

Acute and Chronic Effects of Crude Versus Dispersed Oil on Pre-smolt Chinook Salmon (*Oncorhynchus tshawytscha*)

Data on the health effects of dispersed oil on the life cycle of the Chinook salmon is needed to adequately evaluate the impacts of using oil dispersants in the San Francisco Bay area and inland waters. This presentation gives an assessment of the acute toxic actions of non-dispersed and dispersed water accommodated fractions (WAFs versus CEWAFs) of Prudhoe Bay Crude Oil (PBCO) on Chinook salmon pre-smolts under declining-exposure conditions.

EFFECTS OF CRUDE VERSUS DISPERSED OIL ON PRE-SMOLT CHINOOK SALMON

Ronald S. Tjeerdema, April Van Scoy, Brian S. Anderson, Bryn M.
Phillips, **Ching-Yu Lin**
Department of Environmental Toxicology
University of California, Davis

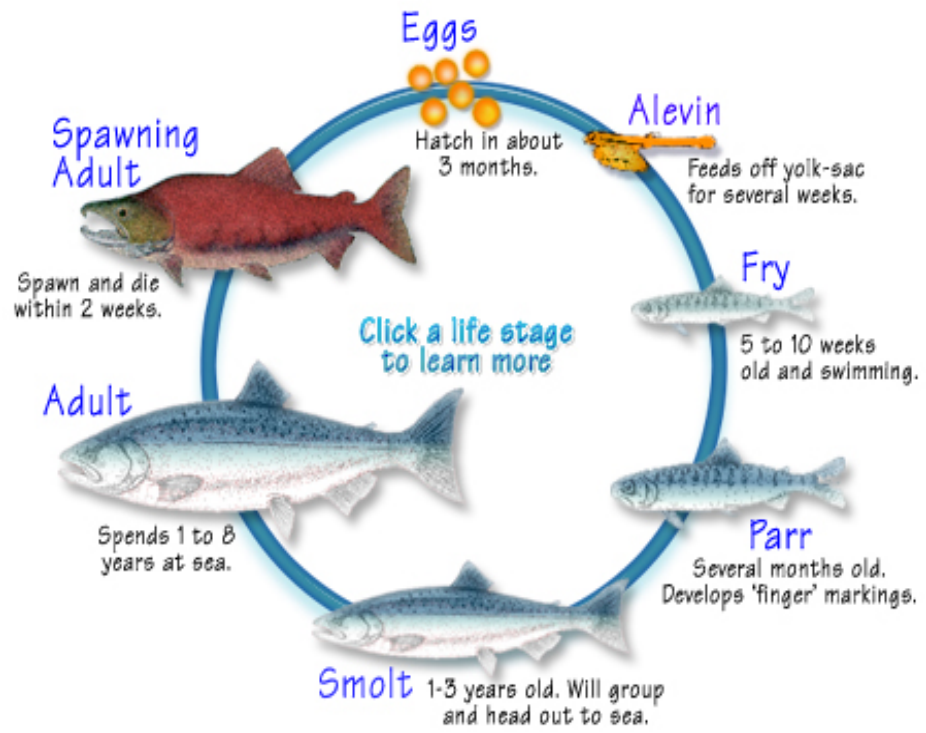
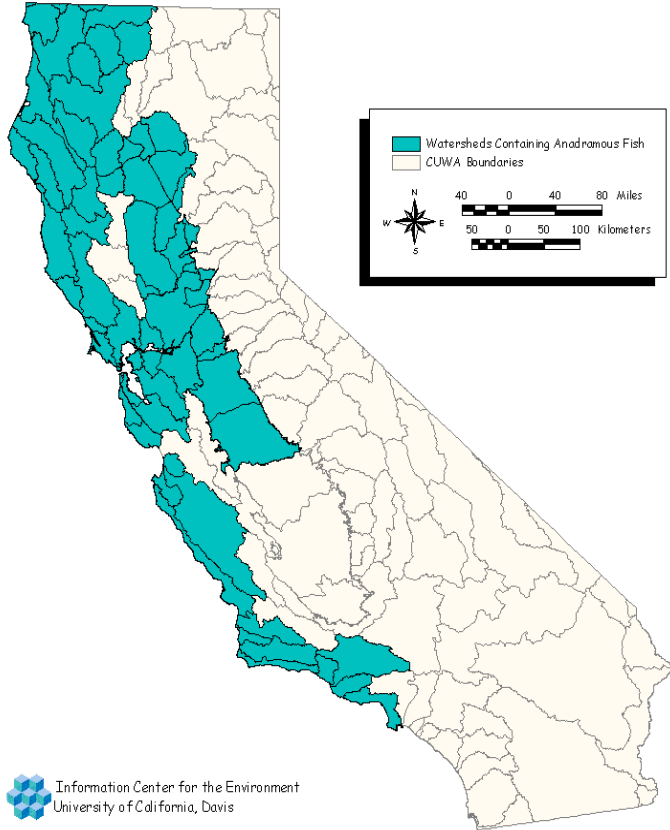
Michael L. Sowby
Office of Spill Prevention and Response
California Department of Fish and Game

David B. Crane, C. R. Todd
Water Pollution Control Laboratory
California Department of Fish and Game



Chinook Salmon – An Anadromous Species

California Unified Watershed Assessment
Presence of Threatened and Endangered Anadromous Salmonids



Objectives

- Assess the **acute toxic actions** of non-dispersed and dispersed water accommodated fractions (**WAFs versus CEWAFs**) of Prudhoe Bay Crude Oil (PBCO) to Chinook salmon pre-smolts under declining-exposure conditions.
- Apply ^1H -nuclear magnetic resonance (**NMR**)-based **metabolomic analysis** to investigate the **sublethal effects** of PBCO WAF and CEWAFs on salmon pre-smolts.

