Using Estuarine and Brackish Water Turtles to Model Potential Long Term Effects of Oiling of Nests During Early Embryonic Development

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Research Questions

- Might water accommodated fractions of crude oil (WAF) or chemically dispersed crude oil (CEWAF) influence embryonic and juvenile traits in sea turtles?
- Do WAF and CEWAF differ in their effects on embryonic and juvenile traits?
- What specific traits are altered by embryonic exposure to WAF and CEWAF?
- Endpoints: DNA damage, developmental rate and hatching success, physiological, morphological, and behavioral traits of juveniles.

- Caveat:
  - Sea turtles are endangered, precluding manipulative experiments with large numbers of eggs
- Solution:
  - Employ model species that:
    1) are largely unprotected
    2) are tractable for use in large scale studies (clutch size, availability)
- Model species: Snapping turtles
  - Original protocol included diamondback terrapins as well, but this species was ultimately removed from study.

The snapping turtle (*Chelydra serpentina*)

Overview of Experimental Design

- Eggs were collected from nests
  - Collections took place in May, 2006 at a commercial turtle farm in Louisiana.
- Eggs were incubated in replicate artificial nests in the laboratory
  - Incubation temperature = expect primarily male hatchlings
  - 6 * 4 design for each species
    - 6 treatments – Control (FW only), WAF Low, WAF High, CEWAF Low, CEWAF High (Low = 0.5, High = 10 g oil/L), or Corexit 9500 dispersant only. An additive (J. Clark, pers. comm.) was used to facilitate dispersal in freshwater.
    - Oil = weathered Arabian Light Crude
    - 1:10 dispersant:oil
    - 4 replicate nests per treatment
    - 12 eggs per nest

Nests – Construction and Dosing

- 15 L, foil lined polyethylene containers filled with a 3:1 medium sand:gravel mix
- Eggs (approximate developmental stage 14) buried 2.5 cm below surface
- Resin (XAD) filled bags and 2 "extra" eggs placed at nest depth in 2 replicates per treatment.
- Prior to dosing, solutions (prepared following CROSERF protocols) were passed through a 15 cm column of nest substrate to simulate percolation of solutions to the depth of a natural nest.

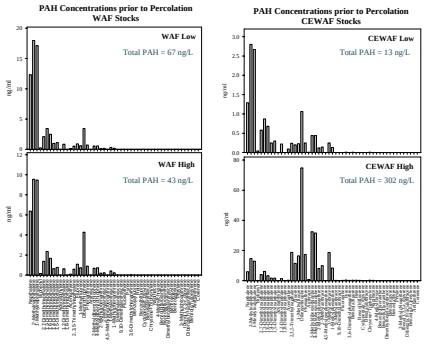
Nests – Construction and Dosing (cont'd)

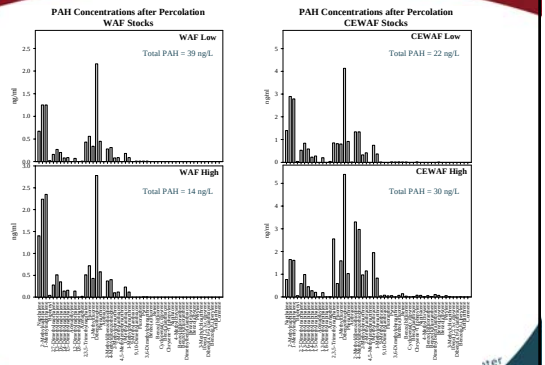
- One XAD bag and 2 "extra" eggs were removed 1 week after dosing when overlying substrate was removed from eggs and replaced with damp sphagnum moss.
- Remaining XAD bags were removed approximately 1 week after hatching began.

Chemical and Biological Endpoints

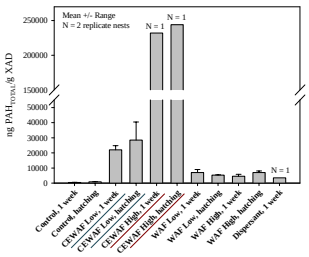
- PAH exposure (Total PAH + 53 individual PAHs; TPH) – Dosing solutions, XAD resin, egg contents
- Mean time to hatching
- Hatching success
- DNA damage in hatchlings (liver)
- Predator avoidance behavior
- Juvenile growth and survival
- Juvenile energetic efficiency (metabolism, lipid production)

Results - Chemistry



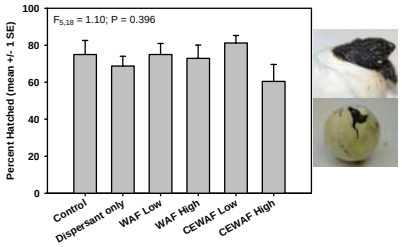


XAD Substrates – Egg Depth
(Collected 1 week after dosing and at hatching)

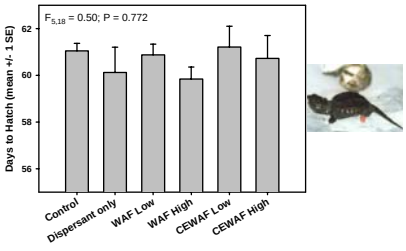


Results - Biotic

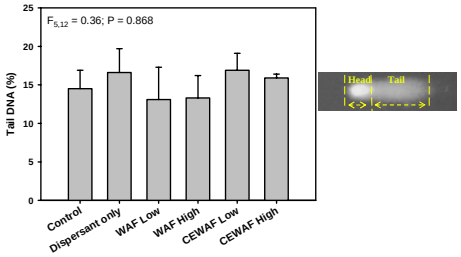
Hatching Success



Time to Hatch (Days Post-Dosing)

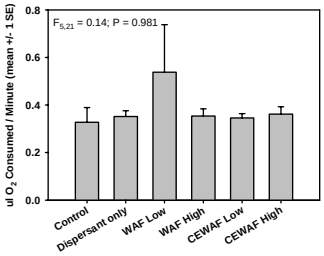


DNA Damage - 1 Week Post-hatching

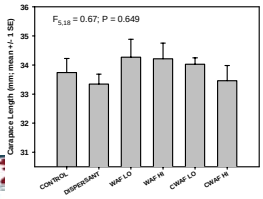


Standard Metabolic Rate - Juveniles

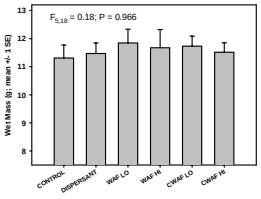
(December 2006, 20°C)

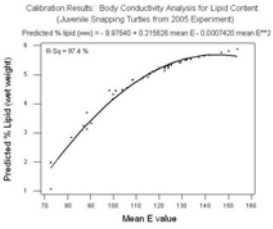


Mean Carapace Length, March 2007



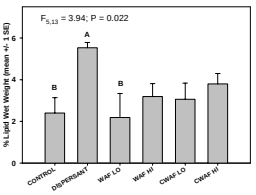
Mean Wet Mass, March 2007





Mean Percent Lipids in Juveniles

(February, 2007)



Predator Response Trials Revealed No Differences in Behaviors

Yet to Complete

- Morphological assessment of gonadal development
- Continued monitoring of growth and physiology
- Complete chemical analyses on XAD and eggs.

Summary

- Even when exposed to WAF and CWAF containing relatively high concentrations of oil derived PAH, no effects on development, DNA integrity, or physiological energetics were detected.
- Using *realistic exposure regimes* via simulating exposure that would occur at the depth of natural nests, these results are likely more suitable for risk assessments that more simplistic studies in which eggs were directly exposed to oil.
- Good news if you're a turtle?

Acknowledgement

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Coastal Response Research Center