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Canada

On-site Monitoring and Laboratory Biotests to Assess the impacts of Fertilizer Treatment on Habitat Recovery in an Oiled Wetland

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Overall Objective

- Determine effectiveness of bioremediation with & without presence of plants (phytoremediation) in restoring contaminated freshwater wetland to pre-spill conditions.
  - Involved intentional release of crude oil onto specified plots.

Biostimulation Strategies

Nutrient enrichment

- **The addition of inorganic nutrients has been demonstrated to be an effective bioremediation strategy for use on oiled beaches.**
- **The advantage of using inorganic agricultural fertilizers as bioremediation agents include low cost, availability and ease of application.**

Biostimulation Strategies

Phytoremediation

- **Plants may stimulate aerobic oil degradation processes by transferring oxygen to the soil rhizosphere.**
- **Plants may take up oil and release exudates/enzymes that stimulate microbial activity.**
- **Vegetative transplantation has been used for cleanup of terrestrial hazardous waste sites.**

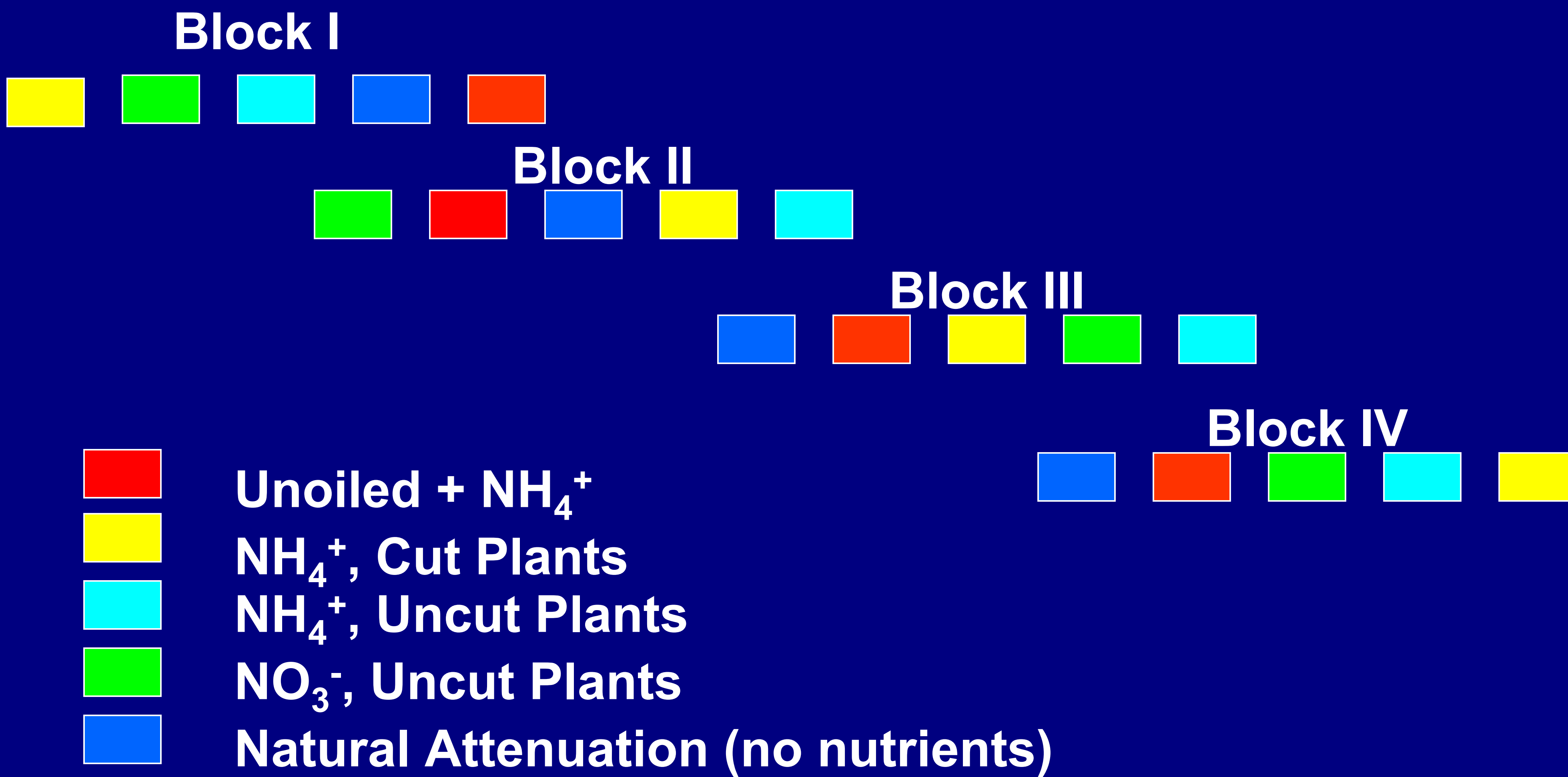
Experimental Design

Randomized Block

Four replicates of five treatments

- **Four oiled treatments**
  - **Natural attenuation**
  - NH_4NO_3 + PO_4^{-3}**, cut plants**
  - NH_4NO_3 + PO_4^{-3}
  - $NaNO_3$ + PO_4^{-3}
- **One unoiled treatment +** NH_4NO_3 + PO_4^{-3}

Plot Layout



Block I
Block II
Block III
Block IV
Unoiled + NH_4^+
NH_4^+, Cut Plants
NH_4^+, Uncut Plants
NO_3^-, Uncut Plants
Natural Attenuation (no nutrients)

Oil and Nutrient Application

- Nutrients applied prior to oil application
  - 1,000 g N and 300 g P/plot.

- Plots raked before and after oiling (top 2-3 cm) to assure oil penetration into the sediment.

- Oil applied via boom with 4 atomizer sprayers attached to hose and pump.
  - 12 L weathered Mesa light crude applied per plot (0.6 L/m^2).

Nutrient Application Strategy

- Objective: to maintain nitrogen in high enough concentration to prevent nutrient limitation.
- Nitrogen monitored twice per week in pore water.
- When concentration fell to below 5-10 mg/L, all plots were re-fertilized.