Methods – Test System and WAF Exposures



- 20-L carboys and 18-L aquaria.
- WAFs spun at low rate with minimal vortex (~150 rpm) for 24 h.
- Each of 3 carboys (33%) distributed to 3 replicate 18-L aquaria (2-cm headspace).
- Aquaria sampled for THC analysis.

Methods – CEWAF Exposures



- Added oil to create vortex size of 20 to 25%.



- Auto pipetted 10% (by oil weight) of **Corexit 9500**.

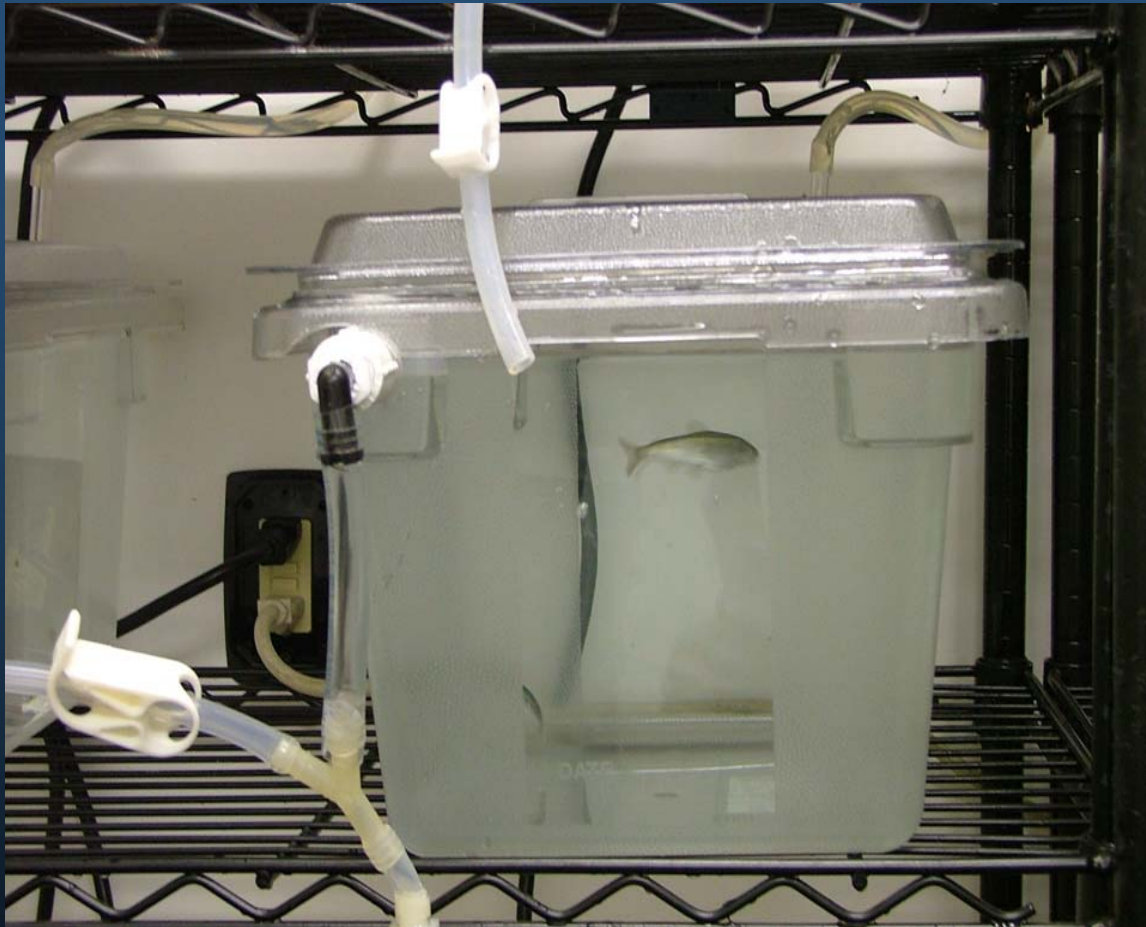


- Spun for 18 h, then settled for 6 h.

