

- ❖ **Overview of PAH ecotoxicology**
- ❖ **Growth effects in juv. chinook from dietary PAHs**
- ❖ **Assessing PAH dose in fish**

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# Short review of Ecotoxicology PAHs

- ❖ **General info / occurrence of PAHs**
- ❖ **Factors that affect bioaccumulation**
- ❖ **Toxicity of PAHs to biota**

# Ecotox review – PAHs

## General info

- ❖ 100's of PAHs. All 2 – 6 rings, 120 – 300 daltons
- ❖ LPAHs (2 – 3) and HPAHs (4 – 6)
- ❖  $K_{ow}$  – about 4 orders of magnitude (3 – 7)
- ❖ Alkylated homologs – more persistent and toxic
  - naph, fluorene, DBT, anthracene, phenanth, pyrene, chrysene
  - C1, C2, C3, C4 – n carbons in alkyl group. e.g. C2: dimethyl- or ethyl-
- ❖ Analysis – usu. 16, 24, or 39+ compounds/classes

# Ecotox review – PAHs

## Factors that affect bioaccumulation and toxicity

- ❖ **Kinetics - uptake and elimination (passive loss, excretion, biotrans)**
  - biotransformation. High variability among taxa
  - inverts, often low metabolism.
  - verts - high biotransformation. Most of dose metabolized quickly, sent to bile, excreted
- ❖ **Previous exposure – naïve Fundulus >> % of abnorm embryos when exposed to PAH contam sed vrs. native fish (heritable)**
- ❖ **Route of exposure - water, diet, contact**
- ❖ **Environmental stressors – pH, temp, D0<sub>2</sub>, salinity, other toxicants. Any factor that affects kinetics or potency**

# Ecotox review – PAHs

## Factors that affect bioaccumulation and toxicity

### ❖ Trophic transfer

- not likely, but depends on species. Metabolites?

### ❖ Lipid

- important for amount bioaccumulated and toxic response

### ❖ Photoactivation

- some PAHs (anth, benz[a]anth, FLA, benz[b]fla)
- bioaccumulation for phototox, environmental relevance?

# **Ecotox review – PAHs**

## **toxicity to biota**

- ❖ **Responses / modes / mechanisms of toxic action**
  - **responses: impairment of growth, development, and reproduction. Also lethal, mutagenic, immunotoxic.**
  - **modes imply impaired function (ACh, uncouplers, narcosis)**
  - **modes: specific and non-specific (narcosis)**
  - **mechanisms are specific biochemical event(s)**
  - **PAH mechanisms: mostly unknown. Some involve AhR, but actual biochemical event unknown. Important for mixtures to determine if additive (dose or response)**

# Ecotox review – PAHs

## toxicity to biota

- ❖ Lethal response - acute (short-term) exposure ( $\leq 96$  h)
  - LC<sub>50</sub> values, water 10 – 100,000 ng/mL, sediment  $> 2$   $\mu\text{g/g}$ , soil LC<sub>50</sub> 50 - 150  $\mu\text{g/g}$
  - mode of action is generally narcosis (high conc in membrane lipids that act by disrupting ion channels)
  - tissue residue, LR<sub>50</sub> usually 2 – 8  $\mu\text{mol/g}$  ( $\approx 400 - 1,600$   $\mu\text{g/g}$ )
  - rat & mouse LD<sub>50</sub> values for various PAHs are 50 – 2000  $\mu\text{g/g}$
  - acute toxic responses are rare in the environment

# **Ecotox review – PAHs**

## **toxicity to biota**

### **❖ Sublethal responses**

- Impaired immune system**
- Inhibition of growth**
- Reproductive effects**
- Developmental impairment**
- Tumors and histological effects**
- Behavior – generally not affected except for narcosis and skeletal abnormalities**
- Others?**

### **❖ Most rapidly proliferating cells susceptible**



# Ecotox review – PAHs immune system

## Specific and non-specific components of immune system

- **non specific** (phagocytic activity, natural killer cells, eosinophils, leukocyte oxidative burst)
- **specific** (antibody response, T-cell and B-cell mediated immunity)

## Few studies on fish and inverts

RBT – LOEC of 17 ppb creosote in water (0.6 ppb PAHs)

mice – ED<sub>50</sub> of 0.03 - 0.14  $\mu\text{g} / \text{g bw} / \text{d}$  (meCholanthrene, DBA, DMBA)

# Ecotox review – PAHs growth and development

## ❖ Growth

- Several studies showing reduced somatic growth
- Inverts – earthworm  $EC_{10}$  30 ppm. Bivalve  $EC_{50}$  for indiv. PAHs with and without UV ( $\Delta$  1000x). Only minor diff for oil exposure

## ❖ Development

- Several studies on vertebrates and many on fish
- Skeletal abnormalities, yolk sac edema, pericardial edema, egg/larval mortality, reduced incubation time

