TOXIC EFFECTS OF CRUDE AND DISPERSED OIL ON CHINOOK SALMON SMOLTS (*ONCORHYNCHUS TSHAWYTSCHA*)

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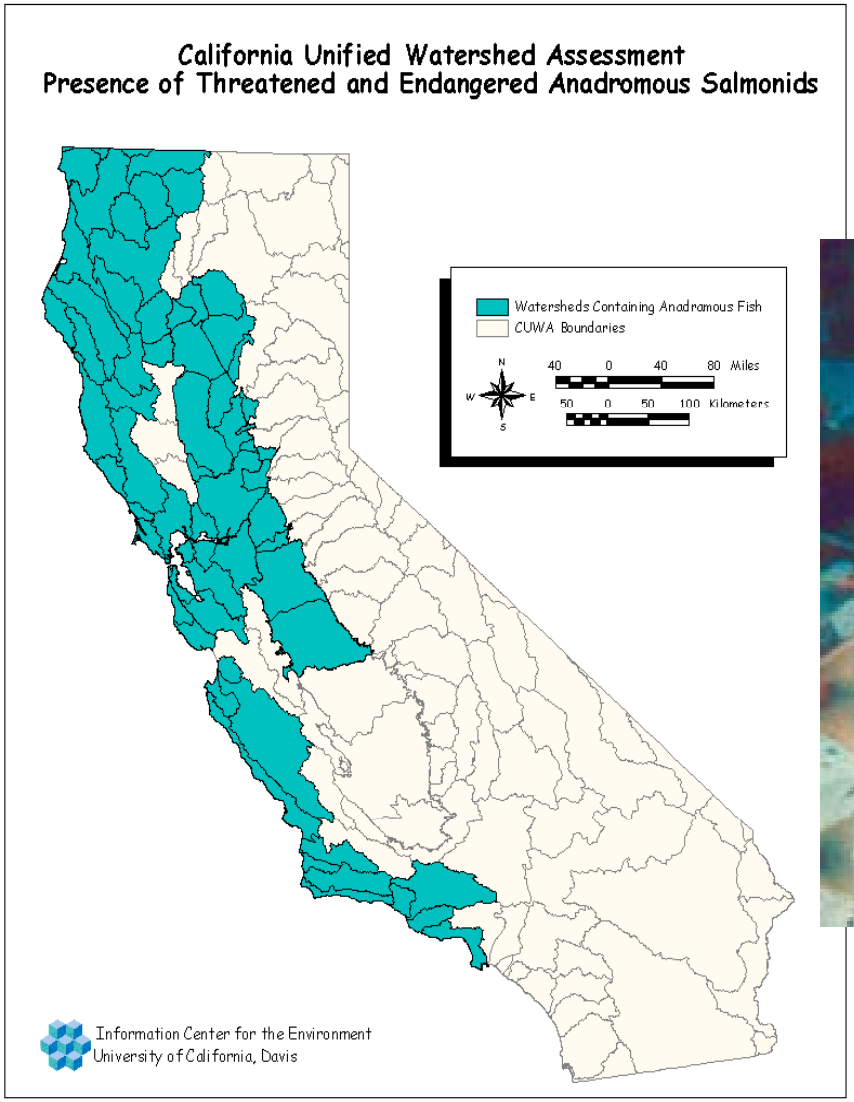
Chinook Salmon – An Anadromous Species



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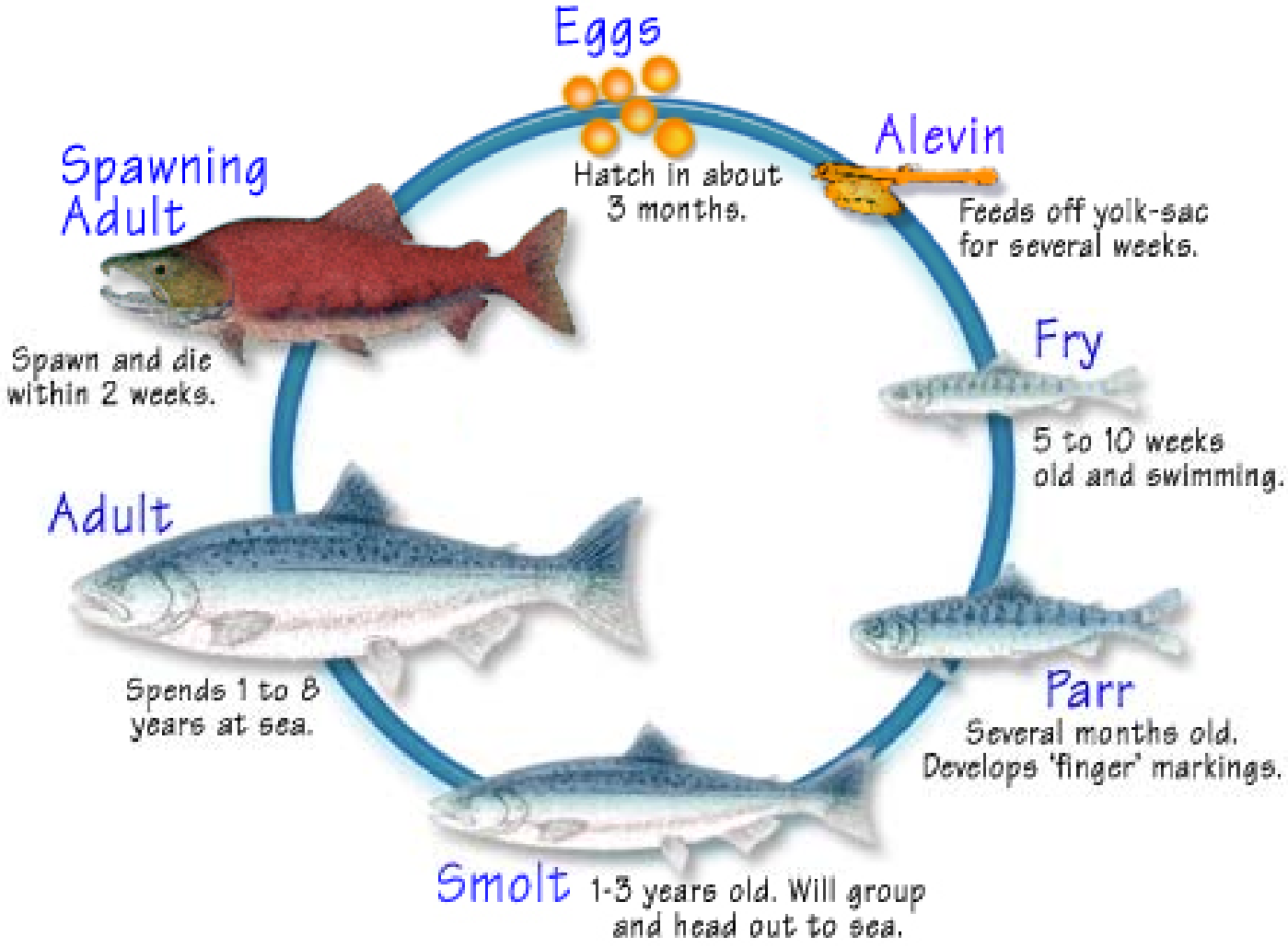
The Salmon Life Cycle



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Objectives

- Assess the relative acute toxic actions of non-dispersed and dispersed water accommodated fractions (WAFs versus CEWAFs) of Prudhoe Bay Crude Oil (PBCO) to Chinook salmon smolts (*Oncorhynchus tshawytscha)* under declining exposure conditions.