Methods – Analytical Chemistry

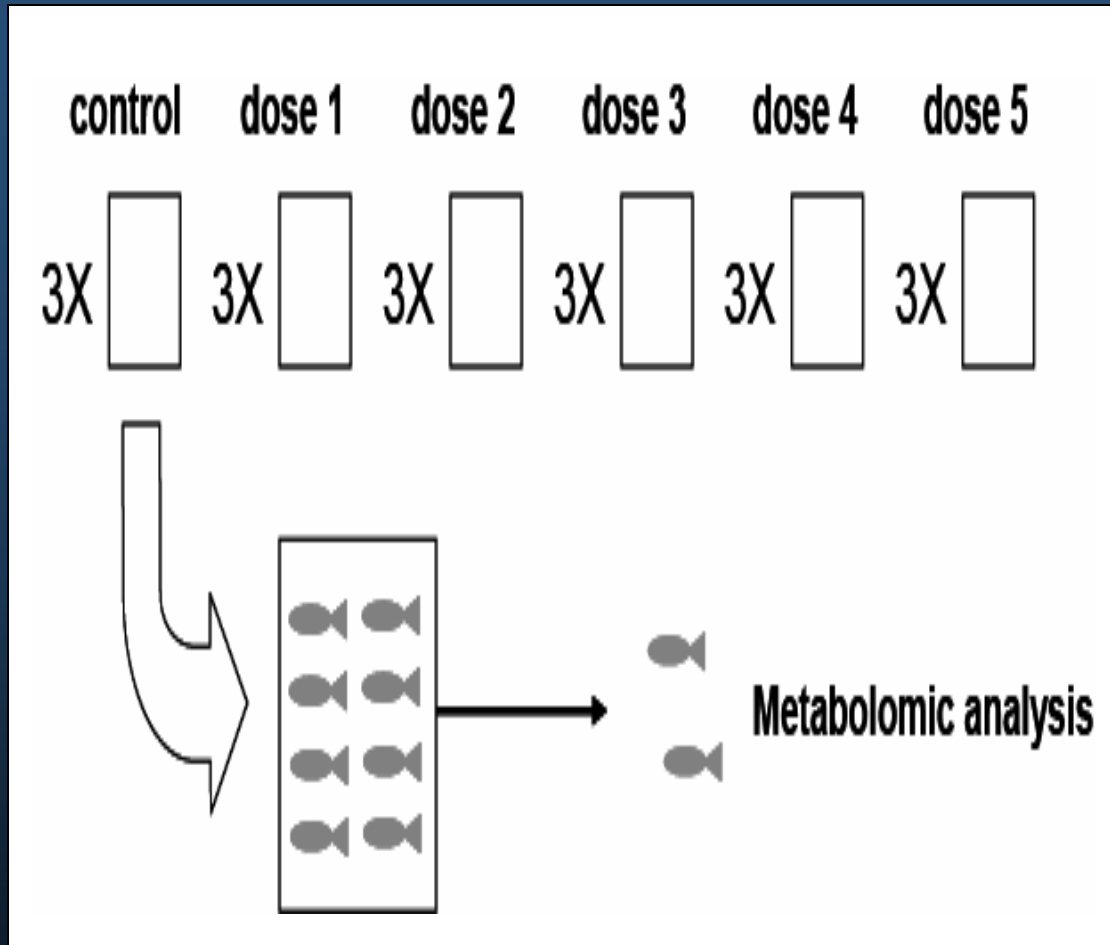
- Total petroleum hydrocarbons (TPH; $C_{10} - C_{36}$): analyzed via GC-FID; PAHs analyzed via GC/MS.
- Volatile hydrocarbons (BTEX; $C_6 - C_9$): analyzed via GC/MS with purge-and-trap extraction.
- Total hydrocarbon content (THC; $C_6 - C_{36}$): Calculated as BTEX + TPH (0, 24 and 96 h).
- Declining exposures confirmed every 2 h for 8 h, then 24 h via TC analysis.

Methods – 96-h Exposures



- Declining freshwater exposures (24 h), static for 72 h.
- Of surviving fish, 2 were sacrificed for metabolomics.
- Remaining survivors cultured for long-term growth effects.

Experimental Design



- Three total tests for each treatment (WAF and CEWAF)

- WAF mean LC50 = 7.56 mg/L THC.

- CEWAF mean LC50 = 48.6 mg/L THC.

Chronic Effects

- Fish from **each concentration per test** placed in one long-term grow-out tank.
- Fish were **acclimated to seawater** by increasing salinity 3-5 ppt daily (hand fed twice daily).
- Fish were **weighed every month** for growth data.