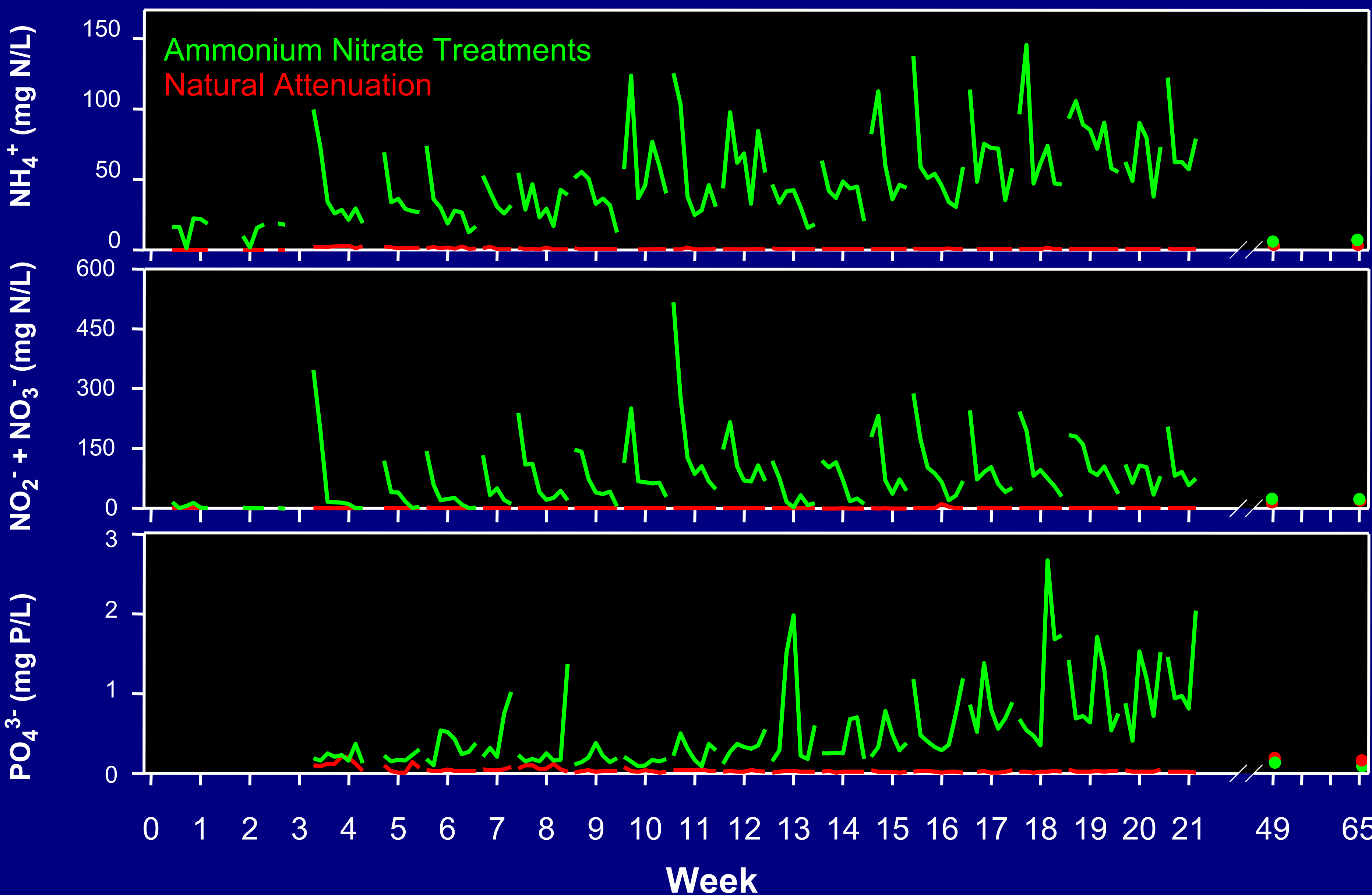
Sampling Strategy

- Plots oiled June 10, 1999 and first samples collected June 11 (week 0).
- Sampling took place at weeks 0, 1, 2, 4, 6, 8, 12, 16, 21, 49, and 65.
- No further fertilization after 21 weeks.
- Samples (~ 6-7 cm deep) collected using tulip bulb planter.
- Sampling crew never walked directly on experimental area.

Analyses

- All composite samples subsampled for oil chemistry, MPN analysis, and ecotoxicity analyses.
  - Oil Chemistry: changes in composition of residual oil by GC/MS-SIM.
  - MPN: microplate MPNs of alkane and PAH degraders (on-site).
  - Ecotoxicity: field surveys and bioassays.

Interstitial Nutrients

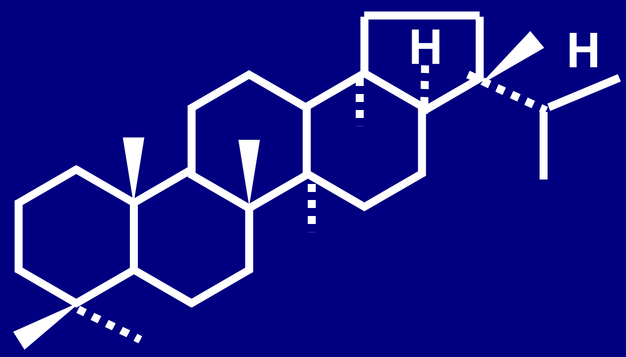


Results - *Nutrients*

- **Interstitial nitrogen concentrations could be maintained above the target level of 5 mg/L by weekly additions.**
- **Semi-continuous fertilizing (1999 field season) caused an accumulation of porewater NH_4^+, NO_2^- + NO_3^- and $(PO_4)^{3-}$.**