- Apply ^{1}H-nuclear magnetic resonance (NMR)-based metabolomic analysis to investigate the sublethal effects of PBCO WAF and CEWAFs on salmon smolts.

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Methods: Test System and WAF Exposures

- Methods of CROSERF (Singer *et al.* 2000).
- Polycarbonate 20-L carboys and 18-L aquaria.
- WAFs spun at low rate with minimal vortex (~150 rpm) for 24 h.

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Methods: WAF Tests (continued)

- Each of 3 carboys (33%) distributed to 3 replicate 18-L aquaria (2-cm headspace).

- Aquaria sampled for TPH and THC analysis, 8 fish introduced, and clean water flushing initiated.

- Flush rate calibrated with flow meters @ 200 mL/min; verified with THC.

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Methods: CEWAF Tests

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- Add oil and create vortex size of 20 to 25%.
- Auto pipet 10% (by oil weight) Corexit 9500.
- Spin for 18 h, then settle for 6 h.
- Stagger the test start times.

Methods: Analytical Chemistry

- Total petroleum hydrocarbons (TPH; C_{10} – C_{36}): Used GC with flame ionization detection.

- Volatile hydrocarbons (BTEX; C_6-C): Benzene, toluene, ethyl benzene and the xylenes analyzed via GC/MS with purge-and-trap extraction.

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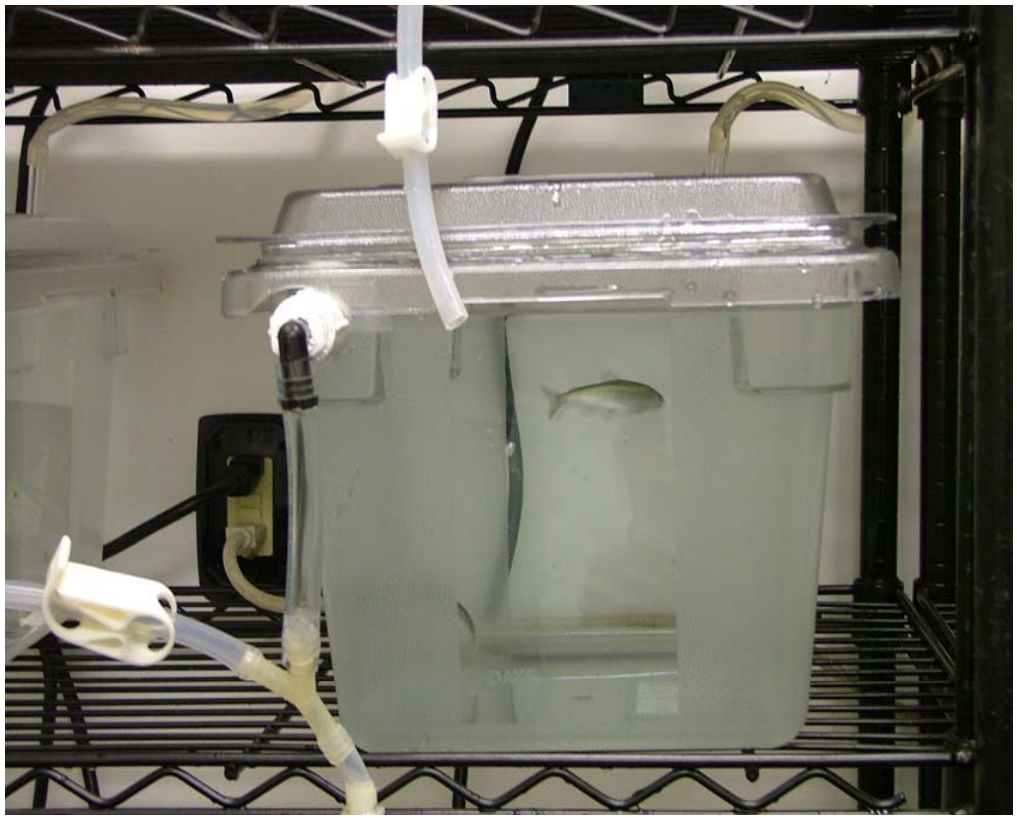
- Total hydrocarbon content (THC; C_6–C_{36}): Calculated as BTEX + TPH.

- Declining exposures confirmed via THC during tests.

Methods: Exposures (96 h)



- Salmon smolts were 8 per aquarium (~ 8 cm).
- Declining seawater exposures (96-h).
- Of the surviving fish, 2 were sacrificed for metabolomics.
- Remaining survivors cultured for long-term growth effects.

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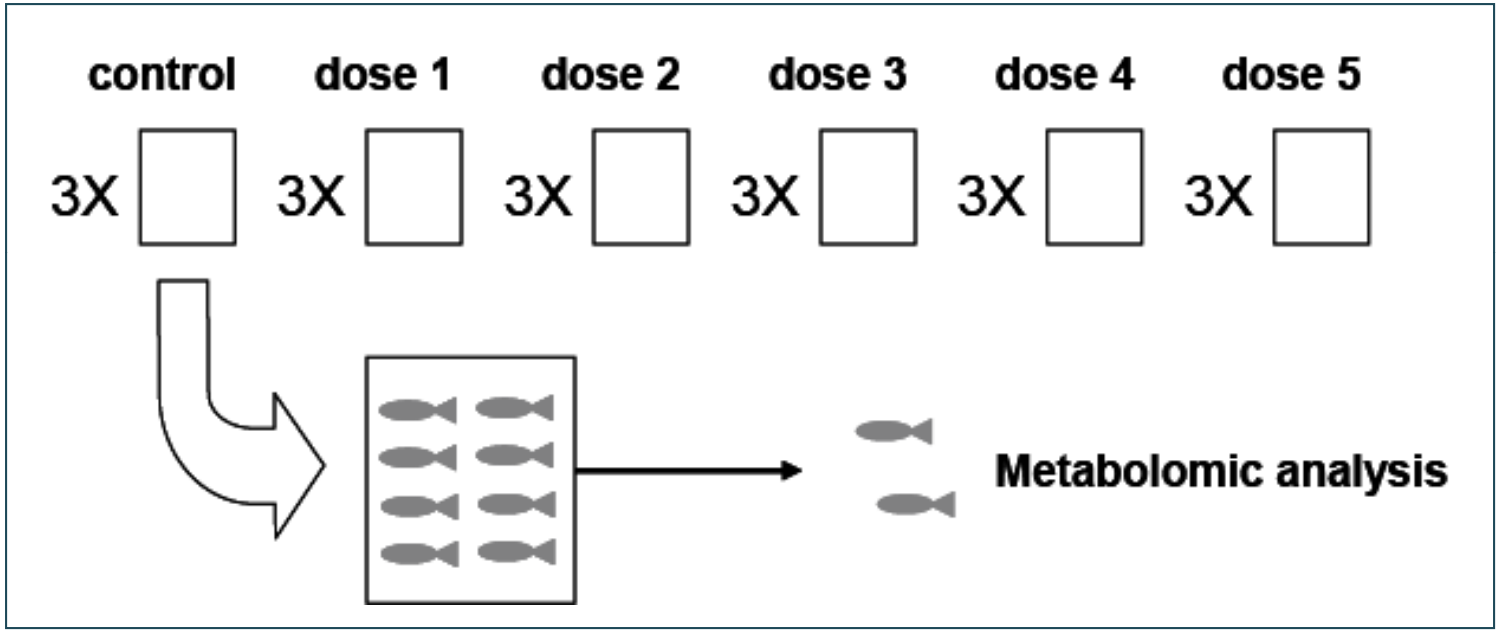
Experimental Design