A reps = anal fin



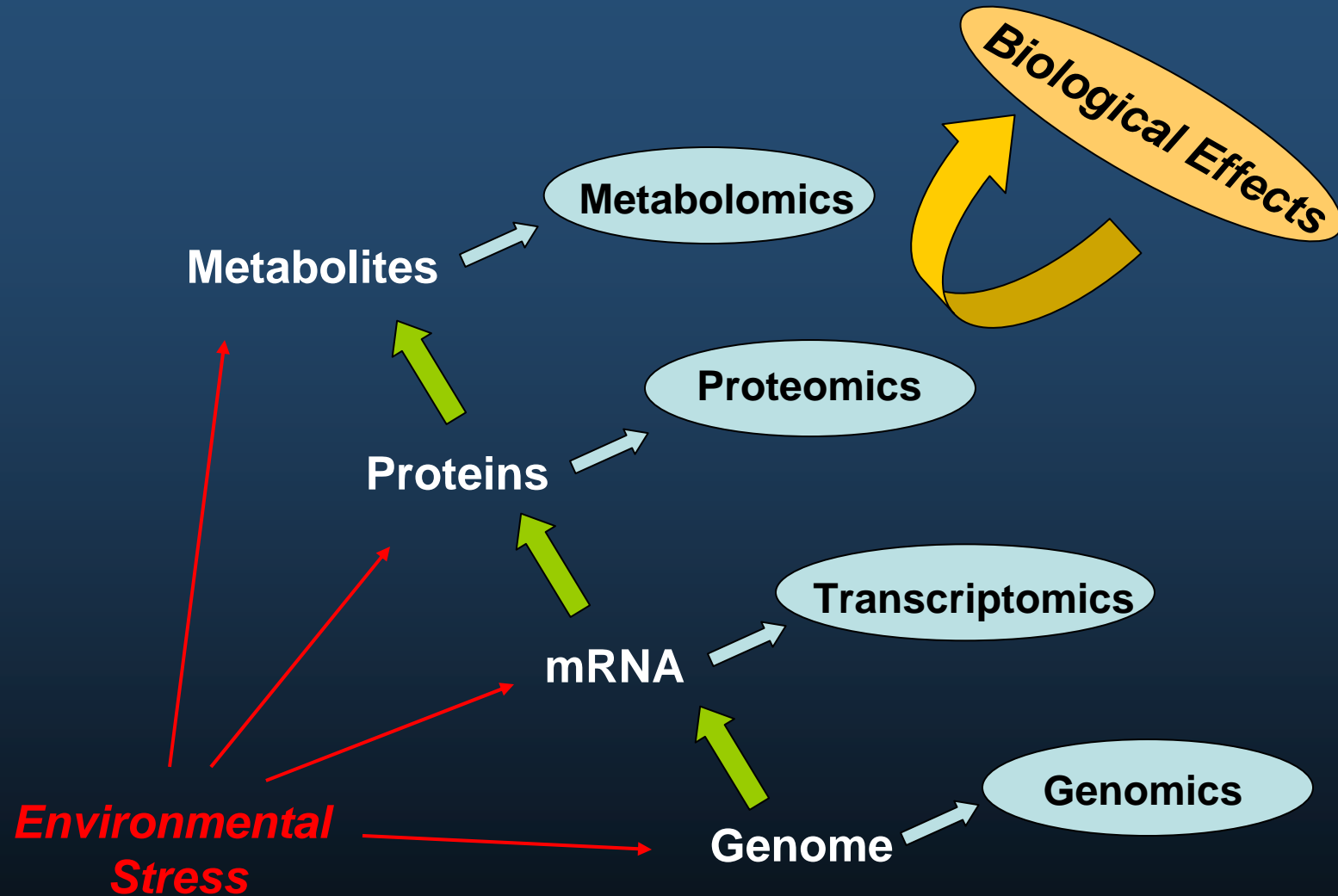
B reps = dorsal fin



C reps = caudal fin



Metabolomics to Characterize the Effects of Stress



Metabolomics Method Overview

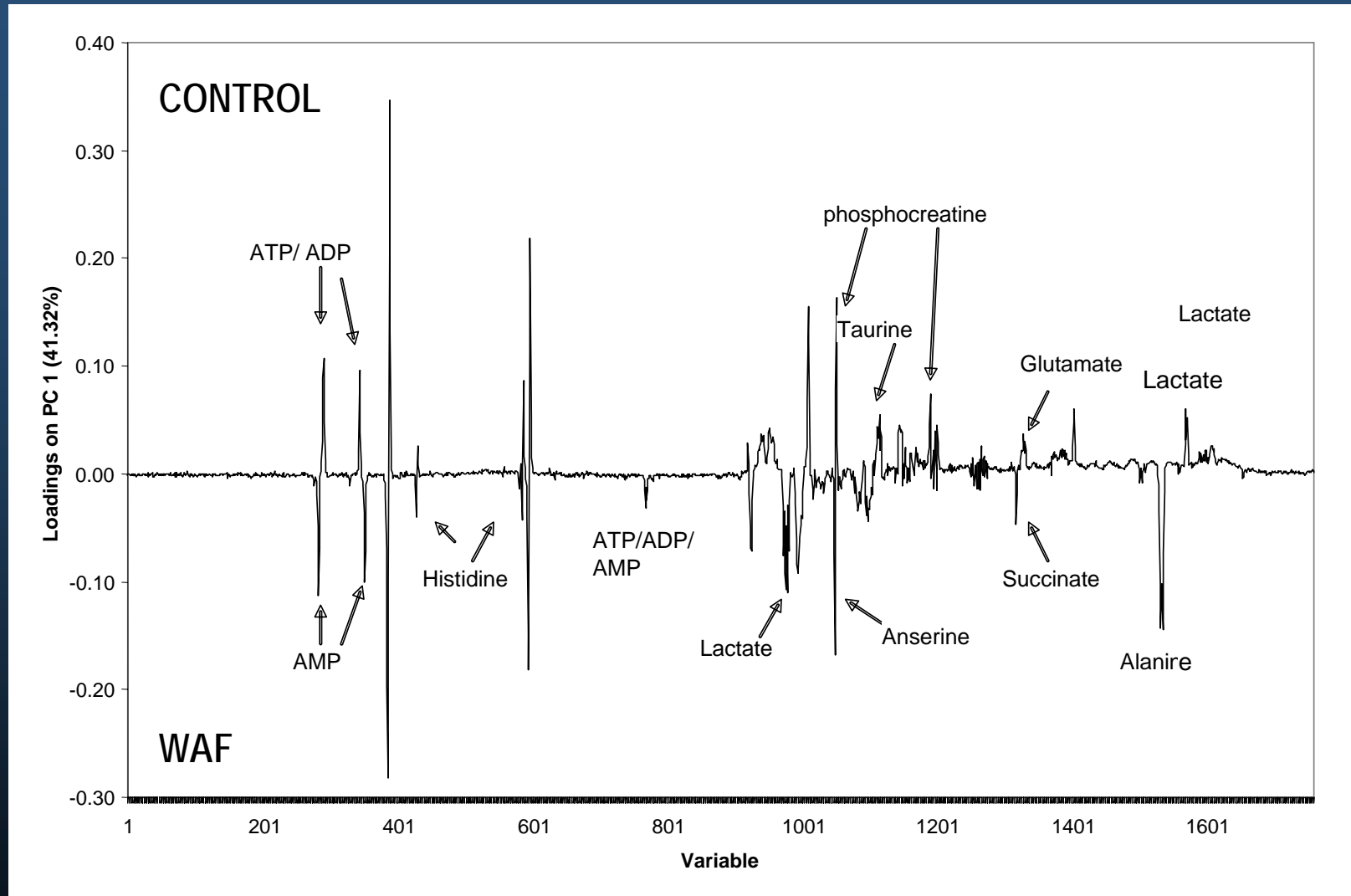


- **Dorsal muscles** from 2 surviving fish from each replicate exposure were flash frozen for metabolomic analysis.

