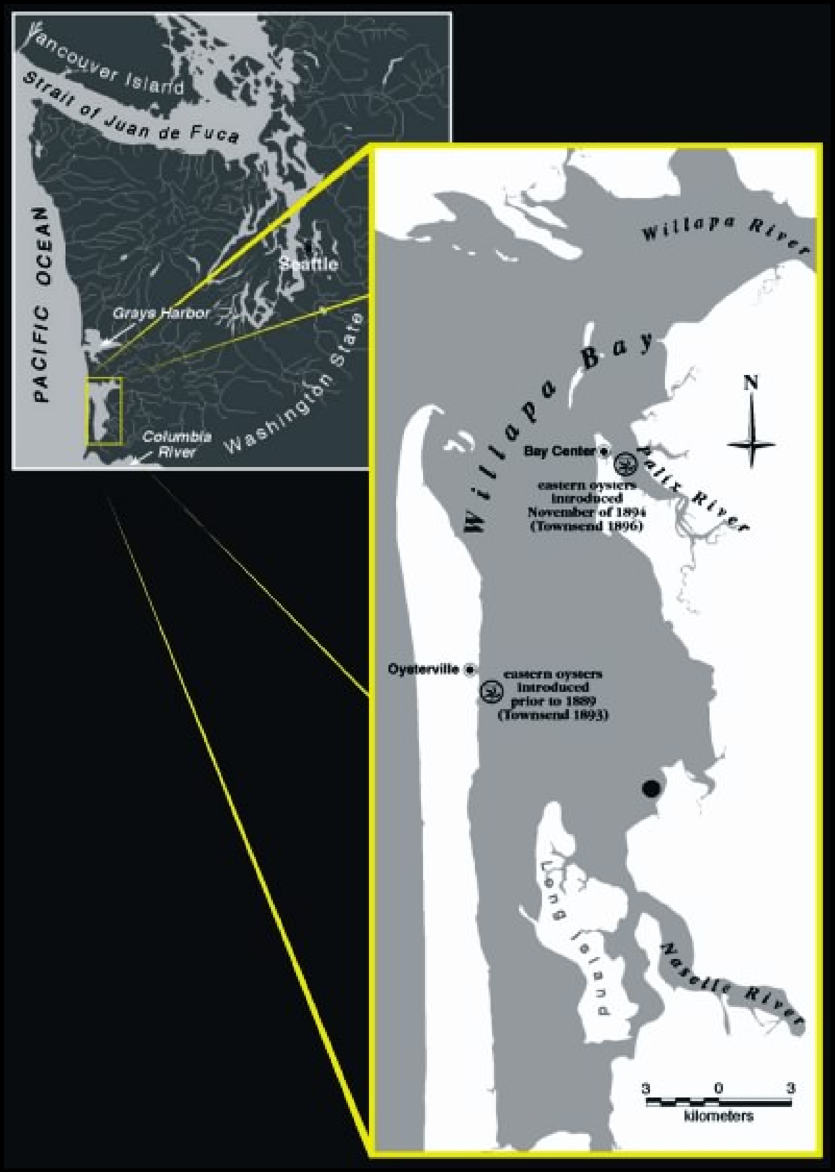




Organophosphorus and Carbamate Insecticides (Anticholinesterases)

Wild Coastal Cutthroat Trout (CCT)

Carbaryl applications to tidelands in Willapa Bay and Grays Harbor, Washington



- seasonal applications to control burrowing/ghost shrimp on oyster beds
- applied by hand and helicopter spray
- surface water concentrations can range from 1 to 1,000 µg/L on the day of spray (>2500 µg/L have been measured)
- Willapa Bay provides summer rearing habitat for several species of anadromous salmonids, including coastal cutthroat trout
- the sublethal effects of carbaryl on salmonids have not been investigated

EPA's Carbaryl IRED – Oct. 2004

- ***Estuarine/Marine Fish*** **– …no data with which to evaluate the chronic toxicity… These data requirements are still outstanding."**
- ***Section 24c Use to Control Burrowing Shrimp*** **– "Although concern has been raised regarding this use and its potential impact to nontarget animals outside the treated areas, very little data have been provided to substantiate these concerns. …. But potential nontarget acute and chronic effects are remote given the relatively small number of acres treated and the rapid degradation of carbaryl from biotic and abiotic factors combined with the dilution from a relatively large influx of water."**
- ***Settlement Agreement*** **– a phase-out (by 2012) of the use of carbaryl on oyster beds in Willapa Bay & Grays Harbor while alternatives are sought.**

Relevant Federal Statutes

Chemical-centric:

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Clean Water Act (CWA)

Species-centric:

Endangered Species Act (ESA)

Magnuson-Stevens Act (Essential Fish Habitat; EFH)

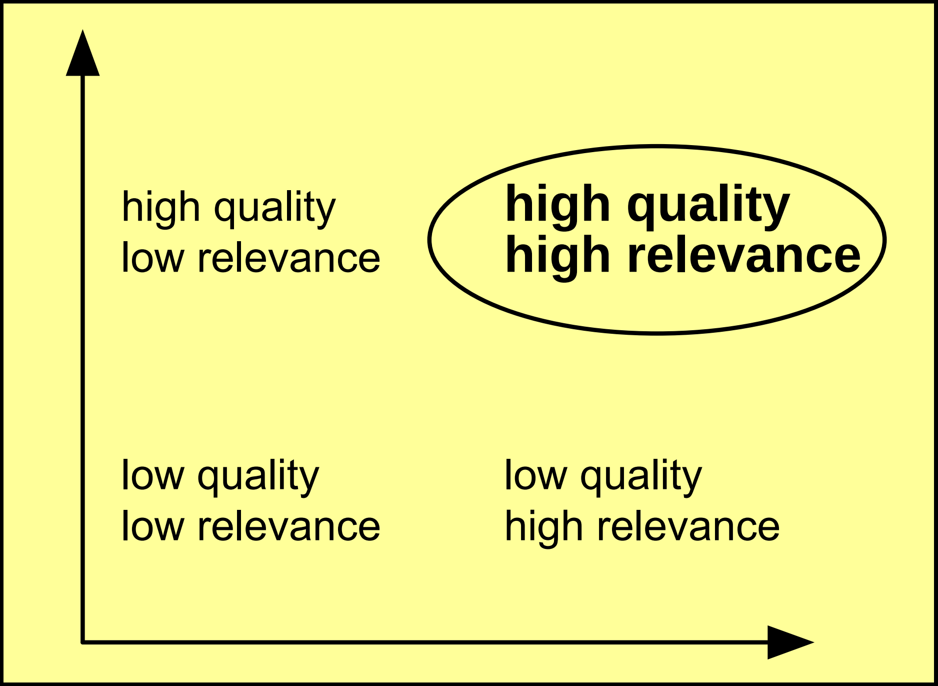
Key considerations under Endangered Species Act

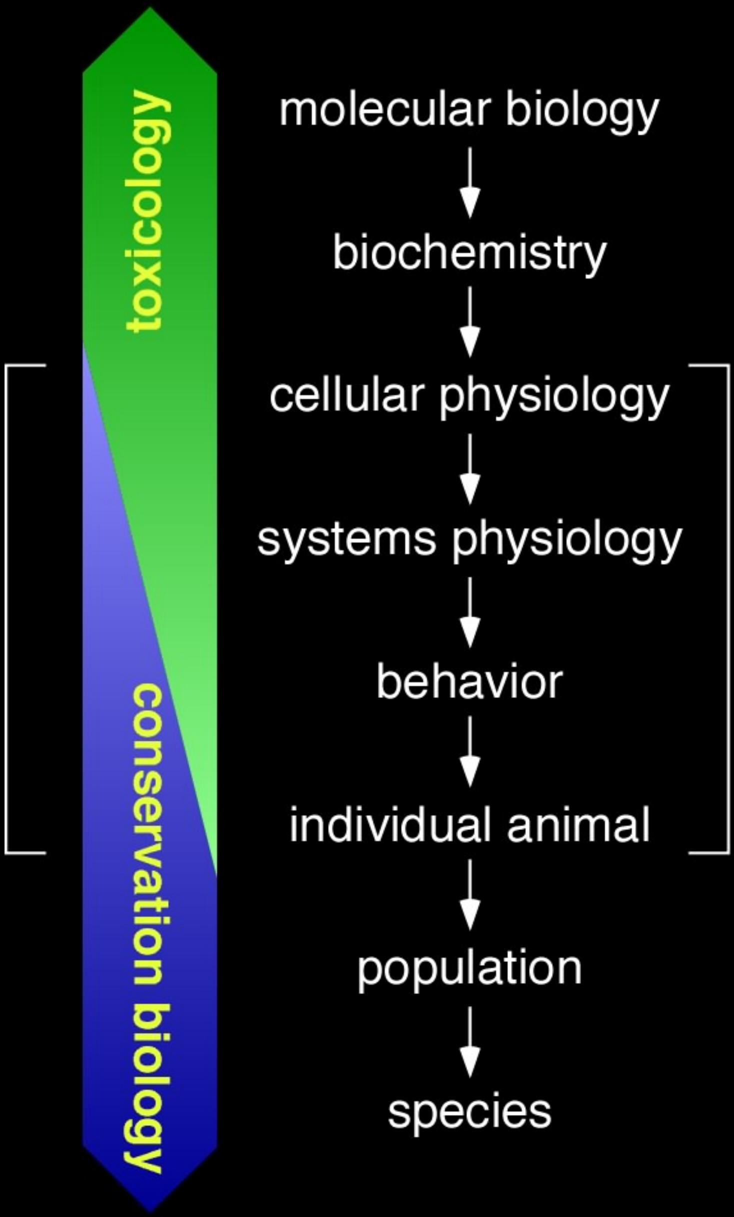
(**Species**-centric)

- **Impacts on salmonid Habitat**
- **Impacts on salmonid Health**

Impacts on salmonid **Health**

Assessment endpoints specific to salmonid health





Cutthroat Trout from WDFW’s Eells Springs Hatchery

Field Station

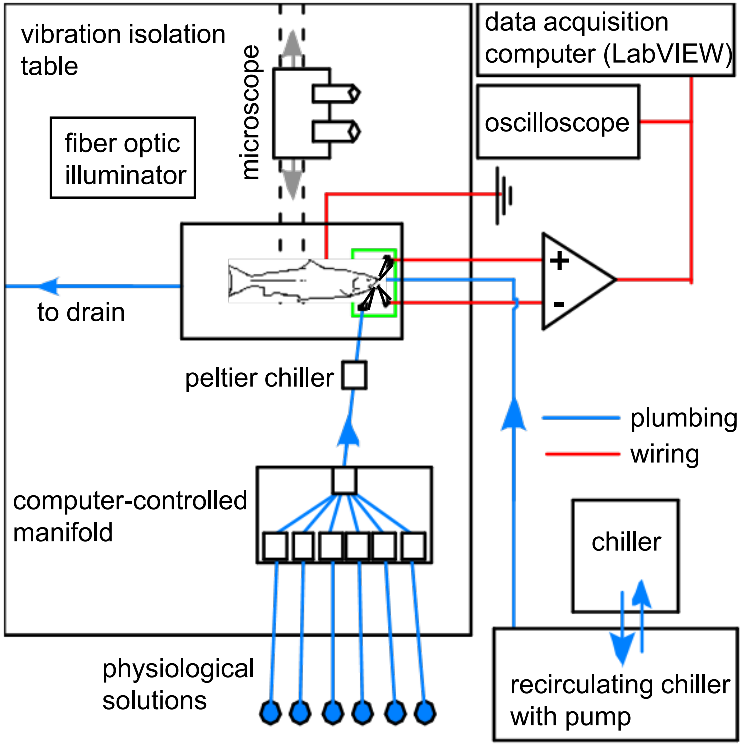
Year 1 Objectives

1. Can salmonids smell carbaryl (do smolts have an olfactory response to carbaryl)?
2. Do salmonids avoid carbaryl-contaminated seawater?
3. What are the effects of short-term exposures on acetylcholinesterase activity in brain and muscle?
4. What is the time course for carbaryl's effects and how long does it take the fish to recover?

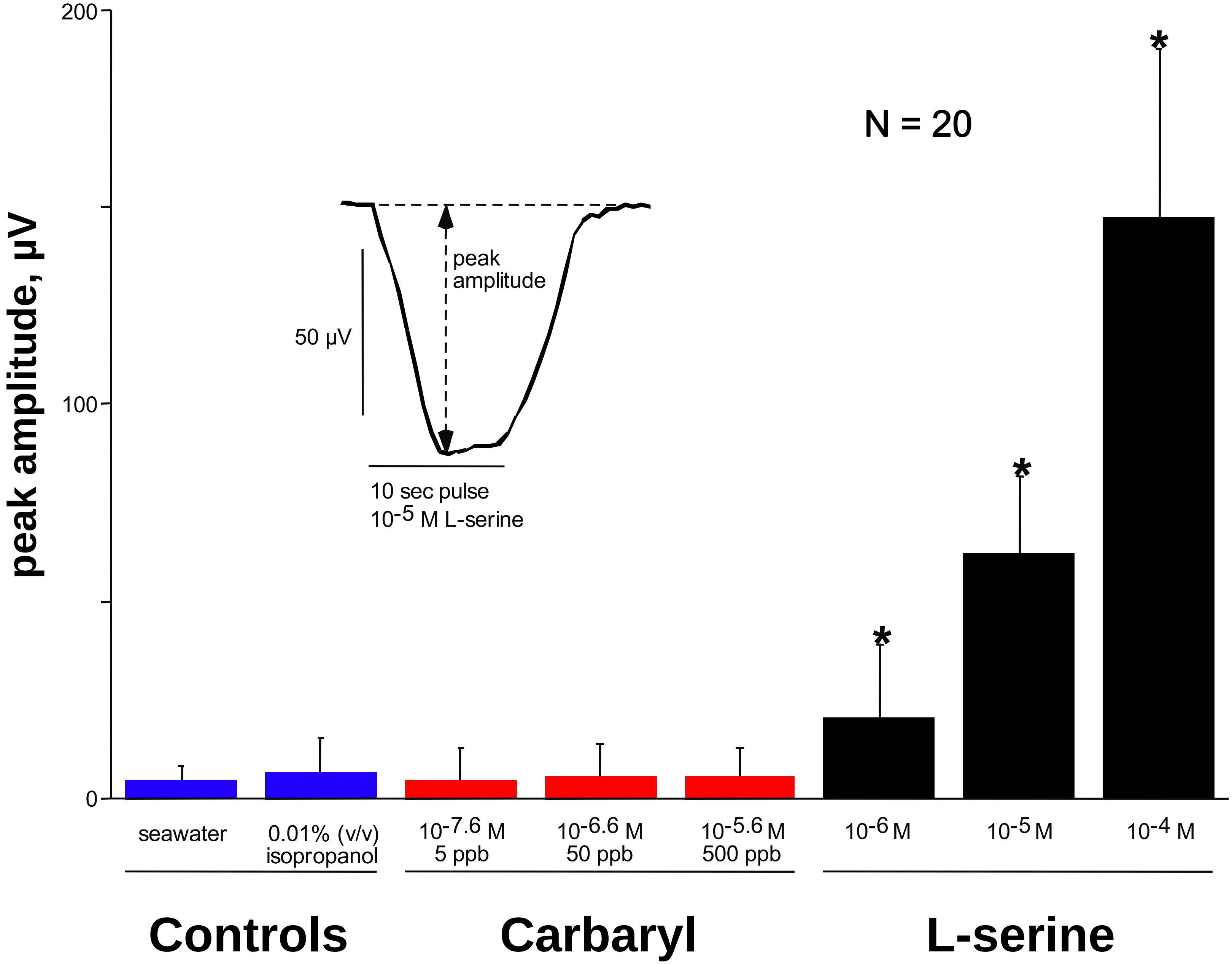
- Fish were age (class) 1

In vivo olfactory recordings

A

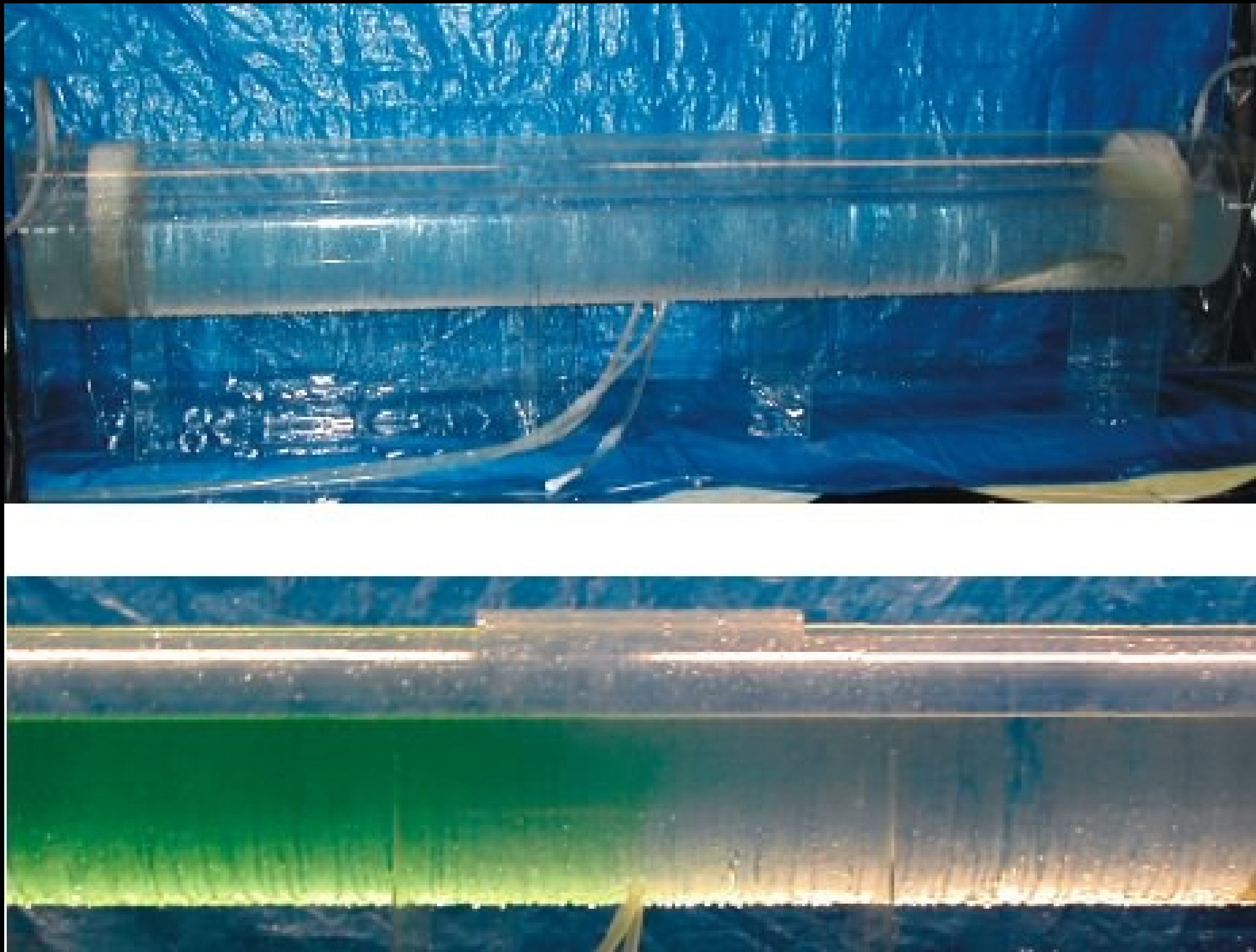


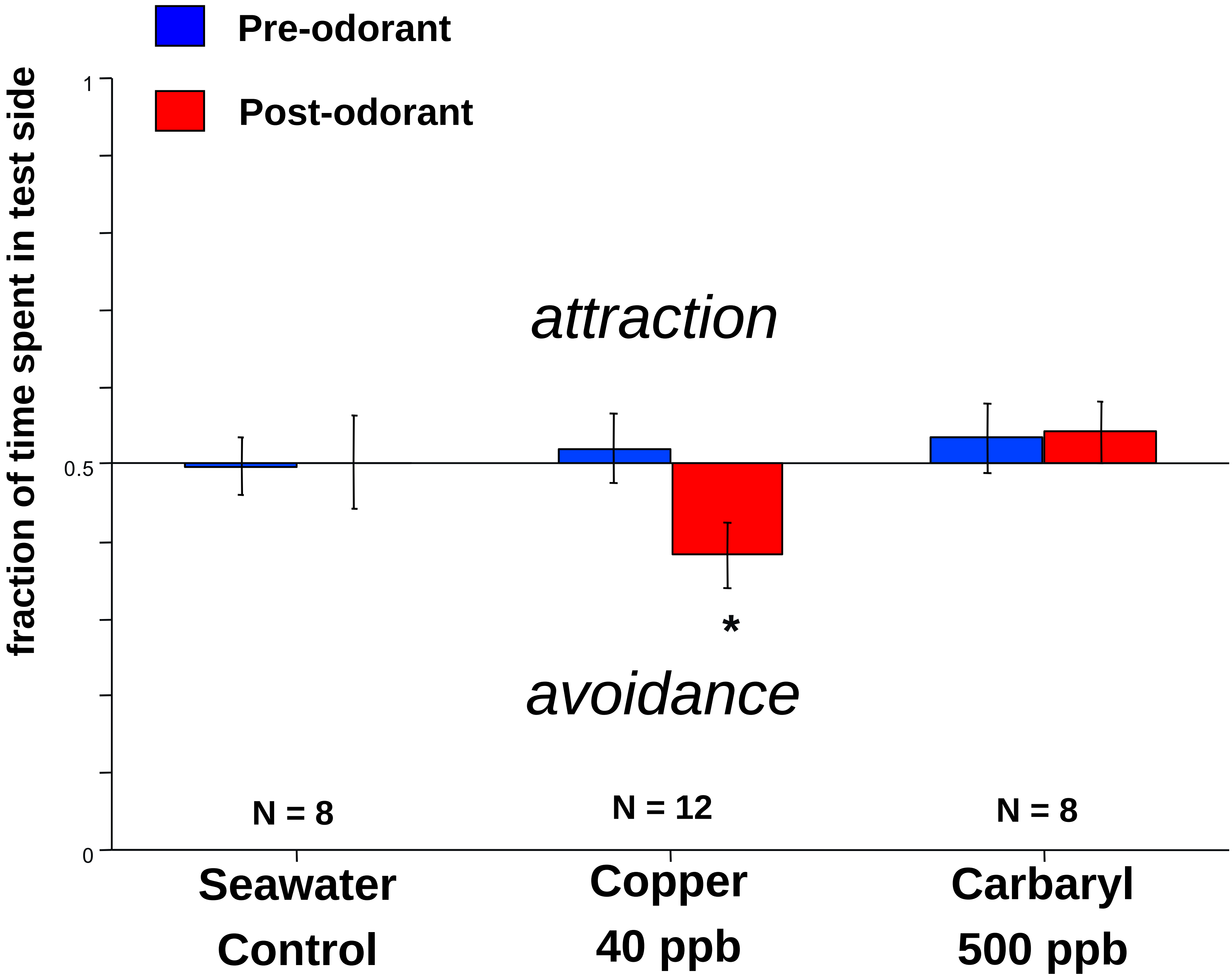
B



200
100
0
peak amplitude, µV
N = 20
peak
amplitude
50 µV
10 sec pulse
10-5 M L-serine
*
*
*
seawater
0.01% (v/v)
isopropanol
10-7.6 M
5 ppb
10-6.6 M
50 ppb
10-5.6 M
500 ppb
10-6 M
10-5 M
10-4 M
Controls
Carbaryl
L-serine

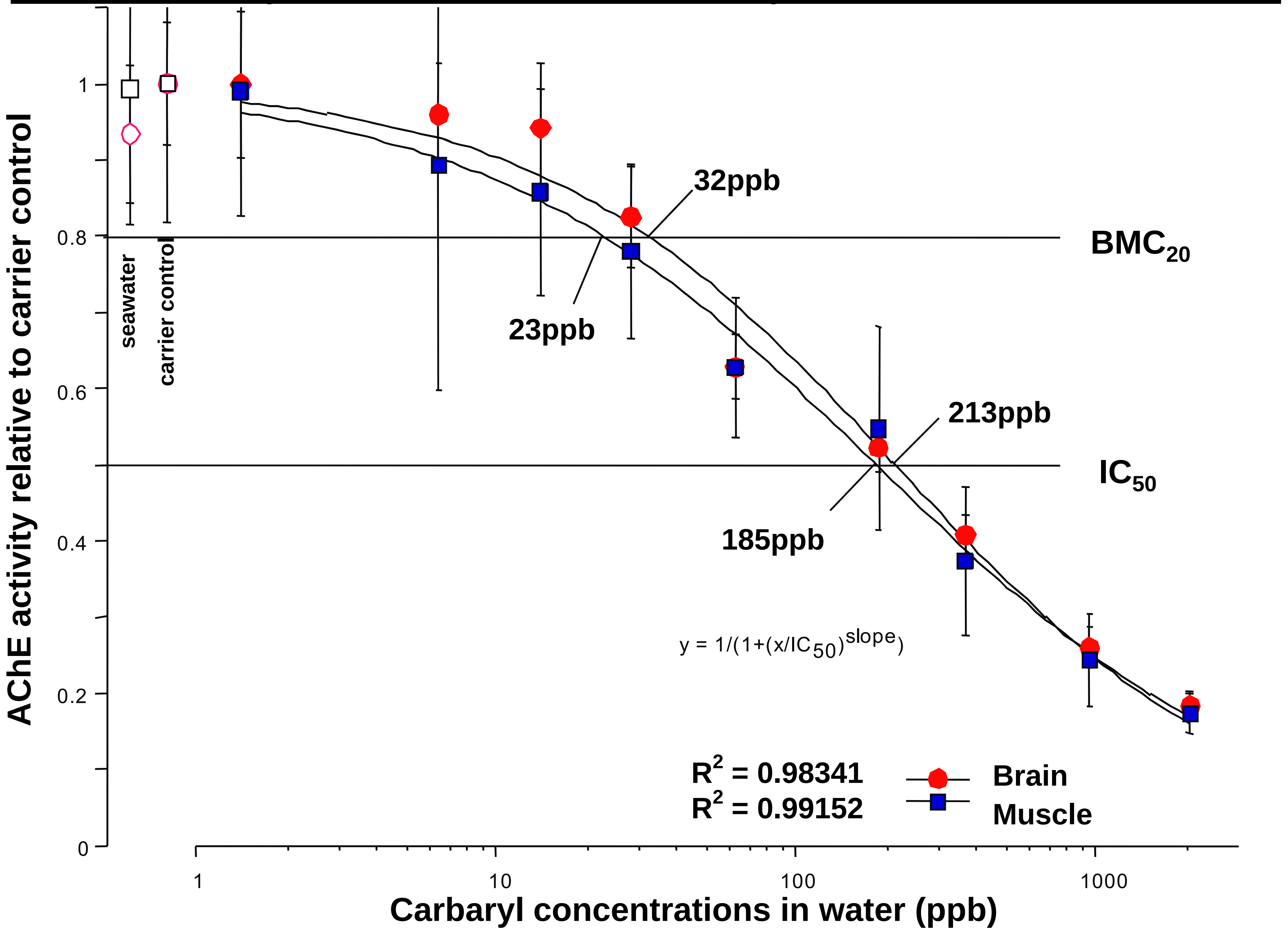
Two-choice Chamber for Measuring Behavioral Avoidance

