Discussion on "Methods to Assess PAH Exposure and Effects II"

Integrating Multiple Endpoints for Understanding Individual and Population Effects on Sensitive Species

Coastal Response Research Center

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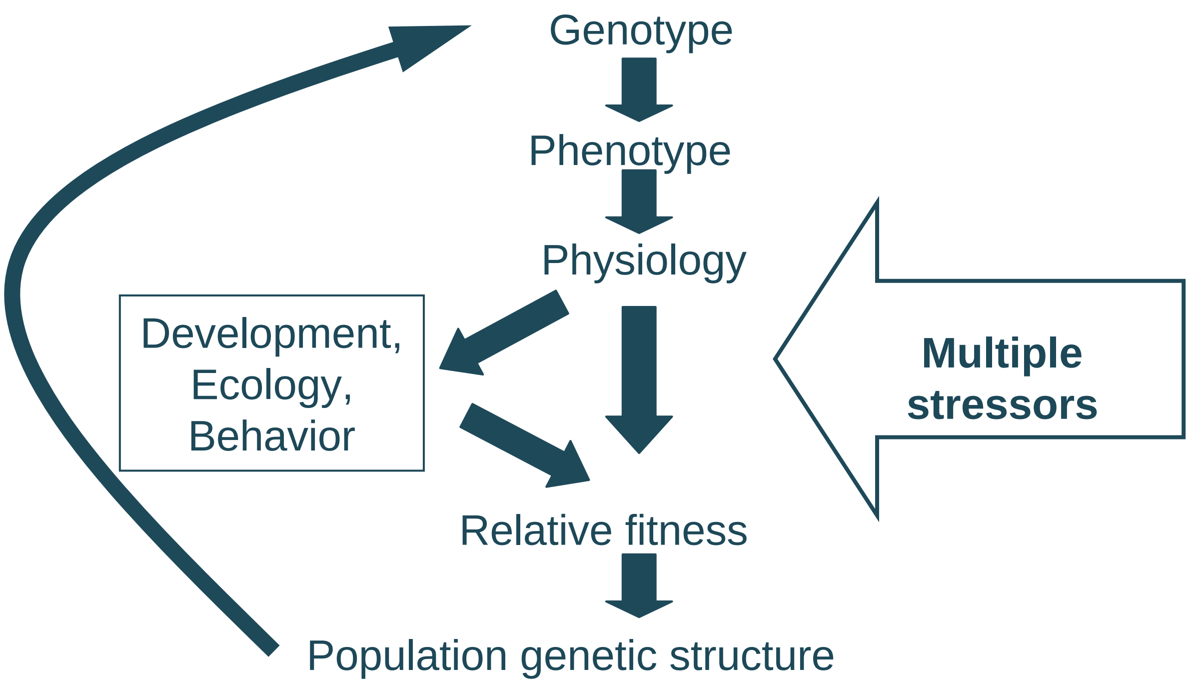
Discussion Points

1. Introduction
2. Anemone and Coral multiple metrics
3. Dispersant toxicity
4. Oil / Dispersed Oil toxicity
5. Reptile multiple endpoints
6. Conclusions

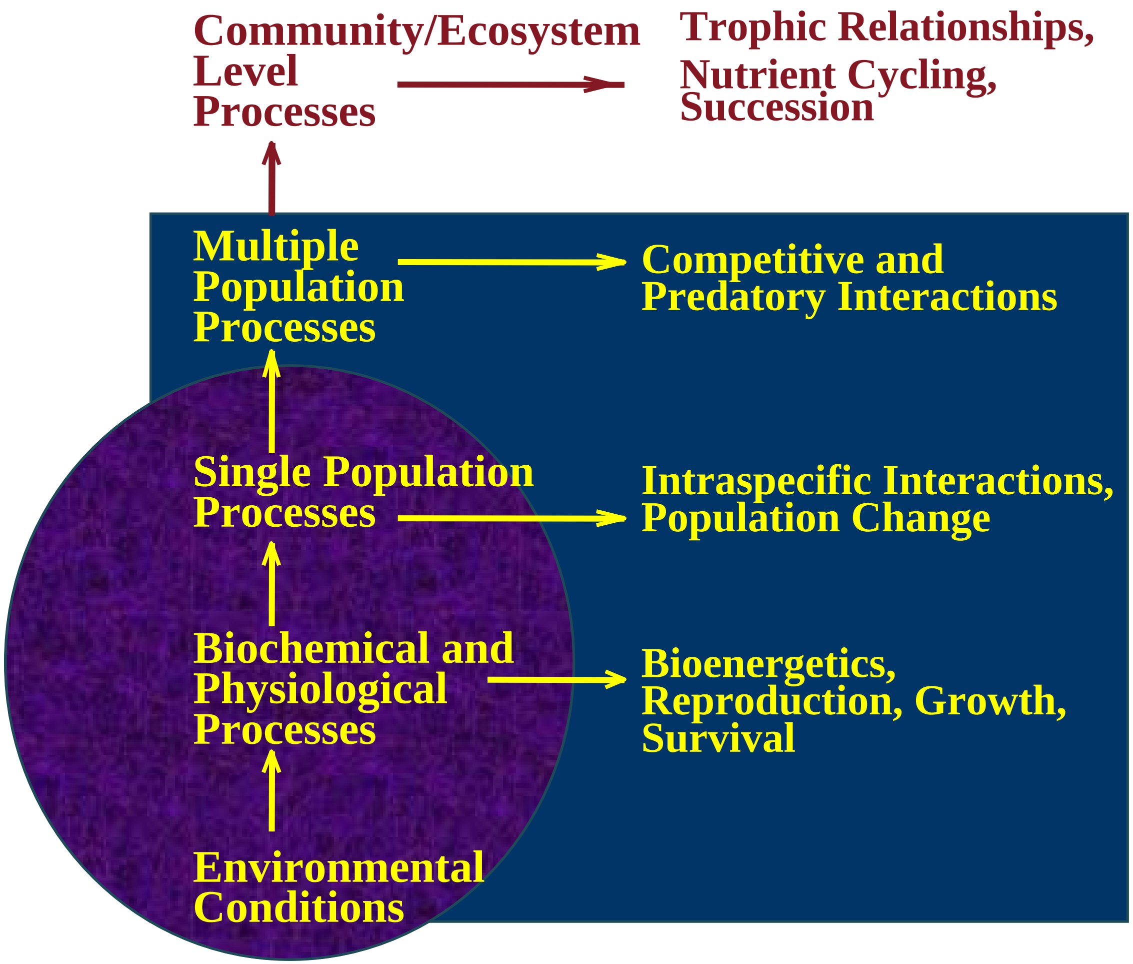
Introduction

- Goals understand impacts at population levels
- In complex ecosystems / multiple populations present
- How assess impact / recovery?