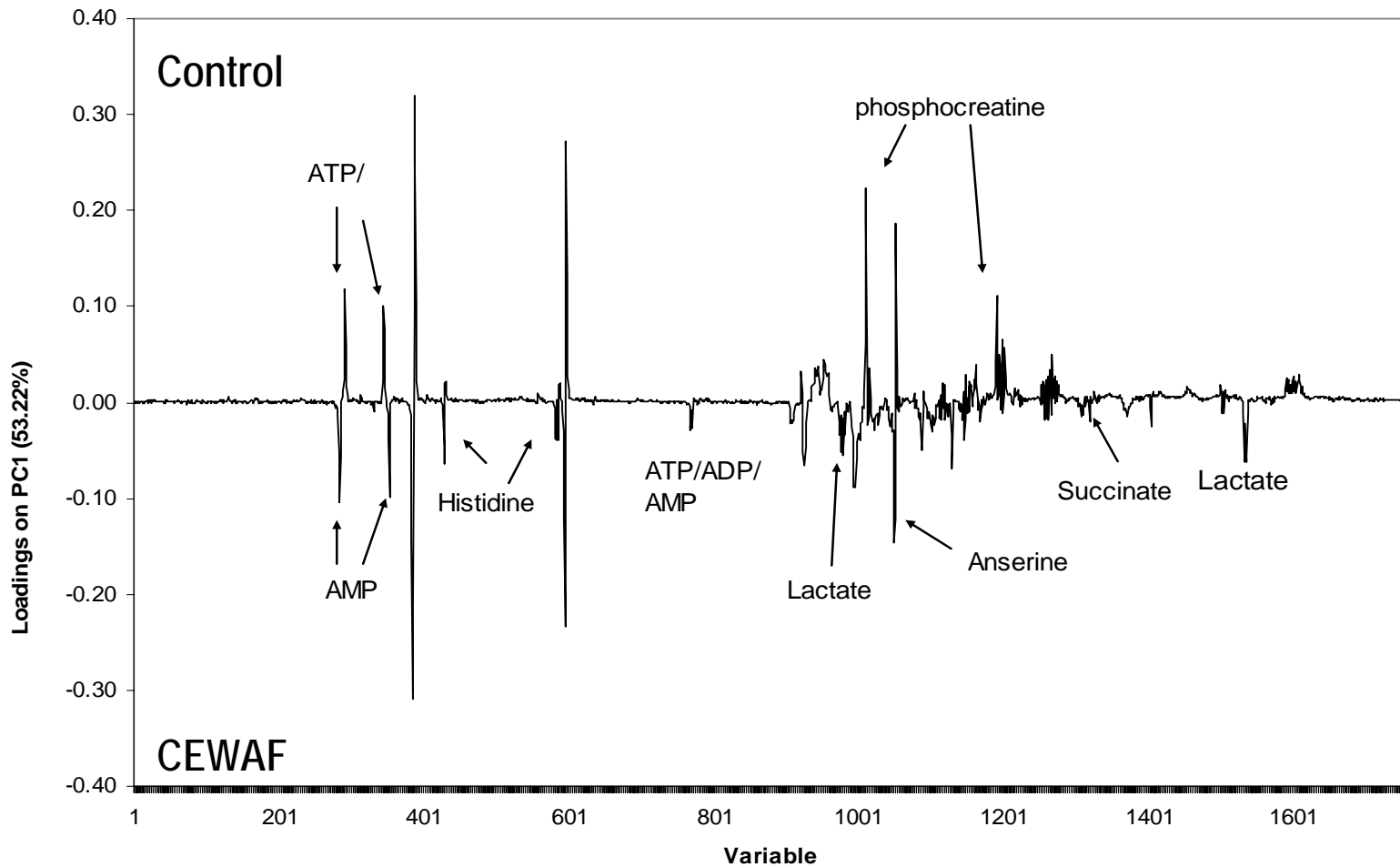


- **Small metabolites** were extracted with MeOH/H₂O.
- ¹H-NMR analysis provides **metabolite profiles**.
- Metabolite profiles are then subjected to **multivariate analyses (PCA)**.