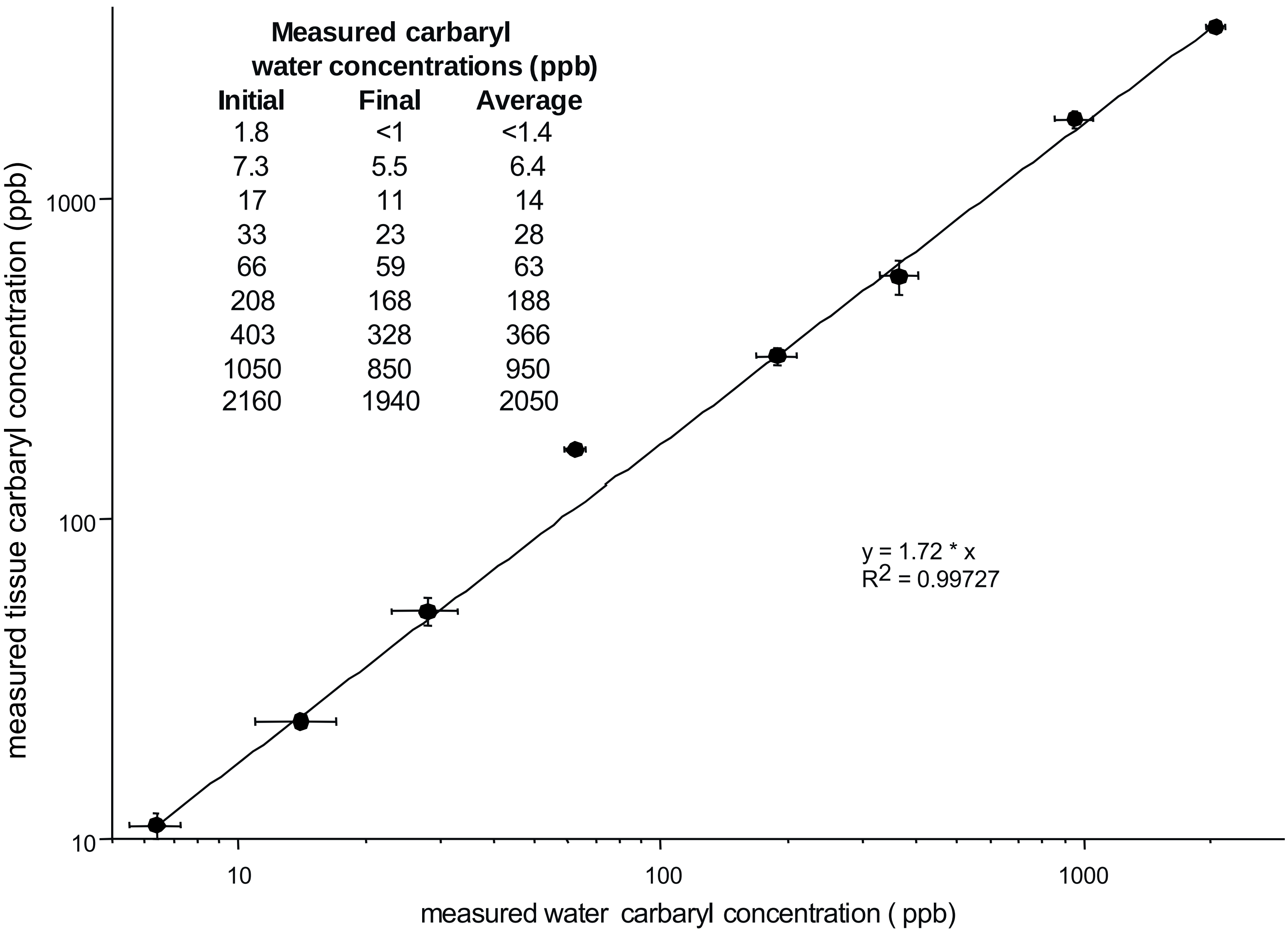



Pre-odorant
Post-odorant
fraction of time spent in test side
1
0.5
0
attraction
avoidance
*
N = 8
N = 12
N = 8
Seawater
Control
Copper
40 ppb
Carbaryl
500 ppb

Short-term Exposure Methodology

AChE Activity versus Measured Carbaryl Concentrations in Water



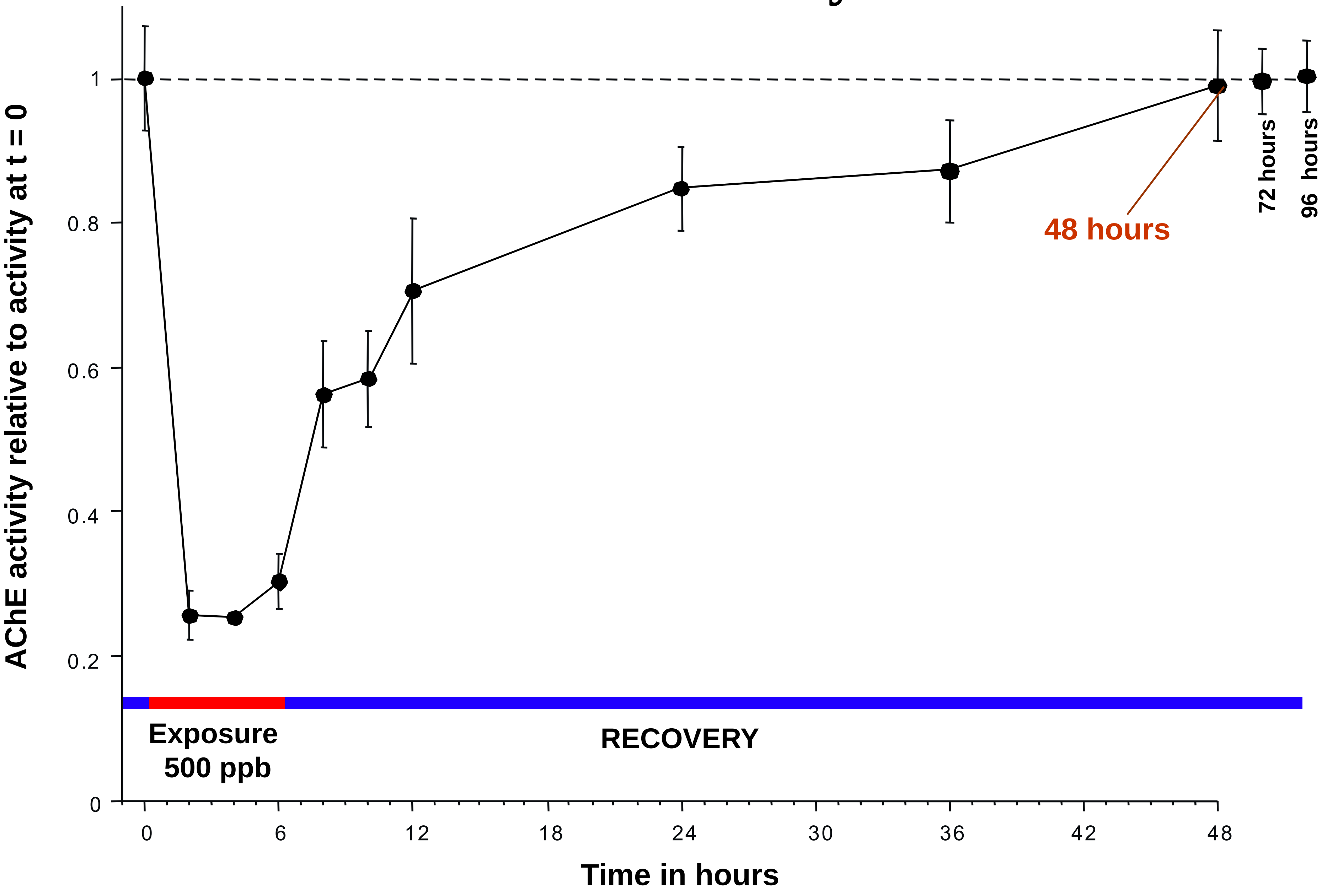


Measured carbaryl
water concentrations (ppb)
Initial Final Average
1.8 <1 <1.4
7.3 5.5 6.4
17 11 14
33 23 28
66 59 63
208 168 188
403 328 366
1050 850 950
2160 1940 2050
y = 1.72 * x
R2 = 0.99727
measured tissue carbaryl concentration (ppb)
measured water carbaryl concentration (ppb)
10
100
1000


B
C


B
C
VWR

Time Course for Carbaryl's Effects



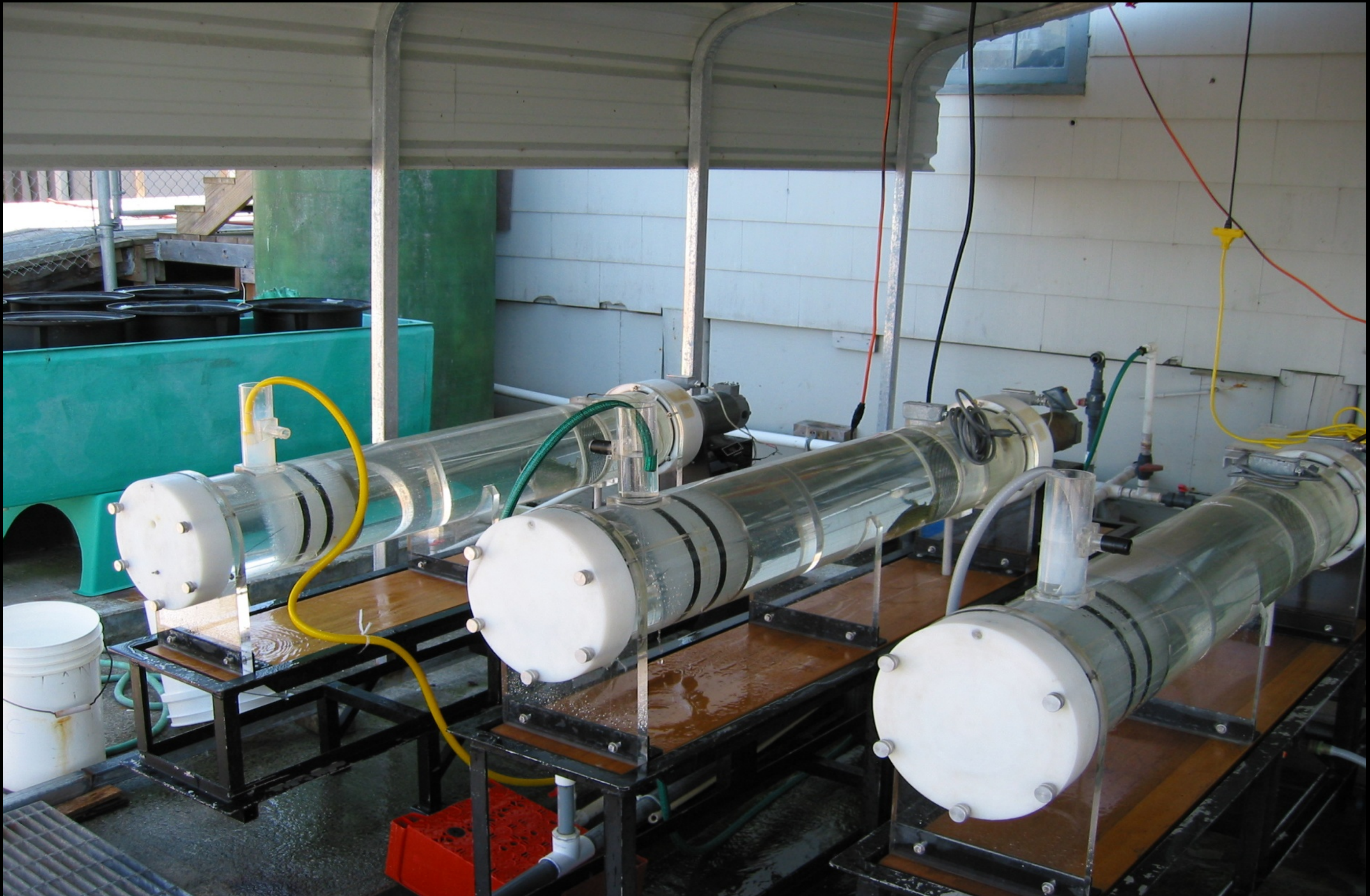
Year 1 results:

- **Cutthroat trout do not show a measurable olfactory response to carbaryl**
- **Animals do not avoid carbaryl in a two-choice test (but they do avoid copper)**
- **A six-hour exposure is sufficient to produce a dose-dependent inhibition of brain and muscle AChE**
- **The onset of cholinesterase inhibition is rapid, and fish gradually recover when returned to clean seawater**

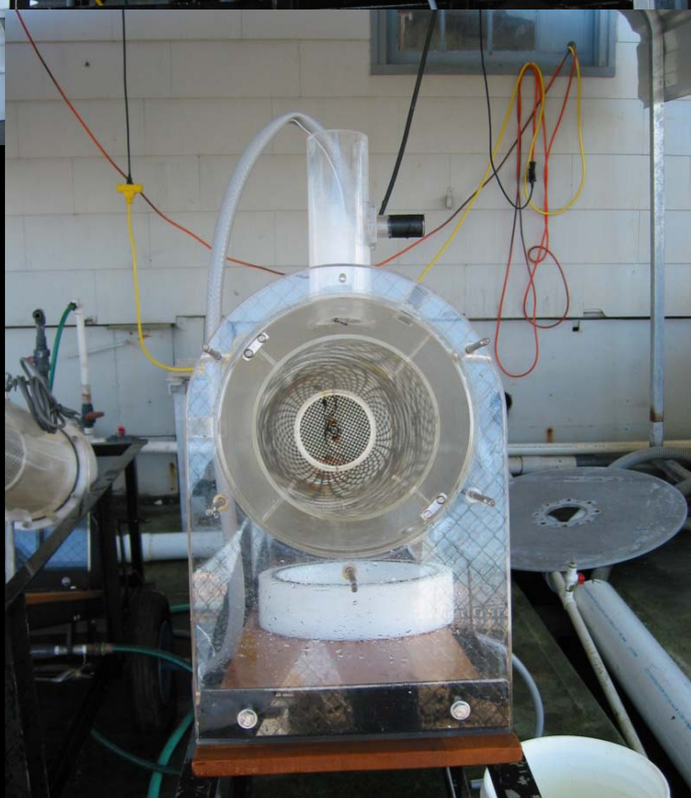
Year 2 & 3 investigations (behavioral and ecological performance)…

Year 2 Objective

Does carbaryl exposure affect the swimming performance of salmonids?



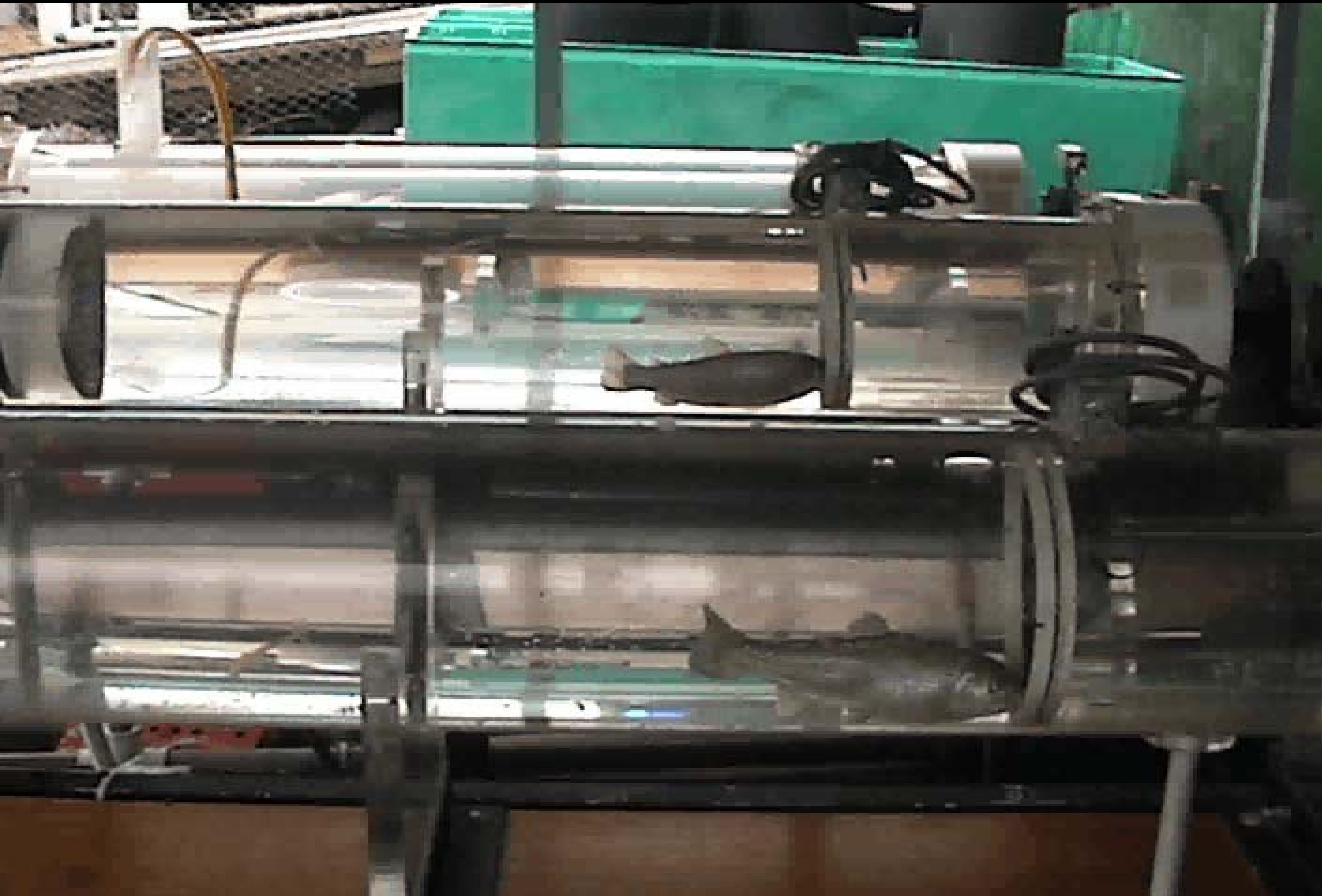
Blazka-style swimming chambers



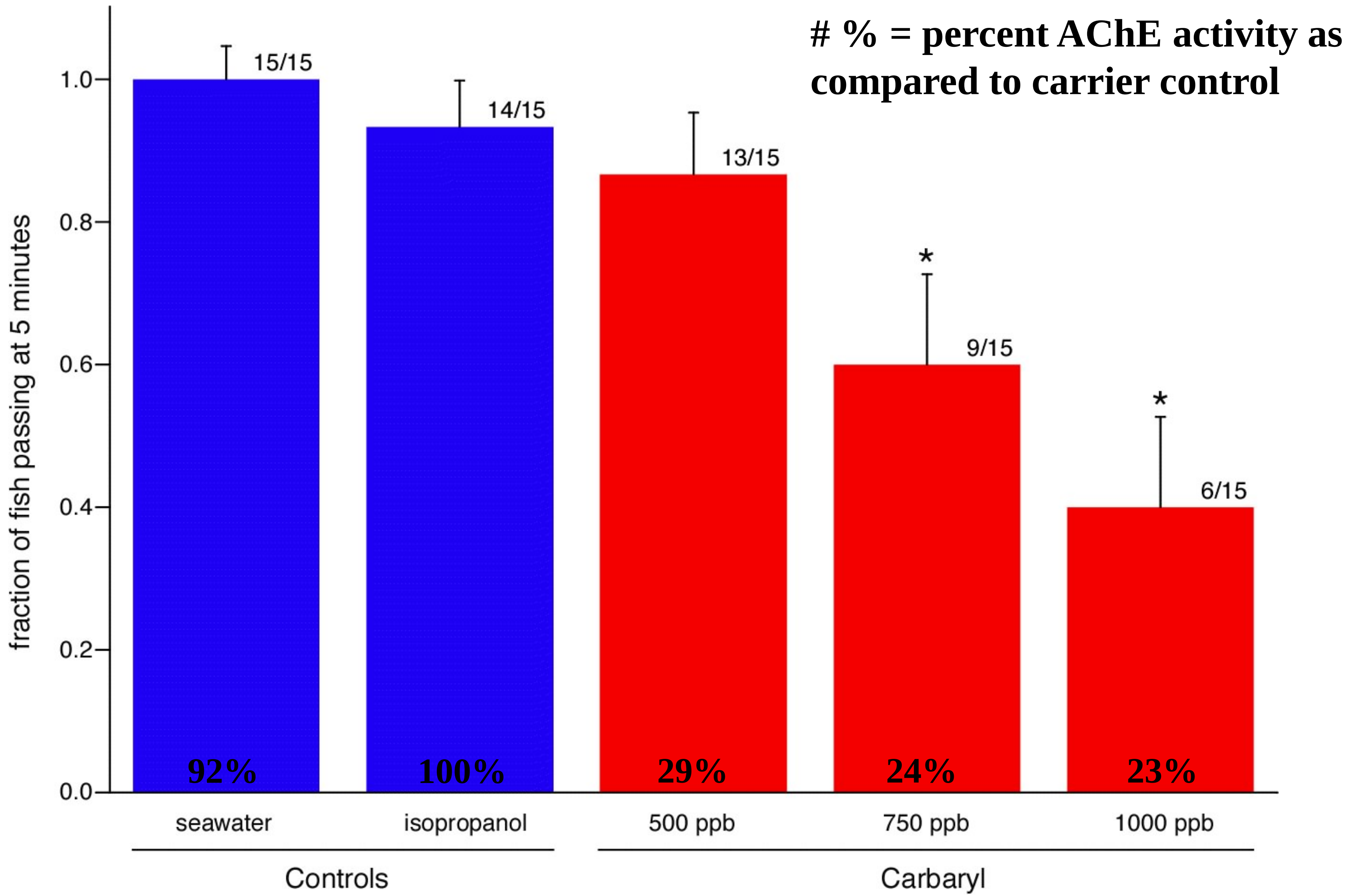
SCORING for Swimming Performance

PASS

FAIL

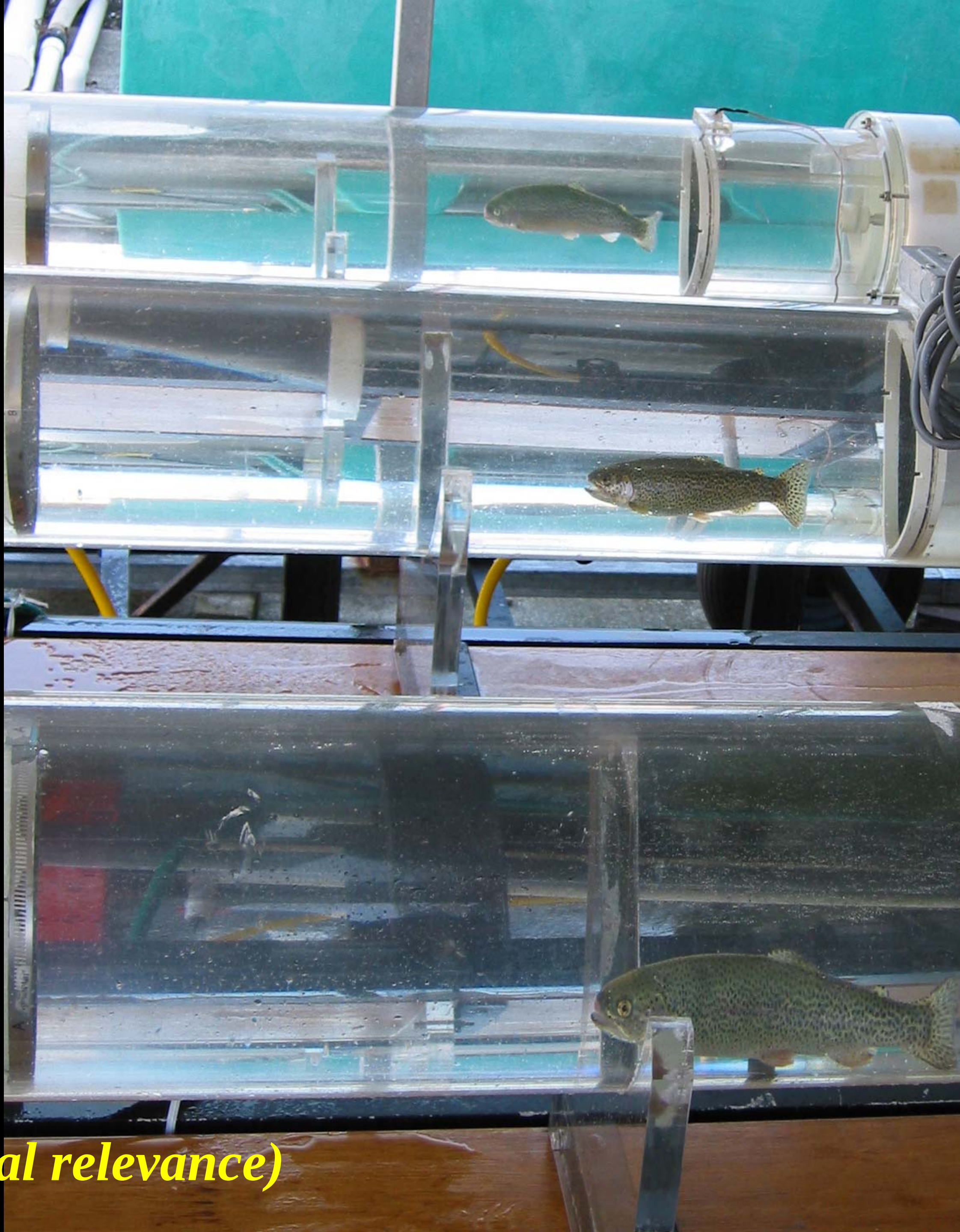


Percent Trout which Passed Swimming Start-up Bioassay



Year 2 results:

- Exposures to nominal carbaryl concentrations $\geq$ 750 ppb did significantly affect swimming performance of cutthroat trout as measured in the swimming start-up bioassay



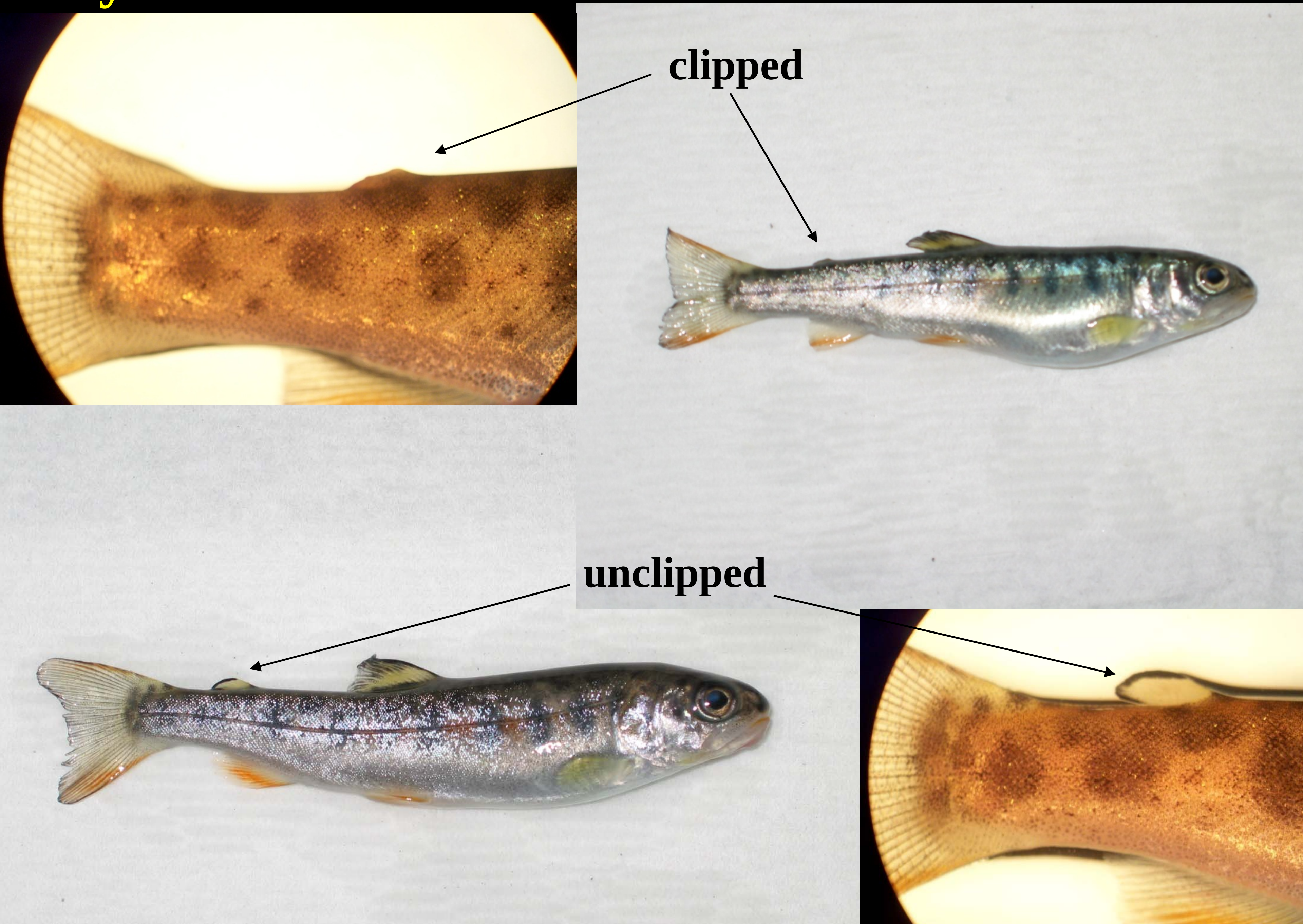
Year 3 investigation (ecological relevance)

Year 3 Objective

Does carbaryl exposure render salmonids more vulnerable to predation?