# Ecotox review – PAHs

## Reproductive effects

### ❖ Endocrine disruption

- PAHs are generally anti-estrogenic (antagonistic), however some OH-metabolites can be estrogenic (agonistic)
- Steroids metabolized via P450; induction may dec steroid hormones
- Anti-estrogenic: One study found 15x ↓ in Vtg in reproductive RBT from creosote exposure (0.11 ng total PAH/mL)
- Estrogenic: metabolites give + on YES assay

# **Ecotox review – PAHs**

## **Reproductive effects**

### **❖ Organismal level**

- Few studies showing organismal effects**
- Several studies showing effects on thymic gluco-corticoid receptors, oocyte and follicle integrity, and placental function in small mammals (mostly BaP).**
- Field studies showing inhibited gonadal growth, precocious female development, and infertile eggs in *E. sole*. Also decreased sperm quality and egg hatchability in plaice**
- Most effects related to depressed estradiol?**

# **Ecotox review – PAHs tumors and histological effects**

- **Overwhelming evidence that PAHs are mutagens**
- **Cause genetic damage that can lead to toxicopathic lesions and tumors**
- **Mostly 4 – 6 ring PAHs. Alkyl moiety can inc mutagenicity**
- **Mutagenicity correlated to phototoxicity (and immunotoxicity)**
- **Adducts correlated to sediment PAHs (E. sole and toadfish)**
- **Neoplasms, foci of cellular alterations, specific degenerative necrosis,  
proliferative lesions are all correlated w sed PAHs for E. sole  
(hockey stick)**

# Ecotox review – PAHs

## toxicity to biota

- ❖ **Plants** – PAHs toxic only at high conc. Could be a source
- ❖ **Birds** – very toxic to embryos. Inc morts, dec embryo wt., inc abnormalities at 1 – 2  $\mu\text{g}$  / egg ( $\approx 0.036 \mu\text{g/g}$  wet wt. for DMBA, BaP, chrysene).
- ❖ **Reptiles and Amphibians** – Biotransformation relatively low, few tumors. Phototoxicity important. Time to death reduced by 20 to 70x for frogs exposed to fluoranthene.

# Ecotox review – PAHs

## Guidelines

### ❖ Water Quality Criteria

- several for human health ranging 0.004 – 960 ng/mL
- none for aquatic life

### ❖ Sediment Criteria

- U.S. EPA, still working on it...
- WA State, 370  $\mu\text{g/g}$  OC LPAHs, 960  $\mu\text{g/g}$  OC HPAHs
- Canada, 0.06 – 0.11  $\mu\text{g/g}$  sed dry wt. depending on PAH

### ❖ Human Health – ingestion RfD; indiv PAHs 0.03 to 0.3 $\mu\text{g} / \text{g bw} / \text{day}$

### ❖ New approaches? Tissue residues and BSAF or BCF for SQG and WQC, Target Lipid Model, dietary and ventilation dose

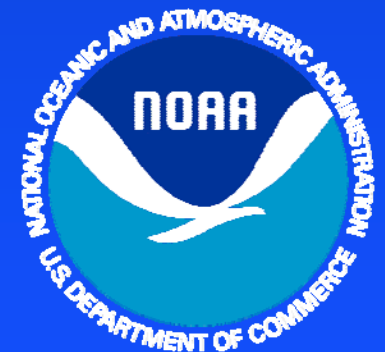
- Development of fish dose database ( $\mu\text{g} / \text{g fish} / \text{day}$ )