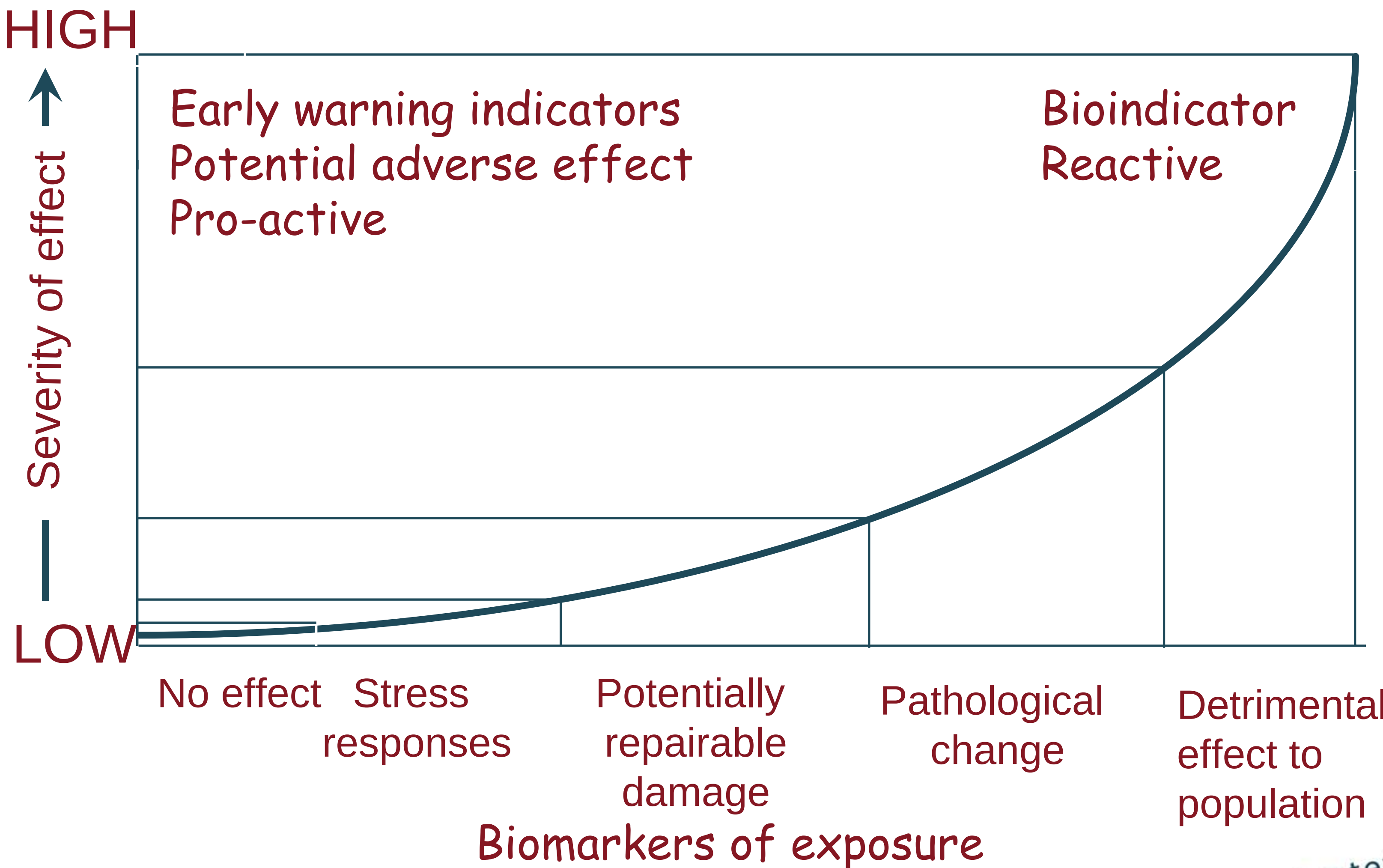
    - Acute …need % population affected (not 100%)
    - Long-term sub lethal (delayed) responses



Introduction



Biomarkers - Individual level responses



Multiple Endpoints

Challenge:

- Choice of endpoints?
- Often species / route of exposure / toxicity mechanism dependent
- Integrating individual endpoints ; systems approach
- Translation to population effects

Cnidarian Research

Approach:

(1) Detailed chemistry
- exposure
  (exposure routes)
- bioaccumulation
  (bioavailability and persistence)

(2) Multiple biological endpoints;

- Acute: mortality
- Sub lethal:
  - Behavioral endpoints
  - Growth
  - Mucus production
  - Algal / chlorophyll content (bleaching)
  - Protein / lipid content
  - DNA damage
  - Dissolved oxygen (photosynthesis)

Cnidarian Research

Questions:

- Sensitivity compared with other species
- Importance of route of exposure?
- Potential for delayed effects; mortality or sub lethal effects?
- Phototoxicity issues - use of natural sunlight conditions
- Bleaching
- Excess mucus production - energetic cost
- DNA damage (PAH metabolism +/- phototoxicity) - death or mutations

Cnidarian Research

Species:

(1) Temperate anemone (*Anthopleura elegantissima*)

- Important primary producer in intertidal zone
- Symbiotic with algae
- ‘Model’ cidarian for corals?

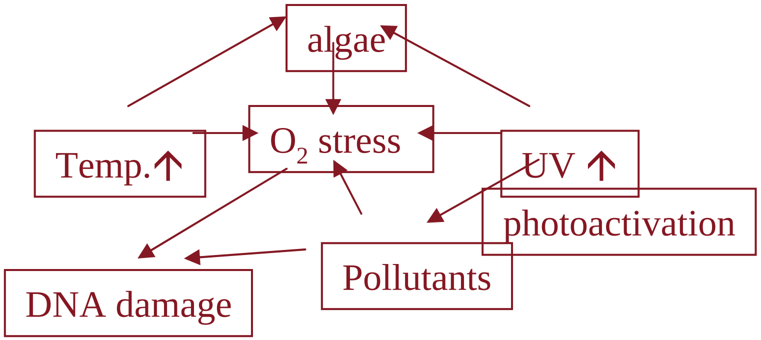
Behavioral Endpoints Studied:

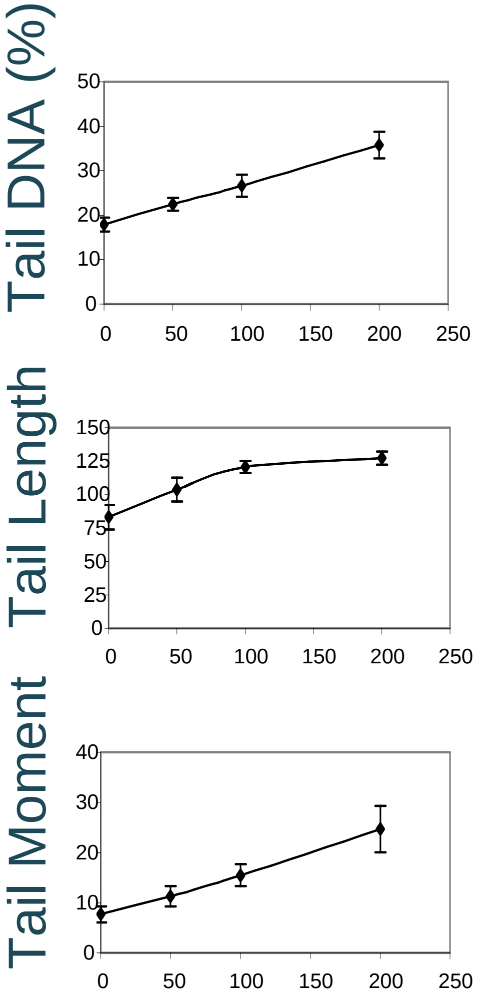
- Tentacle retraction
- Column extension

Cnidarian Research

Additional Endpoints Studied:

- Mucus production
  - Previous work demonstrated excessive production as a protective response
- DNA damage
  - Benzo(a)pyrene dose-dependent increase in DNA damage





Cnidarian Research

Experiments:

(1) 96 hour LC50 dispersant (Corexit 9500) exposure

(2) 8 hour Acute WAF and CEWAF exposures (Arabian light crude)

- variable dilution using 25g/l and 100, 50, 25, 10 and 1% doses
- dispersant:oil ratio (1:10)

(1) Detailed study WAF and CEWAF exposures

- 8 hour exposure
- One month recovery / delayed responses
- Filtered versus non filtered preparations
- Low dose (0.5g/l oil) and high dose (10g/l oil)
- 1:10 dispersant:oil ratio