Muscle Loadings Plot from WAF Exposure



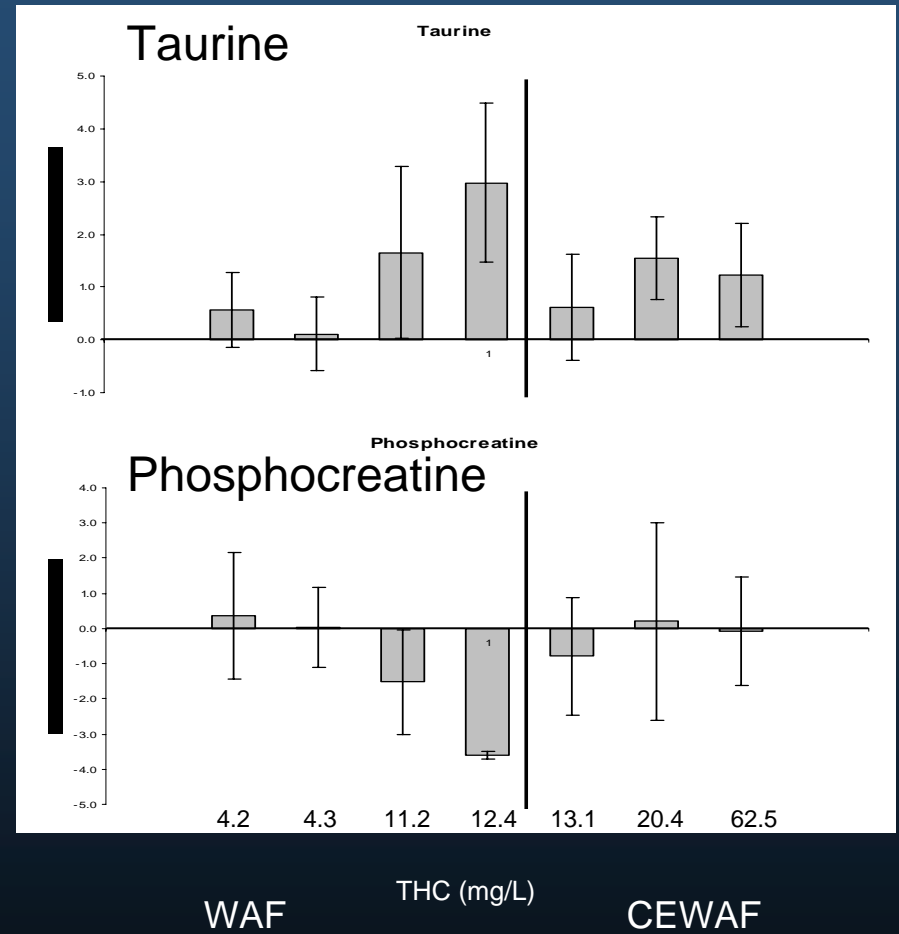
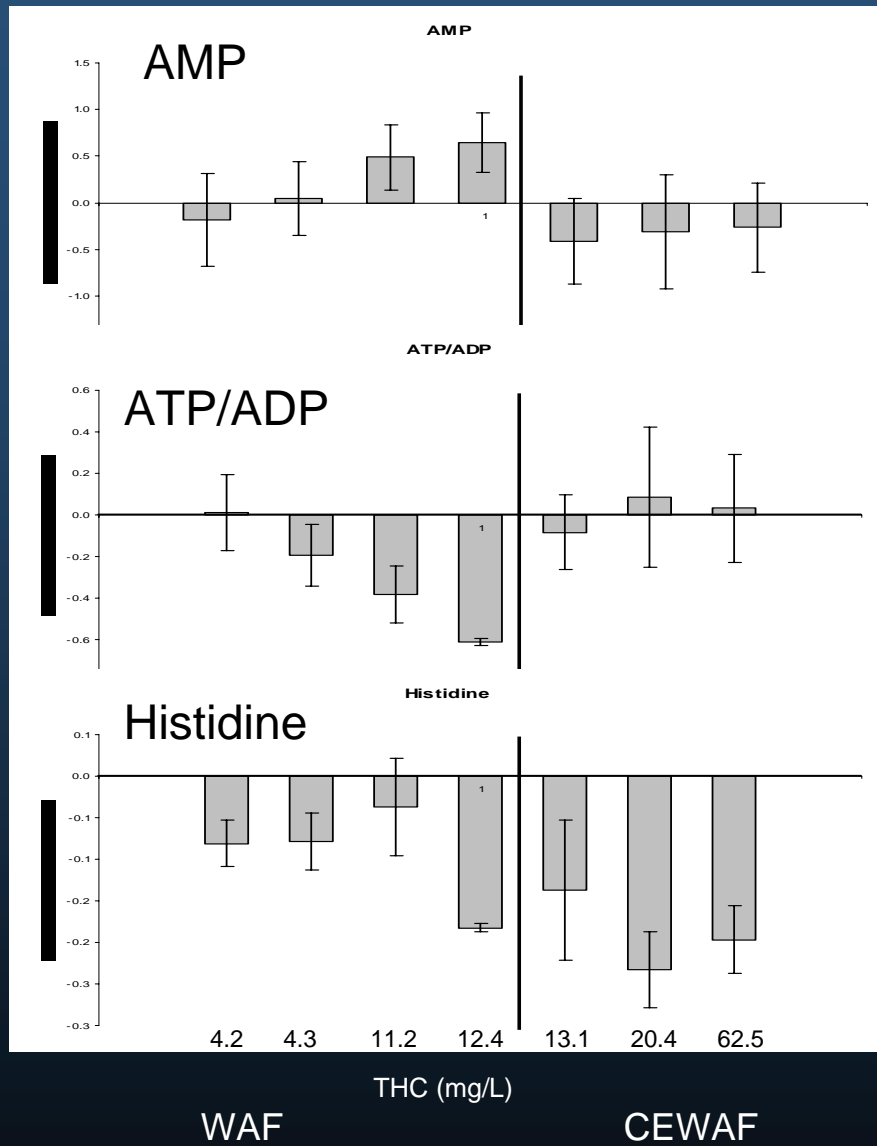
Muscle Loadings Plot from CEWAF Exposure