# Growth effects in juvenile chinook salmon from dietary PAH exposure

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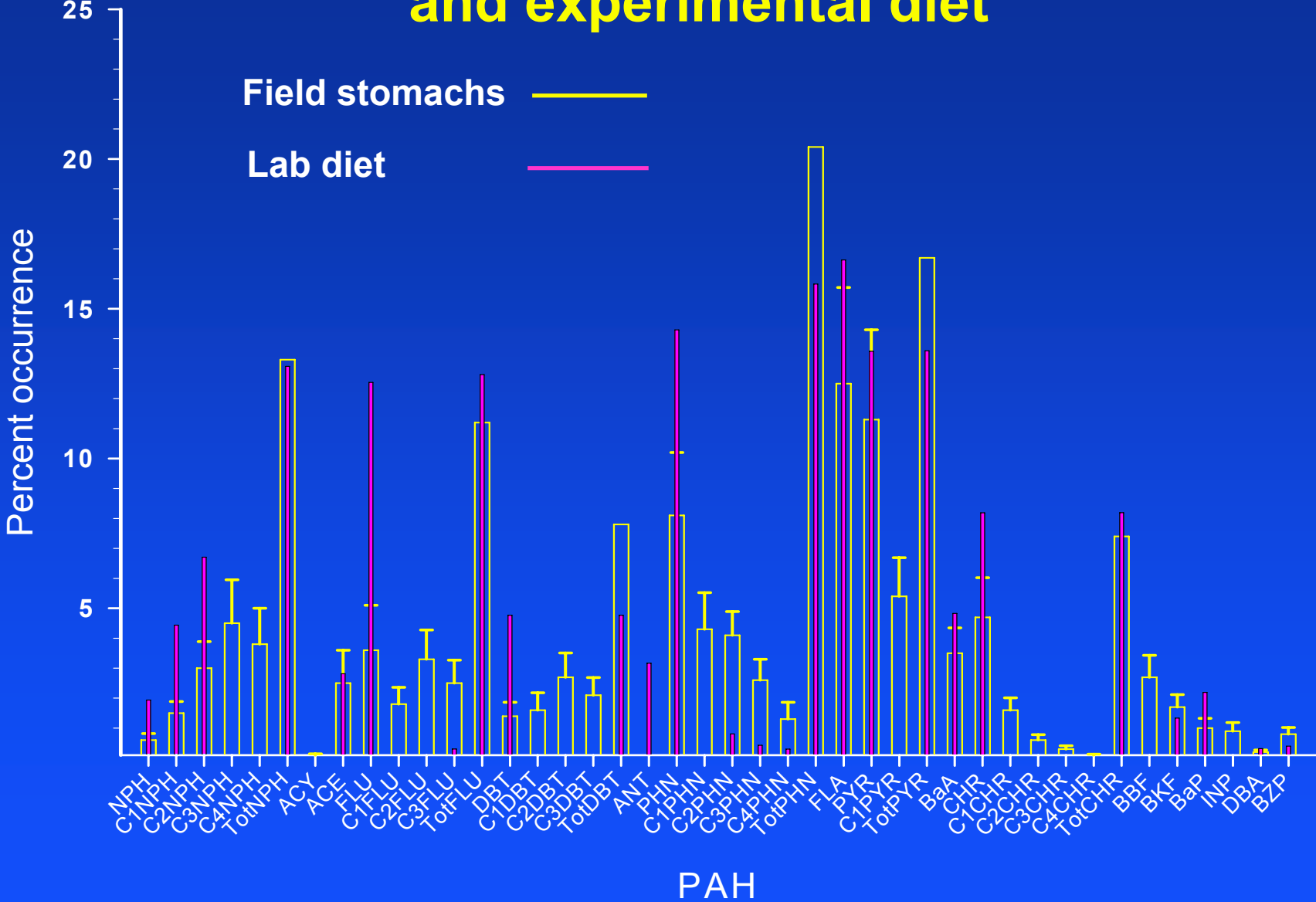
# Methods