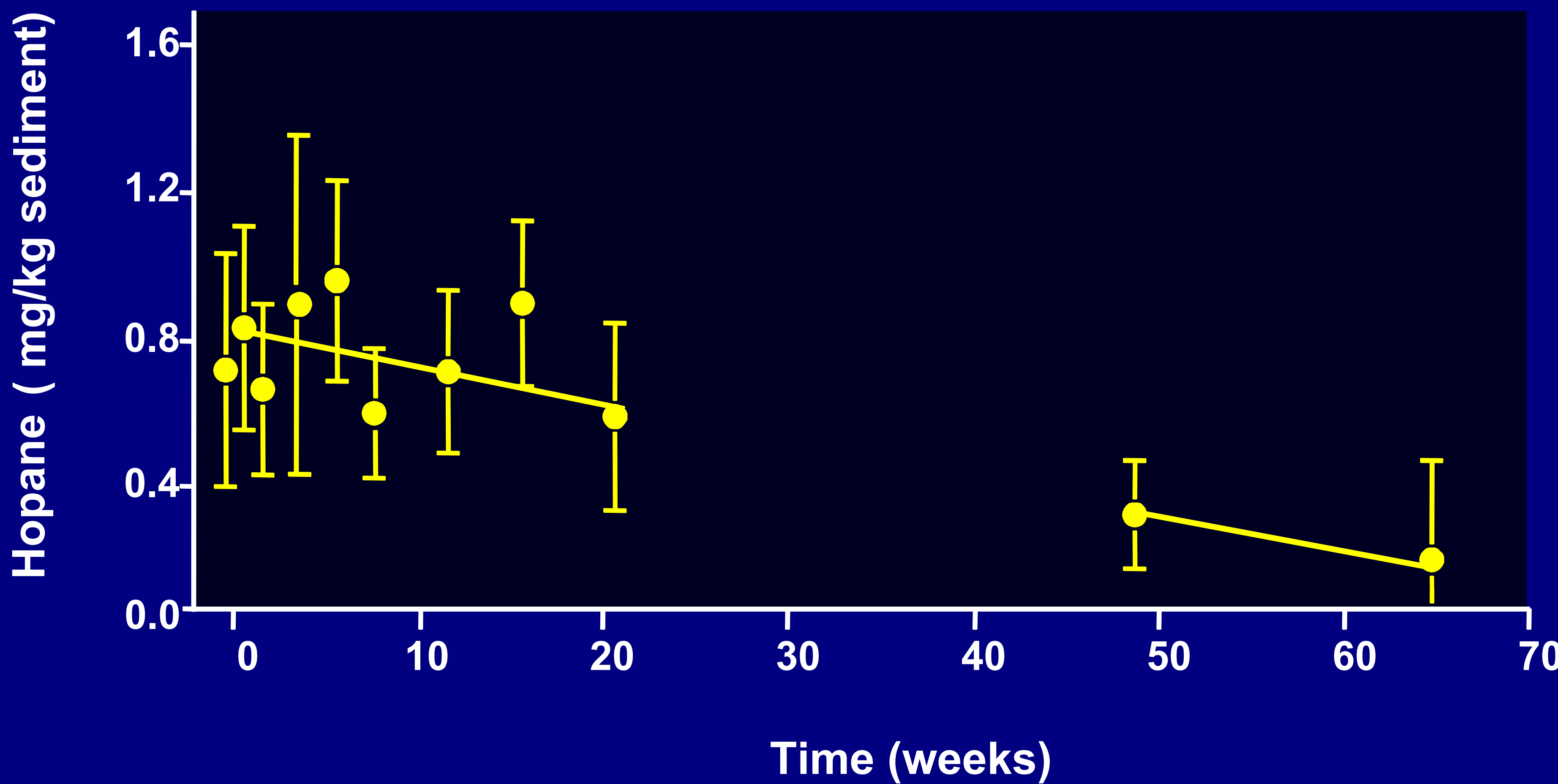
Hopanes

- **Common constituent of crude oils.**
- **17α(H),21β(H)-hopane was used as an internal standard for studying biodegradation of crude oil in the environment as it is neither generated nor biodegraded on time scales relevant to cleansing of oil spills.**