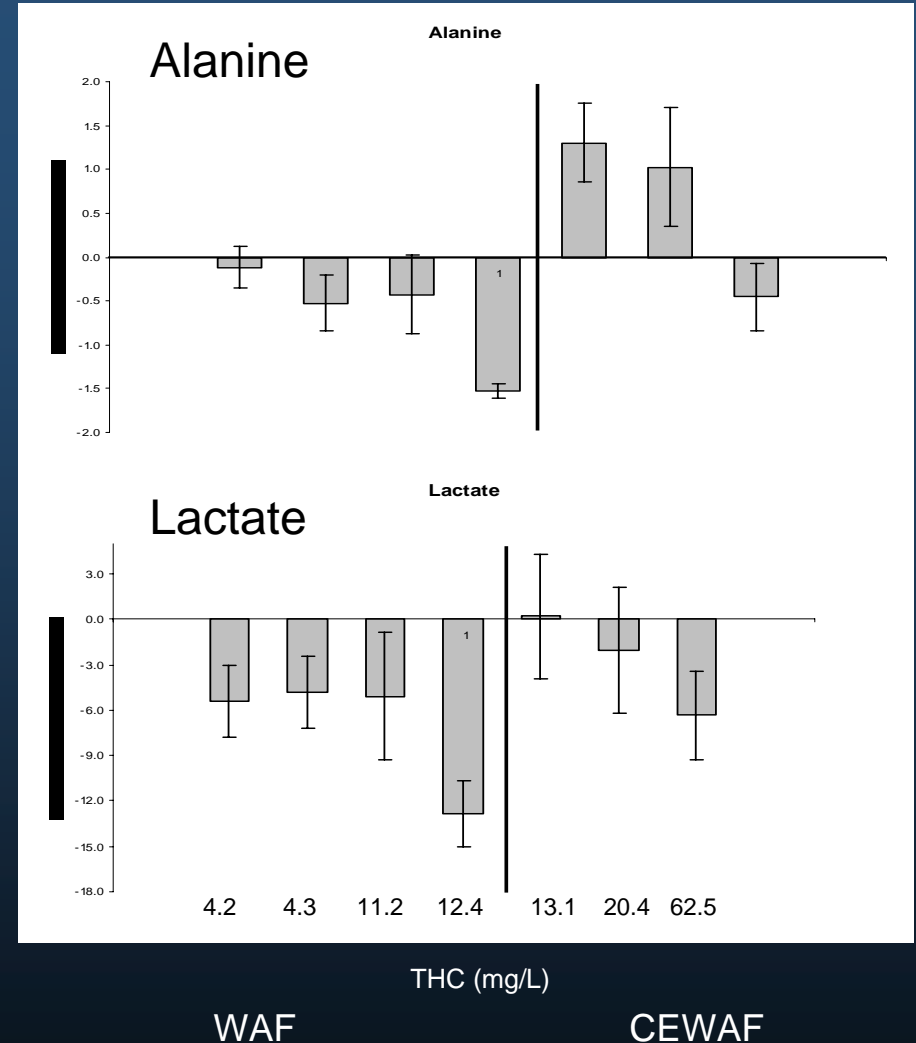
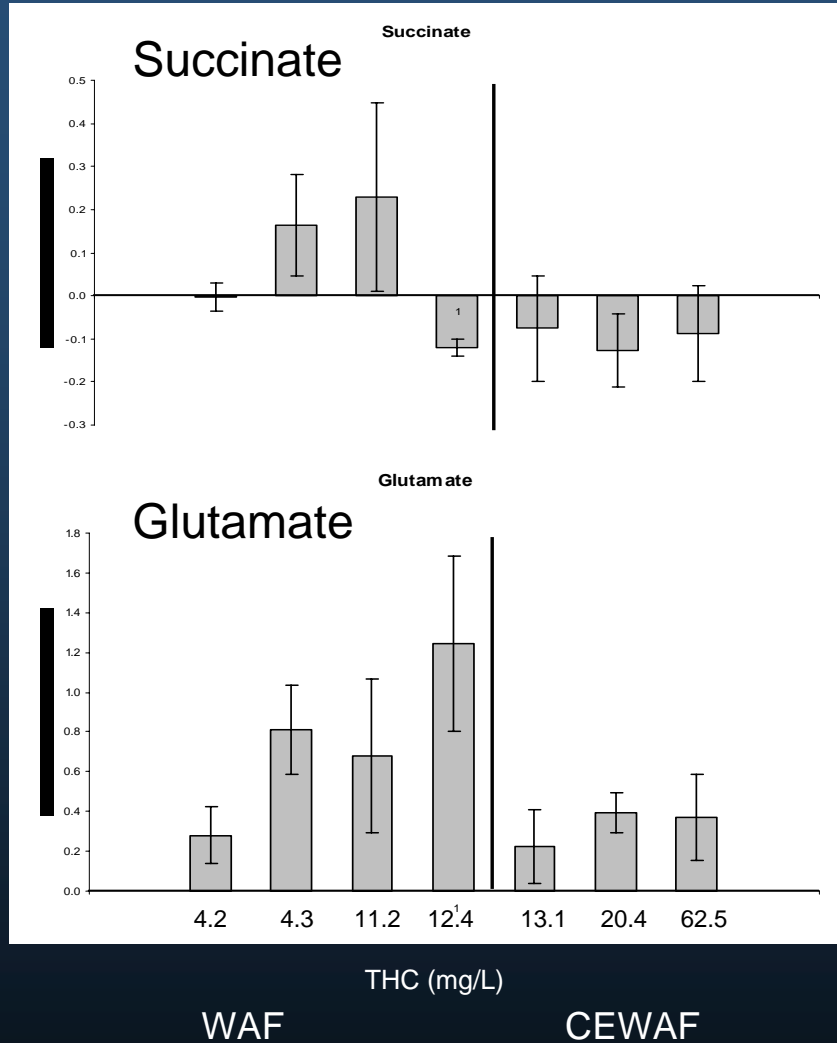
Metabolic Effects In Muscle