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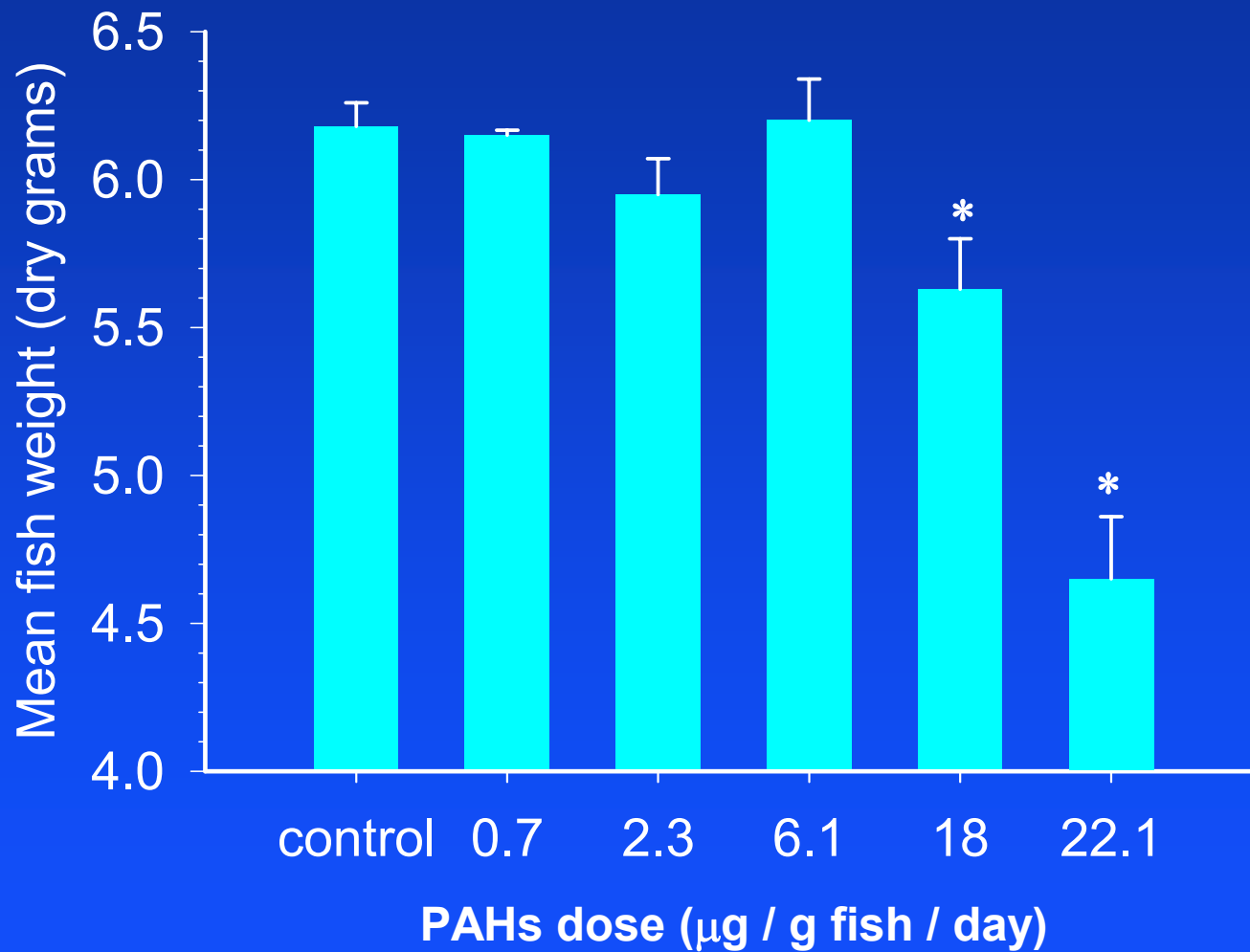
- ✱ Post smolt, in seawater, July – September
- ✱ 5 doses + control, 4 reps/treat, 50 fish/tank
- ✱ Dosed over 53 days with low-fat food to mimic natural prey
- ✱ Fish weight, plasma chemistry, whole-body chem & lipids, bile
- ✱ Meador et al. (2005). *Conducting Dose-Response Feeding Studies with Salmonids: Growth as an Endpoint*. In: *Techniques in Aquatic Toxicology Vol 2*. G.K. Ostrander (ed). Ch 5.