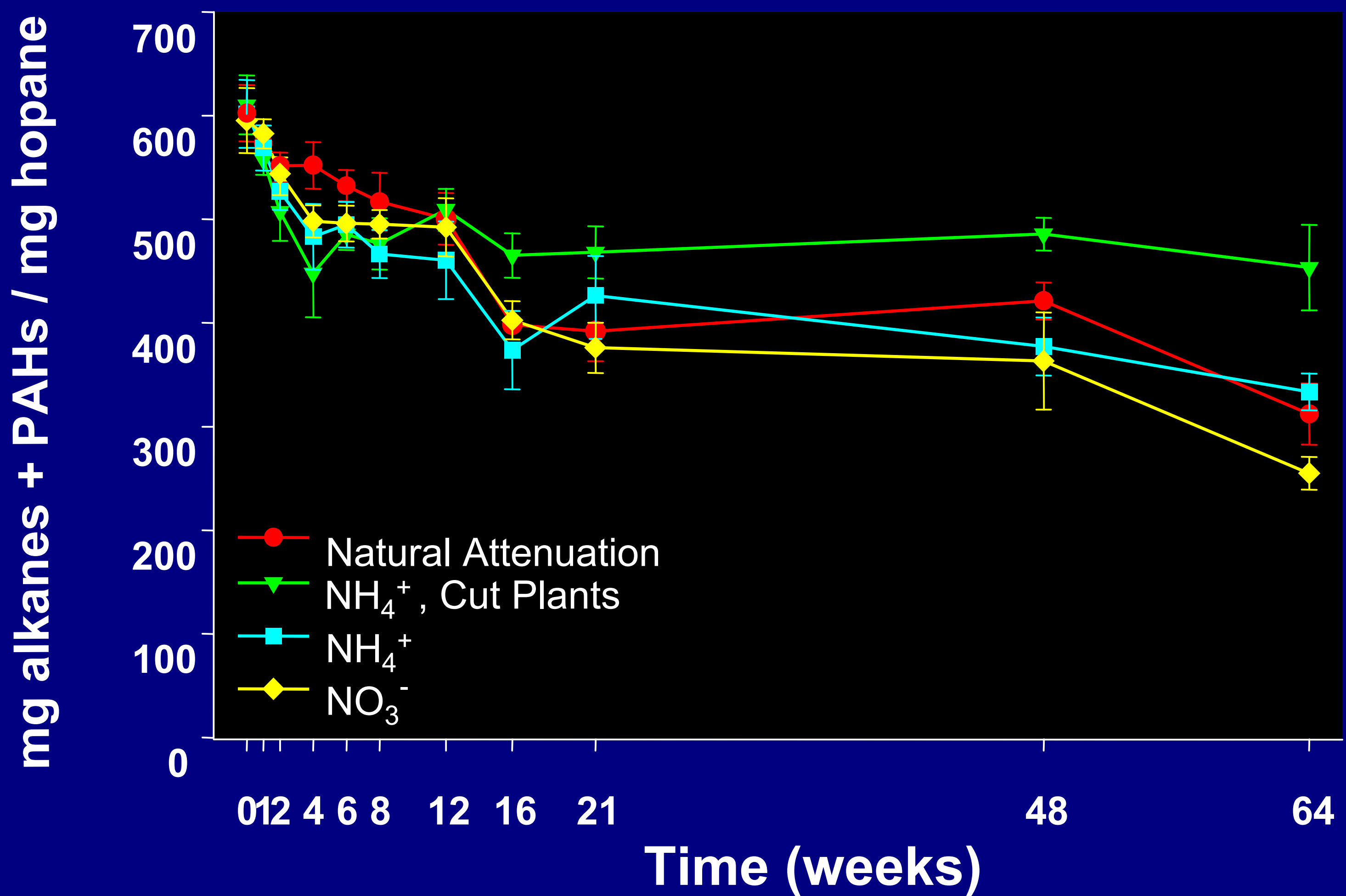


17α(H), 21β(H) - hopane

Loss of Hopane in 65 Weeks Averaged Over All Plots



Total alkanes and PAH's normalized to hopane



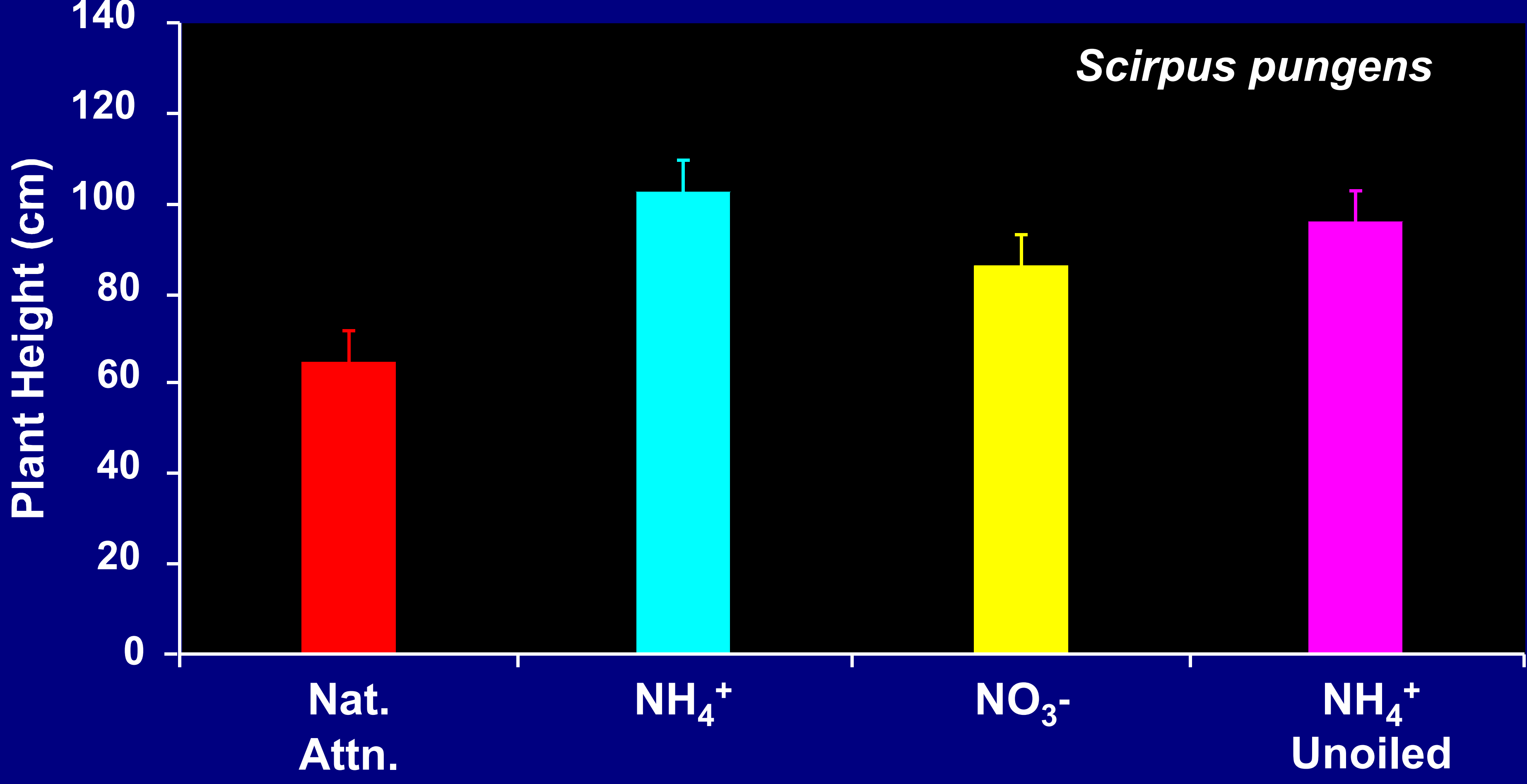
Results - *Oil Composition*

- C_{30}-17α-(H),21β(H)-hopane, a conservative biomarker, provided a means to quantify rates of oil removal by physical processes.
- Physical processes removed a large fraction of the residual oil.
- Experimental treatments were ineffective in accelerating natural biodegradation rates.
- Suppression of plant activity by removal of above-ground growth appeared to diminish the rate of oil loss after week 12.