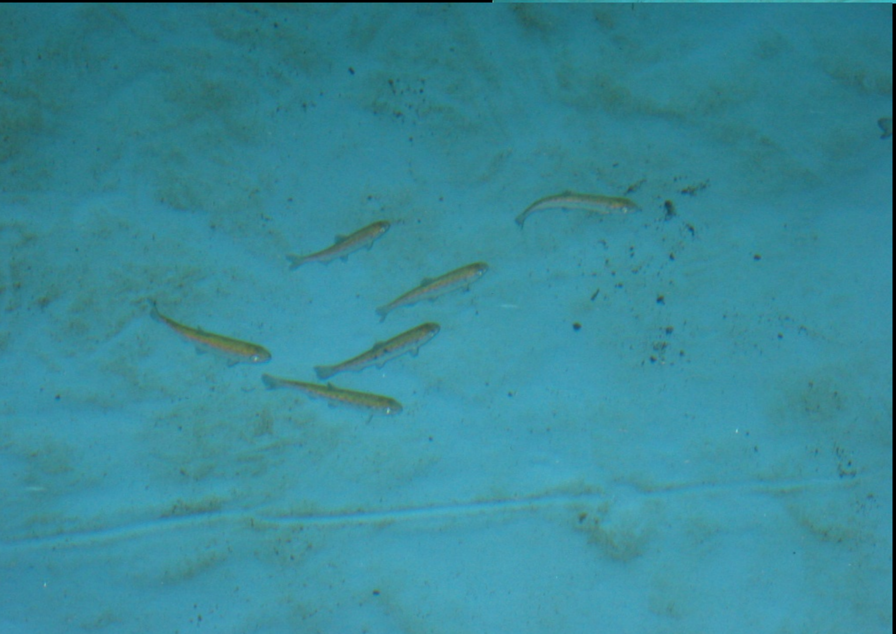
Predator - Lingcod

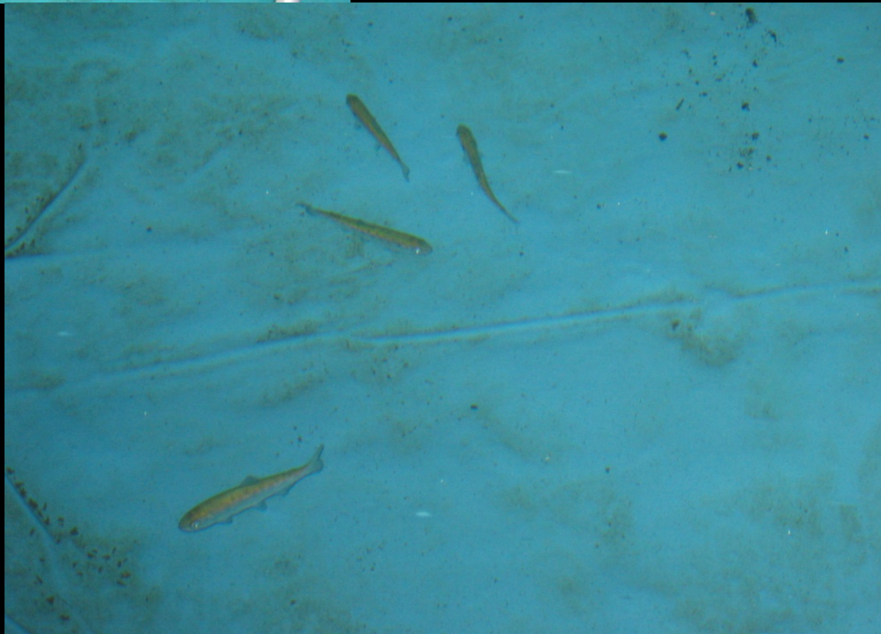
Prey – Cutthroat Trout



Acclimation for Predator Avoidance Assay

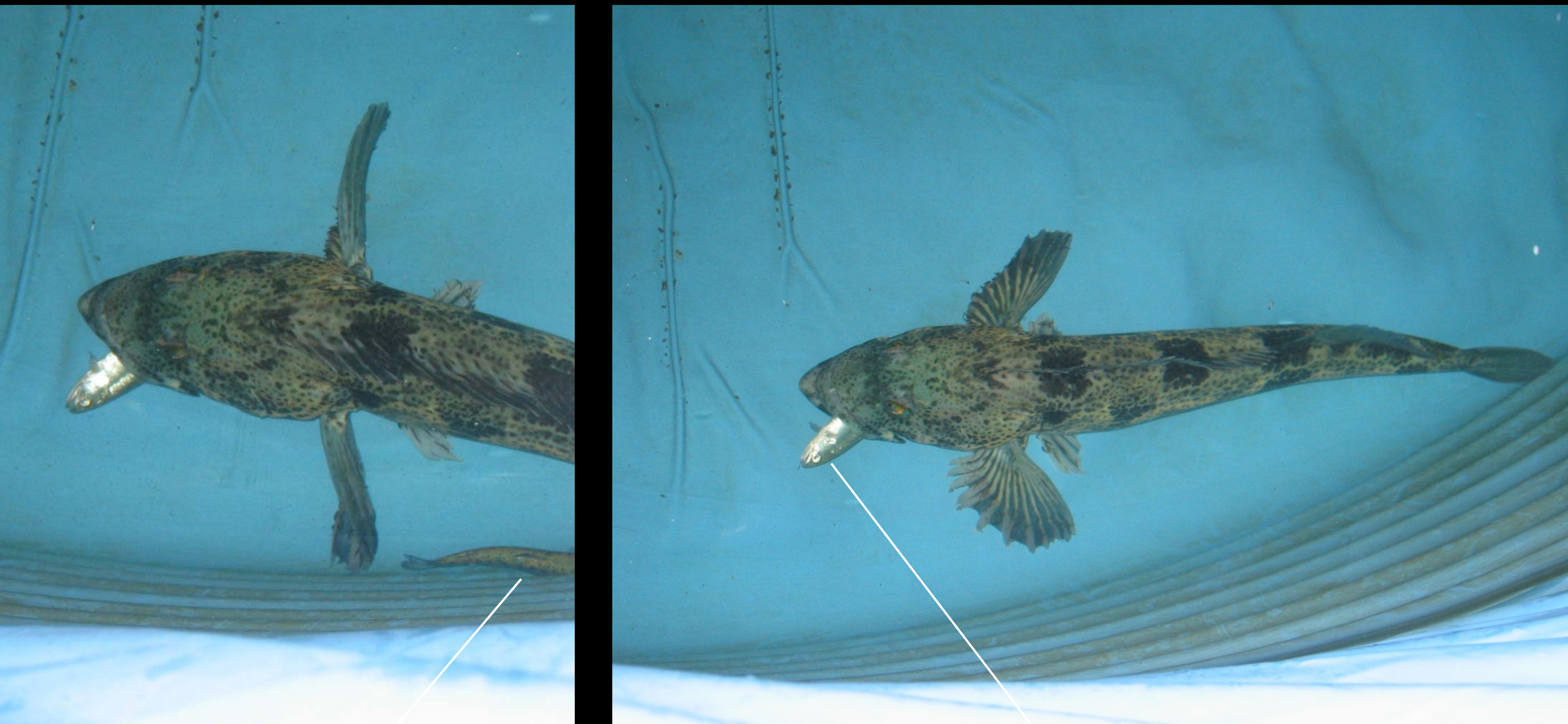
Prey are Released





Prey Meets Predator

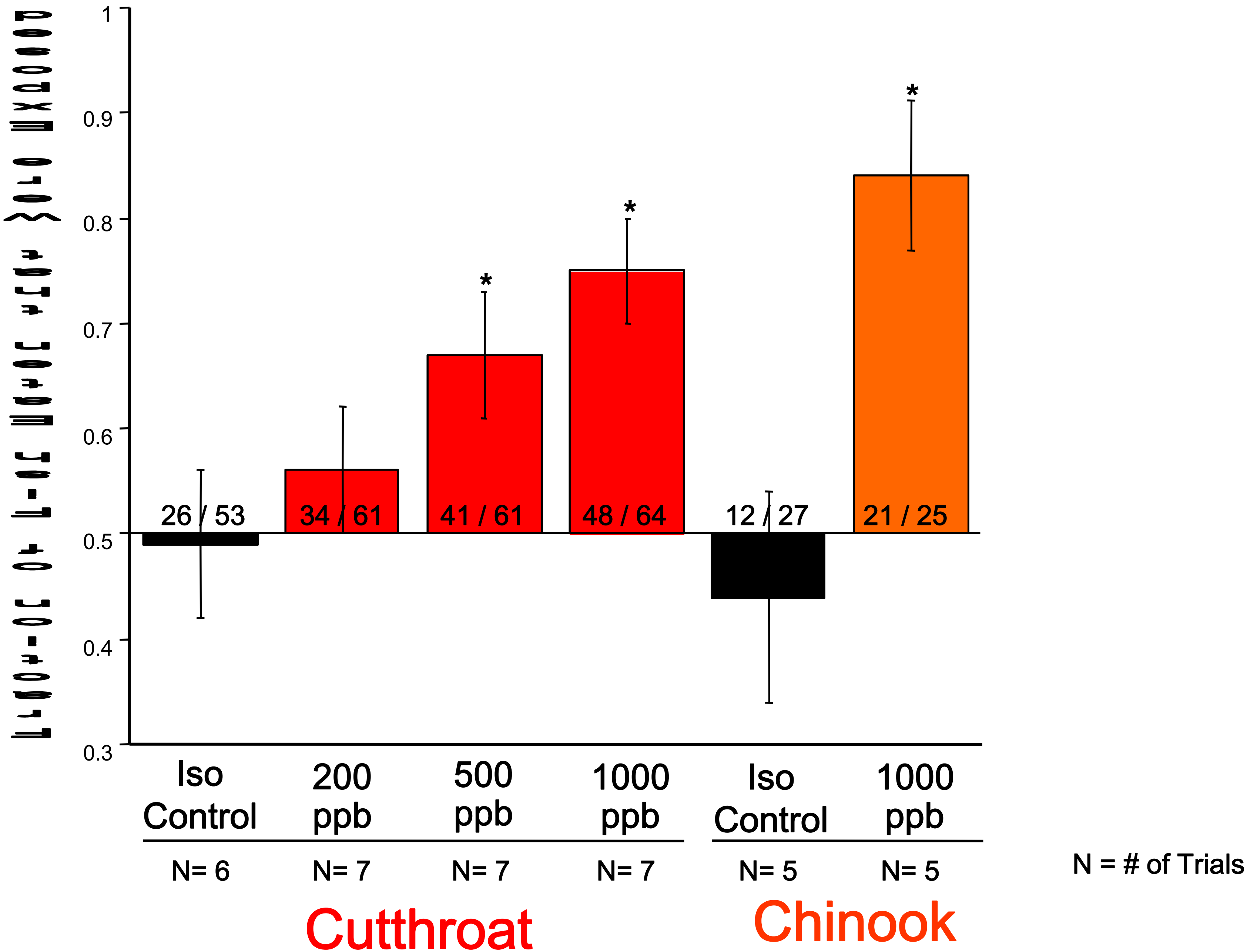
Evade or….be Eaten



Control Fish?

Dosed Fish?

Relative Rates of Predation Versus Control

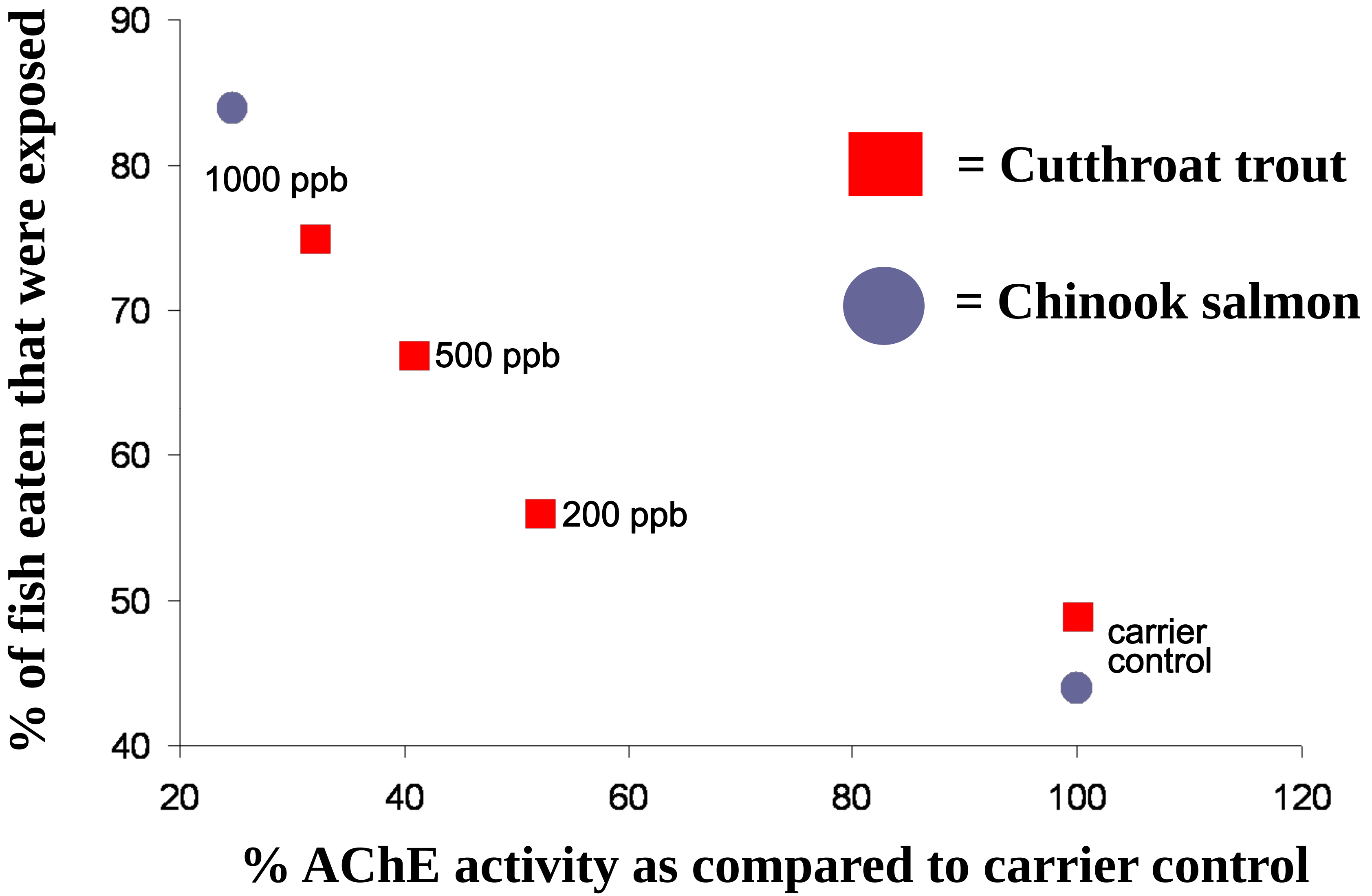


Year 3 results:

- Exposures to nominal carbaryl concentrations $\geq$ 500 ppb did significantly affect the salmonids' vulnerability to predation from lingcod

Relative Rates of Predation Versus AChE Activity

i.e. correlation between neurotoxicity and behavioral impairment



Vol. 329: 1–11, 2007 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 11

http://www.int-res.com/articles/feature/m329p001.pdf

OPEN ACCESS

FEATURE ARTICLE

Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl

Jana S. Labenia[1], David H. Baldwin[1], Barbara L. French[1], Jay W. Davis[2], Nathaniel L. Scholz[1,*]

[1]NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Boulevard E, Seattle, Washington 98112, USA
[2]US Fish and Wildlife Service, Western Washington Fish and Wildlife Office, 510 Desmond Drive SE, Lacey, Washington 98503, USA

3 CASE STUDIES

Act 1 - The sublethal effects of carbaryl on coastal cutthroat trout

Act 2 - The sensory physiology and behavior of juvenile coho exposed to pesticides

Act 3 – Pesticide mixtures and the environmental relevance to fish

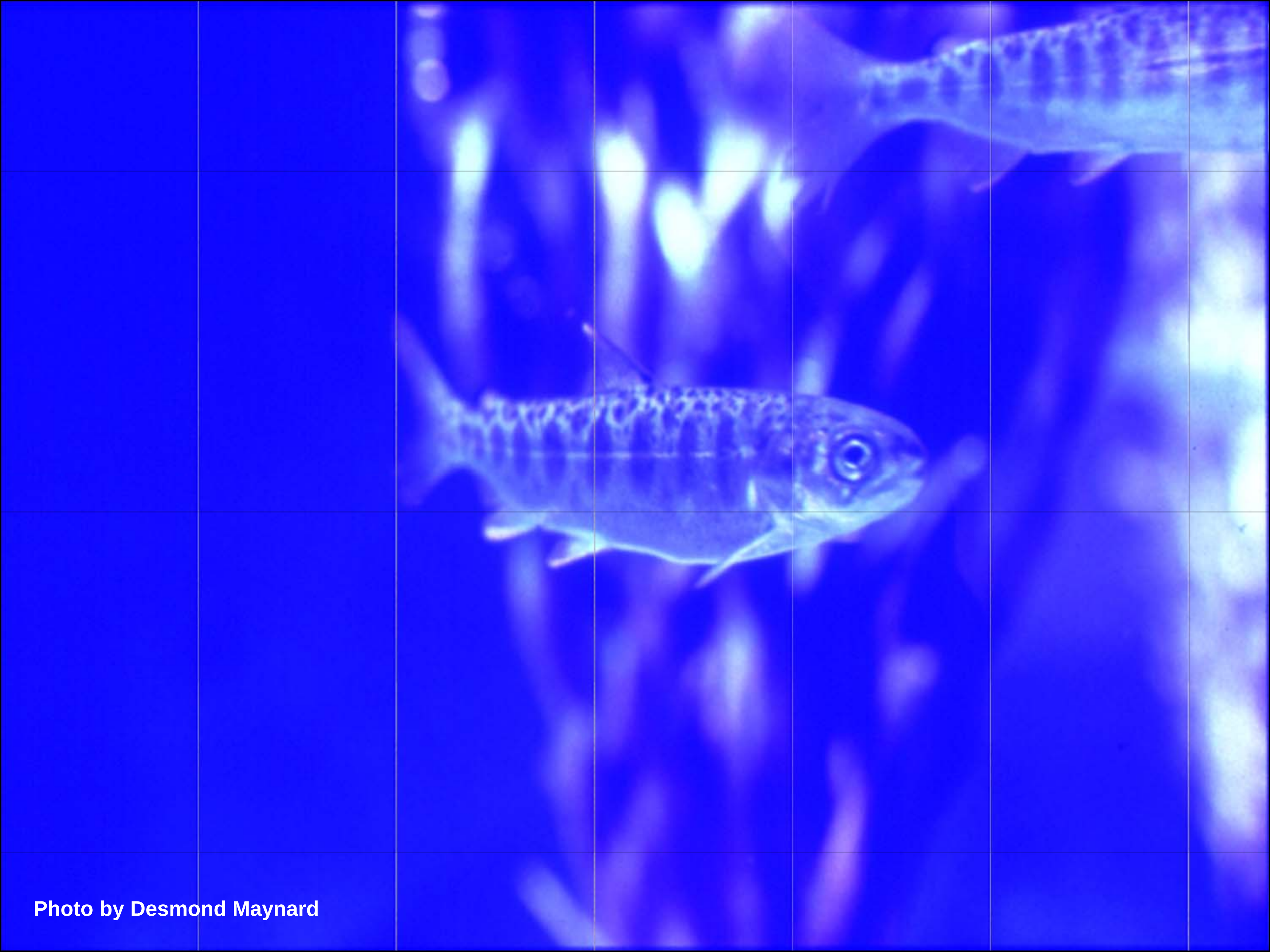
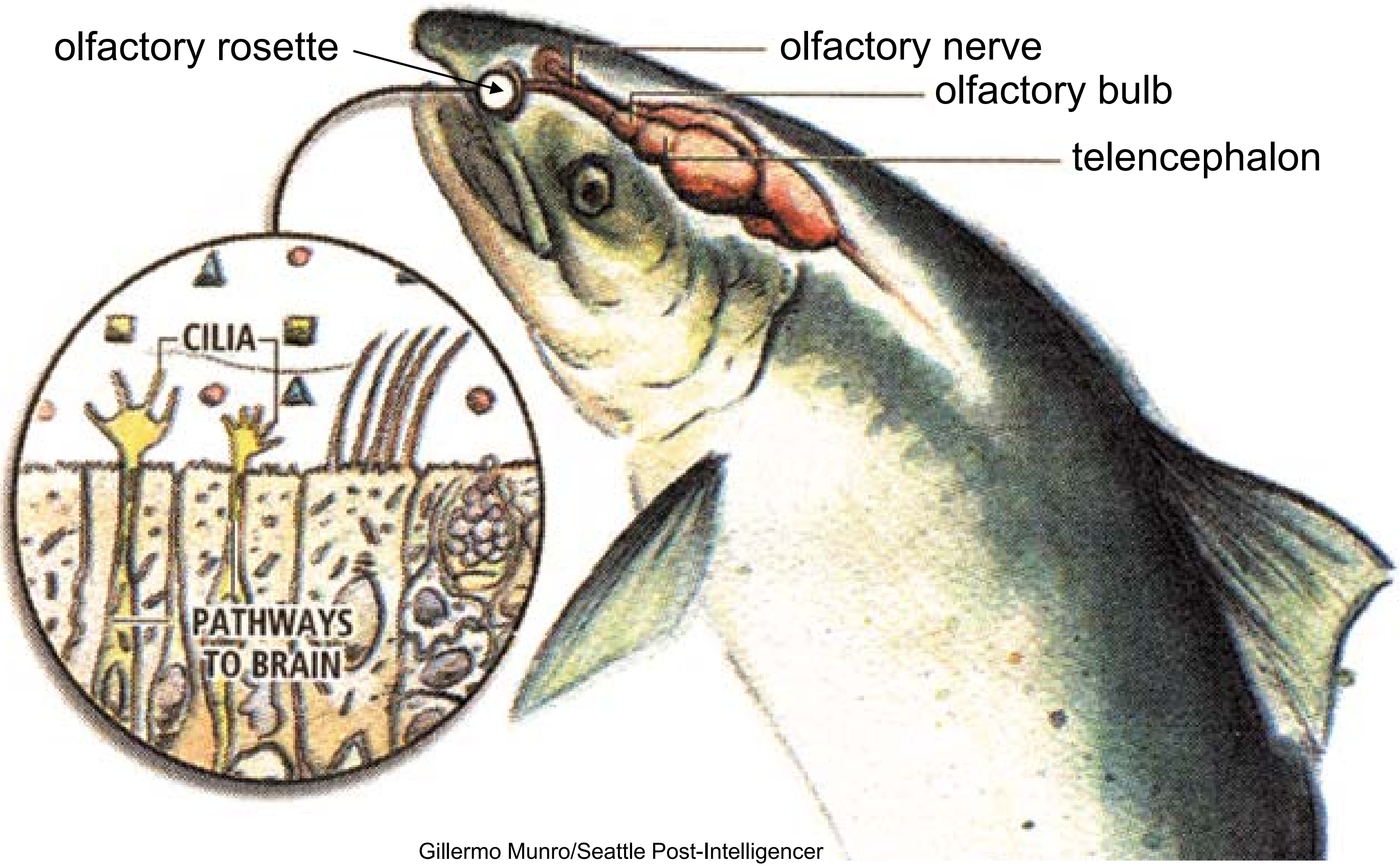


Photo by Desmond Maynard

The salmon olfactory nervous system



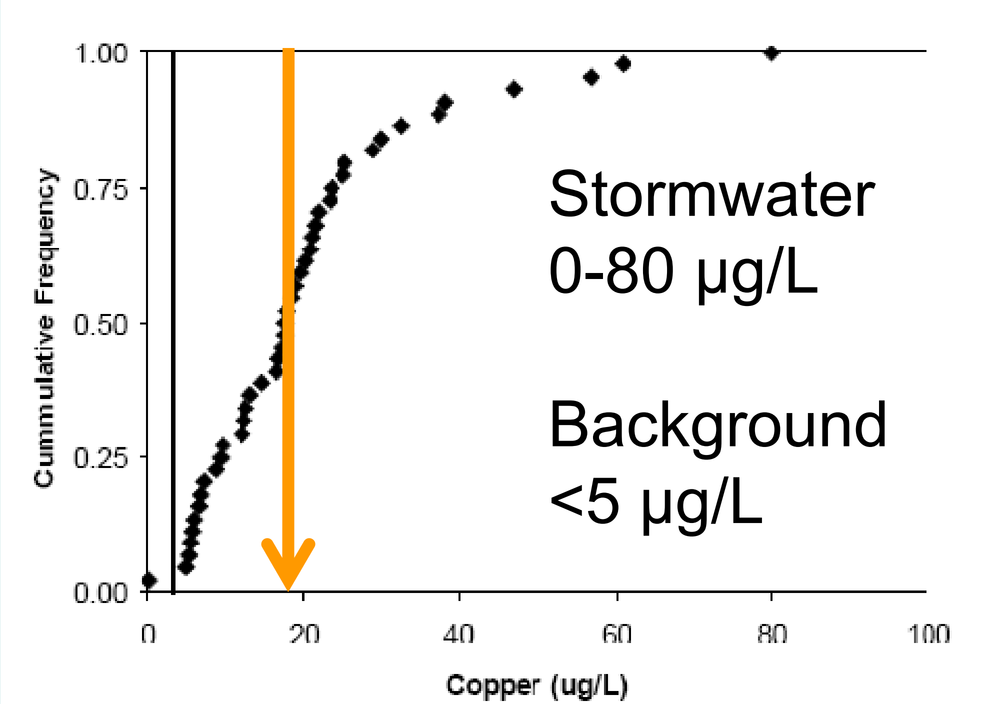
Gillermo Munro/Seattle Post-Intelligencer

Why use the salmon nose as a screen for sublethal neurotoxicity effects?