# PAHs in field stomachs and experimental diet

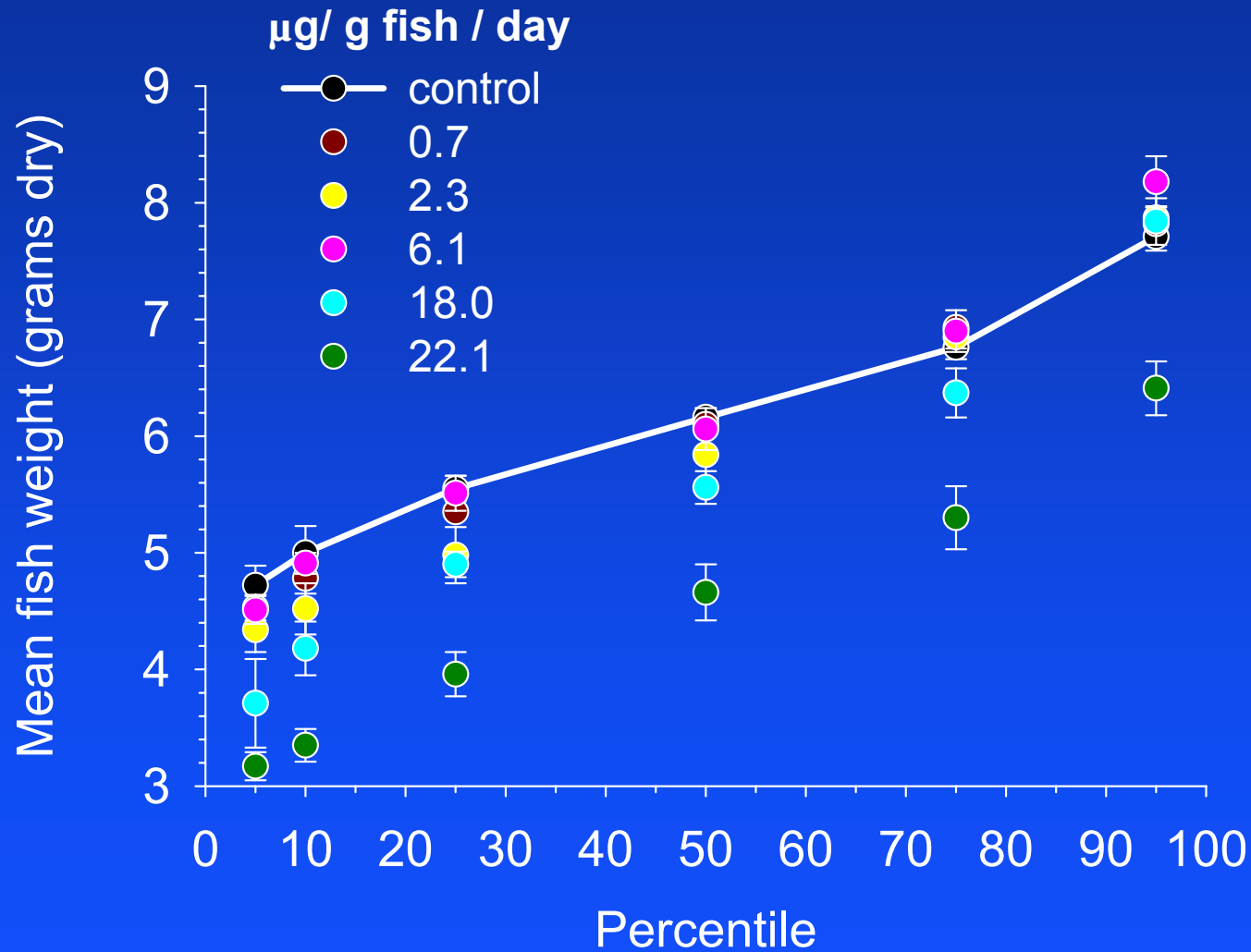


# Chinook mean dry weight PAHs - Day 58





# Fish weight – percentiles – Day 58



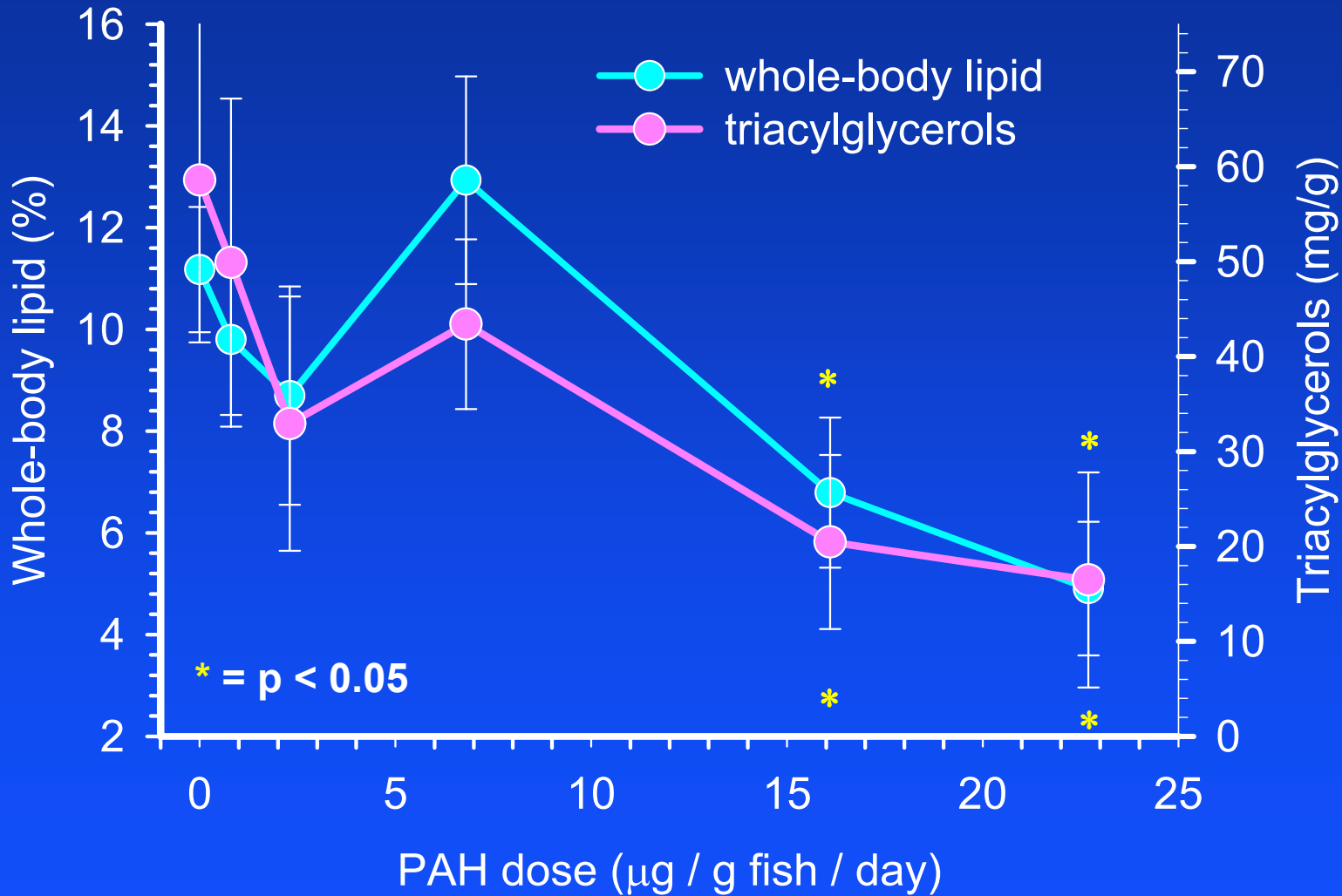
# Summary Stats – Day 58

Treat	Mean			Wet to dry	Morts %
	Wet wt	Dry wt	Tank sd		