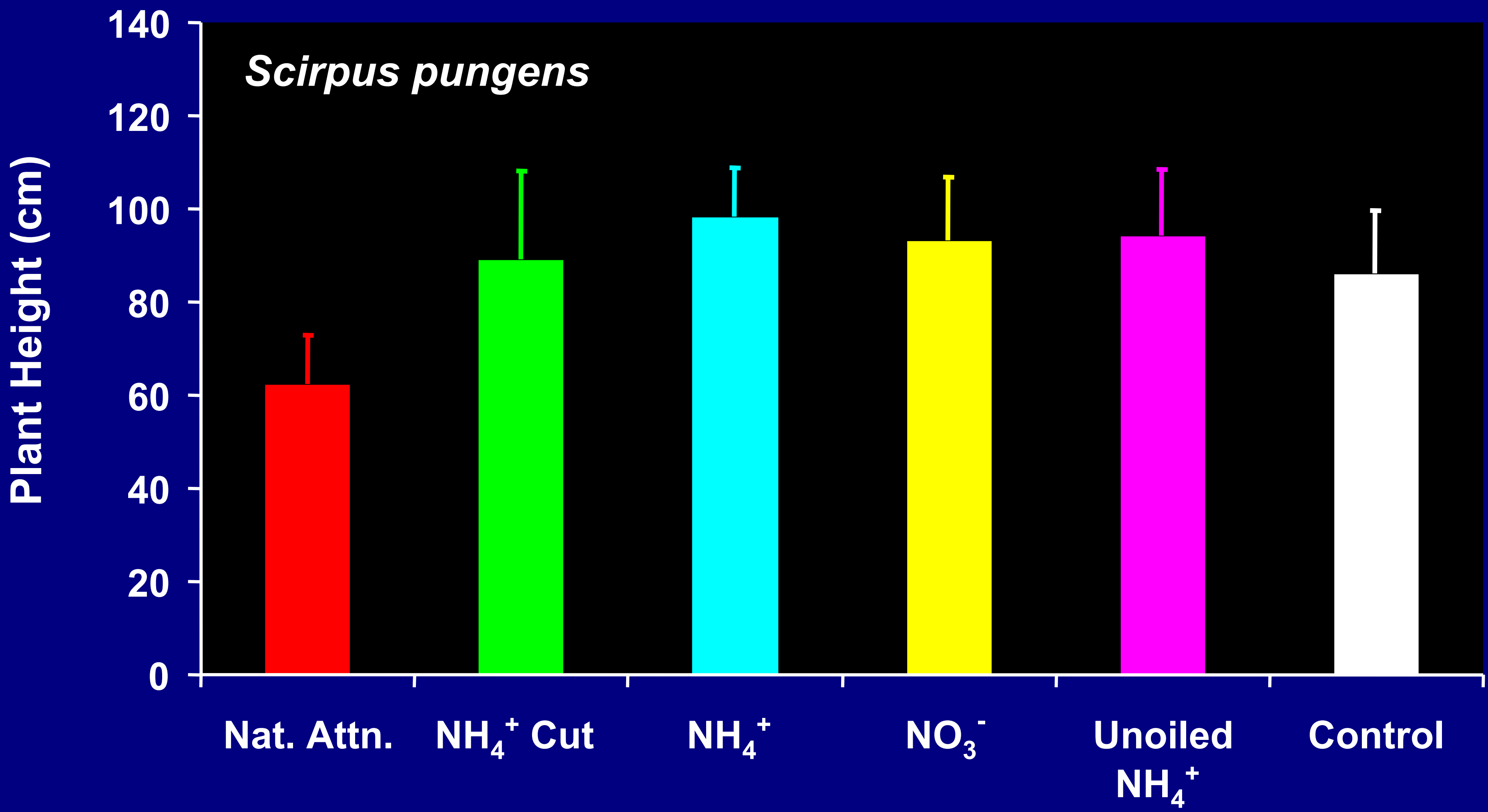
Ecotoxicological Tests

- Quantification of treatment success.
- Identification of operational treatment endpoint(s).
  - Toxicity reduction and habitat recovery.
  - Identification of detrimental effects of treatment.

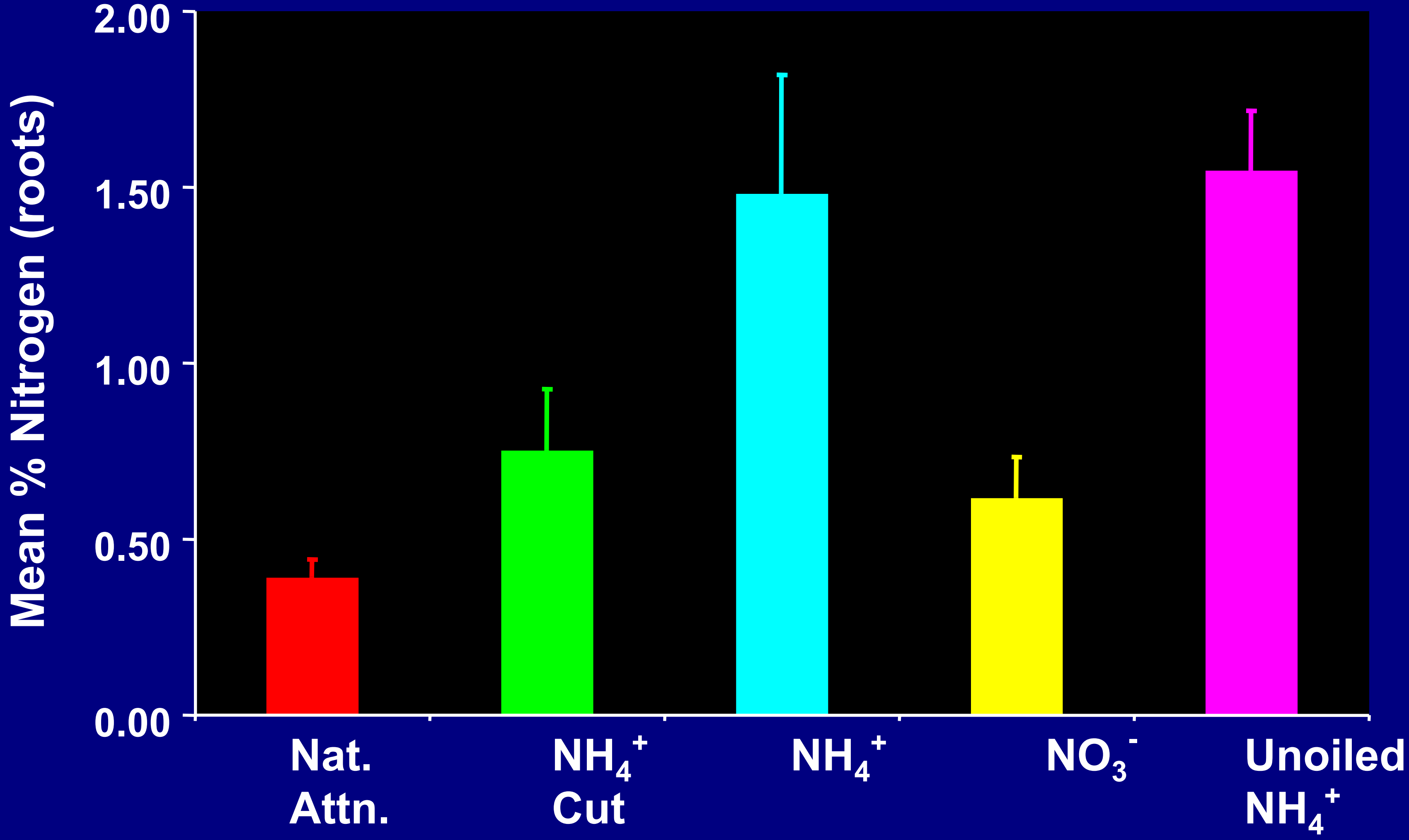
Plant Height at Week 15



Plant Height (65 Weeks)