Three total tests for each (WAF and CEWAF)

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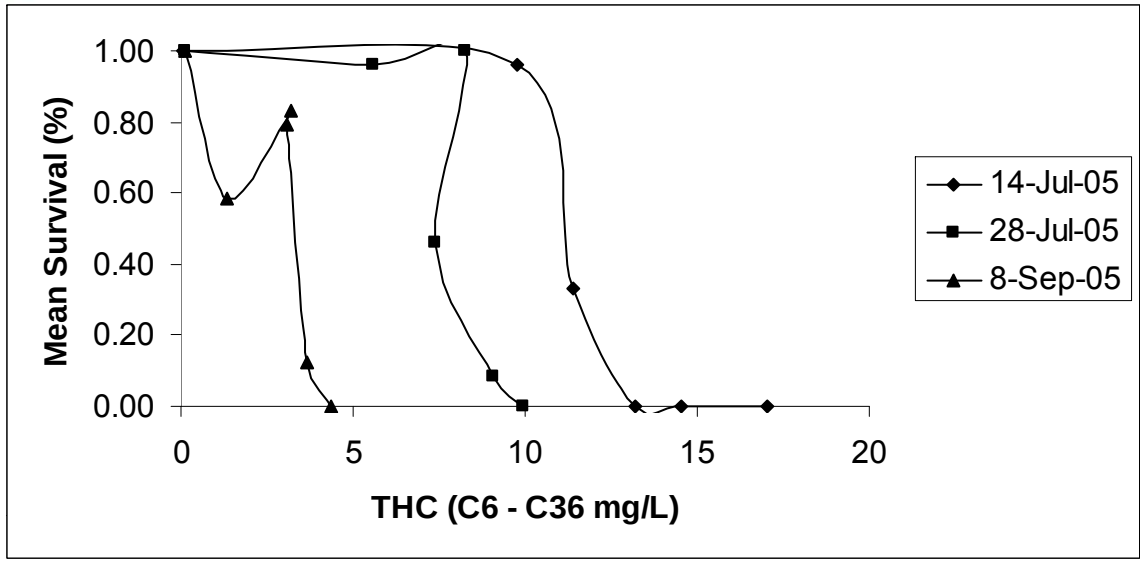
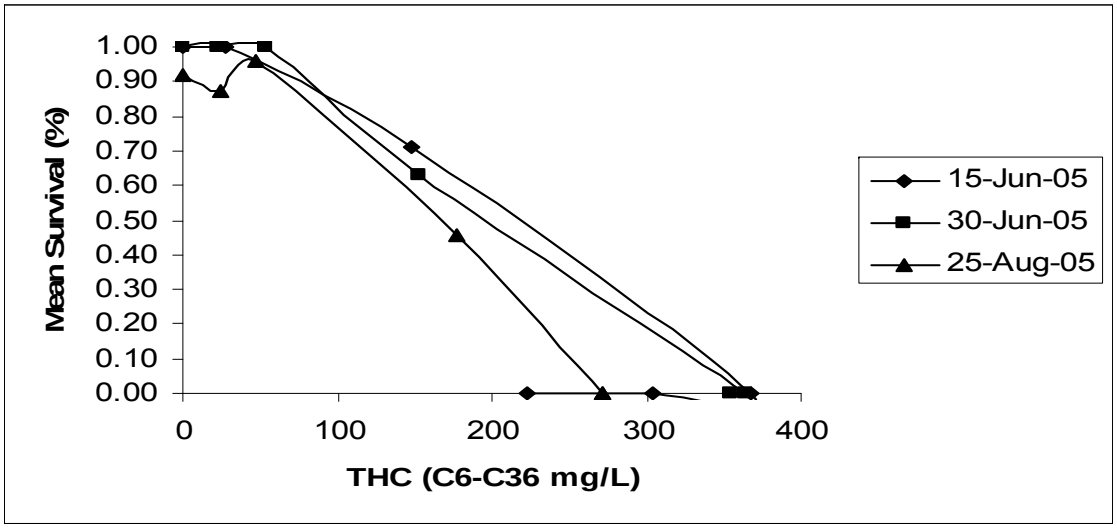
WAF



Mean WAF LC50 = 7.46 mg/L THC.

CEWAF



Mean CEWAF LC50 = 155.93 mg/L THC.

Salmon smolt survival in three WAF and CEWAF tests after 96-h.
THC = total hydrocarbon concentration.

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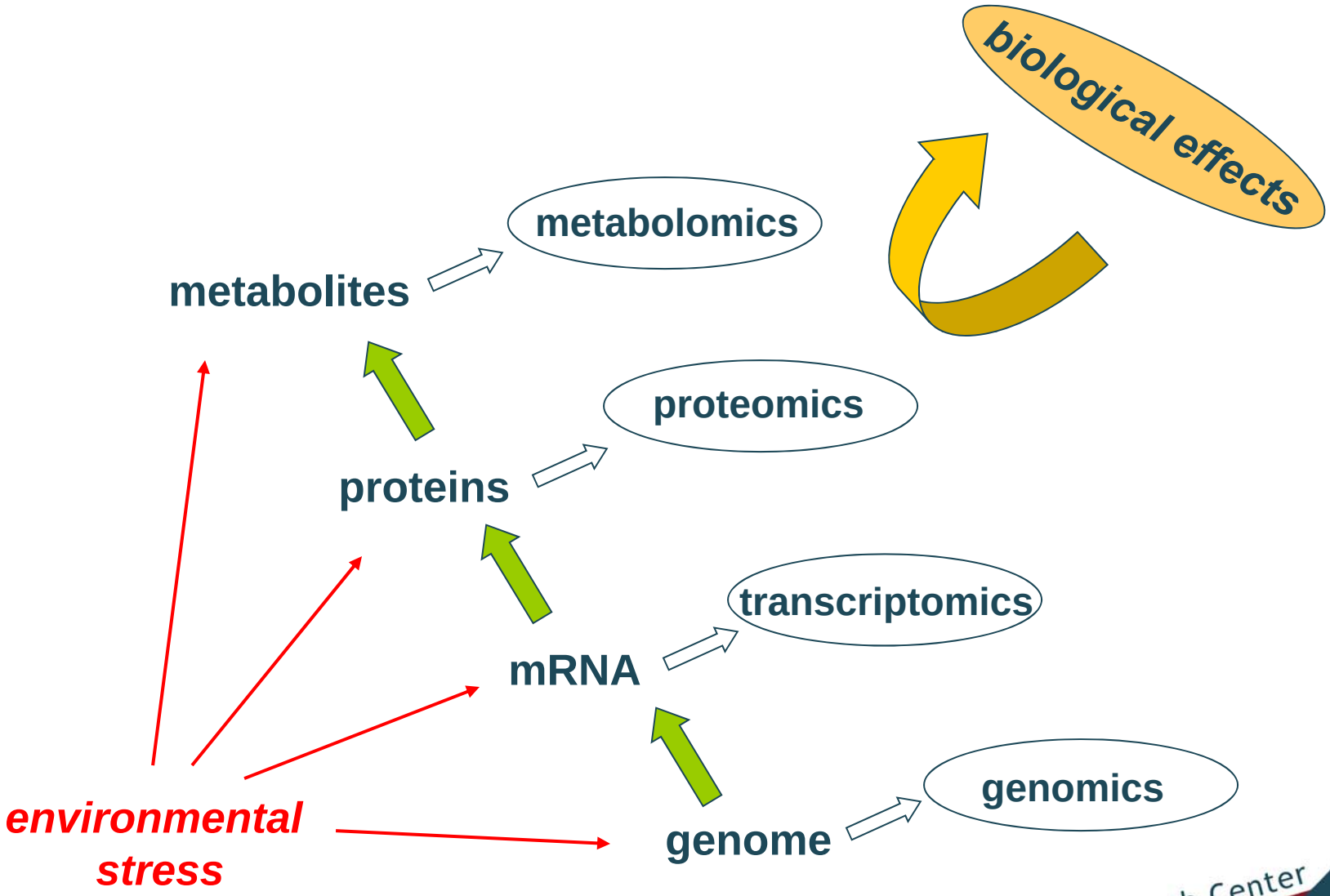
Metabolomics to Characterize Effects of Stress