WAF LC50= 7.56 mg/L

CEWAF LC50= 48.6 mg/L

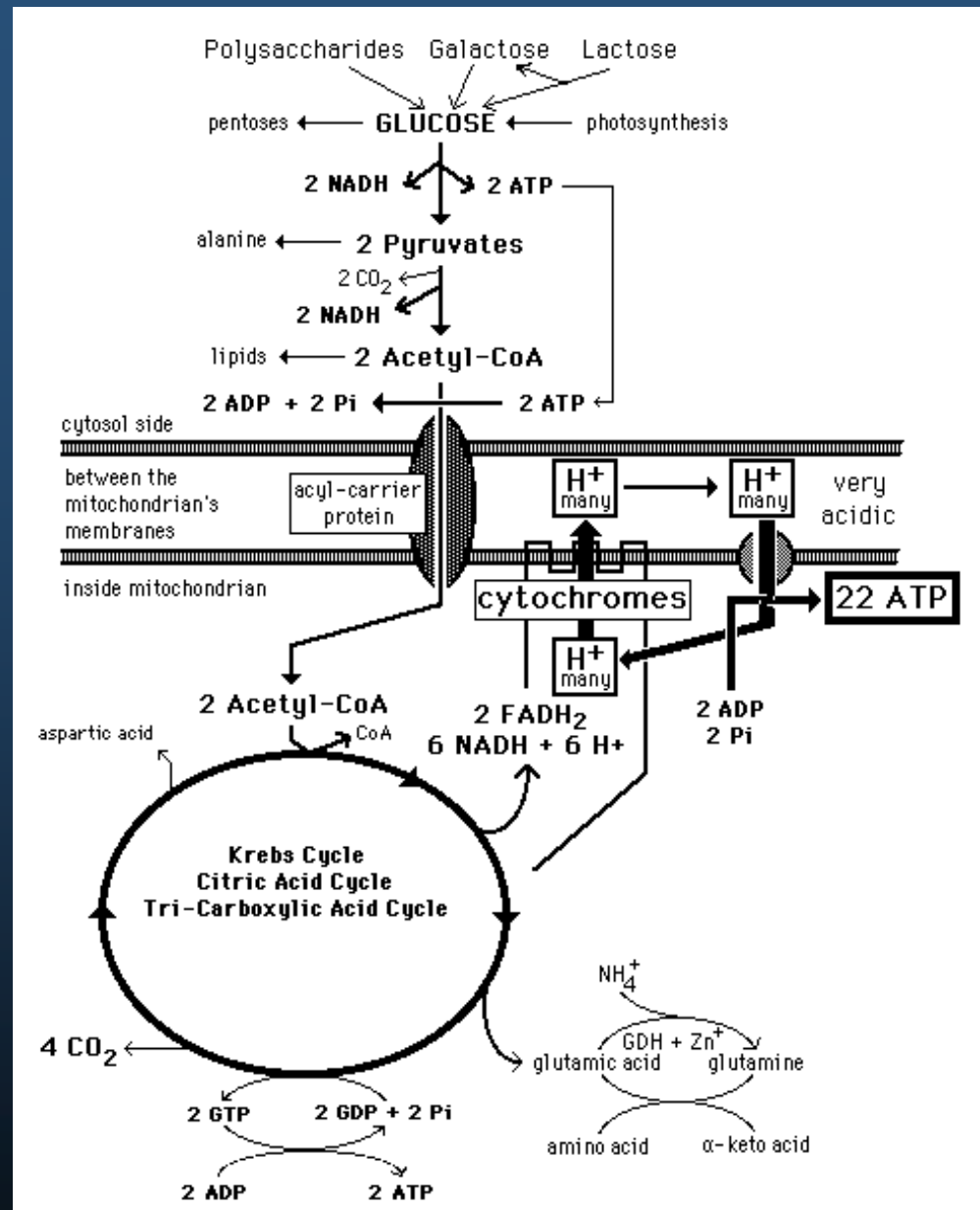


Metabolic Effects in Muscle – Continued



Implications

- WAF and CEWAF both caused increased amino acids.
- Amino acids may result from proteolysis.
- May also be diverted from intermediary metabolism for new protein synthesis.
- Diversion may reduce ATP available for development.



Conclusions

- The CEWAF LC50 was **7-fold higher** than that for WAF, indicating that dispersant presence may **decrease hydrocarbon bioavailability** to salmon pre-smolts.
- Increased free amino acids may **replace damaged proteins and/or enzymes**.
- Loss of energy to damage repair may leave **less for growth** or stress response.
- Metabolic actions are **measurable below traditional lethality endpoints** – metabolomics can provide a more sensitive assessment of toxic impacts.

Supporting Agencies

The California Department of Fish and Game –
Office of Spill Prevention and Response (OSPR)
The UCD Oiled Wildlife Care Network (OWCN)