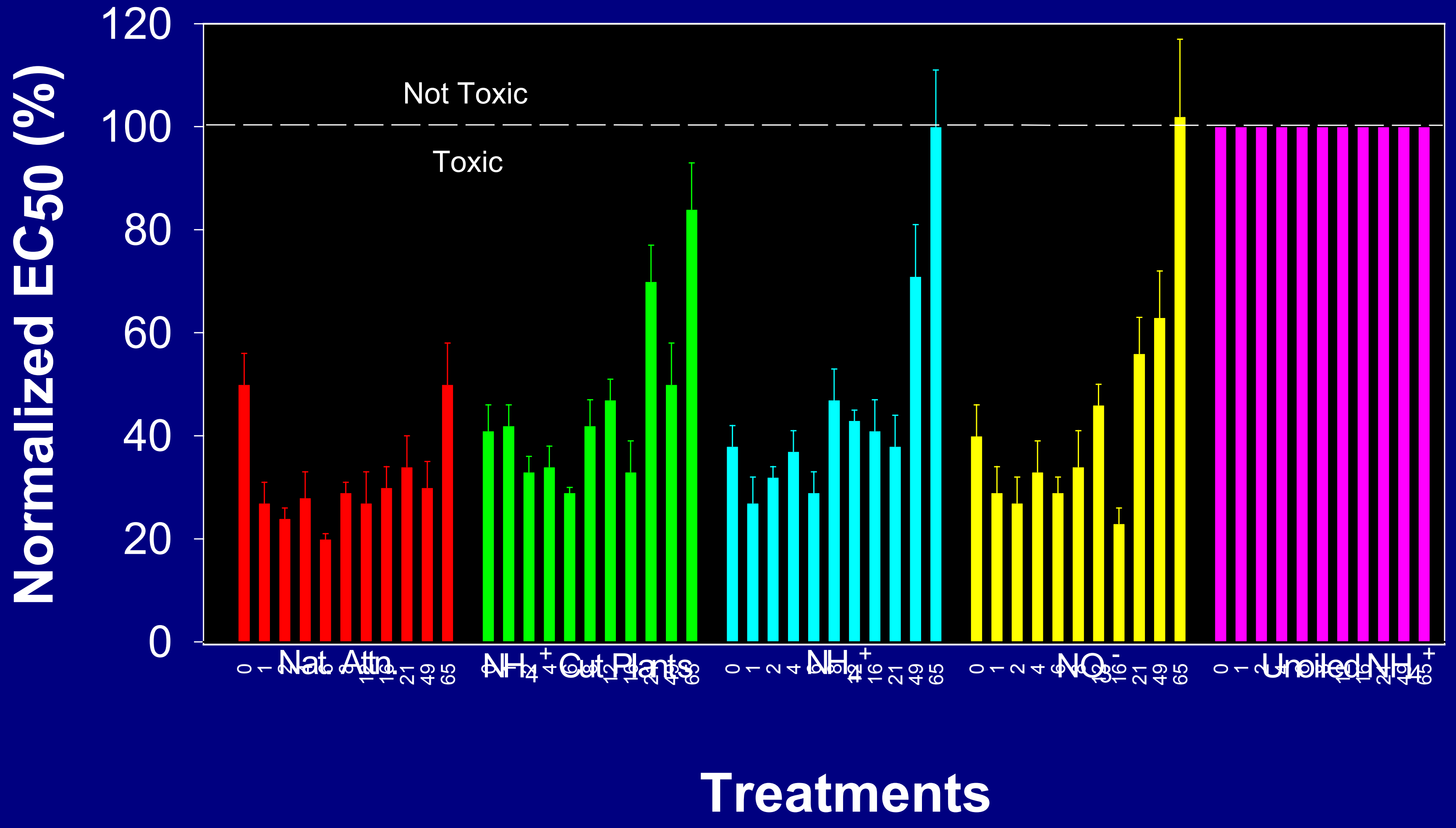
Nitrogen content at week 65



Results - *Plant Growth*

- *Scirpus pungens* tolerated the experimental oil concentrations, but suffered oil-induced growth inhibition, which could be negated by nutrient amendments.
- Stimulated growth was observed in the second year, despite the termination of nutrient additions at the end of the first growing season.
- Analysis of root tissue suggests recycling of stored organic nitrogen accumulated during the first growing season.