- Olfactory receptor neurons (ORNs) are directly exposed to contaminants in salmon habitat.
- Olfactory function has been linked to the performance and fitness of salmonids (e.g., predator avoidance, reproductive priming, etc.).
- A salmon's sense of smell determines, in part, the long-term genetic integrity of wild populations (homing).

Copper is a common aquatic contaminant

E.g. Urban stormwater



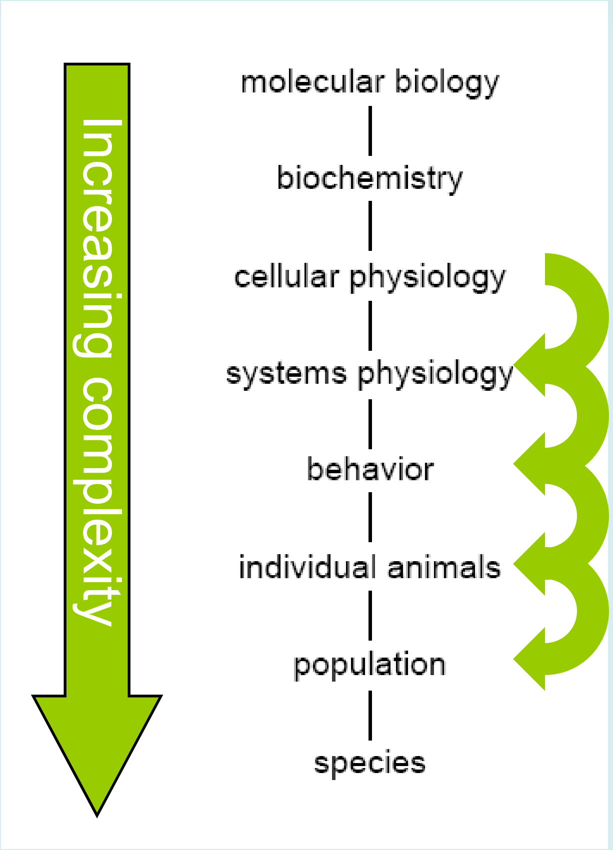
Hwuang et al. UC Davis. Highway runoff.

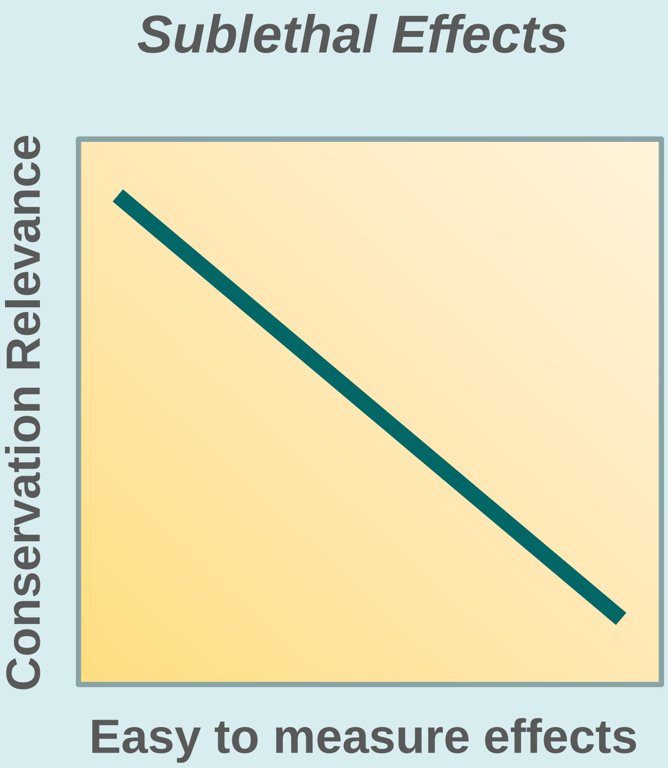
mostly brake pads

Median = 18 µg/L

The Big Picture

Does sublethal copper exposure affect survival?





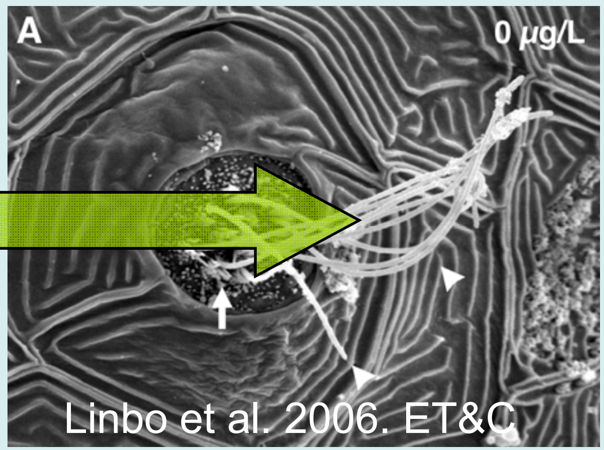
Linking effects across levels of complexity

Copper is neurotoxic

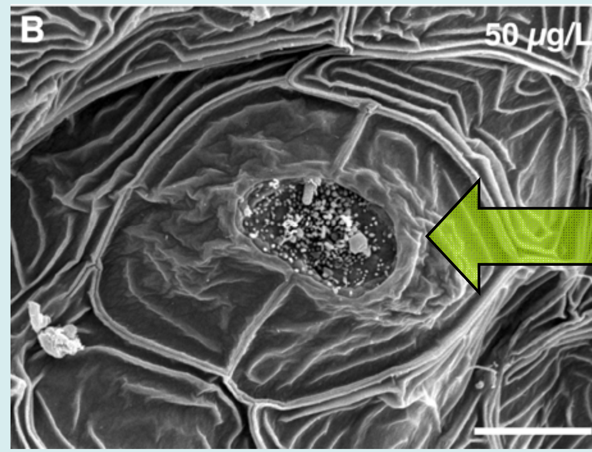
Peripheral sensory system

Control Fish

Hair cells

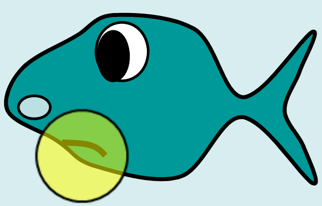


Copper-exposed

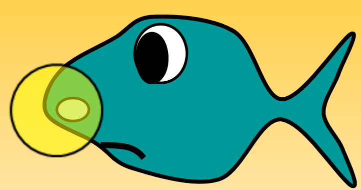


Sensory neurons destroyed

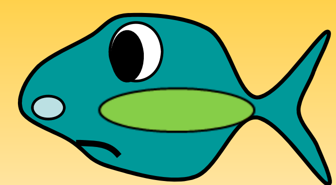
Gustation (taste)



Olfaction (smell)

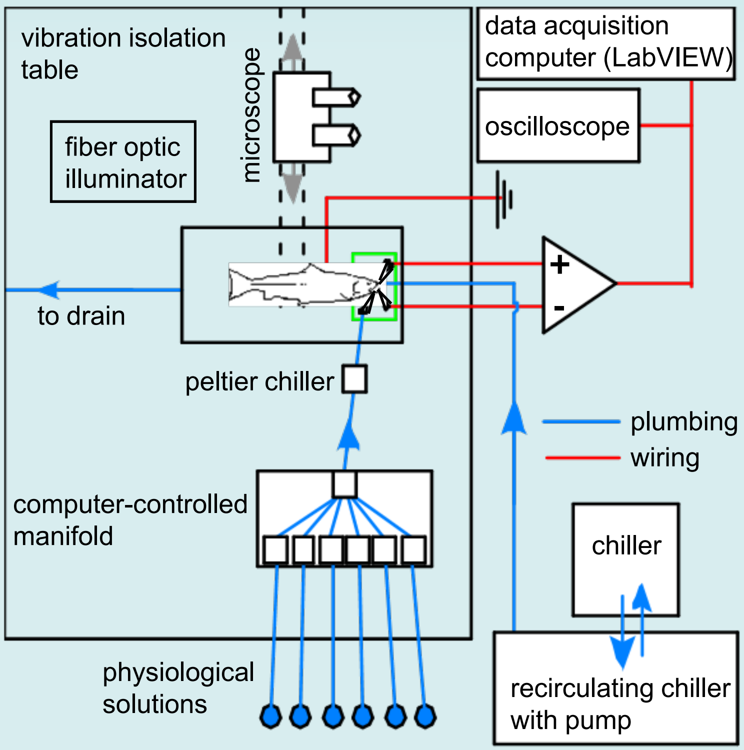


Mechanosensation (lateral line)



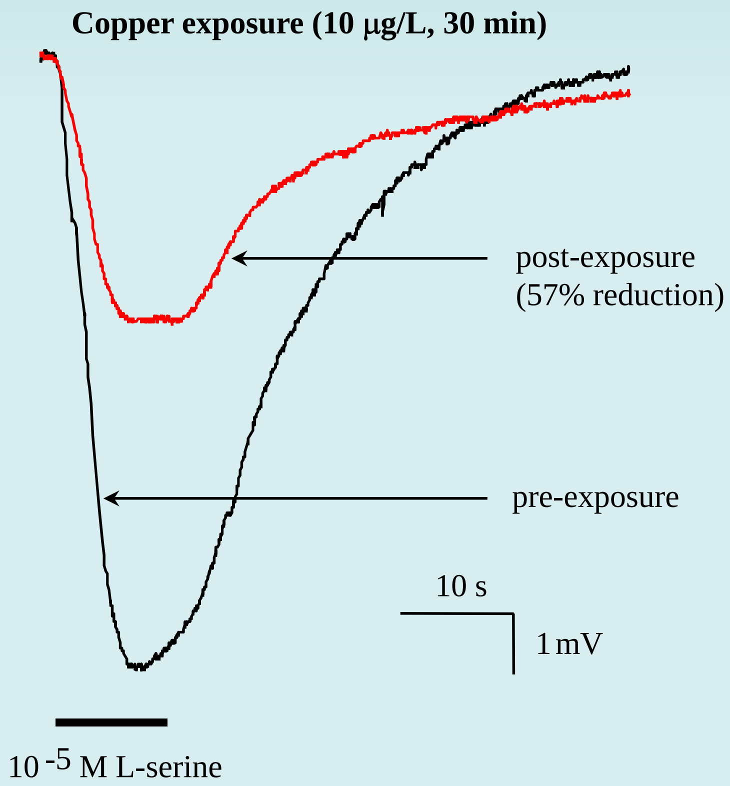
In vivo olfactory recordings

A



B

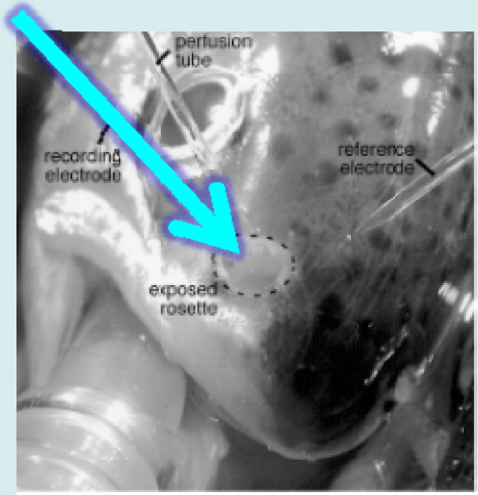
Sublethal copper neurotoxicity in juvenile coho salmon (30 min exposures)

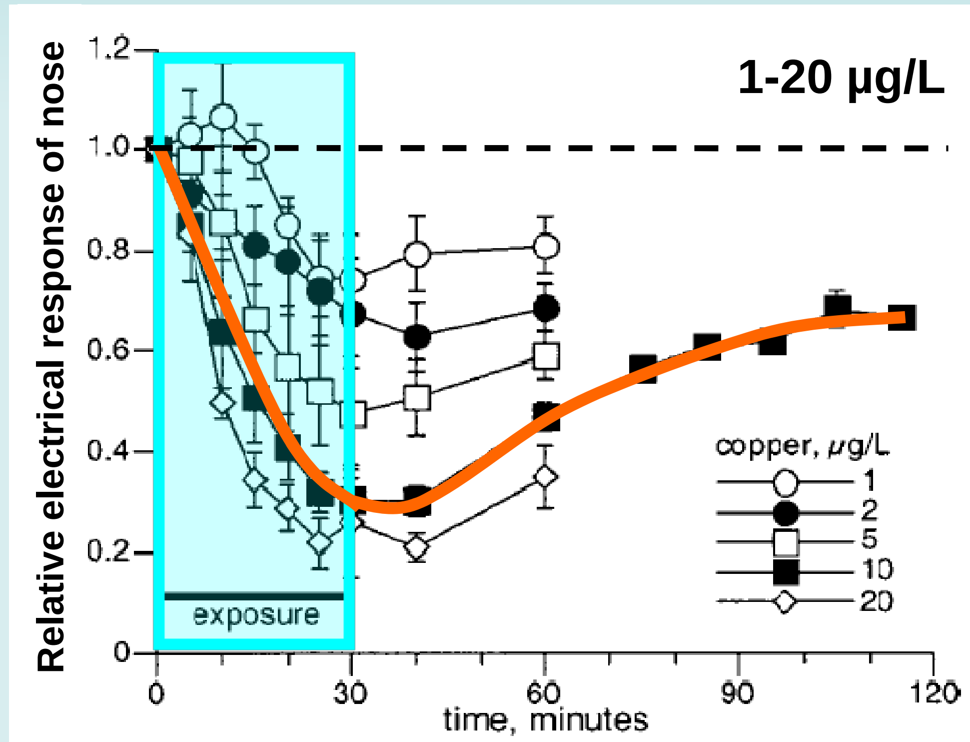


Baldwin. et al., 2003, *Environ. Toxicol. Chem.* 22:2266
Sandahl et al., 2004, *Can. J. Fish. Aquat. Sci.* 61:404

Copper Impacts Olfaction

Recording electrode





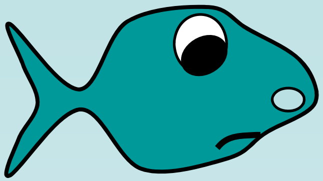
(Baldwin et al. 2003. ETC 22)

Short exposure durations and low concentrations

Sub-Lethal Effects of Copper

Peripheral sensory system

Olfaction



Olfactory-mediated behaviors

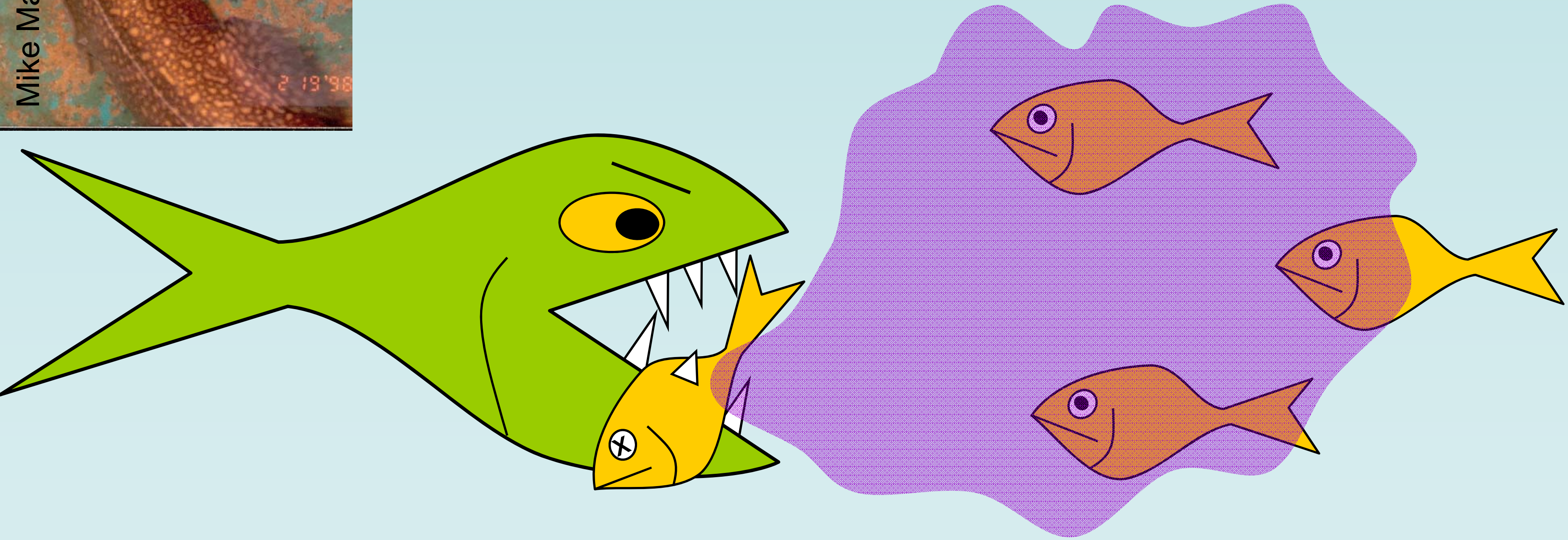
Defense | Feeding | Socializing | Migration | Reproduction