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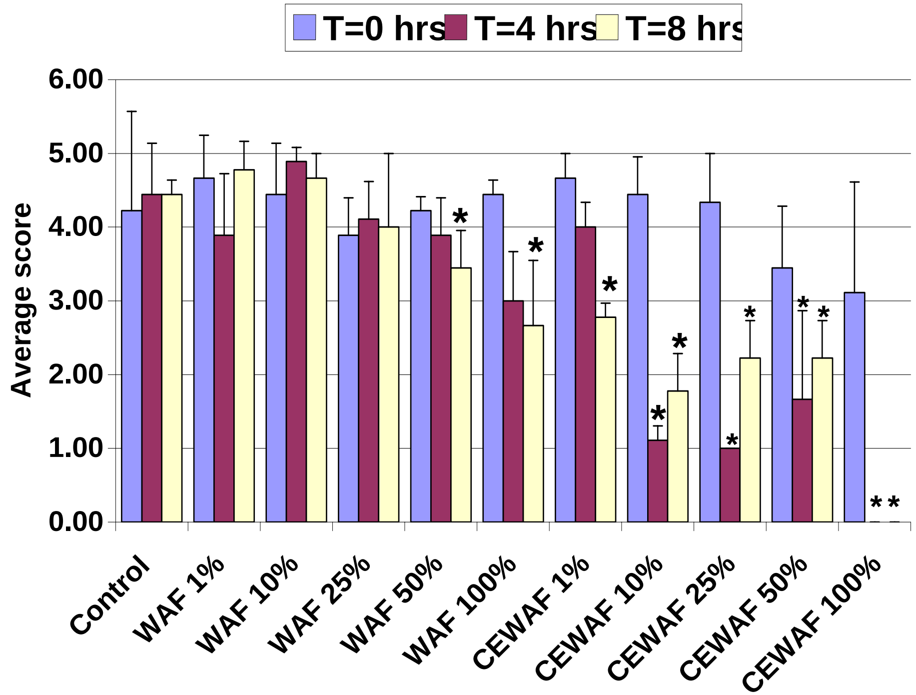
Anemone Results = Acute

(1) Tentacle retraction



t-PAH (ug/l)

%	WAF	CEWAF
1	0.14	52.01
10	1.76	68.60
25	5.42	152.22
50	9.99	343.11
100	19.51	423.03

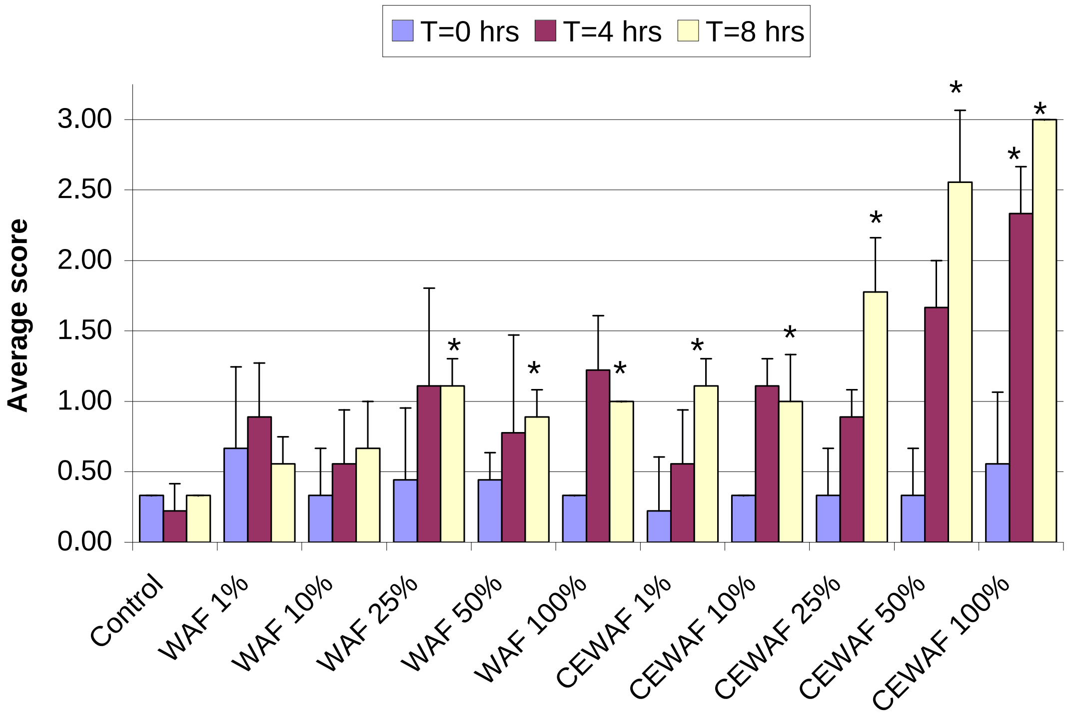
- Effect of WAF at 50 and 100%
- Dose and time dependent effect of CEWAF (from 1%)

Significance:

- Reduces feeding
- Inhibits algal photosynthesis
- Reduction in growth

Anemone Results = Acute

(2) Mucus production



t-PAH (ug/l)

%	WAF	CEWAF
1	0.14	52.01
10	1.76	68.60
25	5.42	152.22
50	9.99	343.11
100	19.51	423.03

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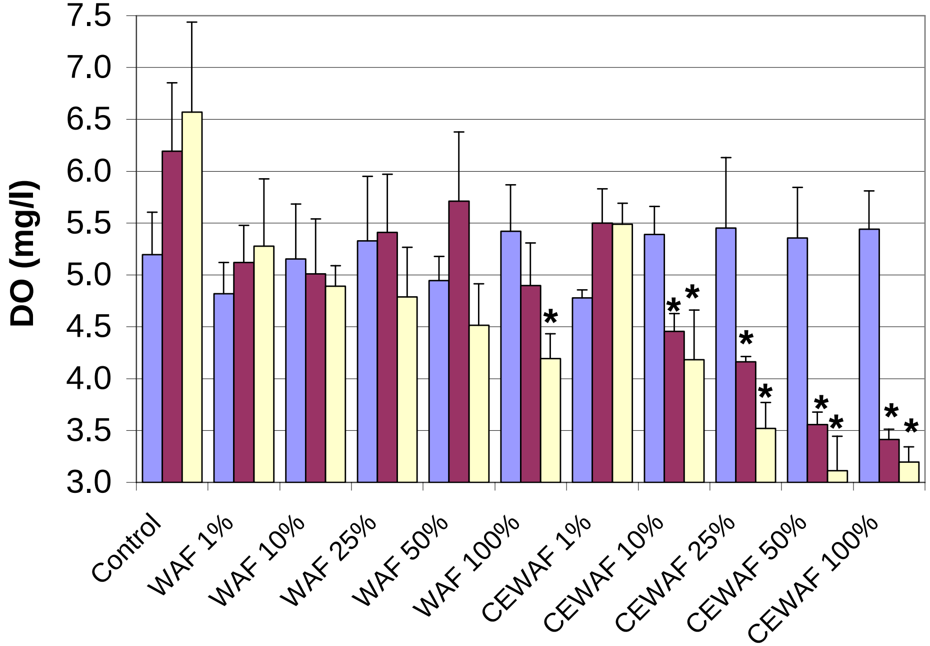
- Effect of WAF 25-100% (more sensitive than tentacle retraction)
- Dose and time dependent effect of CEWAF (from 1%)

Significance:

- Energetic cost and trophic transfer issues
- Needs competent algae = mucus

Anemone Results = Acute

(3) DO production (indirect measure algal photosynthesis)



t-PAH (ug/l)