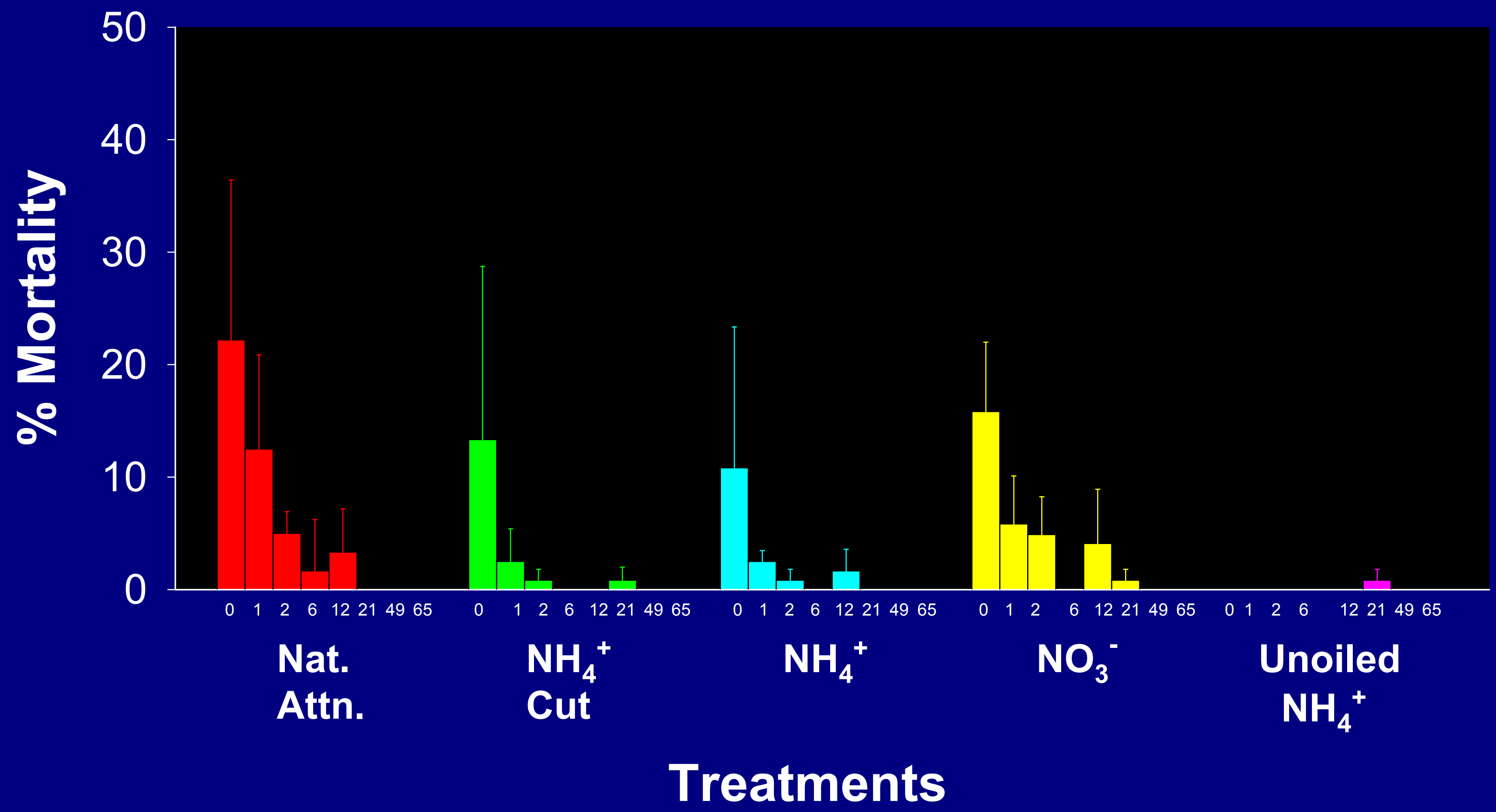
Microtox Solid-Phase Toxicity Test



Results - *Microtox Solid-Phase Tests*

- Comparison of oiled and unoiled plots clearly shows oil toxicity.
- First field season: no major treatment effects observed.
- Second field season: nutrient amended plots with plants, and unoiled controls, had similar relative toxicity.

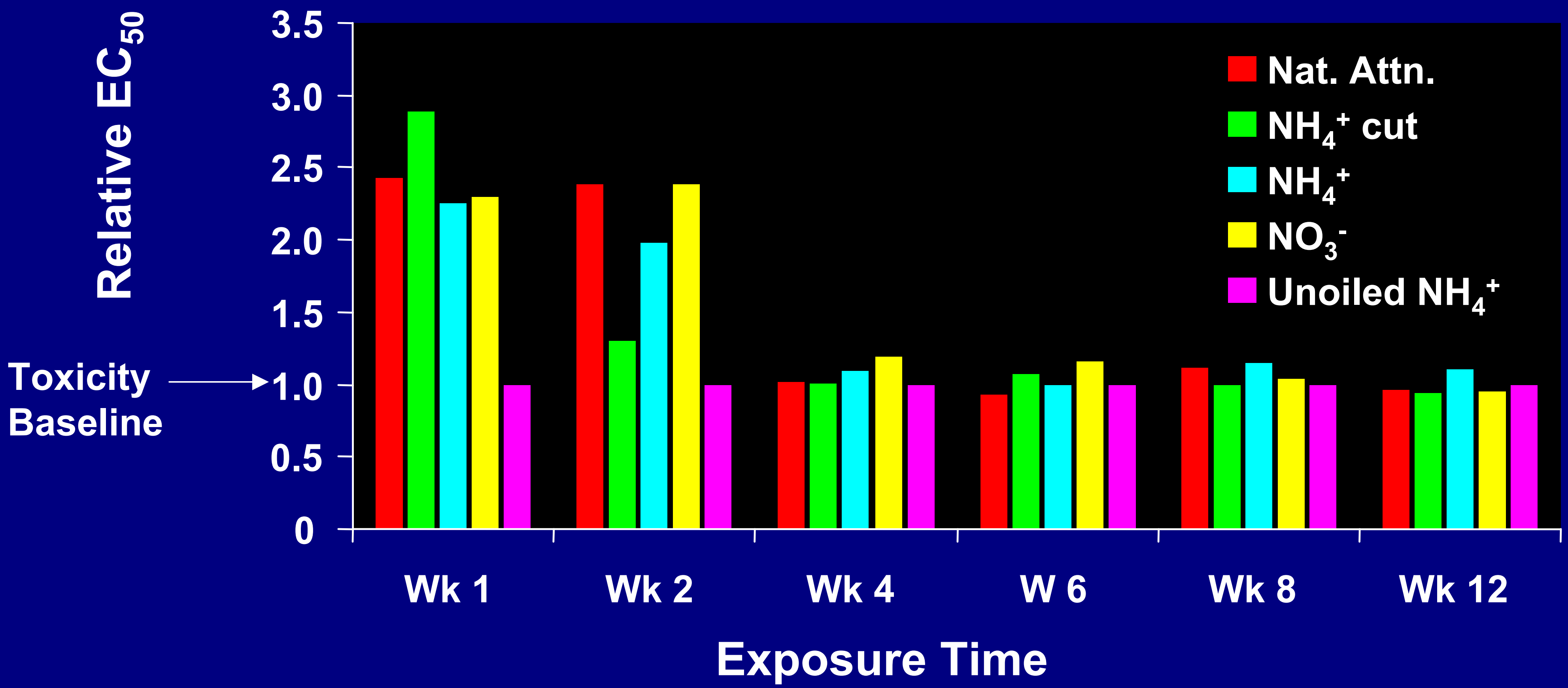
Daphnia magna



Results - *Cladoceran Survival Tests*

- The *Daphnia magna* elutriate assay had limited sensitivity.
- The toxic effect from oiling diminished rapidly (negligible by Week 6).
- Nutrient treatments were not associated with detrimental effects.
  - Use of the protocol was terminated.