E.g. Alarm Response to Olfactory Cue

Olfactory cues ➔ Behaviors

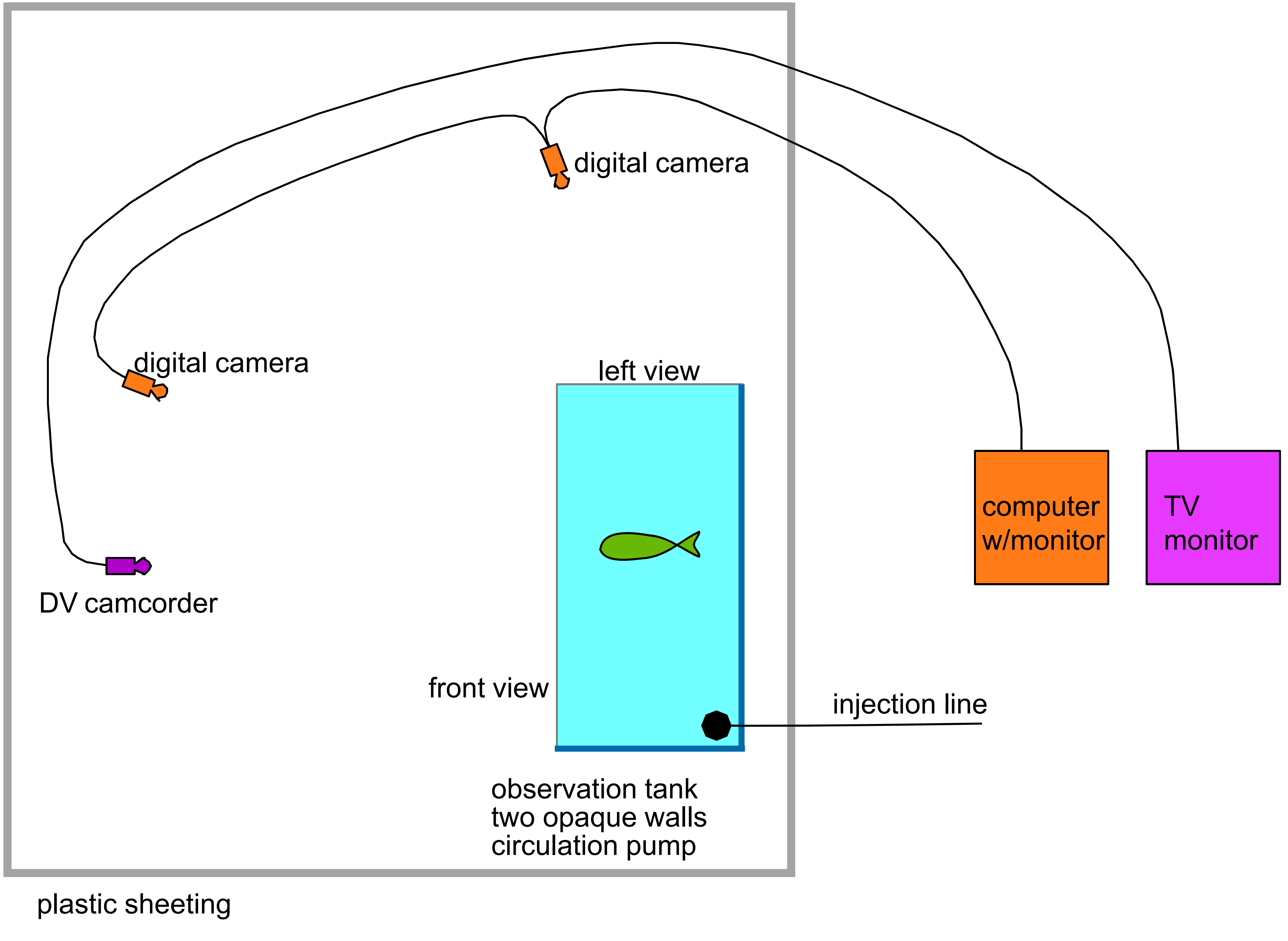
Schreckstoff = alarm cue in fish skin

Released by mechanical damage



Alarm response = freezing

Computer-aided analysis of juvenile coho behaviors



Copper Impacts Alarm Behavior

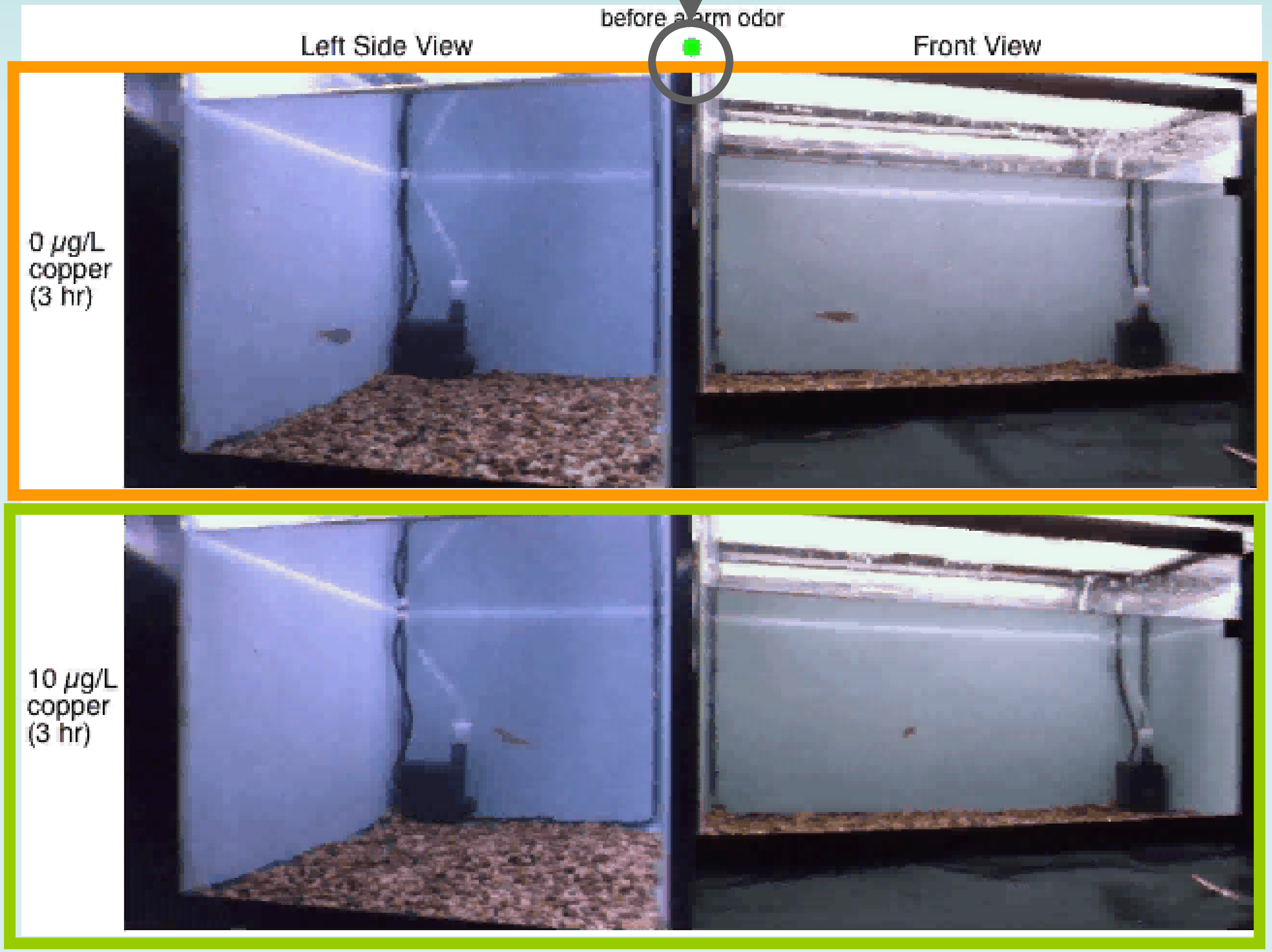
Skin extract added

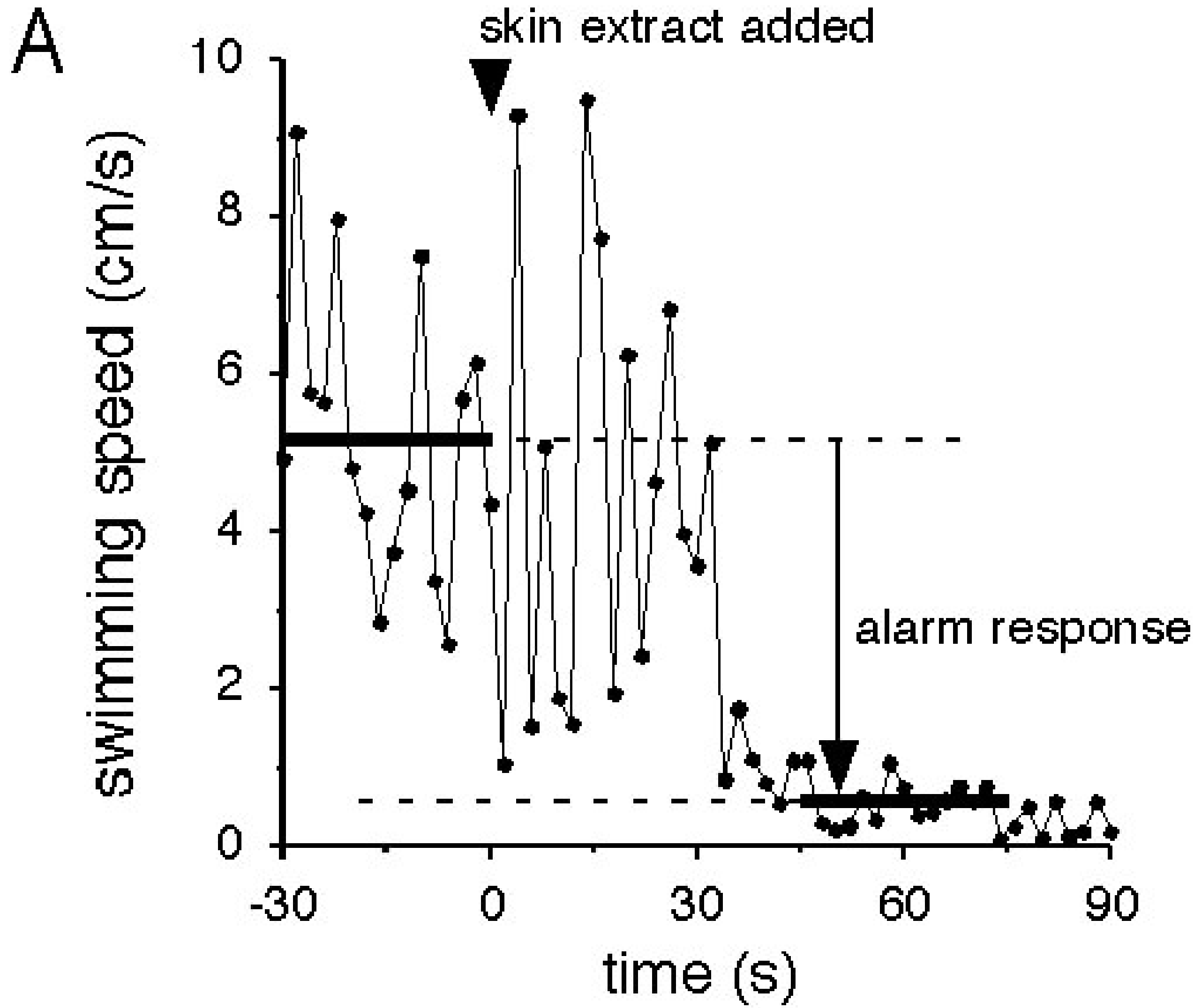
No copper

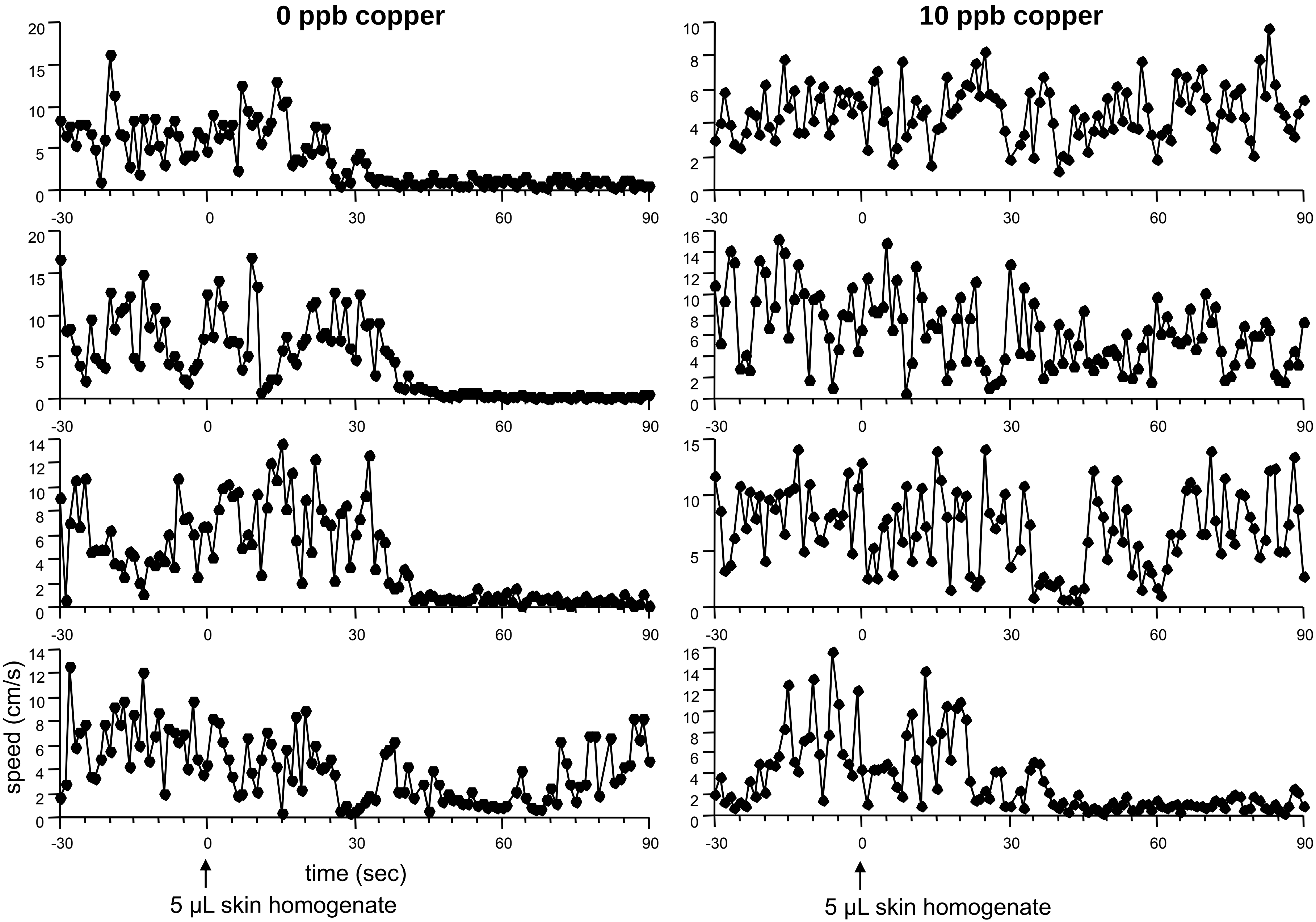
Freeze

Copper

No freeze

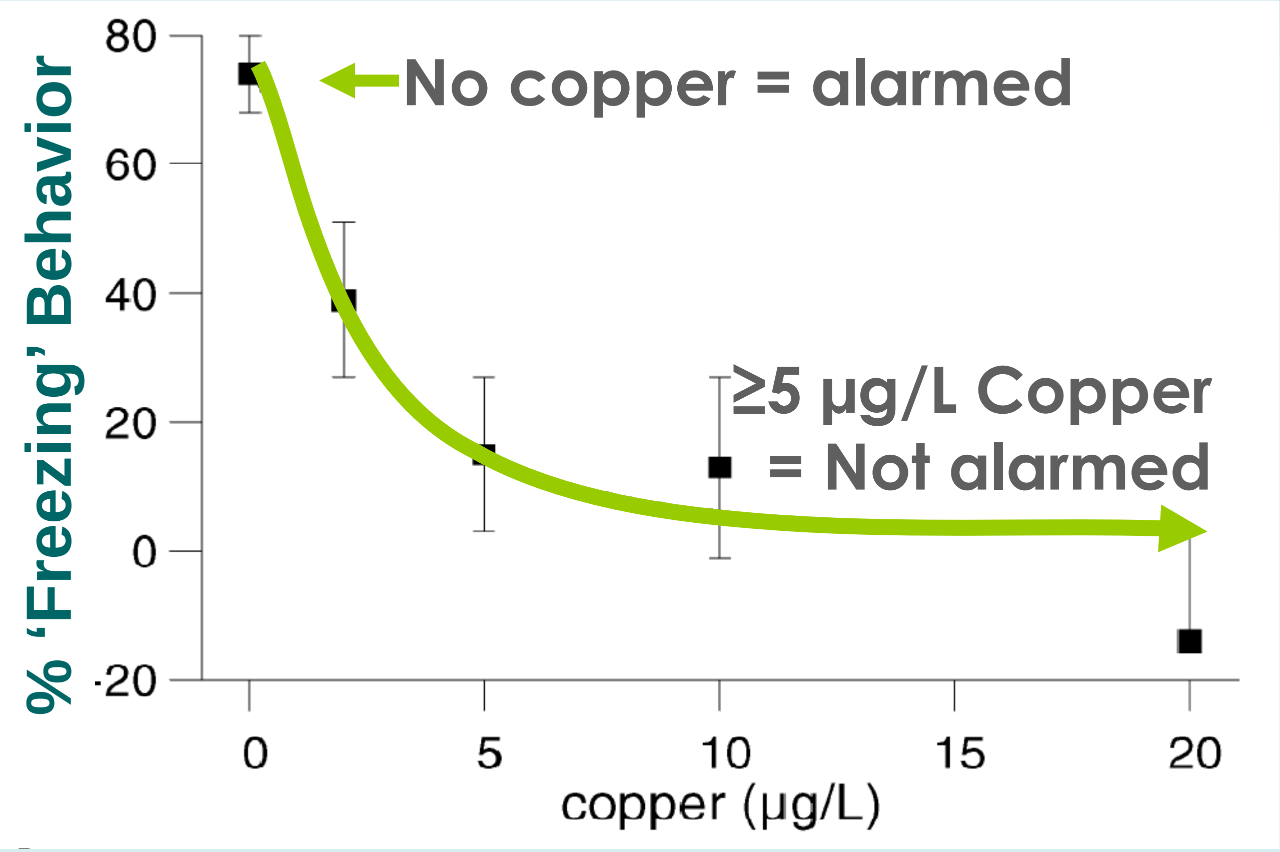



A
skin extract added
alarm response
swimming speed (cm/s)
10
8
6
4
2
0
-30
0
30
60
90
time (s)



0 ppb copper
10 ppb copper
speed (cm/s)
time (sec)
5 µL skin homogenate
5 µL skin homogenate
-30
0
30
60
90

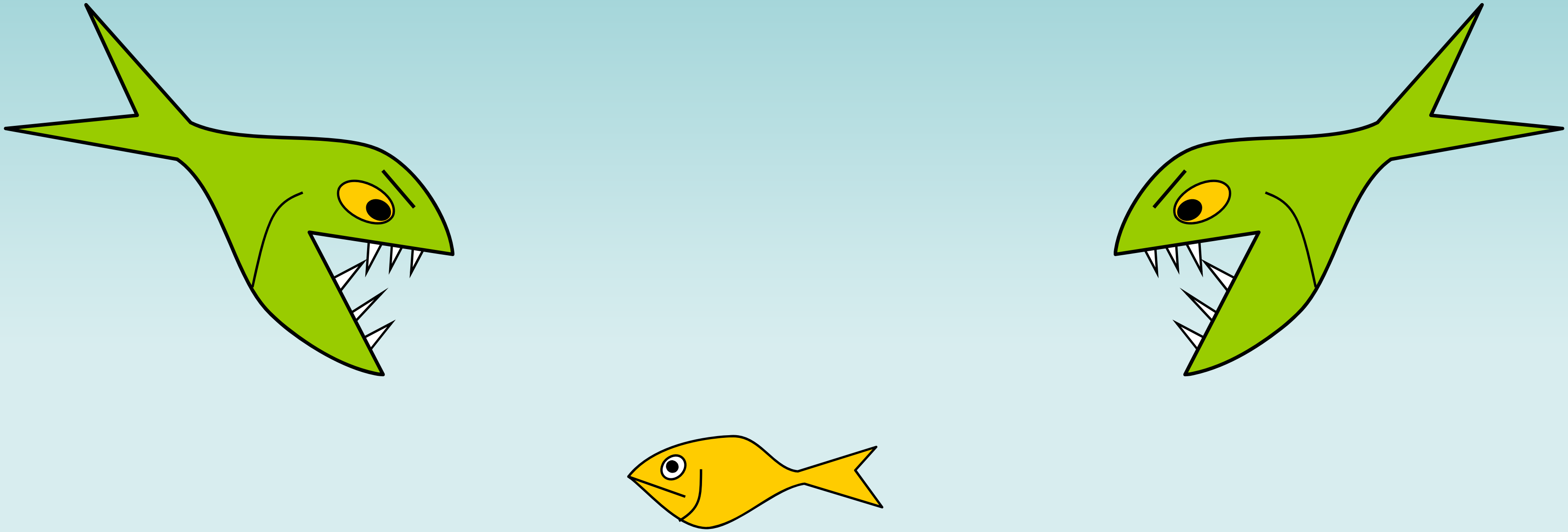
Copper Impacts Alarm Behavior



Sandahl et al. 2007. ES&T 41: 2998

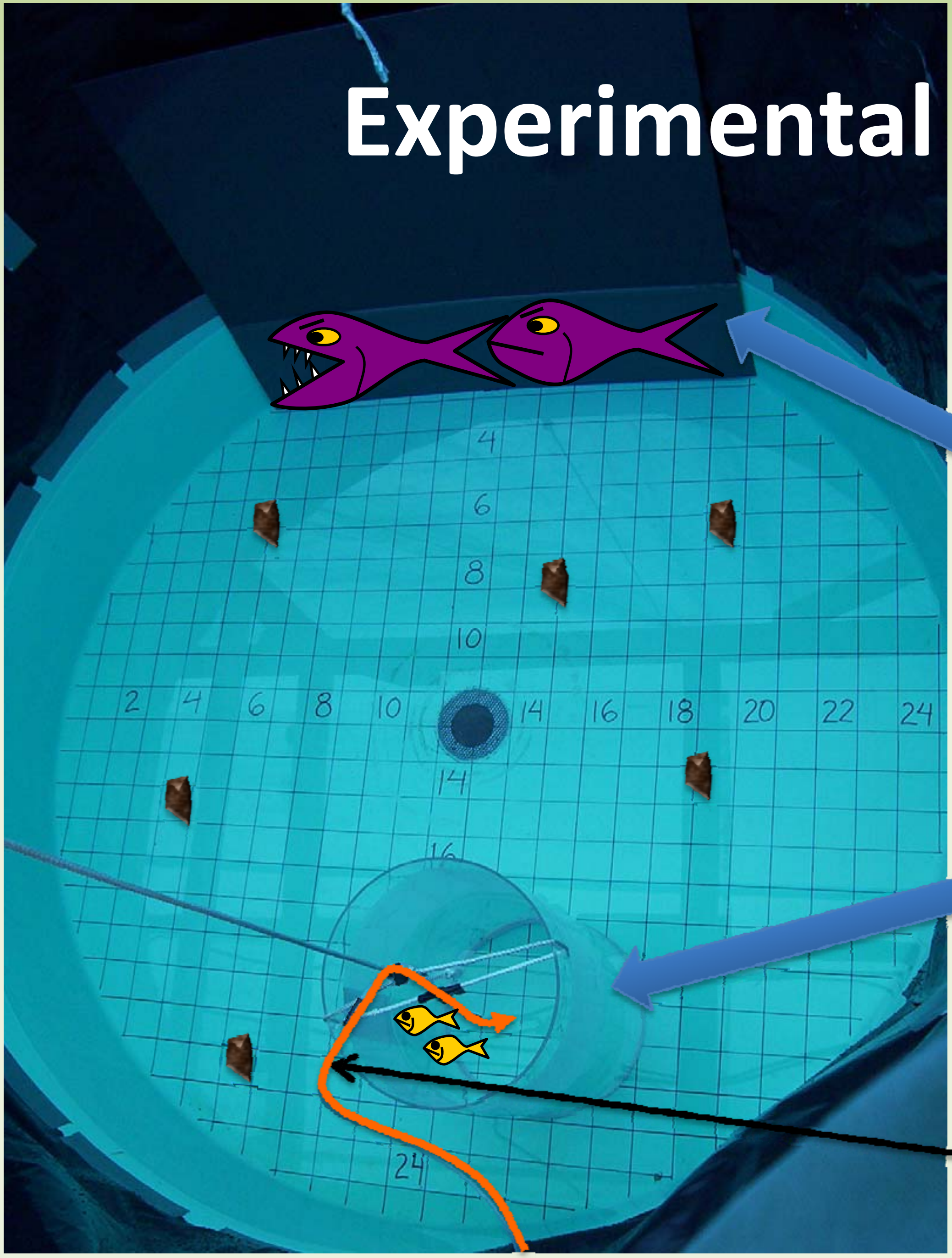
Copper-exposed fish were not alarmed by skin extract

Environmental Relevance?



Linking copper effects on behavior to survival

Experimental Arena 2008

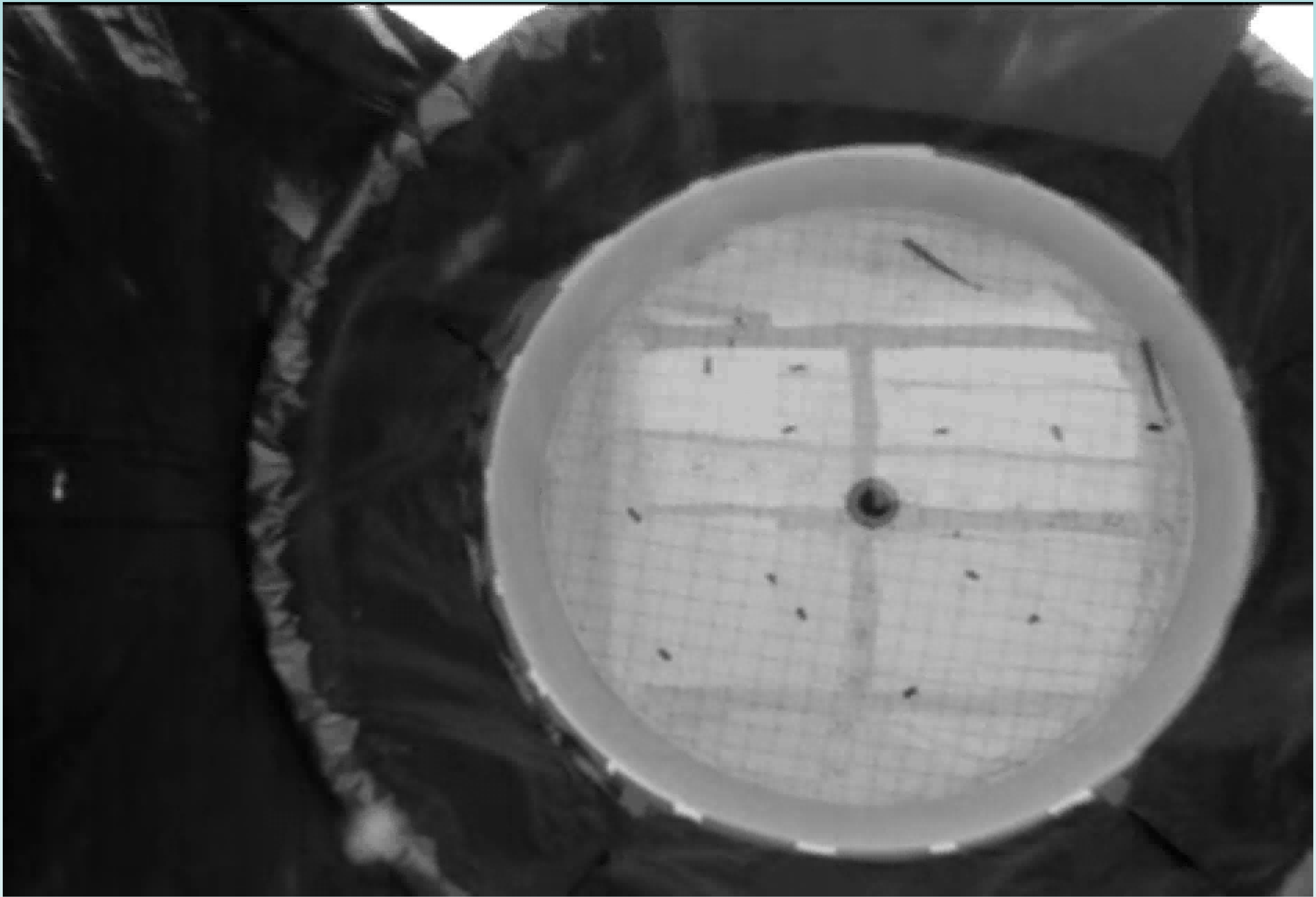


Predator chamber:
2 wild cutthroat (1h)

Prey chamber:
2 juvenile coho (15 min)

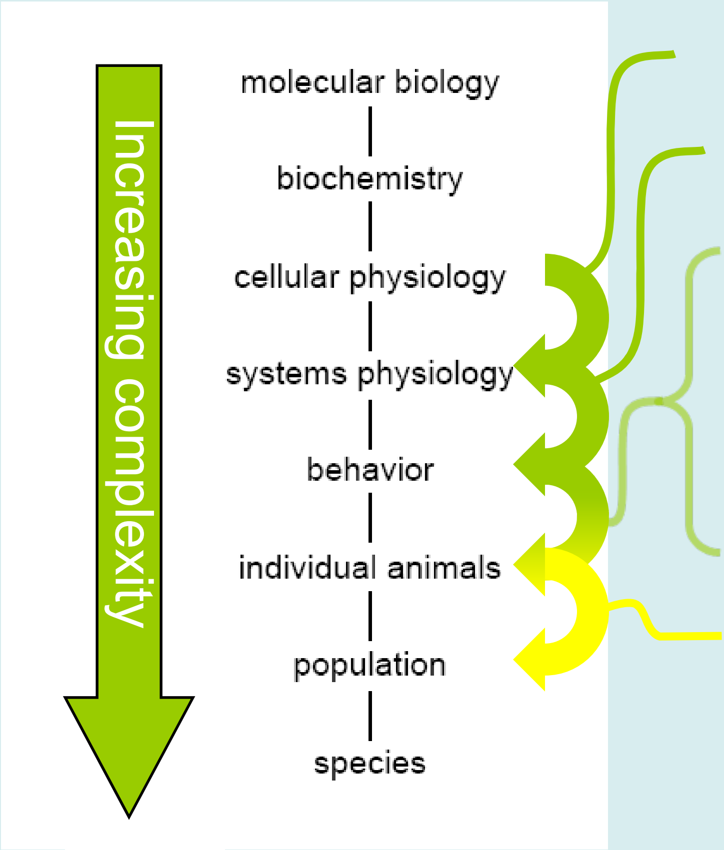
Skin extract tubing

When you move you lose



The Big Picture

Linking sublethal impacts of copper to survival



Hansen et al. 1999; Baldwin et al. 2003

Sandahl et al. 2007. ES&T.

Olfactory-mediated behaviors:

- ✓ **Defense**
- **Imprinting**
- **Homing**
- **Mating**

Next Step: Population Modeling

Major finding: low level exposures to copper in stormwater can have important sublethal impacts on salmon behavior and survival

A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival

JASON F. SANDAHL,[†]
DAVID H. BALDWIN,[‡]
JEFFREY J. JENKINS,[†] AND
NATHANIEL L. SCHOLZ[*,‡]

Oregon State University, Department of Molecular and Environmental Toxicology, 333 Weniger Hall, Corvallis, Oregon 97331, and NOAA Fisheries, Northwest Fisheries Science Center, Ecotoxicology and Environmental Fish Health Program, 2725 Montlake Boulevard East, Seattle, Washington 98112

Motor vehicles are a major source of toxic contaminants such as copper, a metal that originates from vehicle exhaust and brake pad wear. Copper and other pollutants are deposited on roads and other impervious surfaces and then transported to aquatic habitats via stormwater runoff. In the western United States, exposure to non-point source pollutants such as copper is an emerging concern for many populations of threatened and endangered Pacific salmon (*Oncorhynchus* spp.) that spawn and rear in coastal watersheds and estuaries. To address this concern, we used conventional neurophysiological recordings to investigate the impact of ecologically relevant copper exposures (0–20 μg/L for 3 h) on the olfactory system of juvenile coho salmon (*O. kisutch*). These recordings were combined with computer-assisted video analyses of behavior to evaluate the sensitivity and responsiveness of copper-exposed coho to a chemical predation cue (conspecific alarm pheromone). The sensory physiology and predator avoidance behaviors of juvenile coho were both significantly impaired by copper at concentrations as low as 2 μg/L. Therefore, copper-containing stormwater runoff from urban landscapes has the potential to cause chemosensory deprivation and increased predation mortality in exposed salmon.

AQUATIC NON-SCENTS

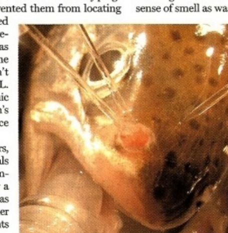
Repercussions of water pollutants that mute smell

BY JANET RALOFF

People complain about the way that fish smell. But it's the fish that should be doing the grumbling. In pristine waters, the animals smell quite well, thank you. Those tiny holes near fishes' mouths are, in fact, nostrils through which the animals draw in water to pump over olfactory nerves. By distinguishing scents, fish find food and mates and avoid predators.

Studies decades ago, for instance, showed that mechanically plugging the nostrils of adult salmon prevented them from locating their natal streams when they attempted to return home to spawn. The fish as juveniles had recorded memories of smells as they went to sea. Without detecting the olfactory signposts, the fish couldn't retrace their routes, says Nathaniel L. Scholz, a zoologist at the National Oceanic and Atmospheric Administration's (NOAA's) Northwest Fisheries Science Center in Seattle.

In a series of studies over the past 6 years, his group has demonstrated that metals and pesticides—at concentrations commonly found in streams—can impair a salmon's sense of smell just as effectively as plugging the nostrils did. Meanwhile, other scientists have shown that such pollutants block the sense of smell in other organisms.



NOSE JOB — Probes in this fish's nostrils measure neurons' ability to pass a scent signal to the brain. Pollutants such as copper and several pesticides shut down that signaling.

"What we're finding," says Scholz, is that "even short-term exposure to many of these pollutants—on the order of hours—can interfere with olfaction."

Researchers have reported that the impairment can disrupt the animals' normal behaviors in several ways. Fish use their keen sense of smell not only to navigate dark and cloudy waters but also to nose out scents indicating danger, such as chemicals from a predators' skin.

The studies are establishing that aquatic animals exposed to pollutants miss chemical cues that have life-and-death consequences, says ecotoxicologist Gregory C. Pyle of Nipissing University in North Bay, Ontario.

PESTICIDAL NOSE PLUGS North America's most widely used herbicide blunts a fish's sense of smell, according to work by Keith Tierney and his colleagues at Simon Frasier University in Burnaby, British Columbia. The herbicide is sold under a number of trade names, including Roundup.

A 30-minute exposure to a 1 parts per billion (ppb) concentration of atrazine reduced the activity of olfactory neurons in coho salmon (*Oncorhynchus kisutch*) by 11 percent, the researchers reported last November at the annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in Montreal. The animals' neural responses to alarm odors dropped by 45 percent. Higher doses of the herbicide triggered greater losses in smell; 100 parts per million atrazine eliminated any response to a predator's scent. River concentrations up to 20 ppb can occur briefly near farms that apply it, says Tierney.

Pure glyphosate, the active ingredient in atrazine, caused similar changes in salmon olfaction, although only at far higher doses than were required of the commercial herbicide formulation. At the November SETAC meeting, these researchers presented data showing that atrazine was 100 times as powerful at blocking fishes' sense of smell as was an equal quantity of pure glyphosate.

Atrazine contains a variety of ingredients added to glyphosate to increase the herbicide's adhesion to leaves and to retard its breakdown. Although these ingredients are listed as inert components on herbicide labels, Tierney's team concludes that they aren't inert as far as fish olfaction is concerned.