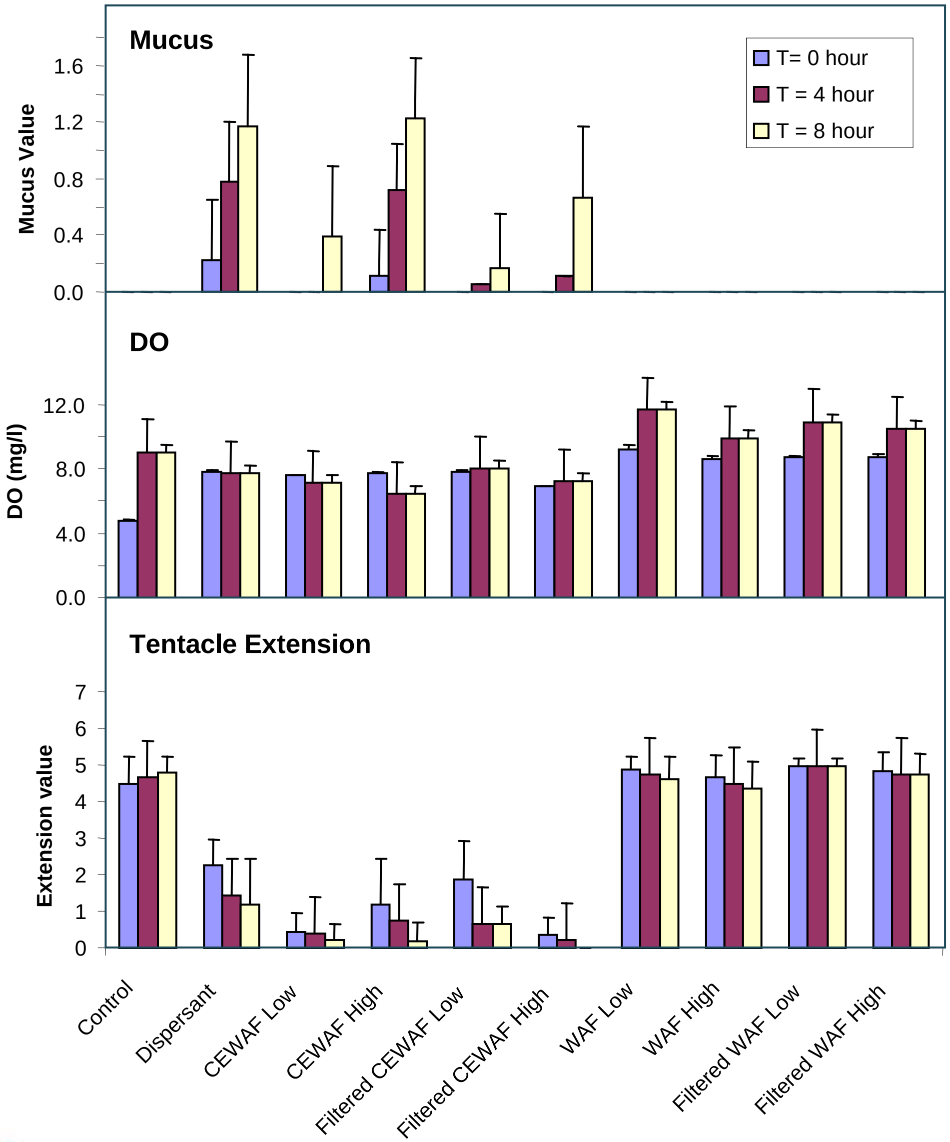
%	WAF	CEWAF
1	0.14	52.01
10	1.76	68.60
25	5.42	152.22
50	9.99	343.11
100	19.51	423.03

- Effect of WAF only at 100%
- Dose and time dependent effect of CEWAF (from 10%)

Significance:

- Algal photosynthesis reduced = reduced supply mucus?
- Control in lab setting for low DO

Anemone Results = Detailed



t-PAH (ug/l)

	WAF	CEWAF
UF low	37	374
F low	23	23
UF high	54	1094
F high	59	115

-Some evidence filtering reduces effects CEWAF

- Mechanism of toxicity? - only small influence of droplets?.

- Dispersant issues!

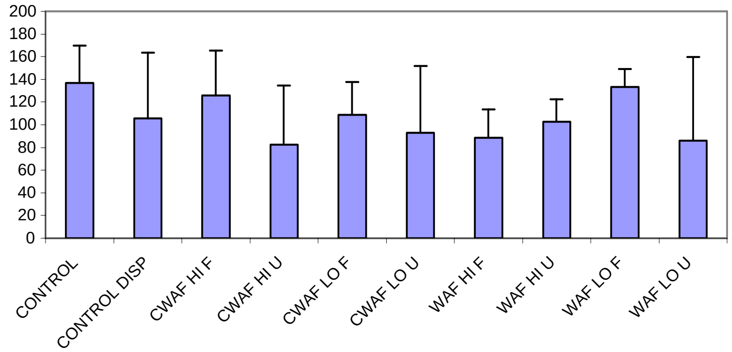
- No mortality in exposure or recovery.

- Two days after exposure no differences in DO, tentacle expansion or mucus production.

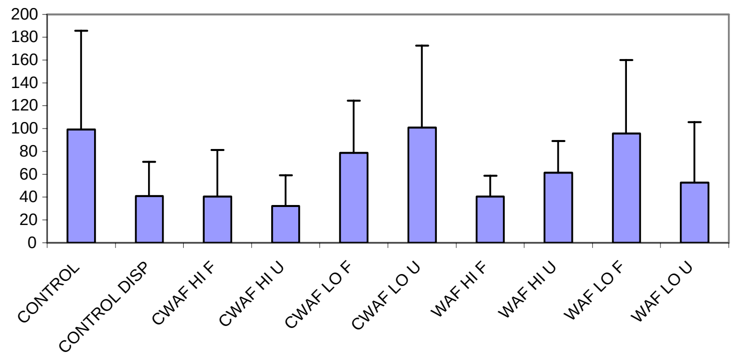
Anemone Results = Detailed

Chlorophyll a content / g wet wt

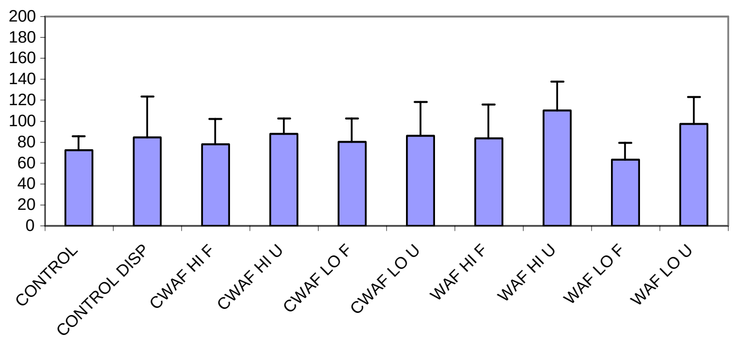
8 hour



7 day



30 day



- No difference in algal cell counts, at any dose or time
- No evidence of bleaching
- Chlorophyll reductions 7 day
- No difference in protein content

For final analyses:

- Need to integrate all multiple metrics to PAH levels in anemones (bioaccumulation)
- Assess bioavailability and persistence
- DNA damage

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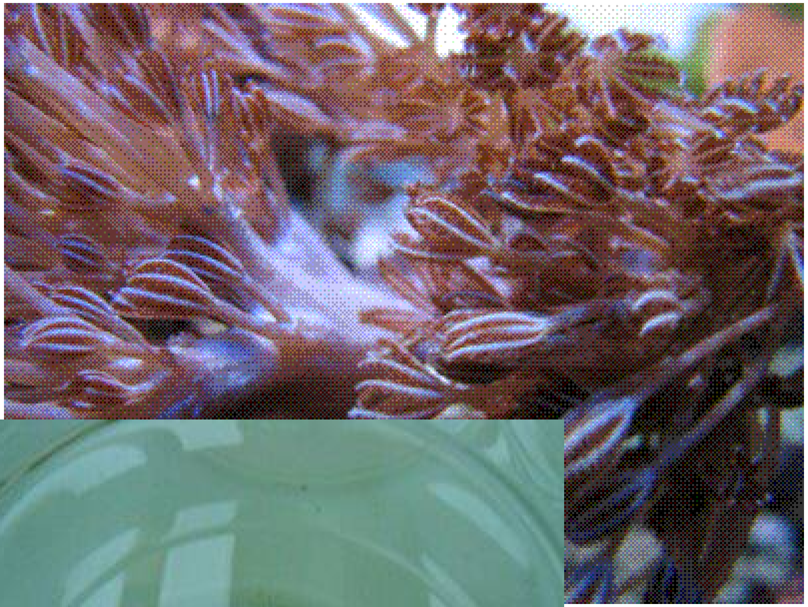
Cidarian Research

Species: (2) Tropical soft coral (*Xenia elongata*)

- Common tropical soft coral
- Obligate symbiont with the sensitive dinoflagellate algae zooxanthellae
- Demonstrated sensitivity to changes in water quality
- Behavioral stress markers such as changes in rigidity and rhythmic pulsing
- Representative of a group of organisms forming basis of complex reef ecosystems



Objectives:

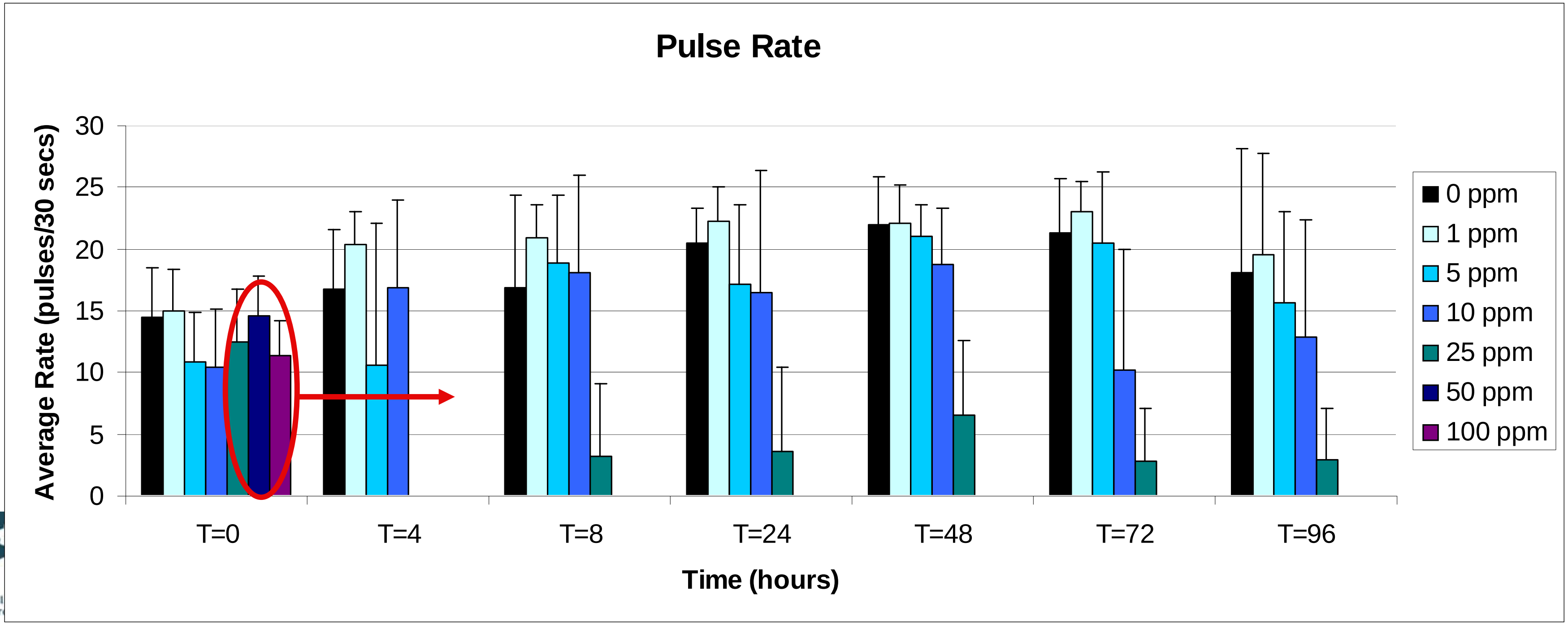
1. Compare acute and sub-lethal effects of short term (8 hour) exposures to various dilutions of physically dispersed oil (WAF) and chemically dispersed oil (CEWAF.)

2. Compare effects of WAF/CEWAF and glass-fiber filtered WAF/CEWAF.

3. Assess long term chronic, sub-lethal effects by monitoring recovery in clean sea water for 28 days.

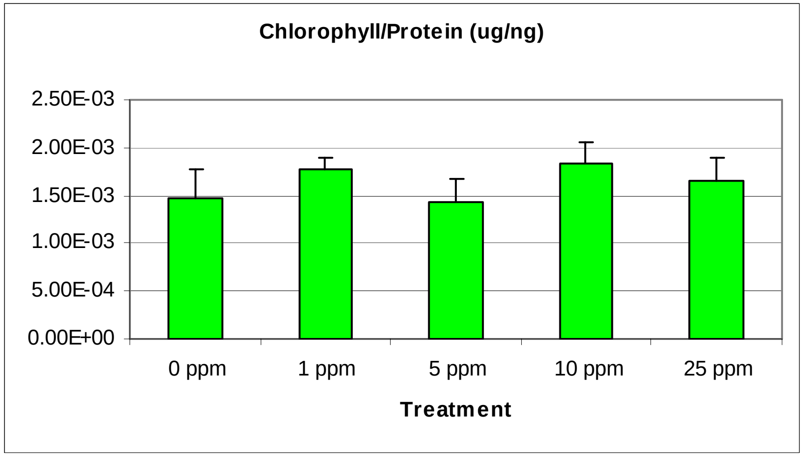
4. Assess a variety of behavioral and molecular endpoints including: pulse rate, rigidity, bleaching, dissolved oxygen, algal cell count, chlorophyll levels, DNA damage, TPH and 53 PAHs.

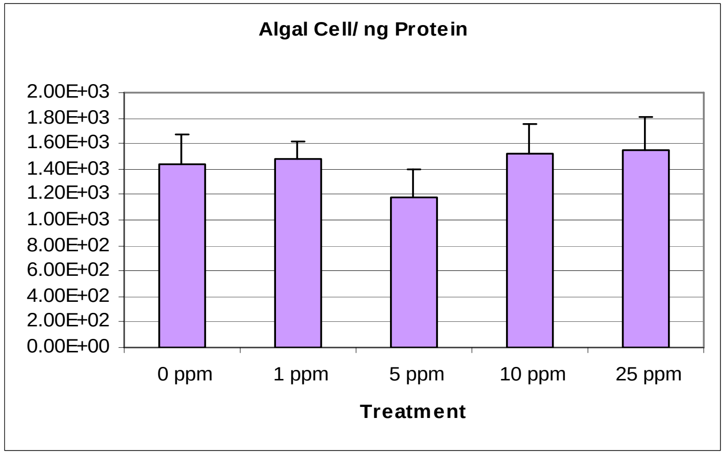
Results: Dispersant exposure



- Mortality 50 ppm and higher after 4 hours
- Pulsing stops completely at levels of 25 ppm and greater.
- Pulsing resumes after 8 hours at 25 ppm, no pulsing at higher concentrations.