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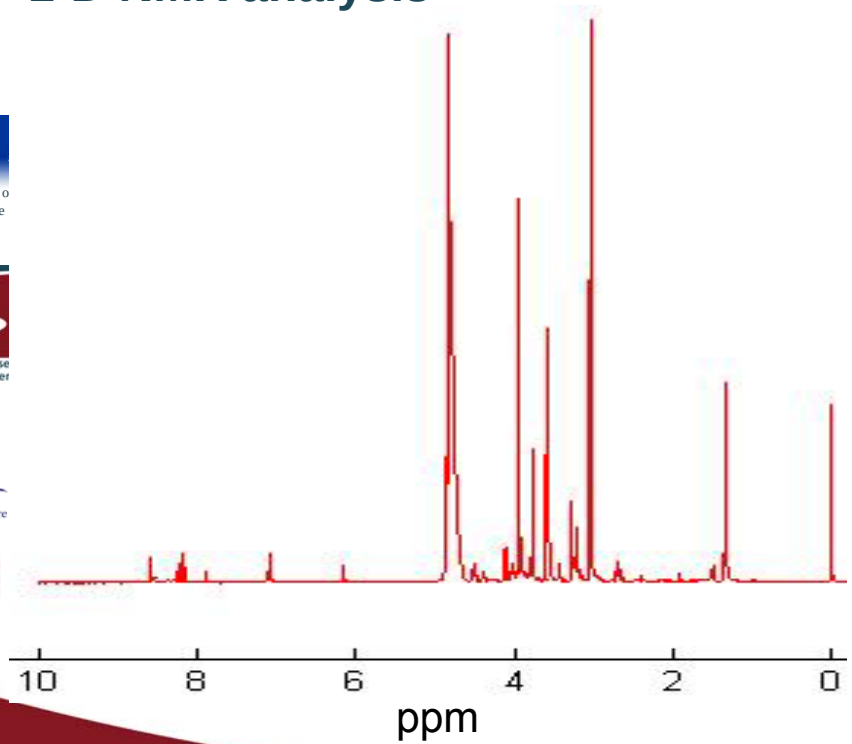
NMR-Based Metabolomics Approach

Sample prep (tissue or biofluid)

1-D NMR analysis



Peak assignment

1-D (^{1}H NMR)

2-D (^{1}H-^{1}H COSY & ^{1}H-^{13}C HSQC)

Multivariate statistical analysis

$$ab = \left\{ \begin{array}{l} a_1b_1,\ a_1b_2,\ a_1b_3 \text{ -------- } a_1b_n \\ a_2b_1,\ a_2b_2,\ a_2b_3 \text{ -------- } a_2b_n \\ a_3b_1,\ a_3b_2,\ a_3b_3 \text{ -------- } a_3b_n \\ \vdots \quad \vdots \quad \vdots \qquad \vdots \\ a_mb_1, a_mb_2,\ a_mb_3 \text{ --------- } a_mb_n \end{array} \right\}$$

Metabolite profiling
(metabolomic classification/
biochemical mechanism)

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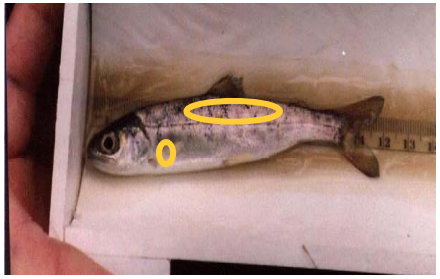
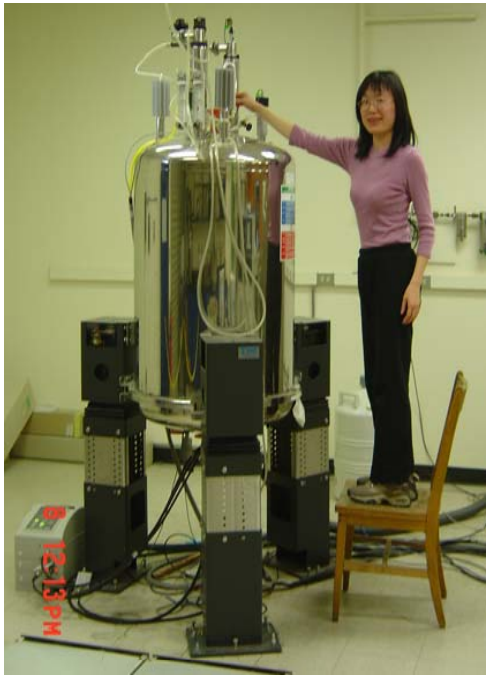
Metabolomics Method Overview



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



- **Small molecular mass metabolites were extracted with MeOH/H_20.**
- **^{1}H-NMR analysis provides metabolite profiles.**
- **Metabolite profiles are then subjected to multivariate analyses (PCA).**