"I'd like to find out what those inerts are," Tierney says, but he notes that pesticide manufacturers regard them as part of their proprietary recipes.

Tierney isn't alone in his concern over supposedly inert ingredients. Some "4.1 billion pounds of inert [pesticide] ingredients are applied annually" to the U.S. environment, Christian E. Grue of the University of Washington in Seattle and his colleagues reported at the SETAC meeting.

Because these compounds aren't lethal to untargeted organisms, they don't require identification on labels, the Seattle researchers note—even though the inerts may exert a subtle but substantial toxic effect on aquatic life. Grue argues that "a new regulatory strategy is needed," which would require toxicity analyses of any supposedly inert ingredients.

Atrazine isn't the only chemical pesticide that can suppress a fish's ability to smell. Tierney's group showed that at exposures of about 10 ppb, the fungicidal wood-preservative known as IPB turned off olfaction in coho salmon. The researchers described that finding in the August 2006 *Aquatic Toxicology*.

They also reported in the October 2006 *Environmental Toxicology and Chemistry* that the insecticide endosulfan and the herbicides trifluralin and 2,4-D can impair a fish's sense of smell.

Scholz' group, too, has made contributions to the list of pesticides that affect fish olfaction. Six years ago, that team showed that diazinon significantly impaired responses by Chinook salmon (*Oncorhynchus tshawytscha*) to alarm scents and reduced their success in finding their natal pools.

Pesticides Detected in Urban Streams During Rainstorms and Relations to Retail Sales of Pesticides in King County, Washington

According to studies conducted in the Puget Sound Basin from 1987 to 1995 and summarized by Bortleson and Davis (1997), more types of pesticides were detected in urban streams than in agricultural streams. As well, in the Puget Sound Basin, more pounds of pesticides were applied in urban than in agricultural areas (Tetra Tech Incorporated, 1988). To provide some insight about sources of pesticides found in urban streams, the U.S. Geological Survey (USGS), the Washington State Department of Ecology, and King County collaborated to study and compare types of pesticides found in urban stream water with pesticide sales information from large home and garden stores.

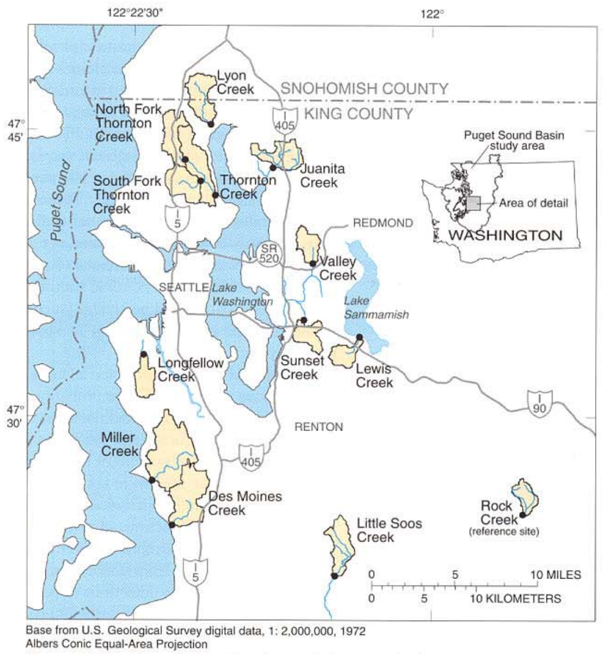


Figure 1. Location of sampling sites within watersheds.

Study Design

The study was designed to detect the largest number of pesticides likely to be transported in surface runoff to urban streams. Sampling occurred when pesticide applications to residential areas were high and when transport of pesticides to surface water would be likely. Sampling was conducted in April and May because data from home and garden stores indicate that pesticide application rates are higher in April and May than in any other months during the year.

Sampling was conducted during storms because previous sampling at Thornton Creek by the USGS showed that pesticide runoff is greatest during storms. Pesticides are not only more likely to be found during storms, but the concentrations of the pesticides found are also more likely to be of ecological concern.

From two to four surface-water samples were collected at each of 12 study sites in 10 urban or suburban watersheds in King County (fig. 1). Rock Creek, in an undeveloped basin, was sampled as a reference site.

Findings

Twenty-three pesticides were detected in water from urban streams during rainstorms, and the concentrations of five of these pesticides exceeded limits set to protect aquatic life.

During rainstorms, 23 of 98 pesticides sampled for were detected in water samples from 12 study sites in 10 urban watersheds. Concentrations of five insecticides exceeded recommended maximum concentrations set by the National Academy of Sciences and National Academy of Engineering (NAS/NAE) (1973). In a few samples, concentrations of Diazinon, carbaryl, and Lindane exceeded U.S. Environmental Protection Agency (USEPA) and other chronic aquatic-life criteria.

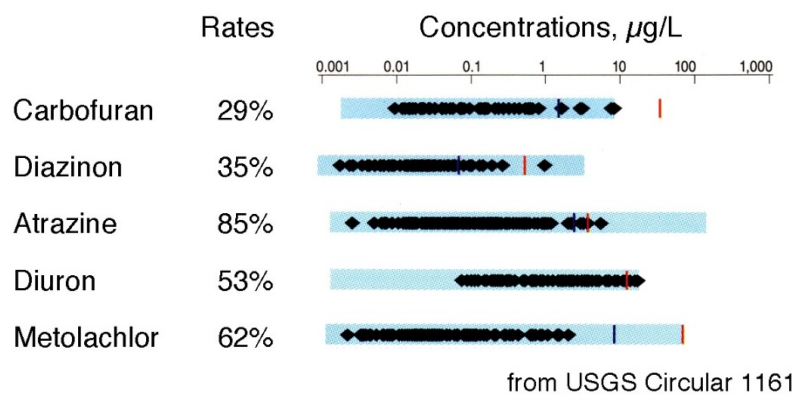
Pesticides used on lawns and gardens contribute to the occurrence of several pesticides in urban streams.

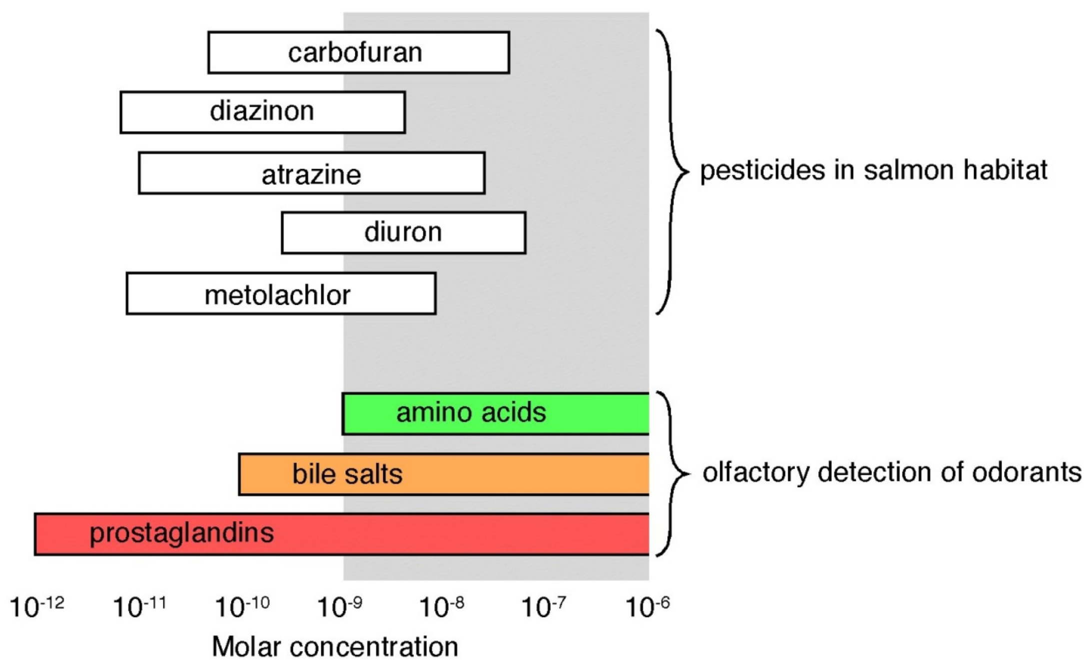
According to 1997 sales data from home and garden stores, of the pesticides sampled for, Diazinon, 2,4-D, and MCPP are the most frequently purchased pesticides by residents of King County. MCPP and 2,4-D are also among those pesticides used by professional applicators for pest control in residential, recreational, and industrial areas. The presence of these pesticides in water samples from all of the 12 study sites shows that their widespread application impacts water quality in urban streams. Also, residents purchased and applied four of the five pesticides that exceeded recommended maximum concentrations set by the NAS/NAE (Diazinon, carbaryl, Malathion, and chlorpyrifos).

Many pesticides found in urban streams might be the result of nonresidential applications.

Almost half of the 23 pesticides detected in stream water had no retail sales according to a 1997 survey of pesticides sales from home and garden stores in King County. Two of these pesticides (atrazine and simazine) were found at more than 60 percent of the study sites. This indicates that these pesticides are being applied to nonresidential areas in urban watersheds such as rights-of-way, parks, and recreational areas.

Surface water pesticide detections in the Willamette Basin





Focus on two major classes of current use insecticides

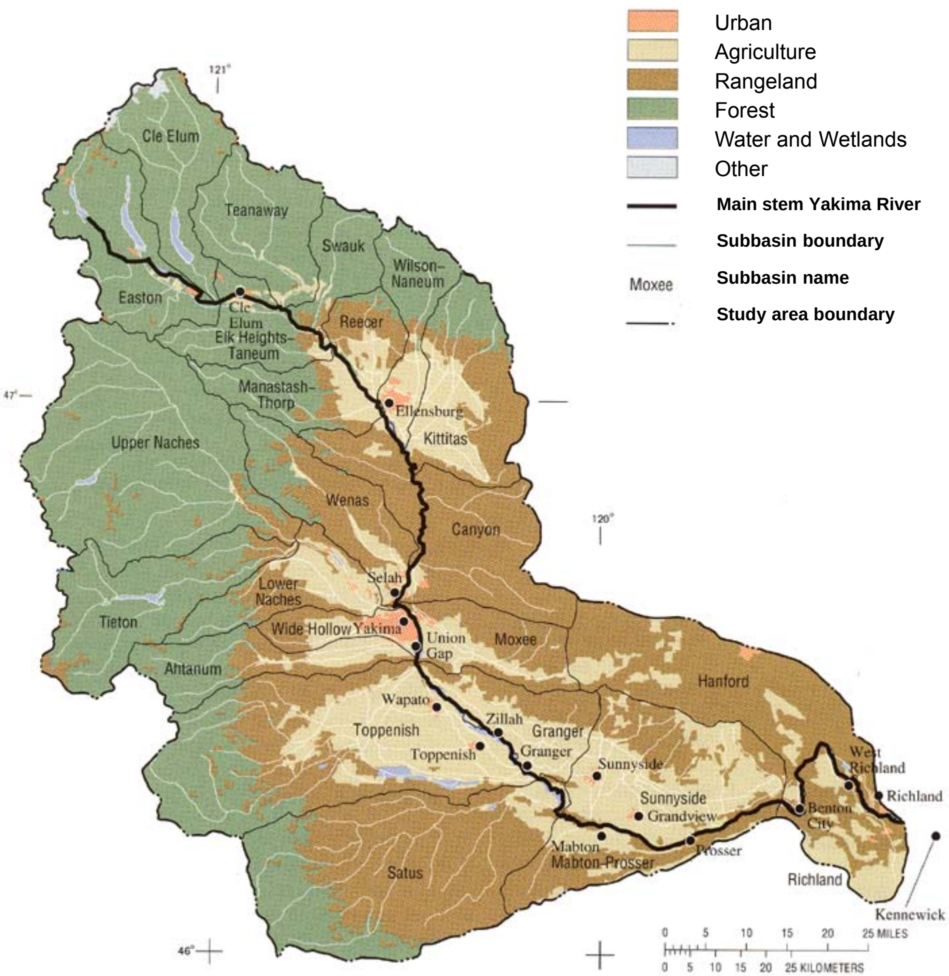
Organophosphates (e.g., chlorpyrifos, diazinon, malathion)

Carbamates (e.g., carbaryl, carbofuran)

- **Neurotoxic to both humans and wildlife**
- **Act by inhibiting the brain enzyme acetylcholinesterase (AChE)**
- **Disrupt several important behaviors in salmonids**

Homeowner use of diazinon and chlorpyrifos was phased out several years ago due to human health concerns (particularly children), but use is still high for many crops in western agricultural watersheds.

Surface water quality in the Yakima River Basin



Land use and land cover for subbasins in the Yakima River Basin (from Rinella et al., 1999).

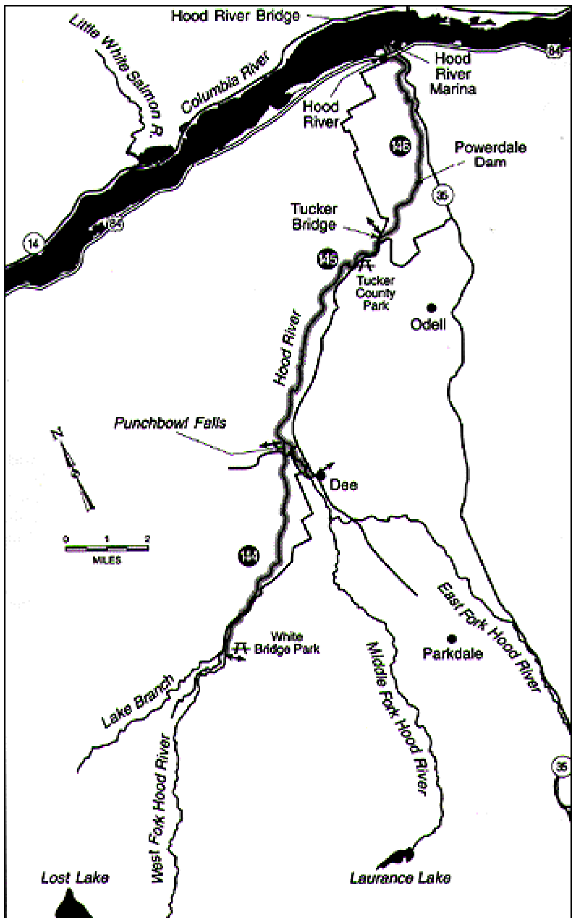
USGS surface water pesticide detections

(Modified from Rinella et al., 1999)

Herbicides		Insecticides	
2,4-D	Metolachlor	Azinphos-methyl	Methidathion
Atrazine	Metribuzin	Carbaryl	Methomyl
Bromacil	Picoloram	Carbofuran	Methyl parathion
Butachlor	Prometon	Chlorpyrifos	Methyl trithion
Cyanazine	Prometryn	Diazinon	Mevinphos
Dicamba	Propazine	Dimethoate	Parathion
EPTC	Simazine	Disulfoton	Permethrin
Fenoprop	Triadimefon	Ethion	Phorate
Hexazinone	Trifluralin	Flucythrinate	Phosphamidon
	Vernolate	Fonofos	Propargite
		Isofenphos	Trithion
		Malathion	

= pesticides with EPA Aquatic Life Criteria

The sublethal impacts of chlorpyrifos on juvenile salmonids - Hood River, OR



2003 laboratory study design:

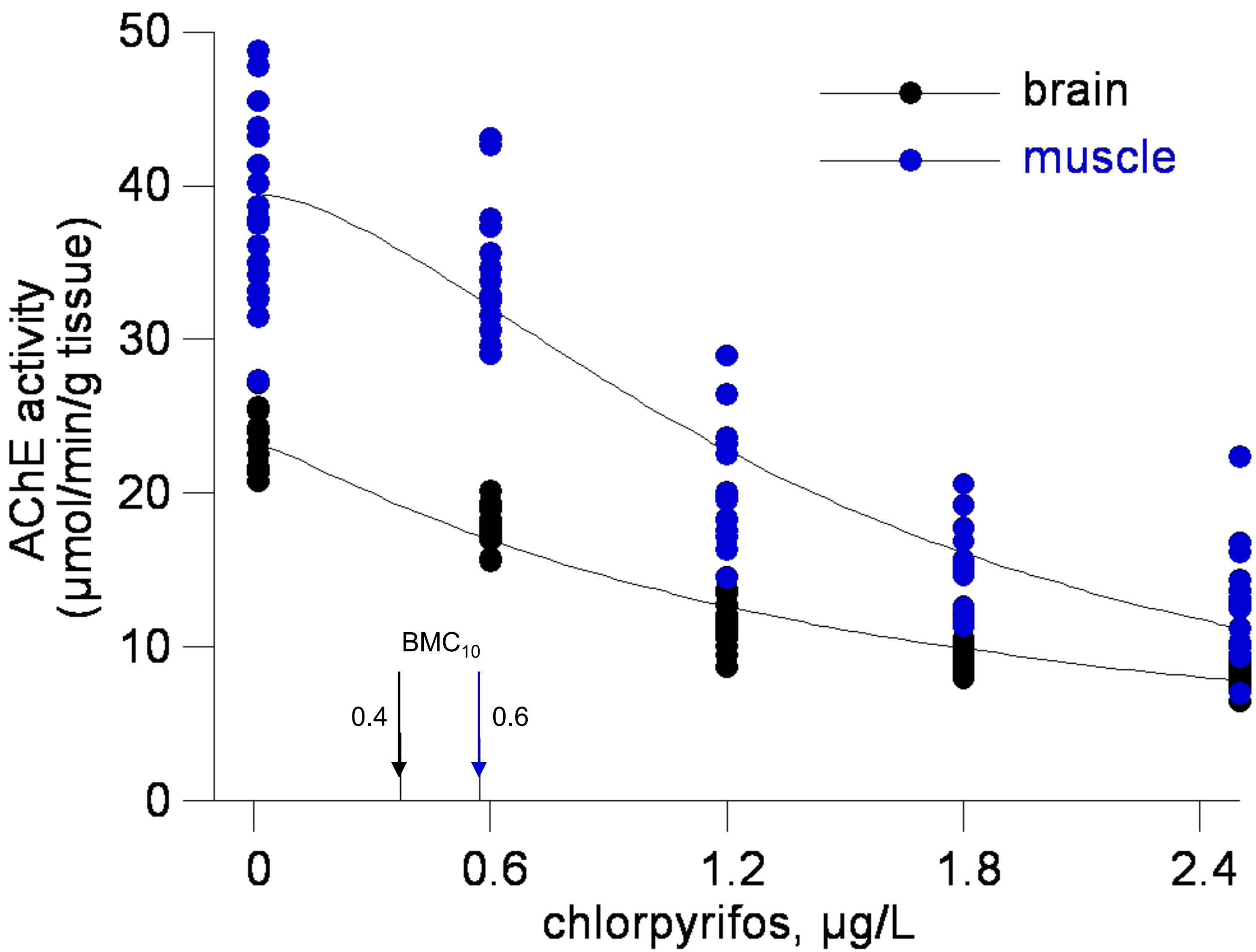
- juvenile coho (parr)
- 96 hr continuous exposures
- 24 hr static renewal
- nominal & measured pesticide concentrations
- computer-driven digital analyses of behavior

Monitored:

- muscle AChE activity
- baseline swimming behavior
- feeding behavior (rate of food strikes)

(Sandahl et al., 2005 Environmental Toxicology and Chemistry, Vol. 24, No. 1, pp. 136–145)

Impact of chlorpyrifos (96 hr) on coho AChE enzymatic activity



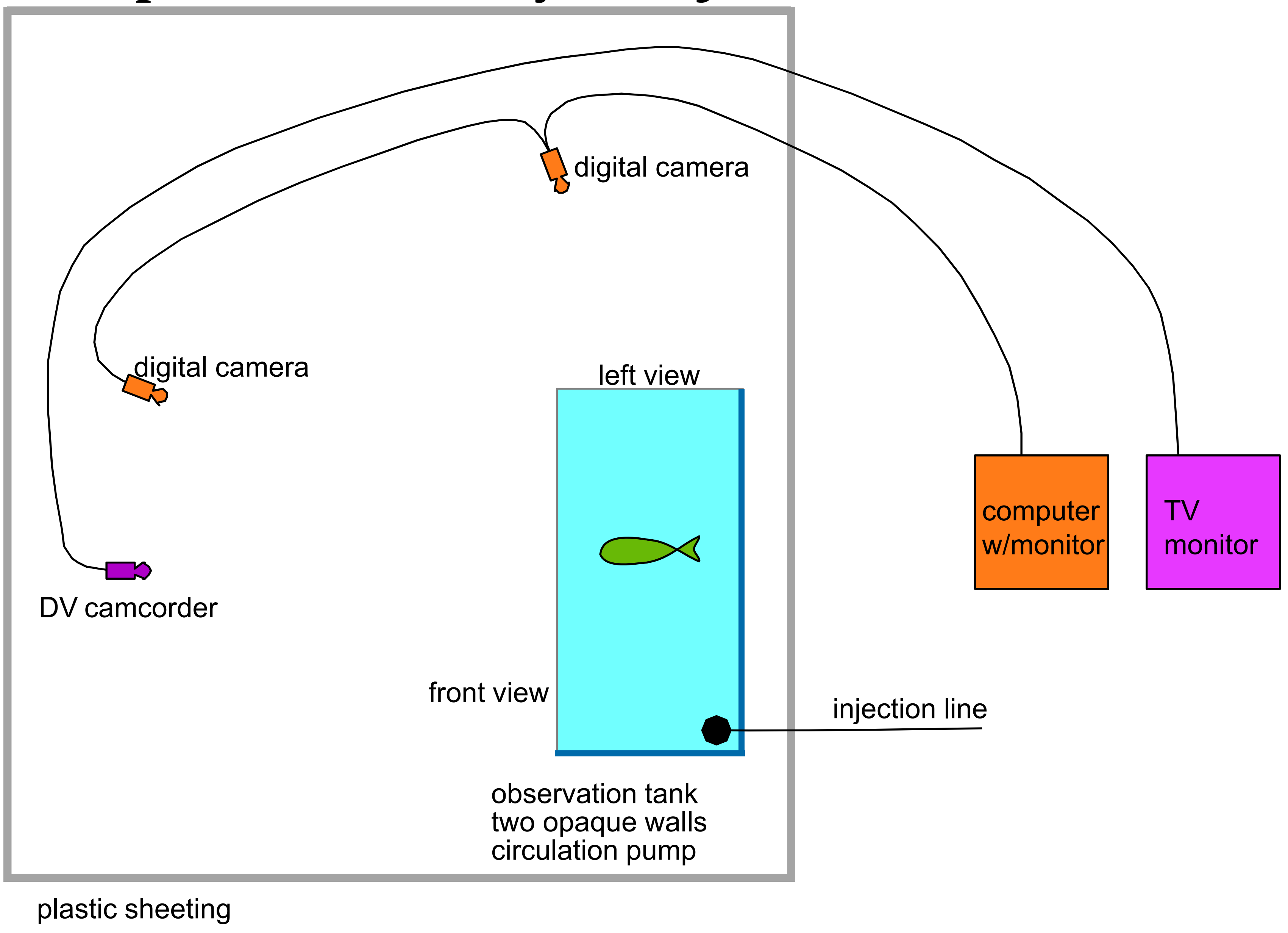
Chlorpyrifos exposure reduces brain and muscle acetylcholinesterase activity in juvenile salmon.

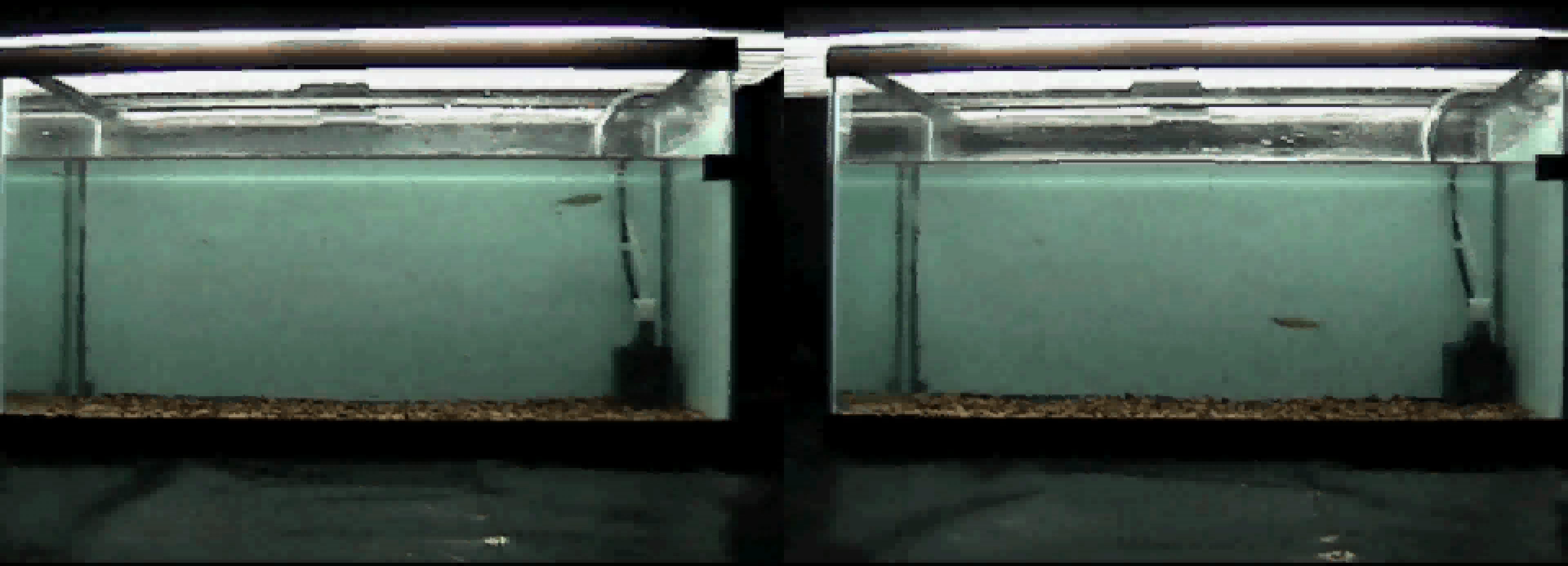
<u>So what?</u>

Are these reductions in enzyme activity correlated with impacts on behavior?

AChE activity as an indicator of exposure vs. an indicator of effect.