Results: Dispersant exposure





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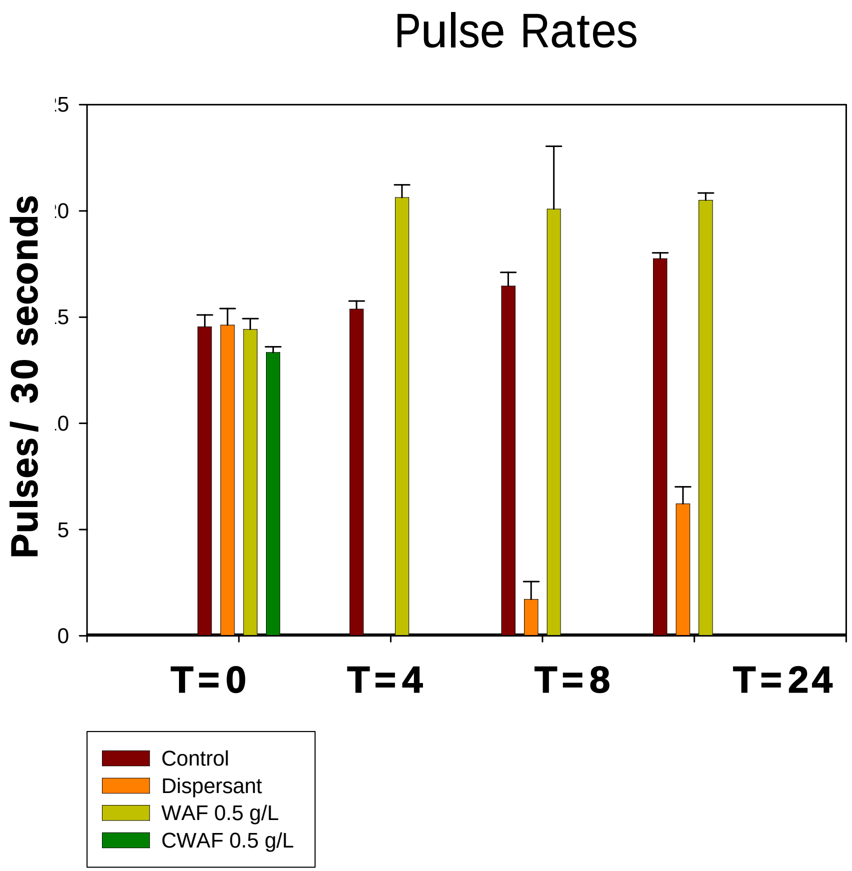
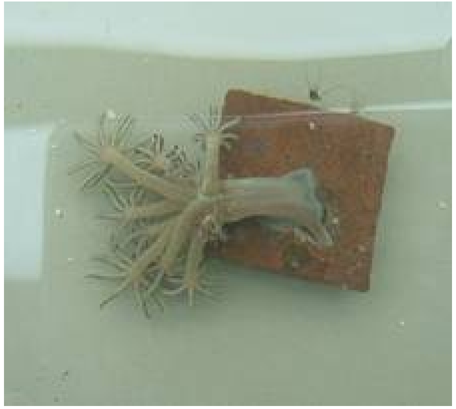
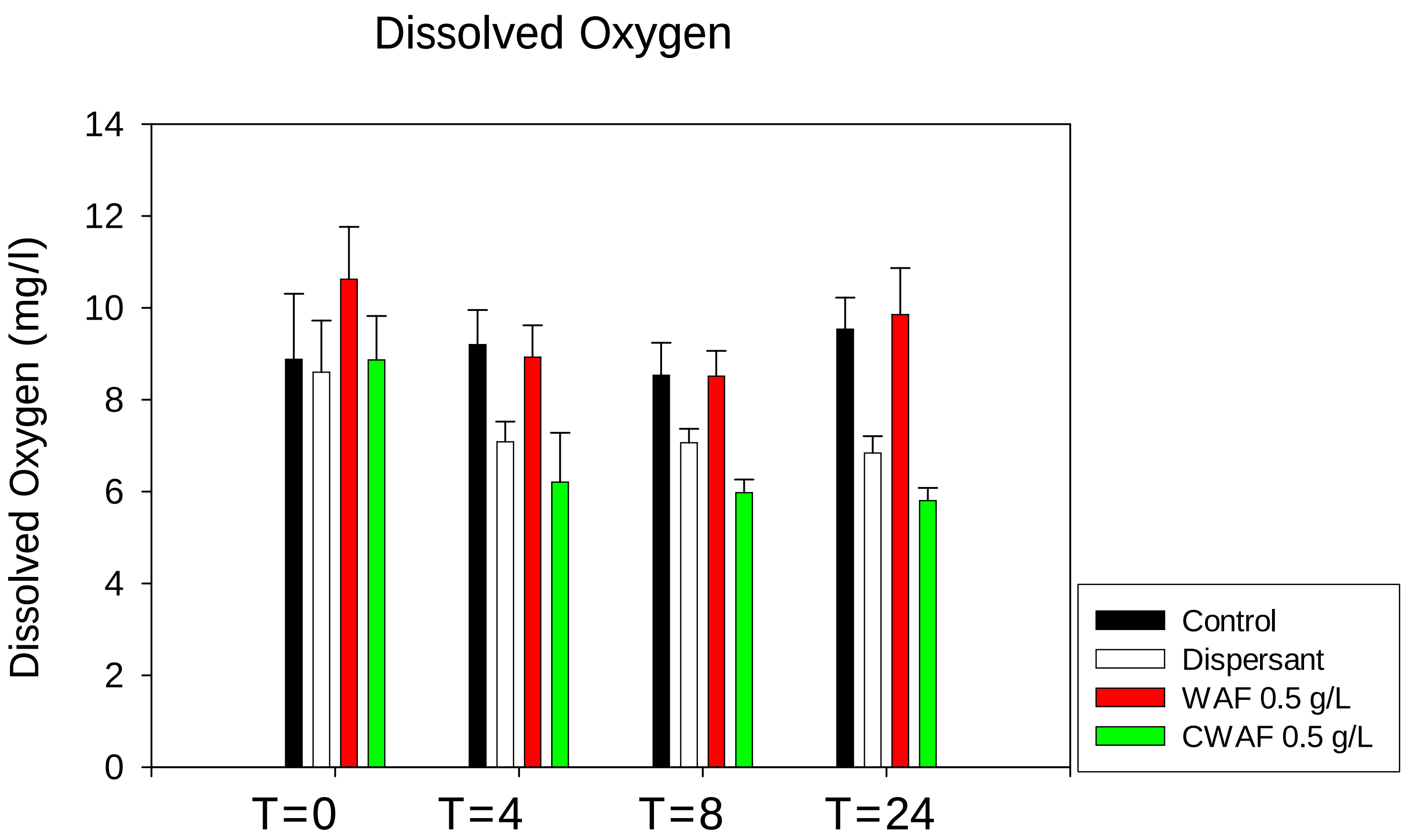
Results: Acute exposure