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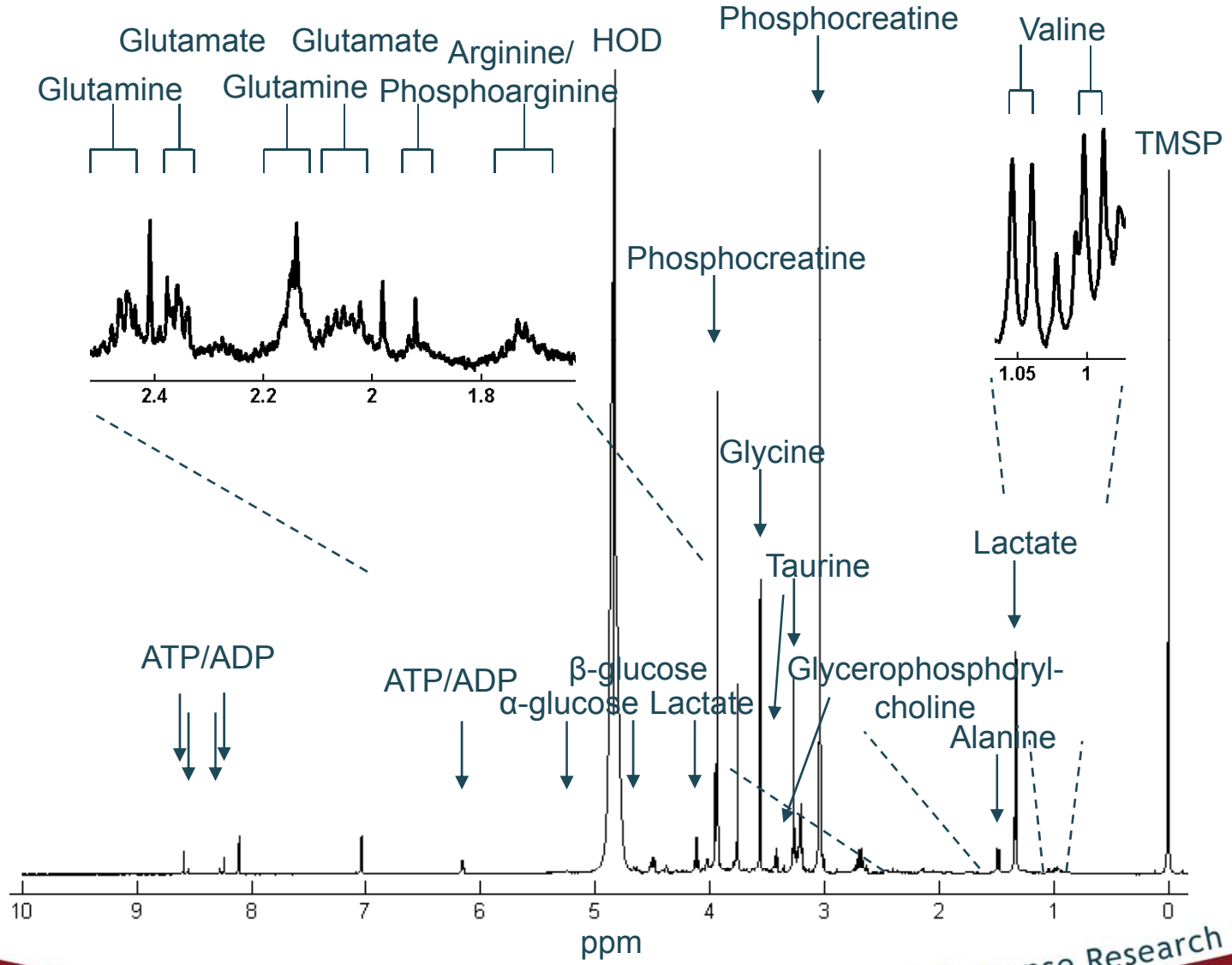
NMR Spectrum of Muscle Extract



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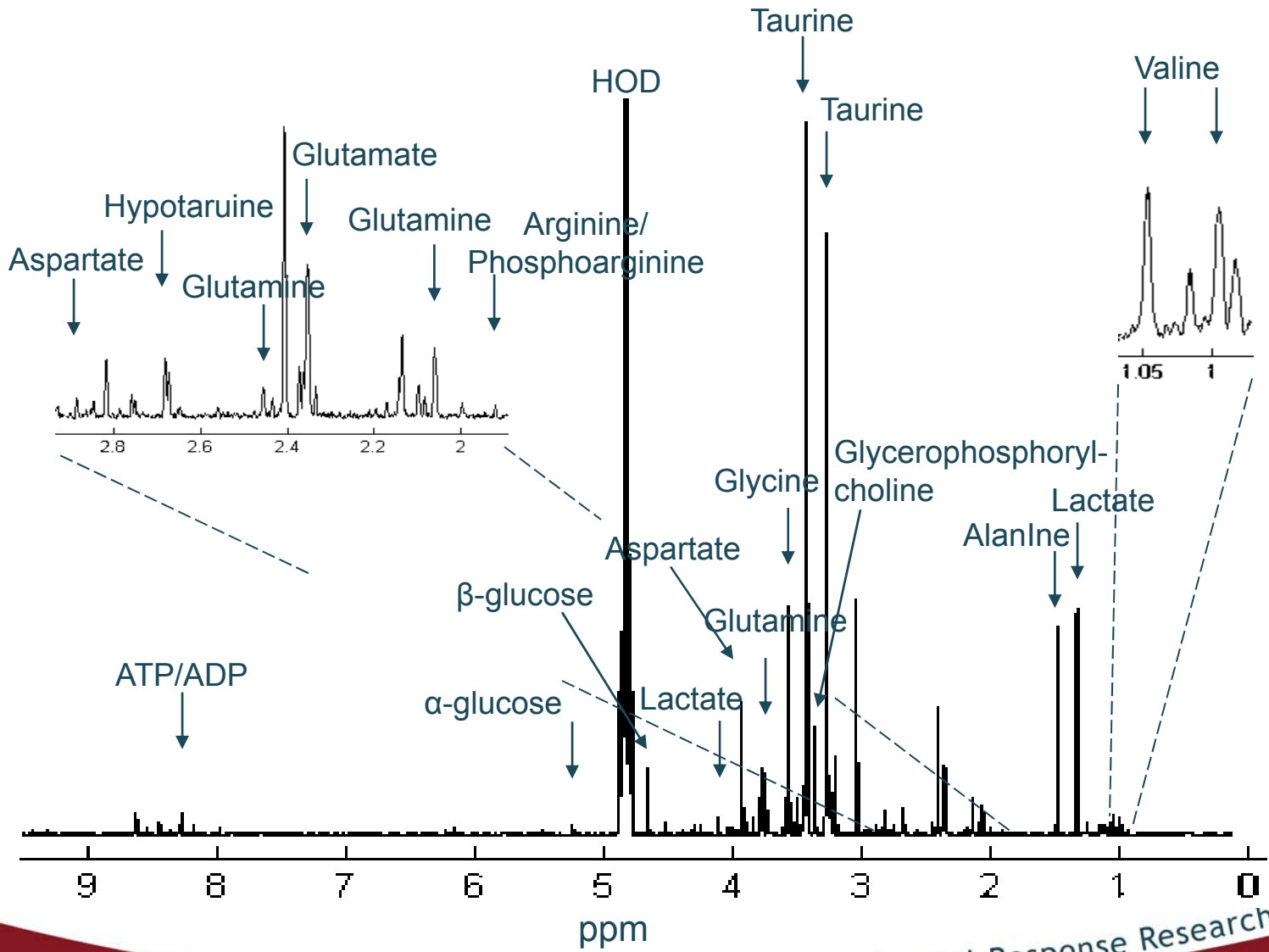
p-JRES NMR of Liver Extract