Computer-aided analysis of juvenile coho behaviors

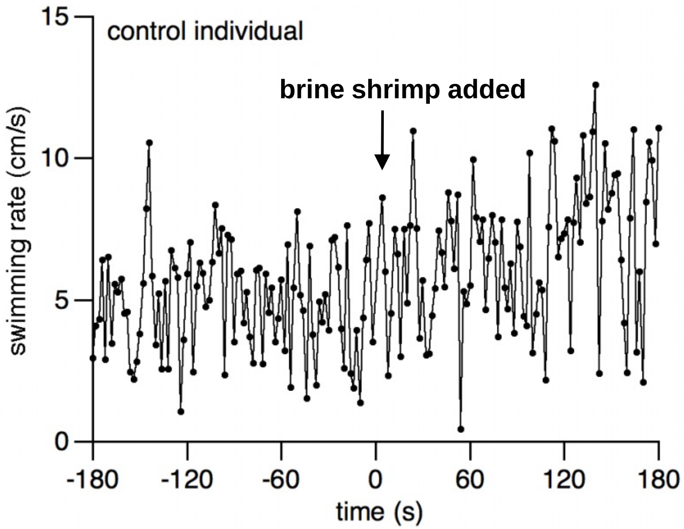


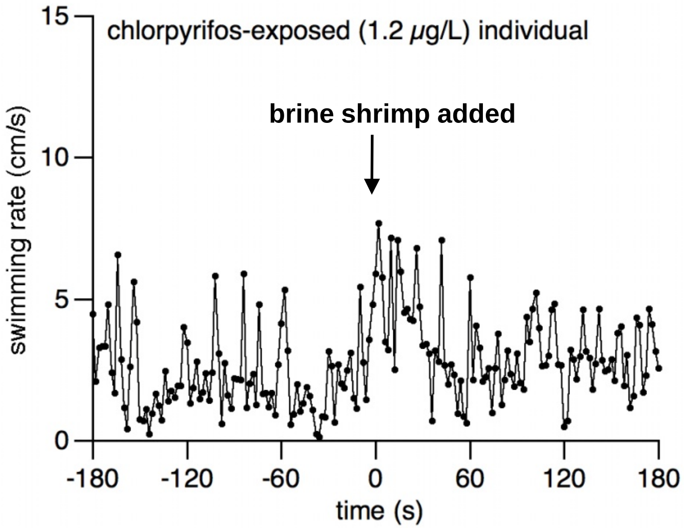


control

1.2 µg/L
chlorpyrifos

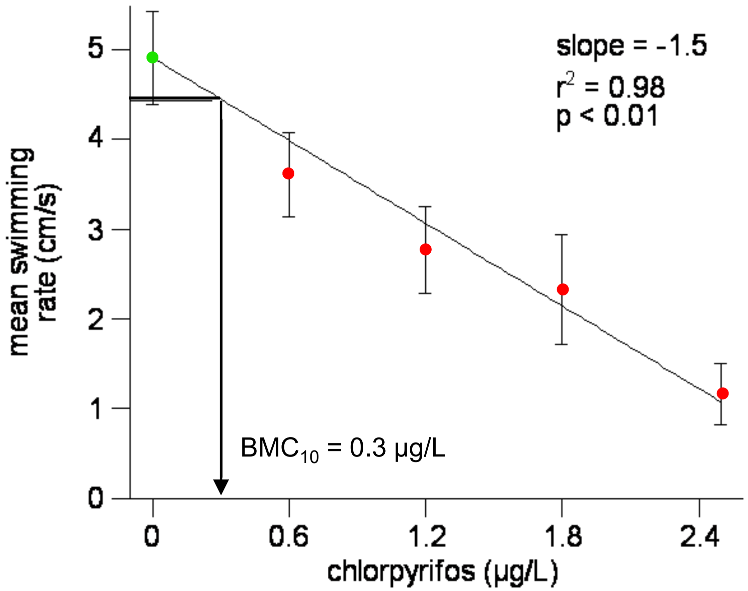
Juvenile salmon exposed to a single pesticide (chlorpyrifos) are lethargic and eat less



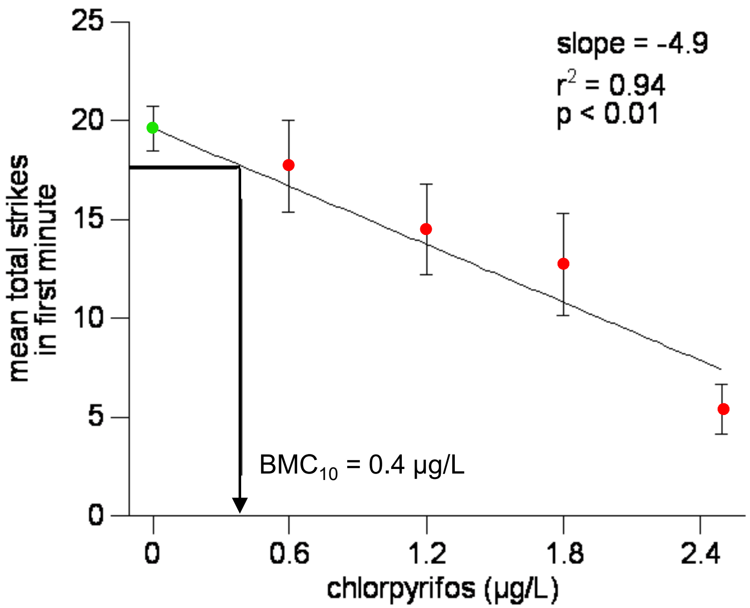


Impacts of chlorpyrifos (96 hr) on the behavior of coho

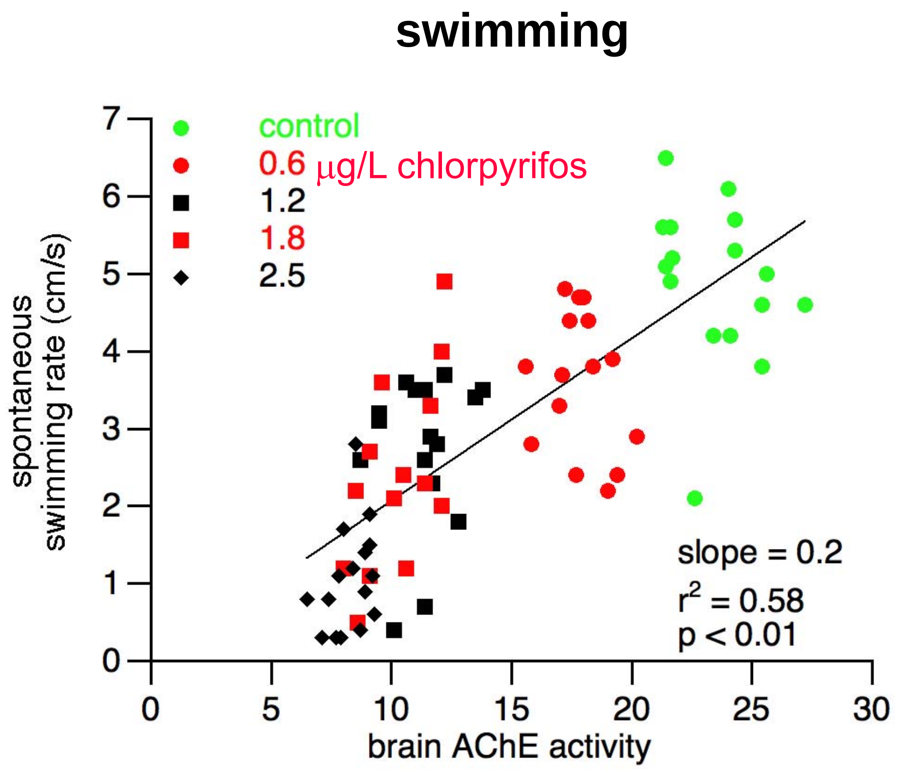
swimming

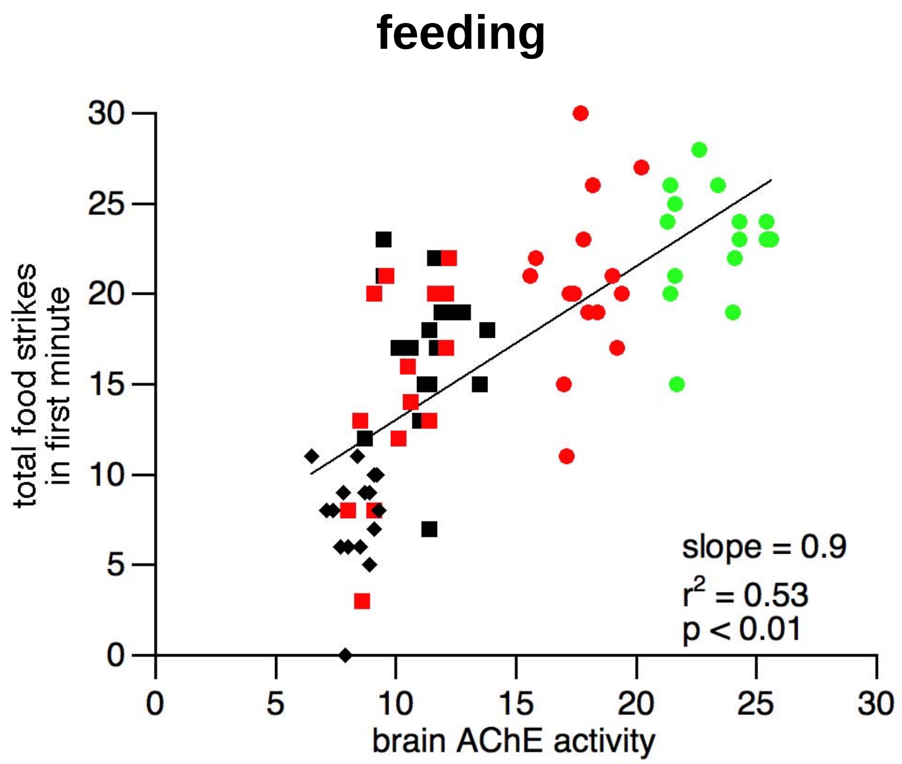


feeding



Swimming and feeding depression are closely correlated with AChE inhibition





Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., and Scholz, N.L. (2005). ***Environmental Toxicology and Chemistry,*** **24:136-145.**

Why do we care about reduced feeding behavior?

reduced feeding behavior

↓

reduced ration

↓

slower juvenile growth

↓

smaller size at ocean migration

↓

reduced ocean survival (size-selective predation)

model

lower individual ocean survival rates

↓

less productive wild populations over time

↓

slower recovery of ESA-listed species

model

3 CASE STUDIES

Act 1 - The sublethal effects of carbaryl on coastal cutthroat trout

Act 2 - The sensory physiology and behavior of juvenile coho exposed to pesticides

Act 3 – Pesticide mixtures and the environmental relevance to fish

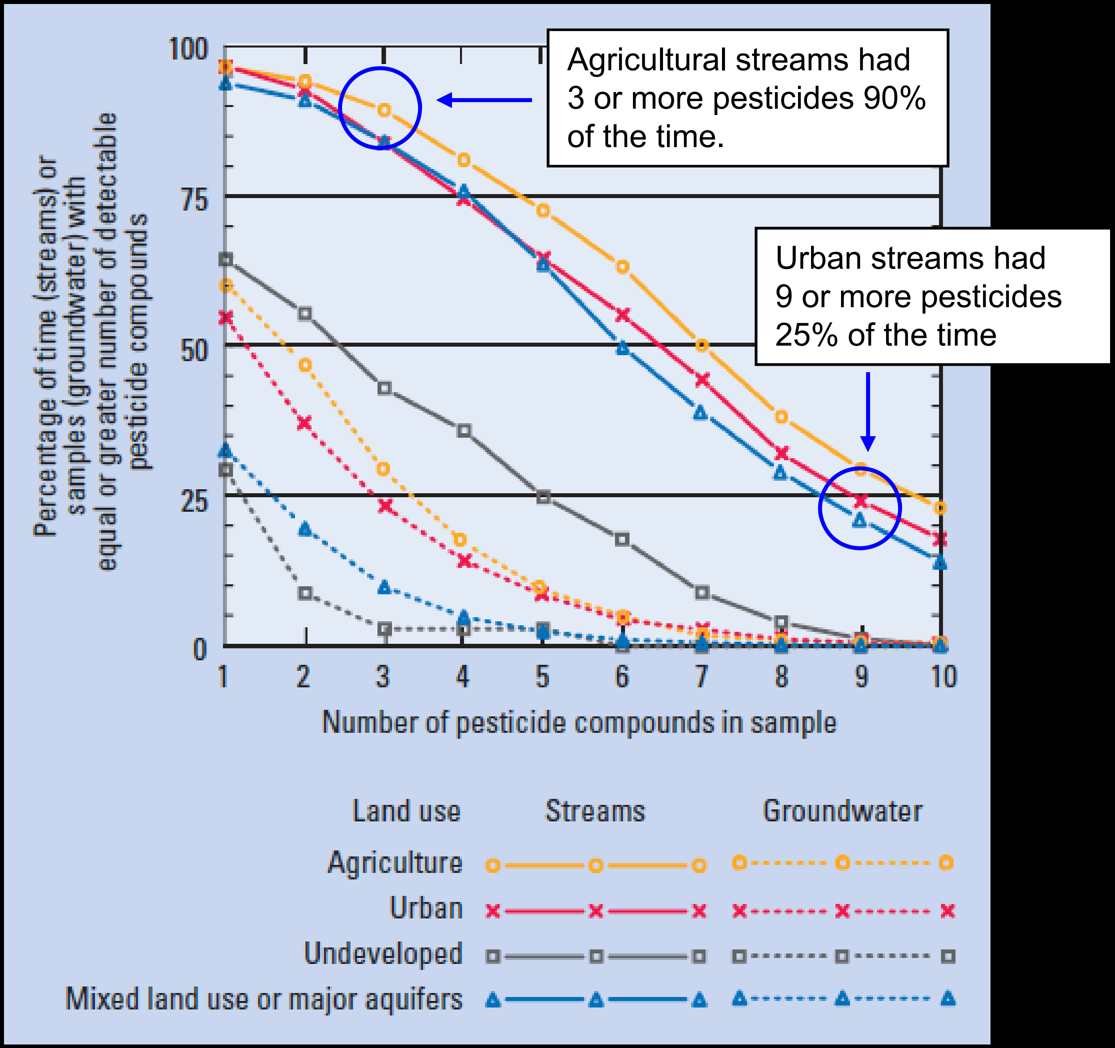
Uncertainty Example
Typical Problem - Mixtures

The disconnect between single-chemical aquatic life criteria and/or toxicological thresholds and actual ecological conditions in salmonid habitat.

The USGS' NAWQA program found that >50% of all surface water samples contain mixtures of five or more pesticides (USGS Circular1225).

Pesticides frequently occur as complex mixtures

Frequency of pesticides in water



Gilliom, RJ. 2007. Pesticides in U.S. Streams and Groundwater.
Environmental Science and Technology 41(10), 3409-3414.

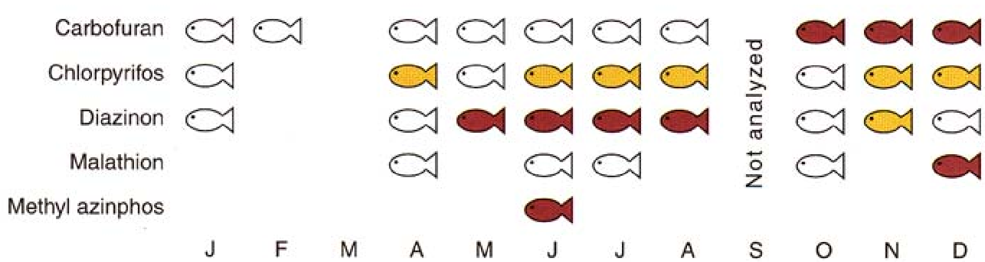
Insecticides are frequently detected in surface waters that also provide salmonid habitat.

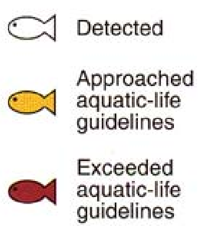
	Frequency of Insecticide Detections in Surface Water				
NAWQA Study Area	**Diazinon**	**Malathion**	**Chlorpyrifos**	**Carbaryl**	**Carbofurar**
Puget Sound	48%	D	3%	D	D
Central Columbia	4%	2%	9%	6%	5%
Yakima River	18%	D	D	90%	ND
Willamette	35%	5%	21%	18%	29%
Sacramento River	75%	33%	38%	60%	36%
San Joaquin-Tulare	71%	8%	52%	25%	5%

D= detected, frequency not reported
ND=not detected

Data from U.S. Geological Survey NAWQA Circulars 1237, 1159, 1161, 1216, 1144 and 1215

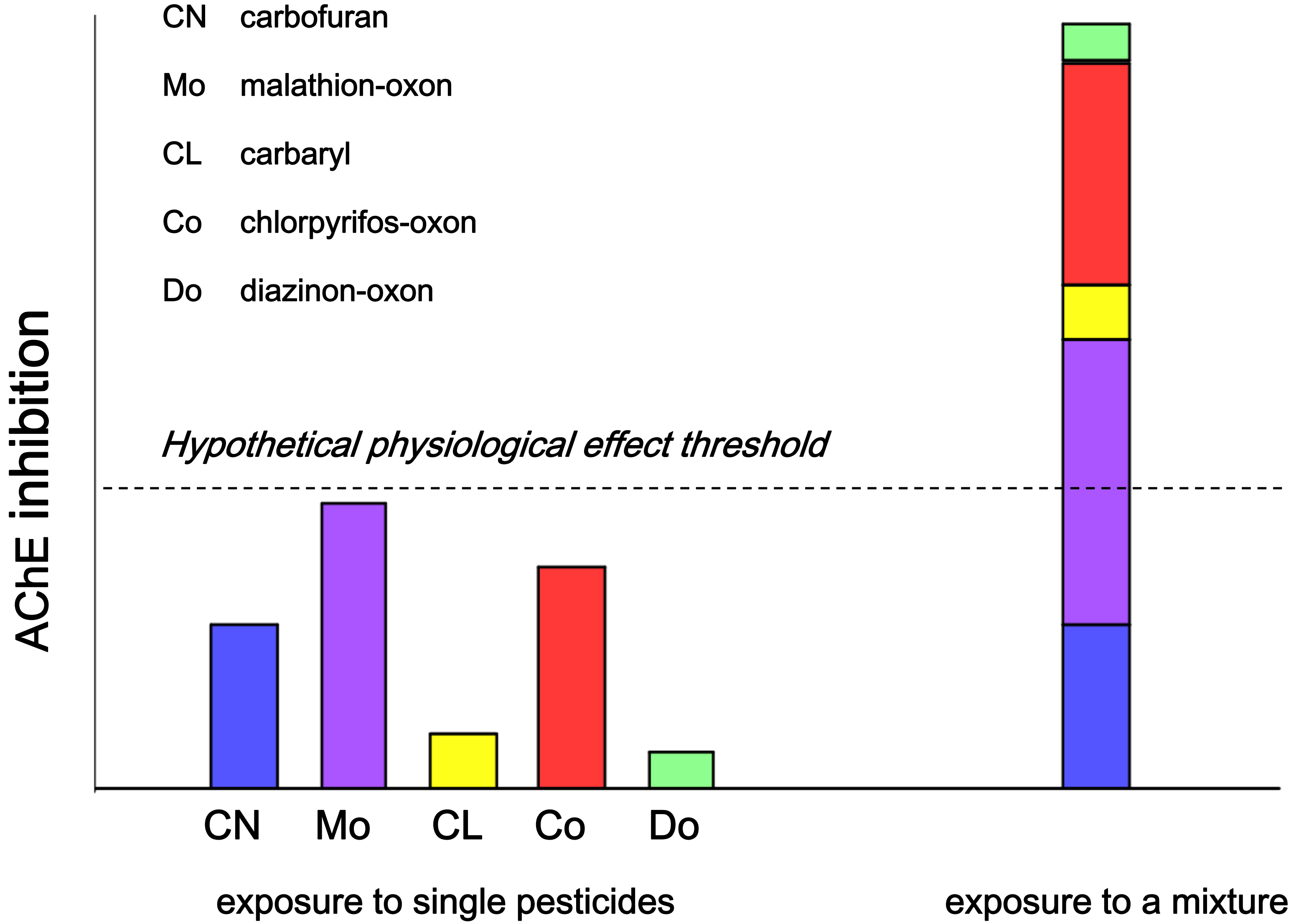
USGS science for a changing world
National Water-Quality Assessment (NAWQA) Program





Zollner Creek in the Willamette Basin receives agricultural runoff from intensively irrigated crops, including row crops, grass, wheat, hops, nurseries, and orchards. A wide variety of insecticides was applied to these crops; the insecticides were transported to the creek by irrigation and stormwater runoff. One or more insecticides were found in most water samples collected during the 2-year period, and several approached or exceeded concentrations that may be harmful to aquatic life, sometimes accurring as mixtures.

Potential Neurobehavioral Toxicity of Pesticide Mixtures

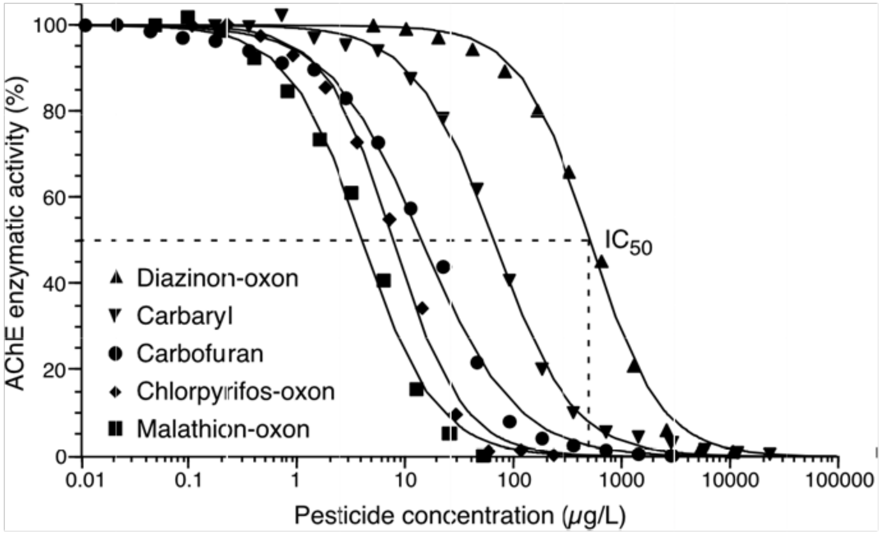


Pesticide Mixtures Alter Neurological Function and Swimming Behavior of Juvenile Coho Salmon

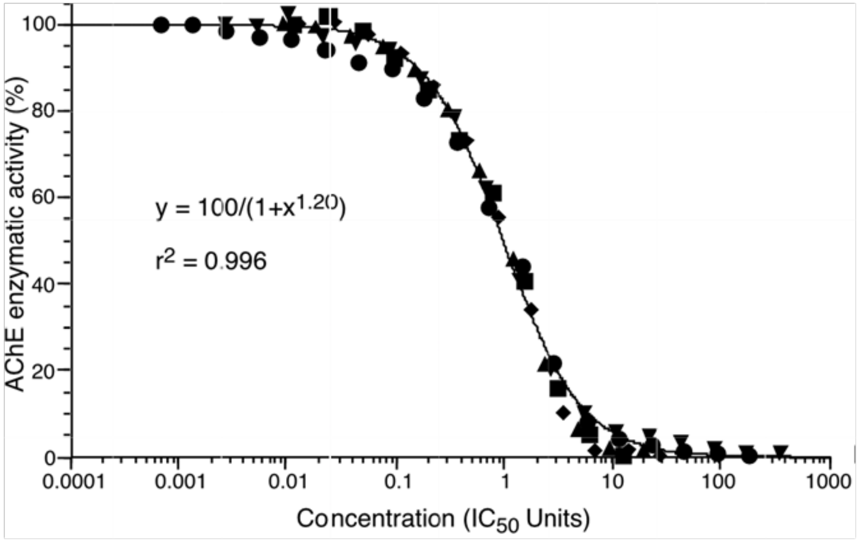
Cathy Laetz, David Baldwin, and Nathaniel Scholz
NOAA Fisheries, NWFSC, Seattle
John Stark
Washington State University, Puyallup

Acetylcholinesterase (AChE) inhibition *in vitro*

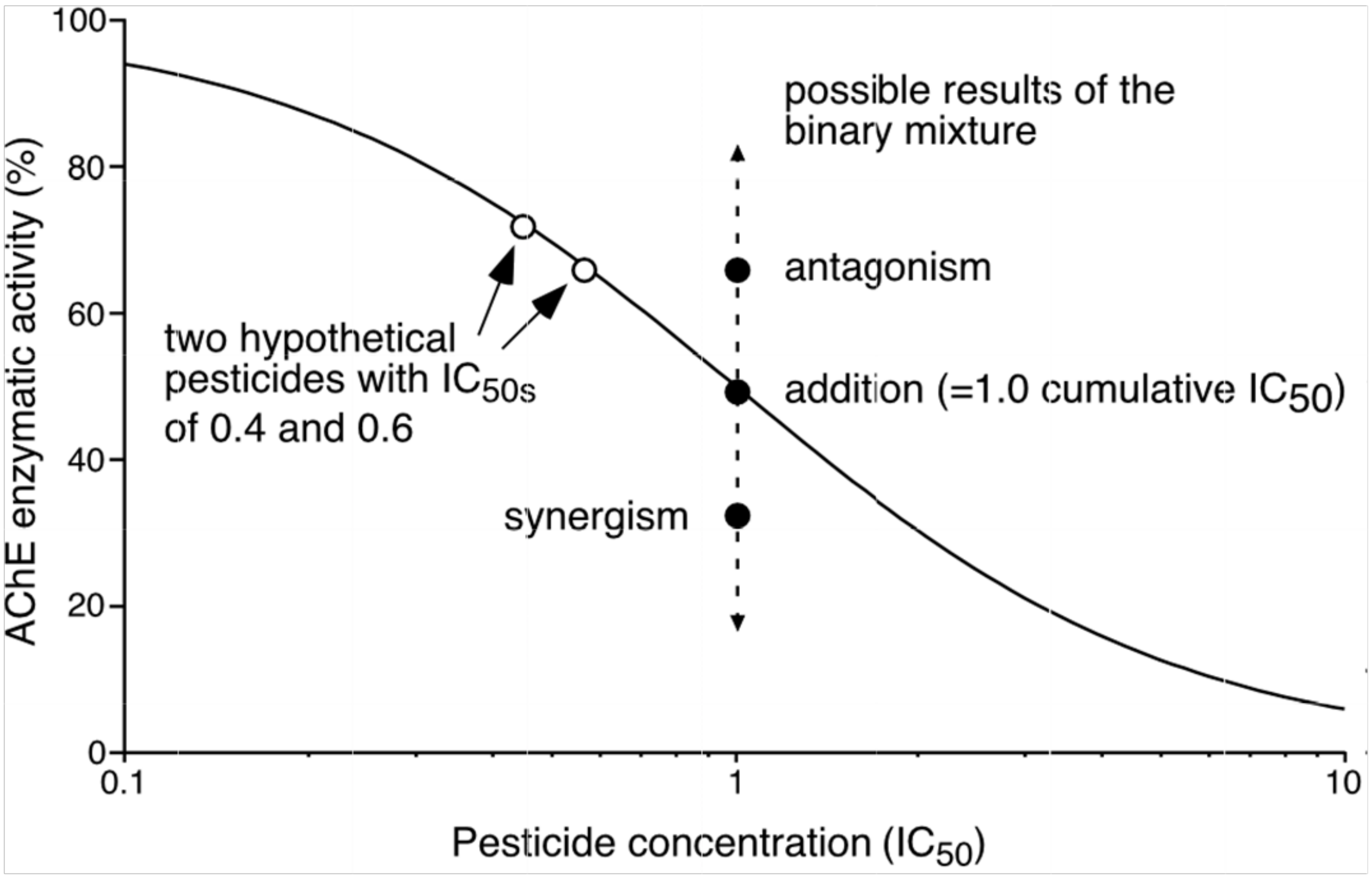
Individual dose-response curves



Pooled data (IC_{50}-normalized)

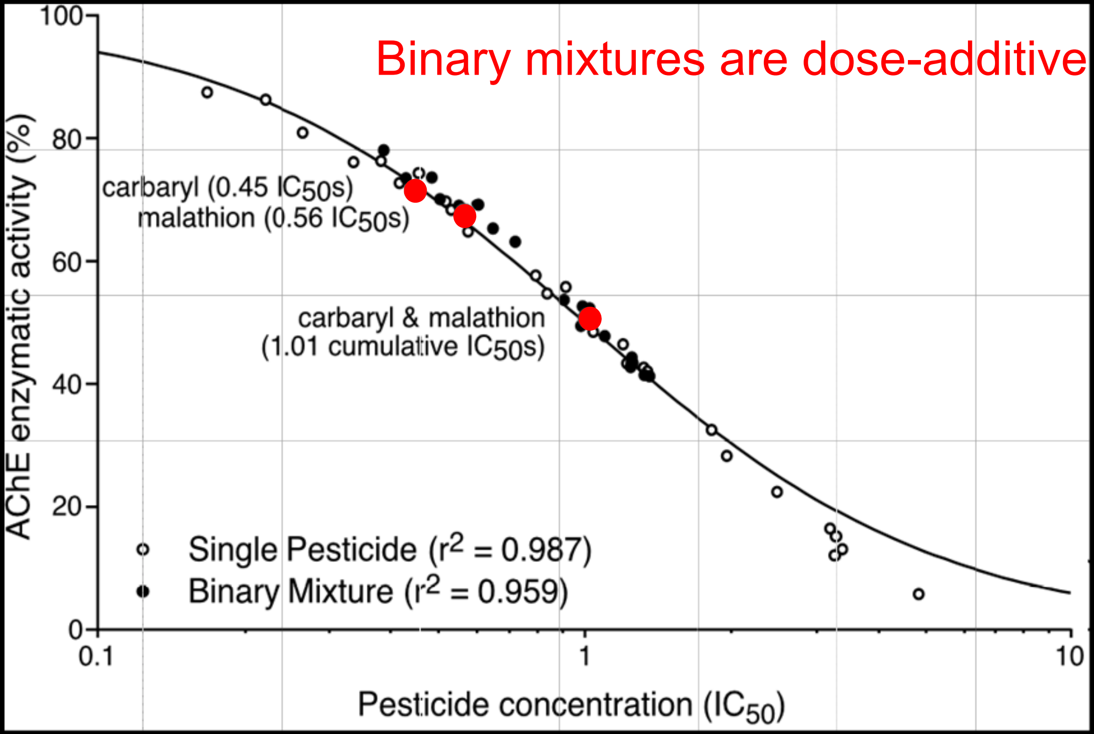


Hypothesis:
Pesticide mixtures produce additive AChE inhibition



Results

Inhibition of AChE is dose-additive after *in vitro* exposure to mixtures of organophosphate and carbamate insecticides.