Control	27.6	6.18	0.91	4.47	0
0.7	28.2	6.15	1.09	4.59	1.5
2.3	26.9	5.95	1.19	4.53	2.5
6.1	28.3	6.20	1.10	4.47	2.0
18.0	26.3	5.63	1.28	4.67	0
22.1	22.3	4.66	1.09	4.80	0

Treatment in  $\mu\text{g}$  / g fish / day

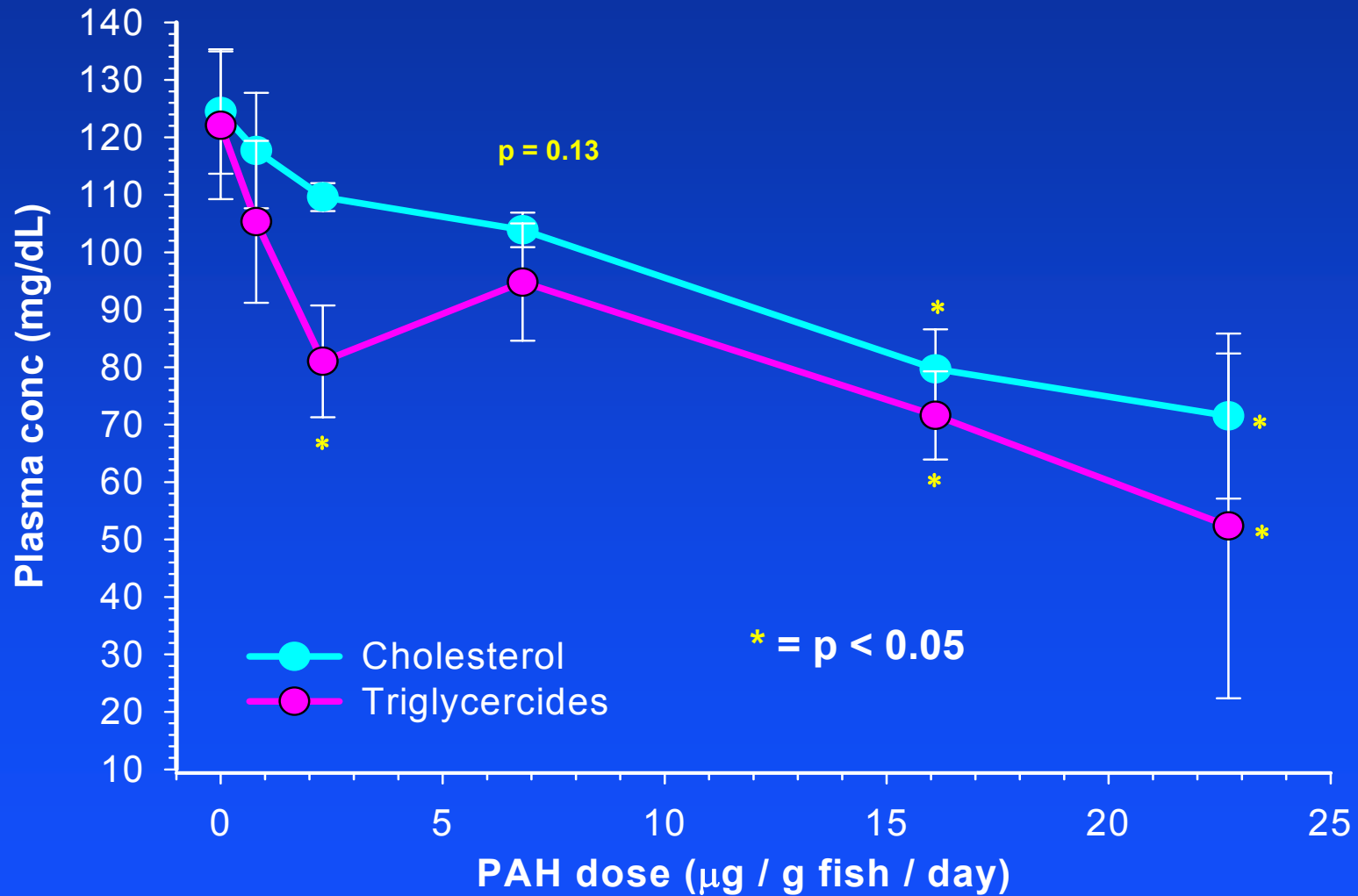
Yellow =  $p < 0.05$  from ANOVA and posthoc test