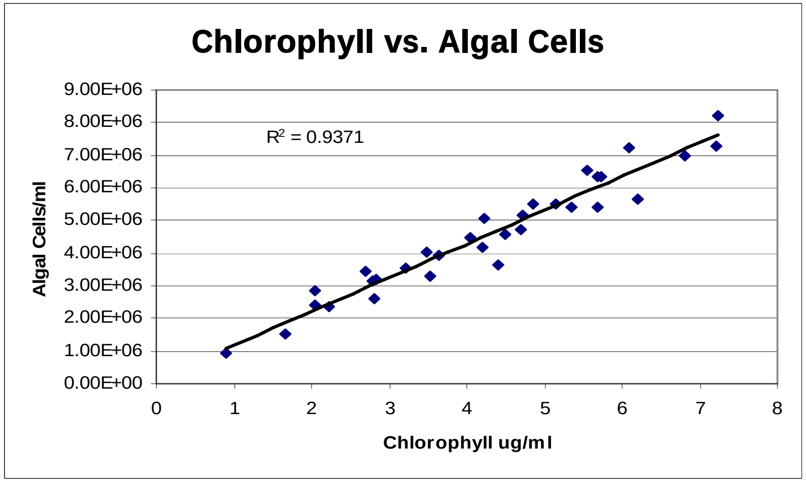


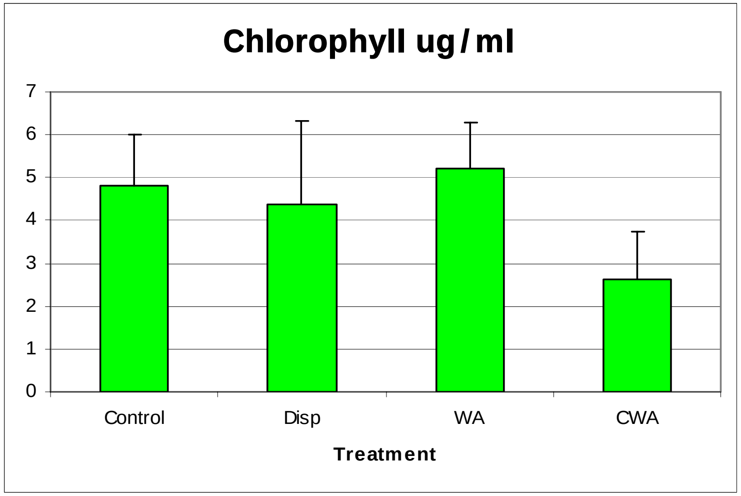


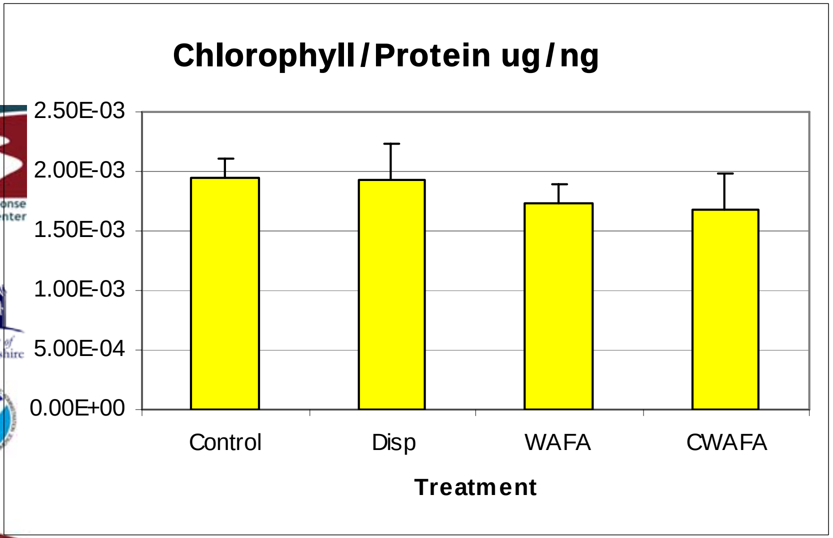
Results: Acute exposure

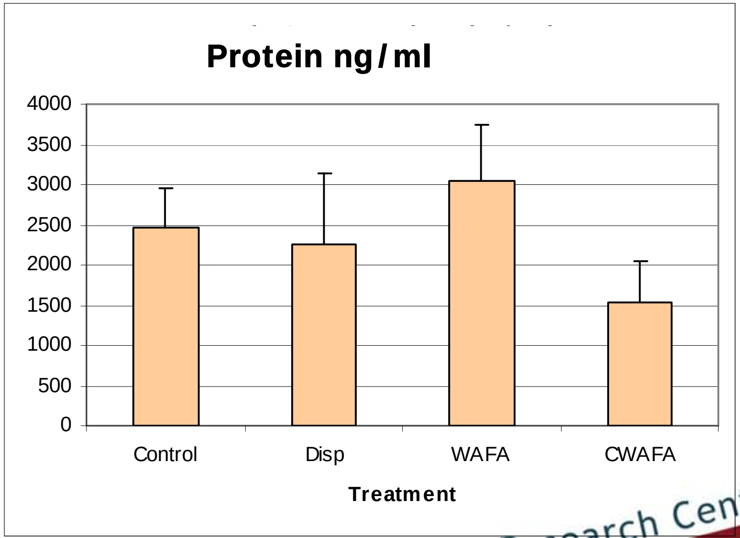


Results: Acute exposure









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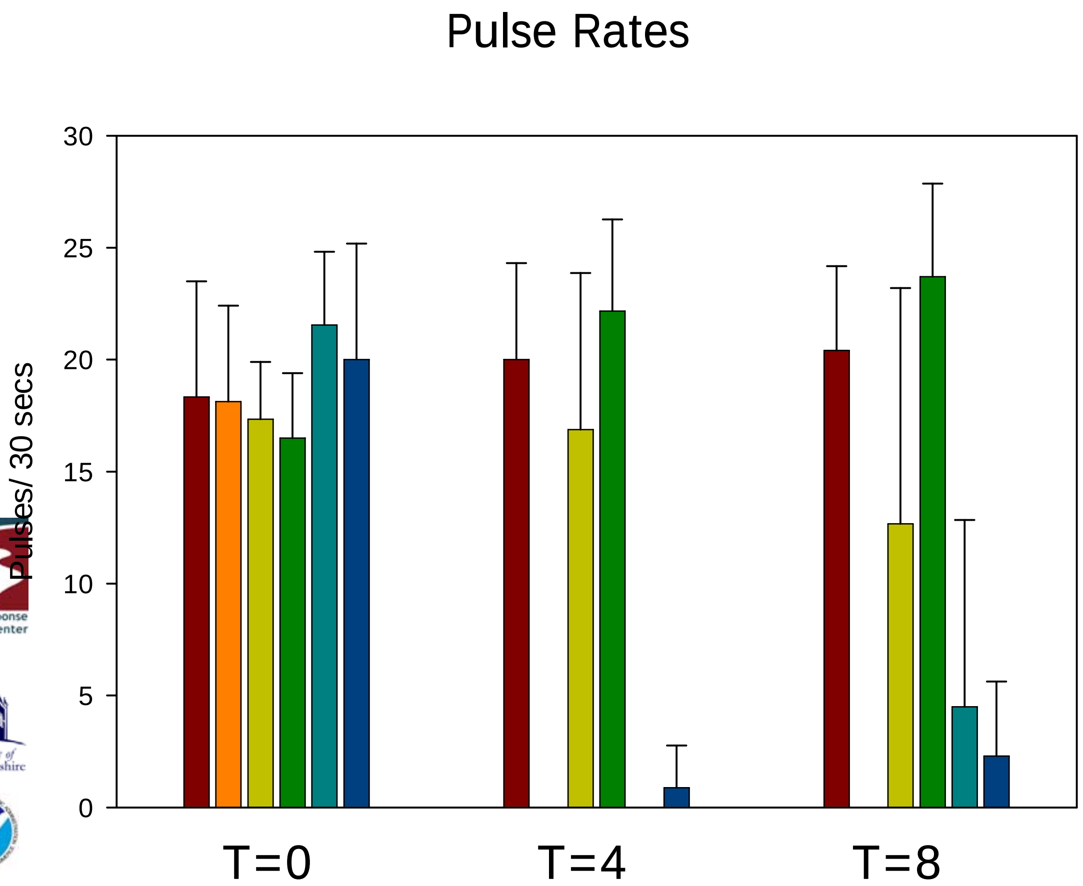
Results: Detailed exposure



CWU – after 1 week in recovery

Xenia study

- Strong behavioral traits, sensitive
- Sensitive to dispersant (LC50 24hr <50ppm)
  Green hydra (160ppm 96hr LC50)
- Algal cell loss / chlorophyll reduction / protein loss

- Last detailed time point ;
  - In recovery delayed mortality observed in UF CEWAF
  - Control and WAFs growth, and all metrics same
  - Dispersant, F CEWAF and UF CEWAF impacted (in that order), show much reduced growth, no up-regulation of GFP

- More results to come:
  - TPH and PAH
  - DNA analysis
  - Recovery rates

Reptile Studies

Multiple metrics of chemical and biological endpoints

Will be used in population models to forecast effects of impacted traits on future population size

Dosed: At critical reproductive period…assessed for delayed responses

Endpoints:

- Chemistry (detailed 53PAHs, TPH)
- Bioaccumulation (bioavailability)
- Hatching success
- Hatchling size
- Metabolism (metabolic rate)
- Behavioral studies; foraging behavior
  Predator response
- Growth
- Morphology (gonads etc)
- Mortality …. Over hatchling, and juvenille stage

Reptile Studies

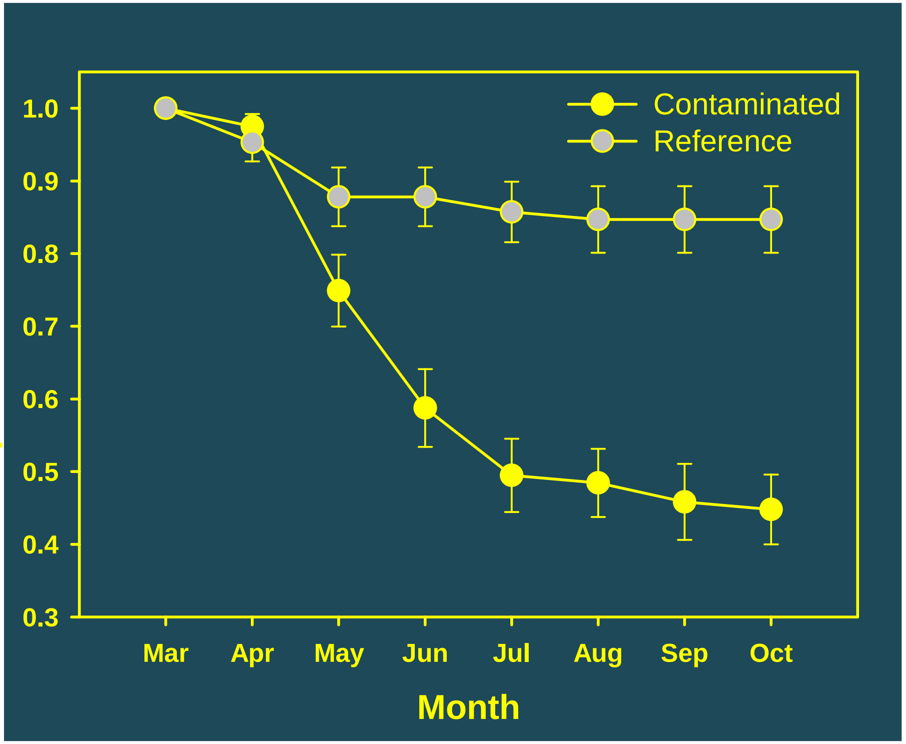
Survival to 13 Months of Age Post-hatching PCB treatments

Delayed Response : Latency period

No discernible differences in survival were observed during first 7 months post-hatching.