Scholz et al., 2006, *Environ. Toxicol. Chem.*, 25(5)

What about *in vivo* exposure to mixtures?

New Hypothesis

<u>Study Objective</u>

Determine whether brain AChE activities in juvenile coho salmon *(Oncorhynchus kisutch)* are dose-additive after *in vivo* exposure to binary mixtures of carbamate and organophosphate insecticides.

C. Laetz, NOAA-Fisheries

NW Fisheries Science Center

Seattle, WA

Methods - Study Design

In vivo exposures to anti-cholinesterase insecticides

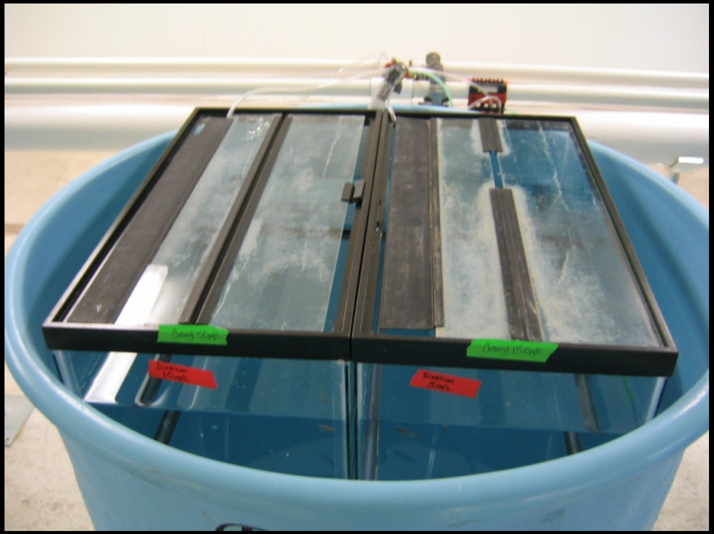
Washington State University, Puyallup

Study design:

- juvenile coho (age 0+)
- 96 hr exposures (n=8 fish at each concentration)
- 24 hr static-renewal
- individual pesticides and binary mixtures
- water and vehicle controls
- measured exposure concentrations
- AChE activity measured using modified Ellman procedure

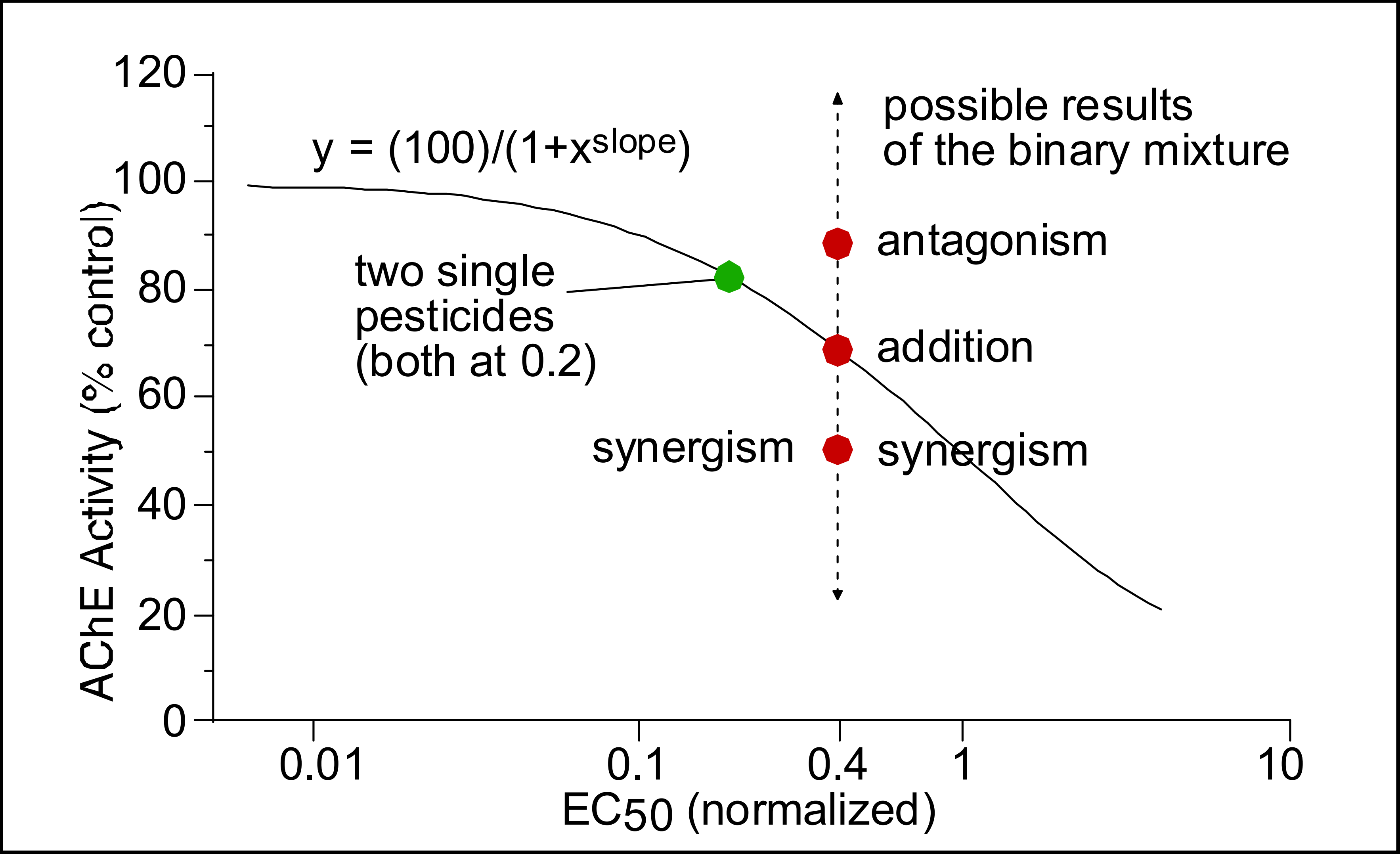
Insecticides:

- carbaryl
- carbofuran
- diazinon
- chlorpyrifos
- malathion



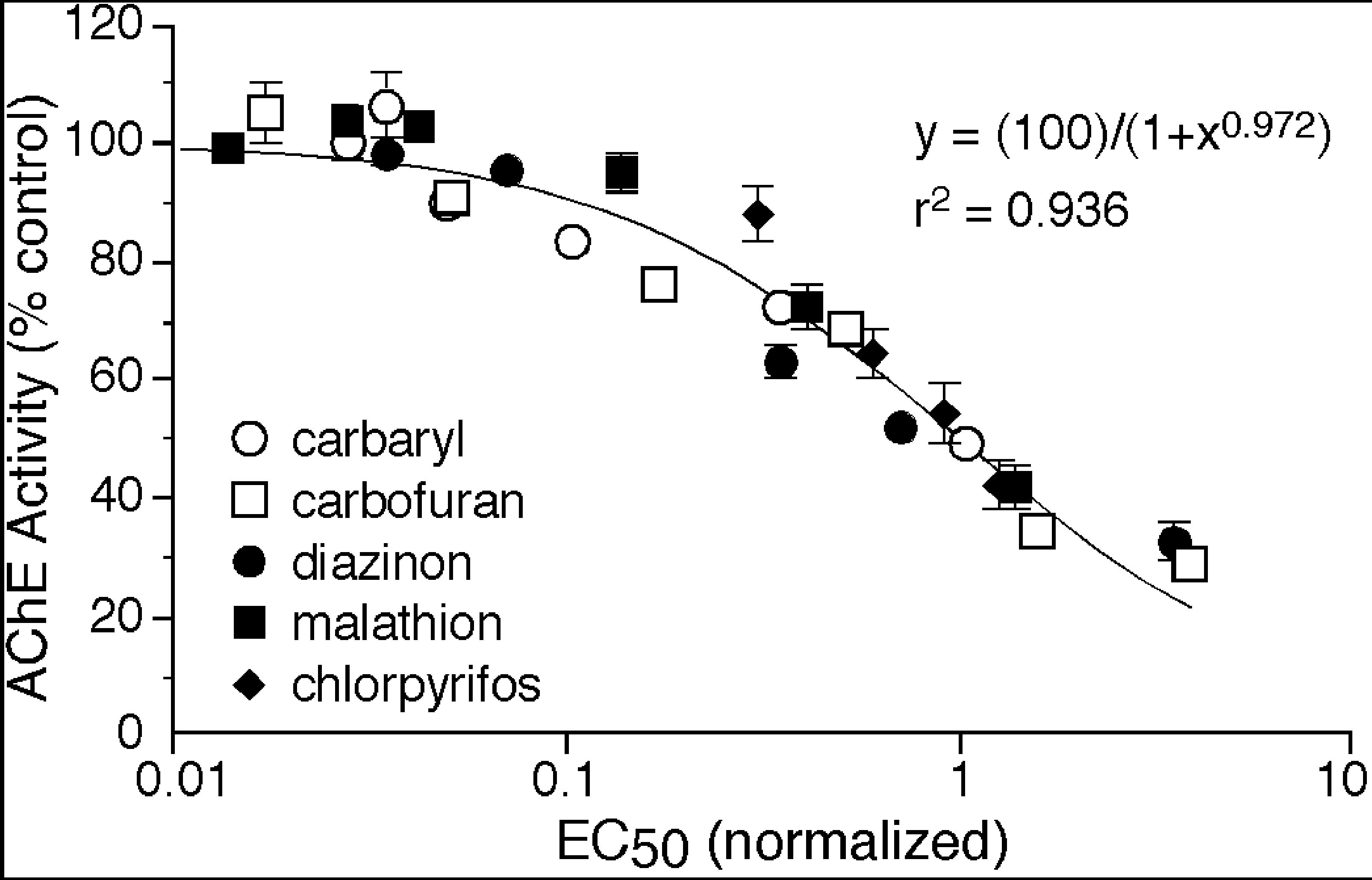
Results - Possible Mixtures Outcomes

Quantitative basis for determining dose-addition



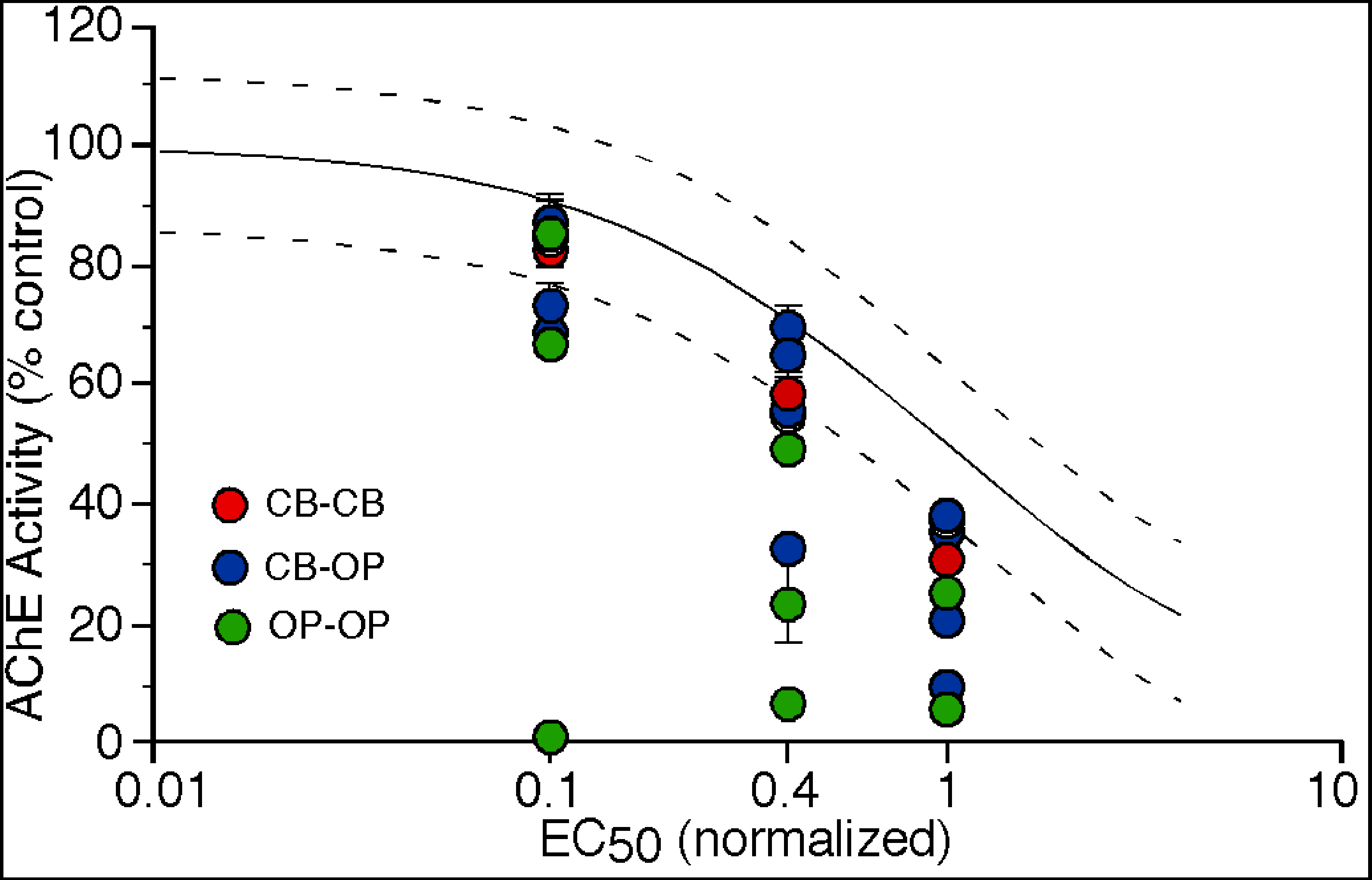
Results

Single pesticides produce dose-dependent inhibition of AChE

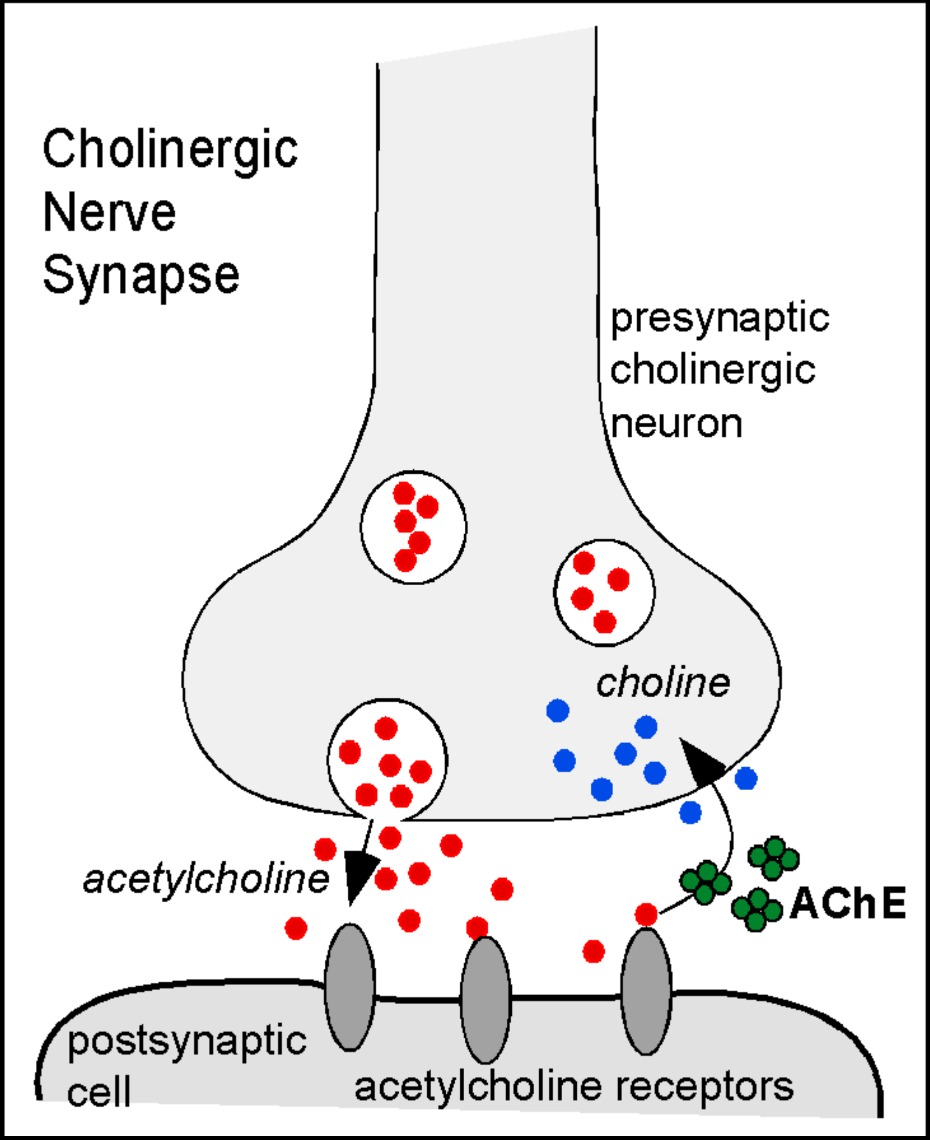


Results

Pesticide mixtures are additive or synergistic



Results



Carbamate (CB) and organophosphate (OP) insecticides bind to AChE, inhibiting its ability to terminate synaptic transmission.

In fish, symptoms of AChE inhibition include lethargy, loss of orientation, increased mucus production, and excitability.

Results

Mortality at sublethal concentrations

All fish exposed to the highest concentrations of the diazinon-malathion mixtures **died within 24 hours**.

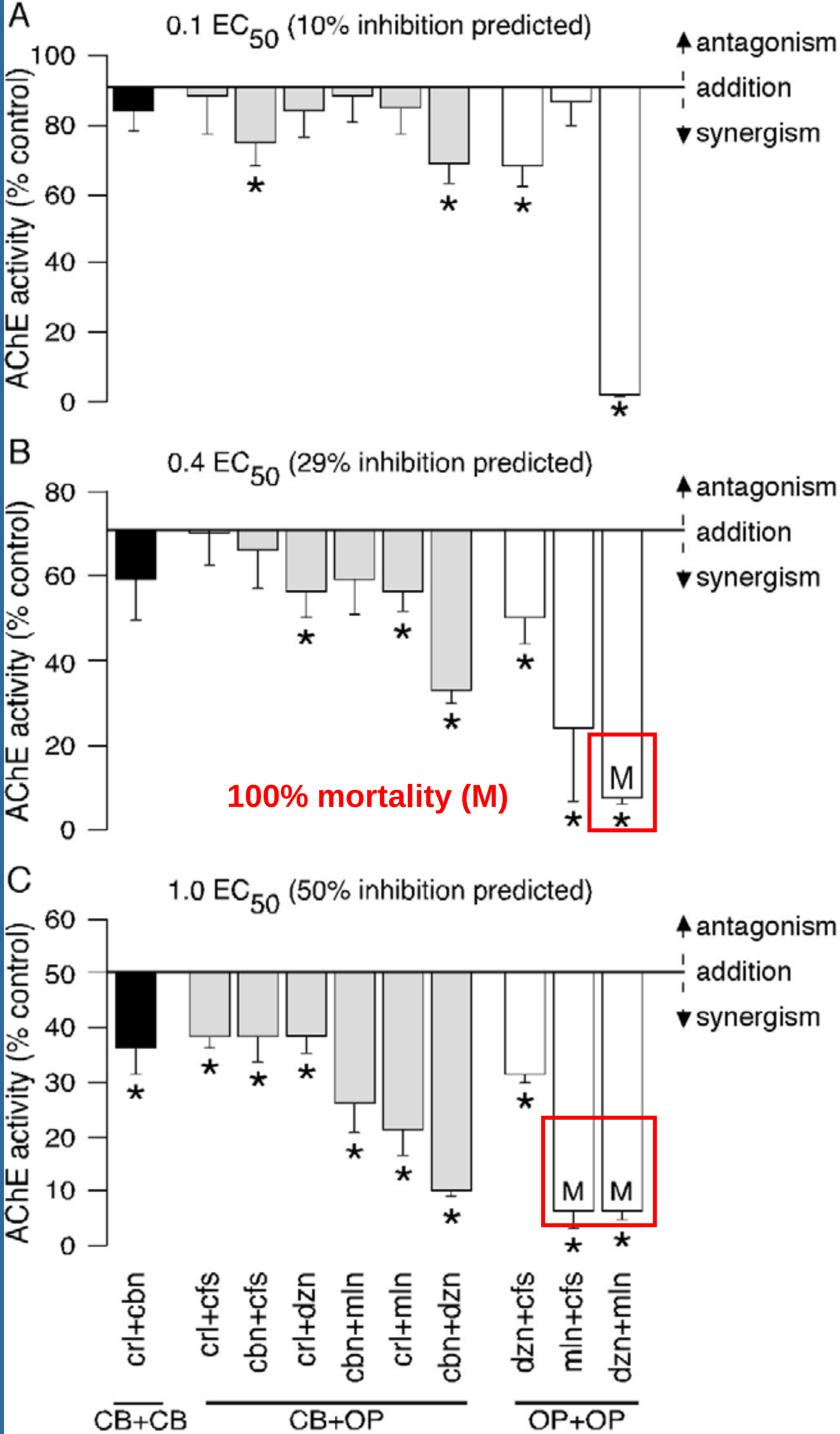
Fish survived exposure to the lowest concentrations of that mixture, but displayed loss of equilibrium, lethargy, and AChE inhibition > 80%.

Pesticide mixtures can be synergistic

Several mixtures showed greater inhibition than expected for dose addition.

Some mixtures of OP insecticides were lethal at concentrations that were sublethal when applied singly.

Laetz et al., 2009. Environmental Health Perspectives 117(3), 348-353.



Summary

Mixtures of neurotoxic pesticides are common in fresh waters that provide habitats for threatened salmonid species.

Exposures to pesticide mixtures produced either additive or synergistic toxicity, with some combinations being unexpectedly lethal.

Fish behavior, as measured by spontaneous swimming speed, was significantly impacted at low pesticide concentrations.

Implications

Single chemical ecological risk assessments will underestimate actual impacts on ESA-listed salmonids where mixtures occur.

Due to a potential for synergism, pesticide mixtures may be a more important obstacle to salmonid recovery than previously appreciated.

Relevant Papers by NOAA’s NWFSC in Seattle, WA

The Synergistic Toxicity of Pesticide Mixtures:

Implications for Risk Assessment and the Conservation of Endangered Pacific Salmon

Environmental Health Perspectives Vol. 117, No. 3, pp. 348-353, 2009

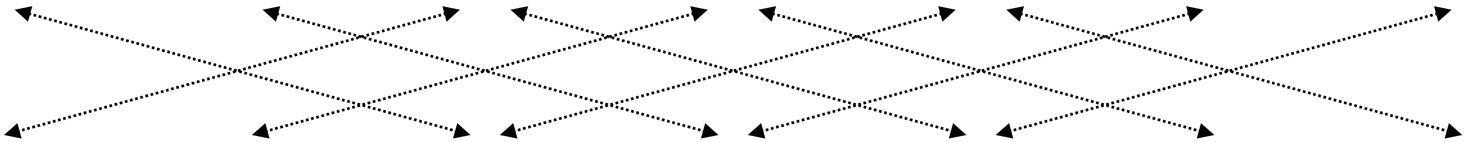
http://www.ehponline.org/docs/2008/0800096/abstract.html

Dose-additive Inhibition of Chinook Salmon Acetylcholinesterase Activity by Mixtures of Organophosphate and Carbamate Insecticides

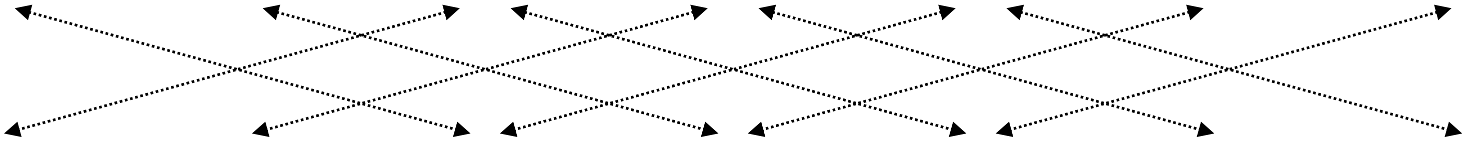
Environmental Toxicology and Chemistry, Vol. 25, No. 5, pp. 1200–1207, 2006

Chemical Habitat Quality and Fish Health

PAHs
organochlorines
dioxins
phthalates
DDTs
antibiotics
metals
PCBs
synthetic hormones
pharmaceuticals
current use pesticides
organobromines



nervous system function and behavior
immune function and pathogen resistance
reproductive and endocrine function
pathology and disease
early development
hazard assessment and risk modeling



ling cod
canary rockfish
coho salmon
steelhead
Pacific herring
surf smelt
delta smelt
northern anchovy

Questions?