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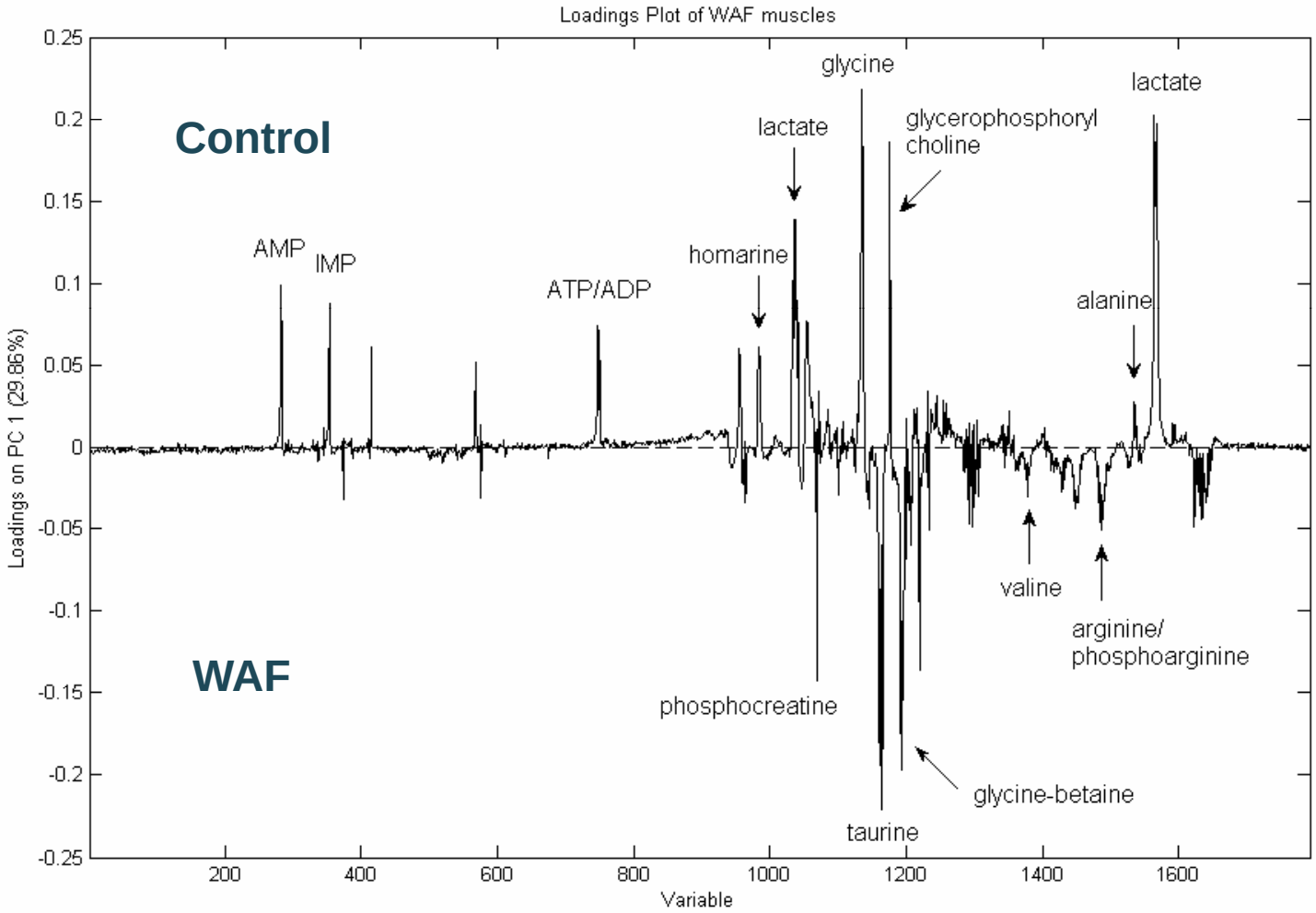
Muscle Loadings Plot from WAF Exposure



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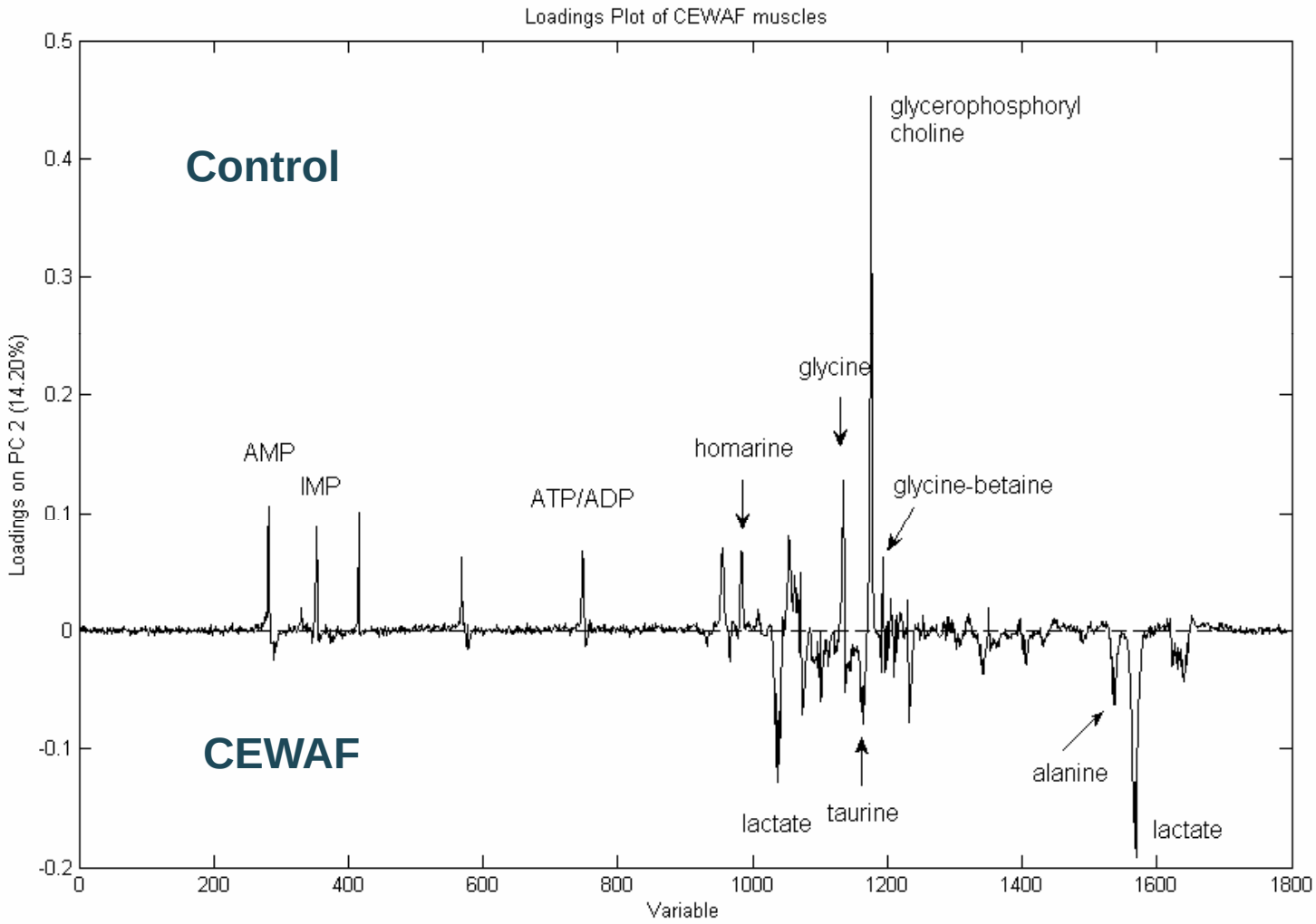
Muscle Loadings Plot from CEWAF Exposure