However, during the final 6 months, survival of individuals from the contaminated site began to decline rapidly.

Average survival at 13 months:
Reference: 85 +/- 5 %
Contaminated: 51 +/- 5 %



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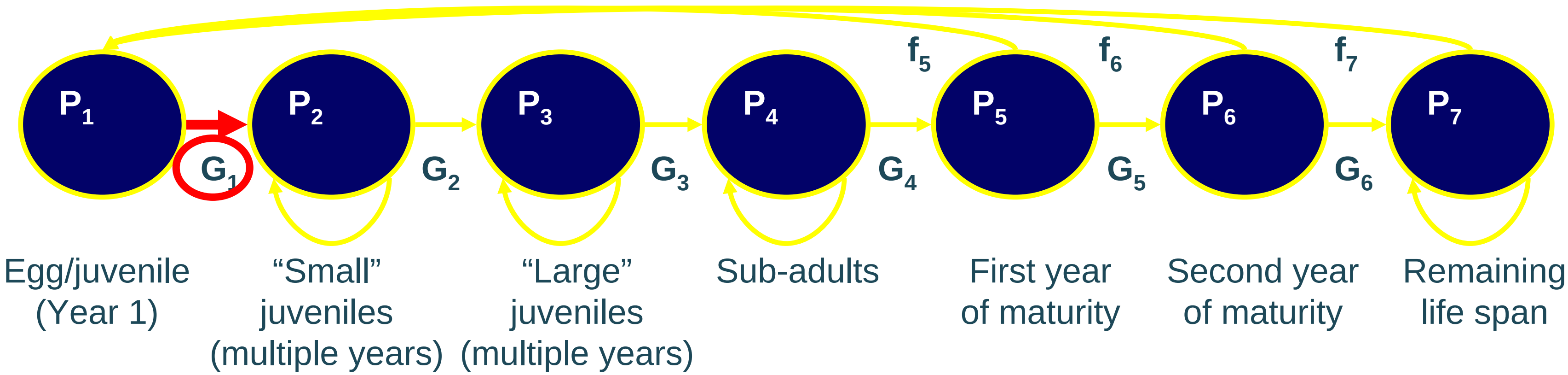
Projecting Population Growth Rates



P_i = age/stage-specific survival probability (e.g. surviving and remaining in stage i)

G_i = age/stage-specific transition probability (e.g. surviving and growing into the next stage).

f_i = age/stage-specific fertility

Models are based on a 1 year time step

Models developed based upon the framework of Connington and Brooks (1996) reflecting data from Brooks et al. (1988) and Congdon et al. (1994)

$$
A = \begin{pmatrix}
0 & 0 & 0 & 0 & f_5 & f_6 & f_7 \\
G_1 & P_2 & 0 & 0 & 0 & 0 & 0 \\
0 & G_2 & P_3 & 0 & 0 & 0 & 0 \\
0 & 0 & G_3 & P_4 & 0 & 0 & 0 \\
0 & 0 & 0 & G_4 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & G_5 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & G_6 & P_6
\end{pmatrix}
$$

$$
\begin{pmatrix}
0 & 0 & 0 & 0 & 15 & 15 & 15 \\
0.053 & 0.698 & 0 & 0 & 0 & 0 & 0 \\
0 & 0.055 & 0.698 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.055 & 0.674 & 0 & 0 & 0 \\
0 & 0 & 0 & 0.079 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.97 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0.97 & 0.96
\end{pmatrix}
$$

Base model matrix reflecting survival of hatchlings derived from reference sites.

$$
\begin{pmatrix}
0 & 0 & 0 & 0 & 15 & 15 & 15 \\
0.032 & 0.698 & 0 & 0 & 0 & 0 & 0 \\
0 & 0.055 & 0.698 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.055 & 0.674 & 0 & 0 & 0 \\
0 & 0 & 0 & 0.079 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.97 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0.97 & 0.96
\end{pmatrix}
$$

Comparative model matrix reflecting survival of hatchlings derived from contaminated sites.

As predicted based upon turtle life histories, model outputs suggest that:

1. Large numbers of juveniles are produced, but exceptionally high mortality rates diminish their per capita reproductive value.

2. Adult survival is almost exclusively responsible for population dynamics.

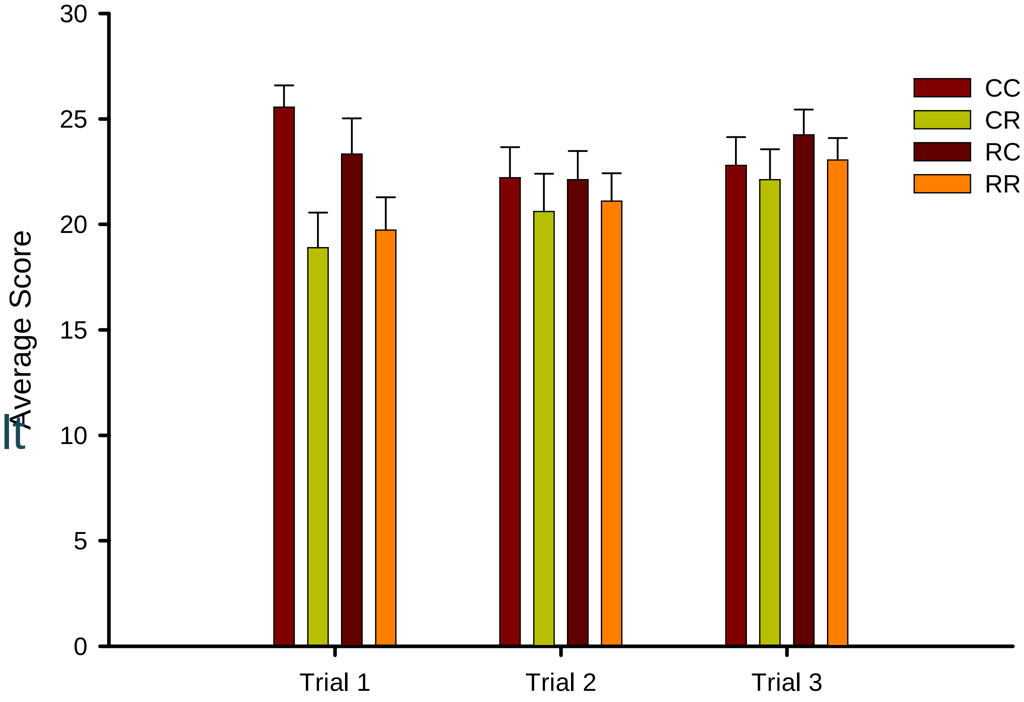
But, mortality during early life stages is not entirely unimportant

Projections based upon our experiments:

Population growth rate (intrinsic rate of increase):
Reduced by **9 %** for contaminant-exposed populations relative to reference populations.

Population size projections (10 years):
Reduced by **15 %** in contaminated areas relative to reference areas (all else being equal).

Predator response



Why Important?

-Survive acute chemical insult
-But run away from predator
-So easier catch

-Death ultimately!

Conclusions

- Anemones hardy species , probably due mucus production

- Longer exposures may not sustain excess mucus production

- Do show significant effect of dispersant alone (>10ppm)

- Impact to photosynthesis sensitive endpoint, problems with anoxia (mucus)

- Xenia sensitive to dispersant

- Xenia exhibited delayed responses in CEWAF (UF),no mucus protection?

- Xenia CEWAF and dispersant growth and recovery significantly reduced

-All studies require final synthesis and integration of all chemical / biological data and endpoints (acute, sub lethal, delayed)

- Comparison of data with other species (exposure time comparable?)

- How fit in with models?

-

Finally…..

We have LOADS of hatchlings this year …..just started!

Acknowledgements

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NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

U.S. DEPARTMENT OF COMMERCE

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National Science Foundation

WHERE DISCOVERIES BEGIN

Maryland Sea Grant

Further Information

www.mitchelmore-lab.com