# Total whole-body lipids (dry wt.)

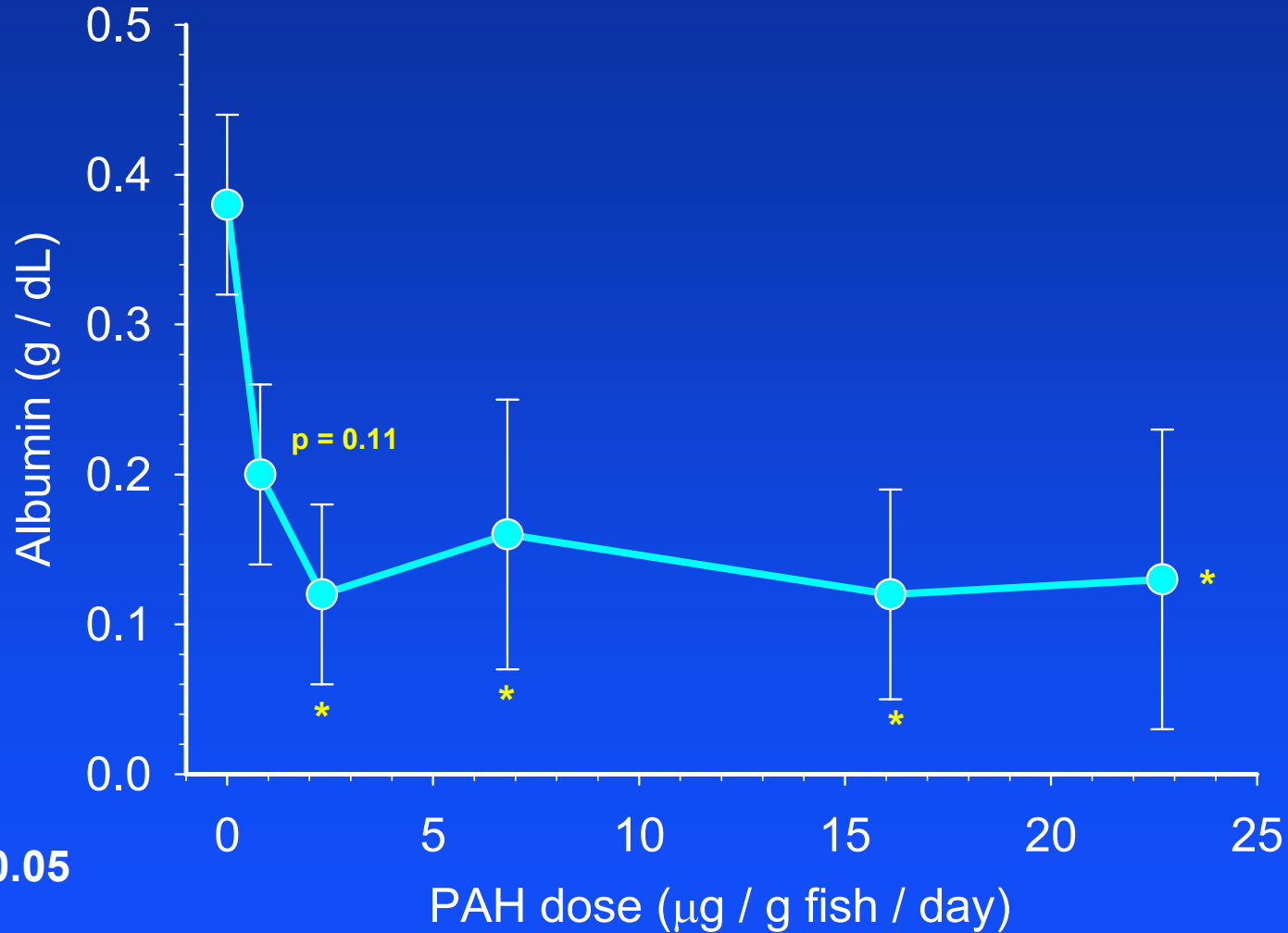




# Plasma lipids – Day 56

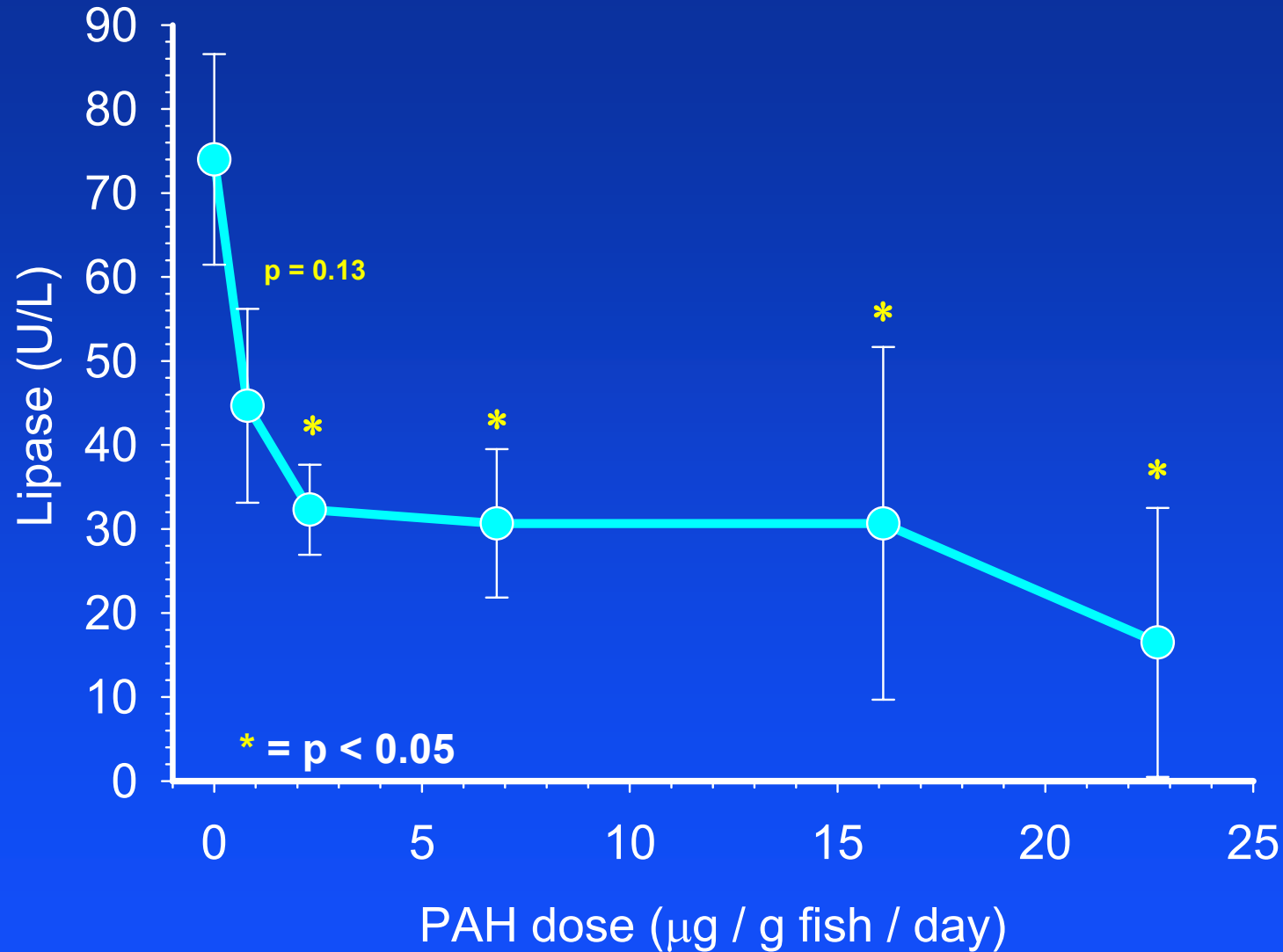


# Plasma albumin – Day 56



\* = p < 0.05

# Plasma lipase – Day 56



# Conclusions

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- ✦ **Growth or physiology affected in all treatments**
- ✦ **Growth effects supported by altered physiological parameters, which mimic starvation**
- ✦ **Conclude that PAHs are highly toxic to juvenile chinook at relatively low dose**
- ✦ **What happens in the estuary doesn't stay in the estuary**

# Implications

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- \* First winter at sea crucial for these fish. Lipid content & size determine survival
- \* **Lipids:** Two high dose treatments close to lethal levels. Low lipid = low winter survival
- \* **Fish weight:** smaller fish less likely to survive first winter due to 1. increased predation and 2. higher metabolism.
- \* For salmonids at this life stage, these factors are critical for survival

# Research Goals

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- ✱ Our group focuses on juvenile salmon outmigrants and the effects of contaminants in urban estuaries
- ✱ Our target species is chinook (*Oncorhynchus tshawytscha*)
- ✱ Mainly assess growth and metabolic effects
- ✱ Attempt to characterize toxic effects to individuals and determine impacts to populations
- ✱ Results are likely applicable to other fish species