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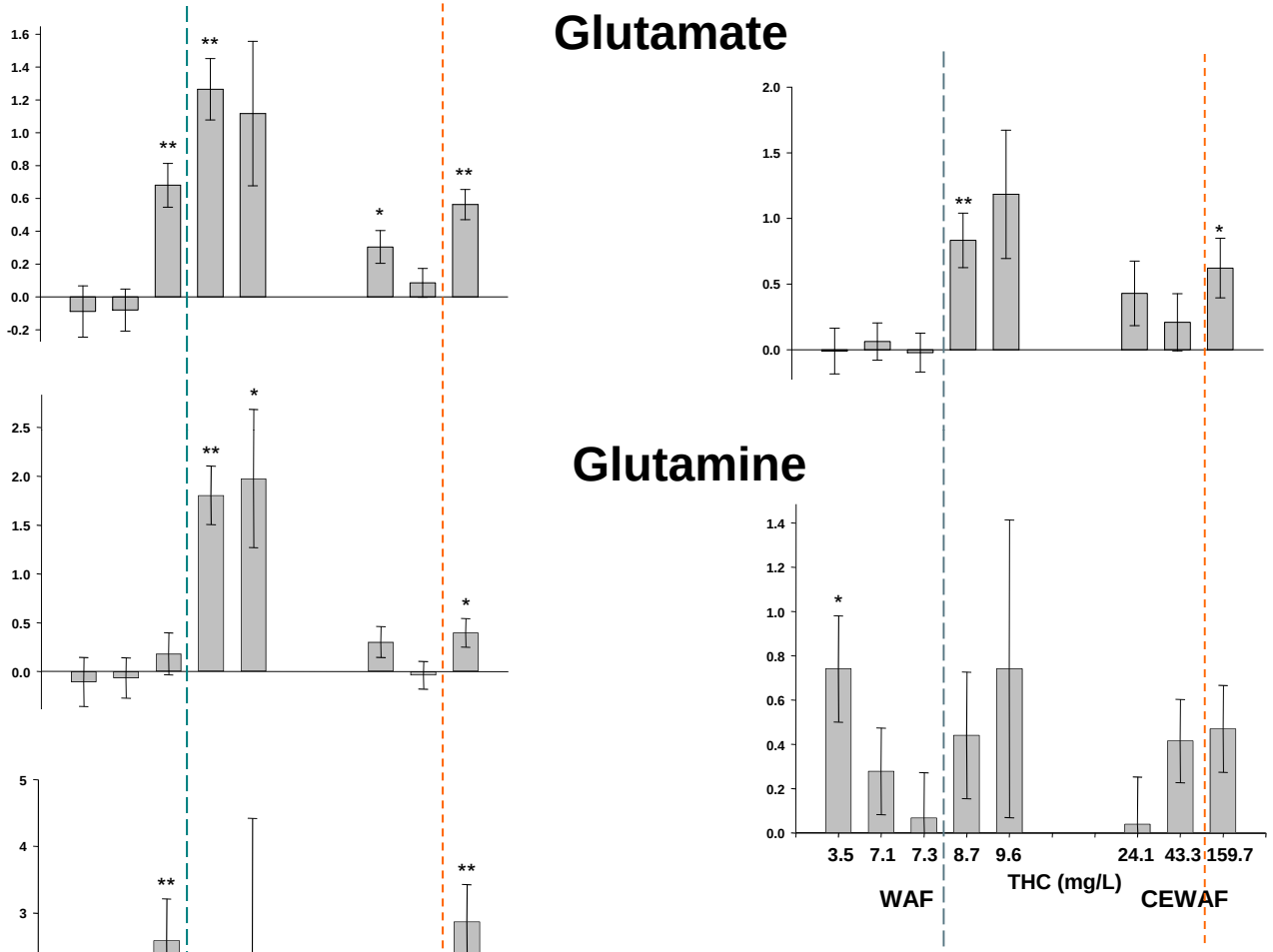
Metabolic Effects in Muscle

Valine

Glutamate



Arginine/ phosphoarginine

Glutamine

Alanine

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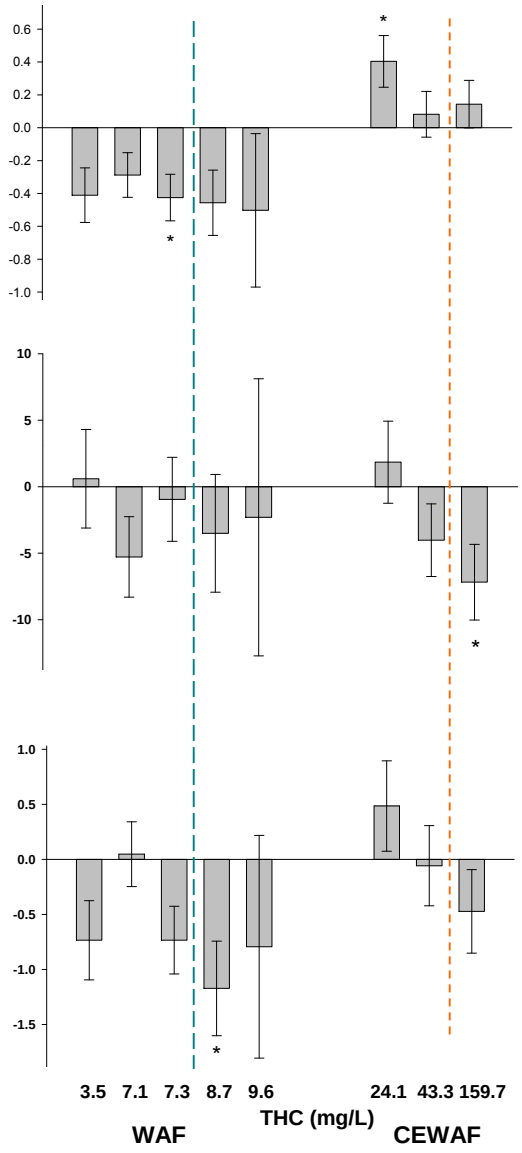
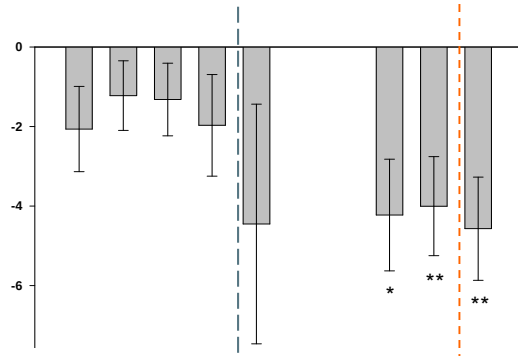
Succinate

Phosphocreatine

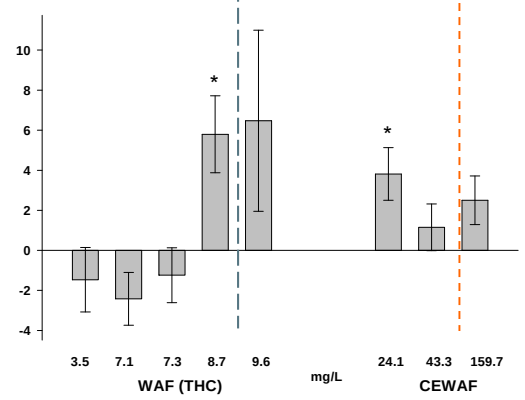
ATP/ADP



Glycerophosphorylcholine



Taurine



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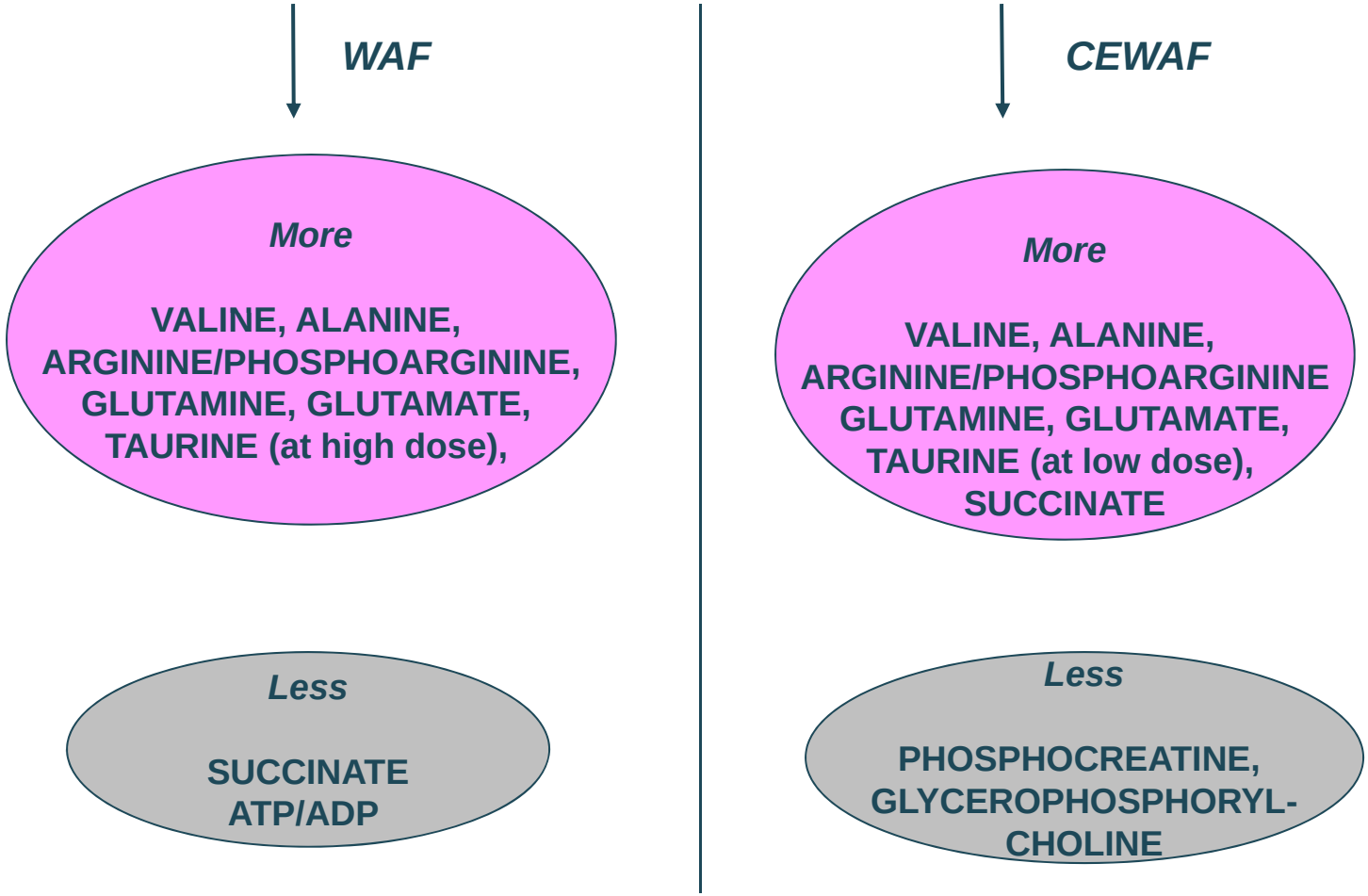
Summary of Metabolic Results in Muscle



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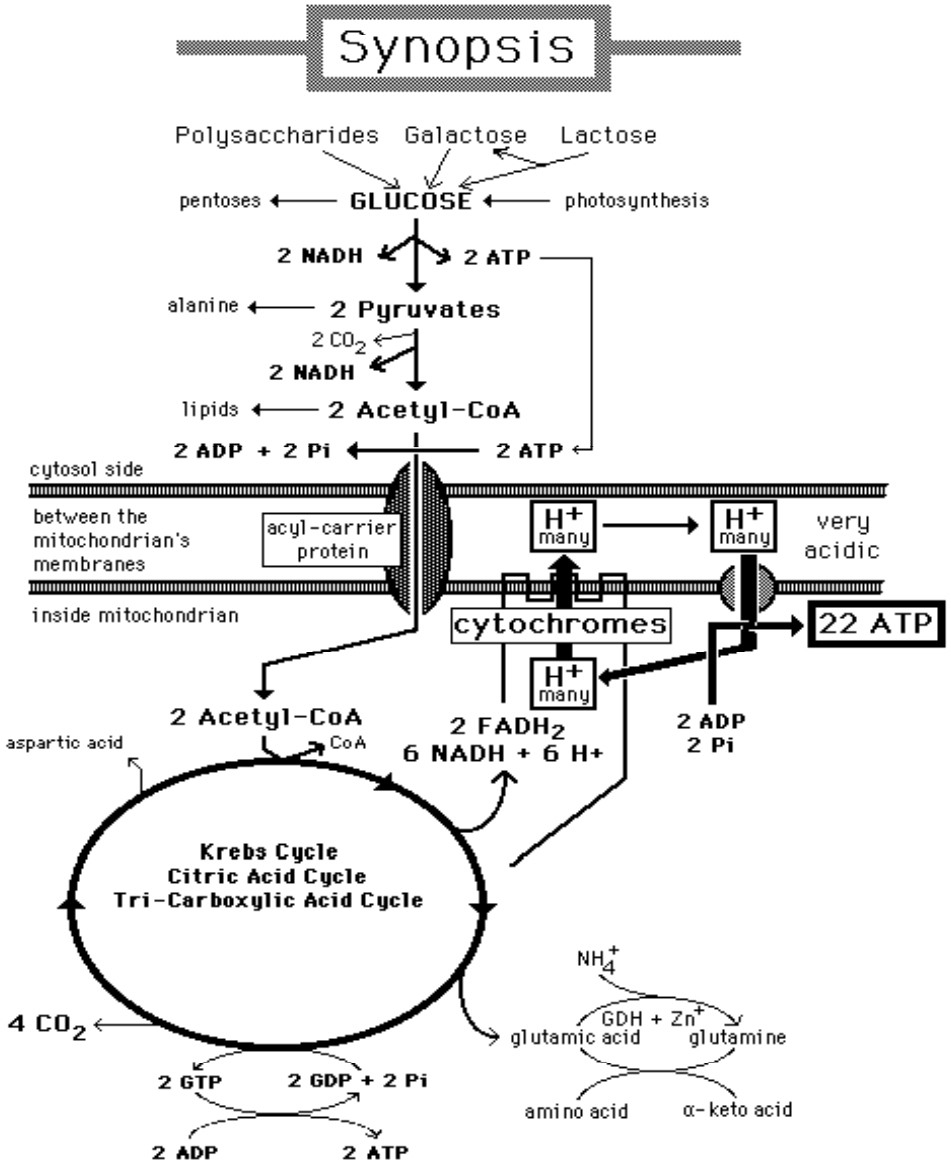
Implications



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Conclusions

- Dispersant treatment may decrease oil toxicity to smolts some 20-fold – a consideration for spill response.
- Both WAF and CEWAF cause an increase in amino acids – potentially to repair damage to proteins and enzymes (via increased protein synthesis).
- Loss of energy for damage repair may leave less for growth, stress response, or future reproduction.

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