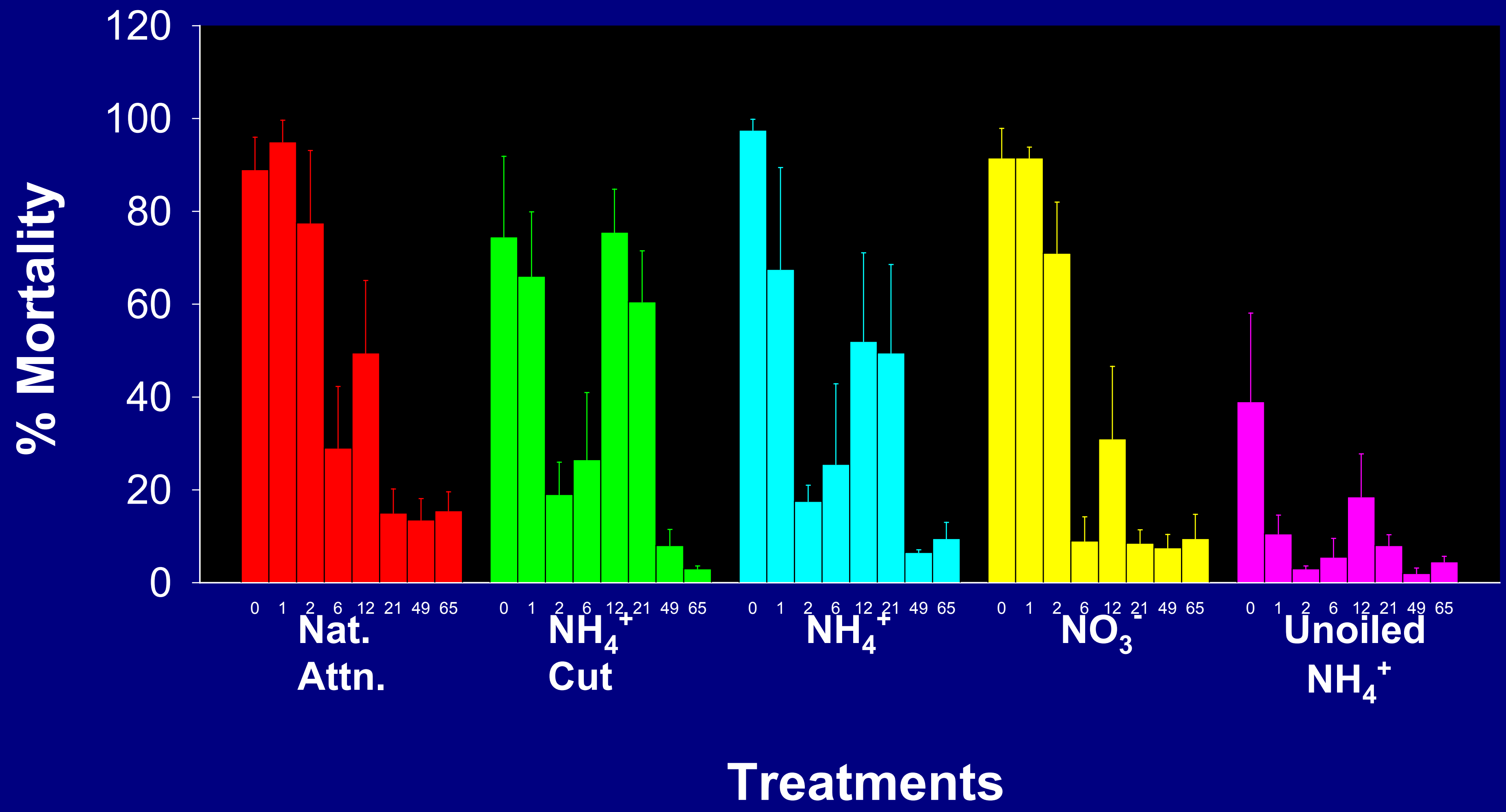
St. Lawrence River Simulated Oil Spill: SPMD-TOXicity Index



SPMD-TOX

- In surface sediments, the bioavailable fraction of the oil dissipated rapidly.
- After 4 weeks of exposure, bioavailable oil products within SPMDs recovered from the experimental plots was similar to background levels in the unoiled control.

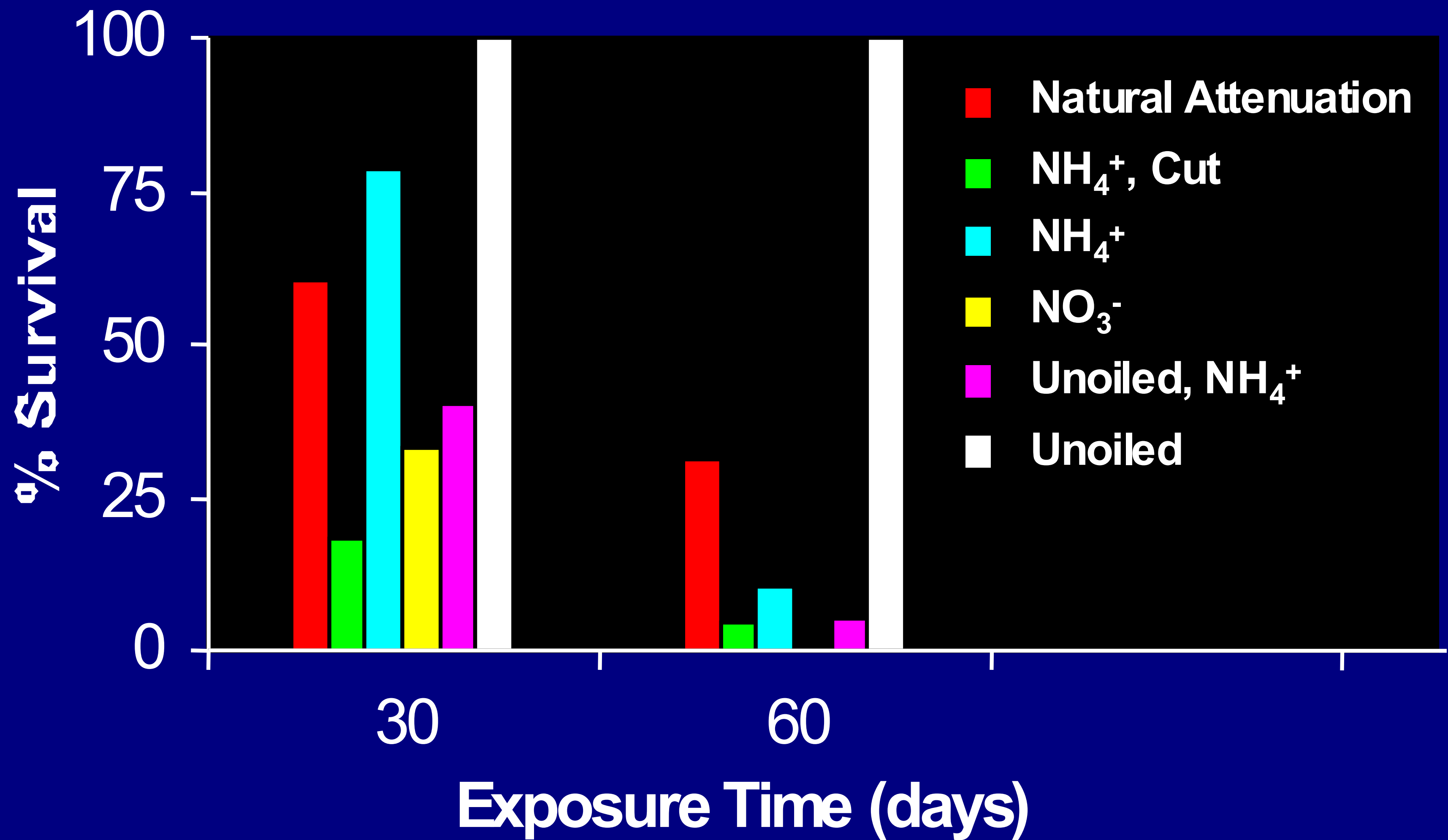
Hyalella azteca



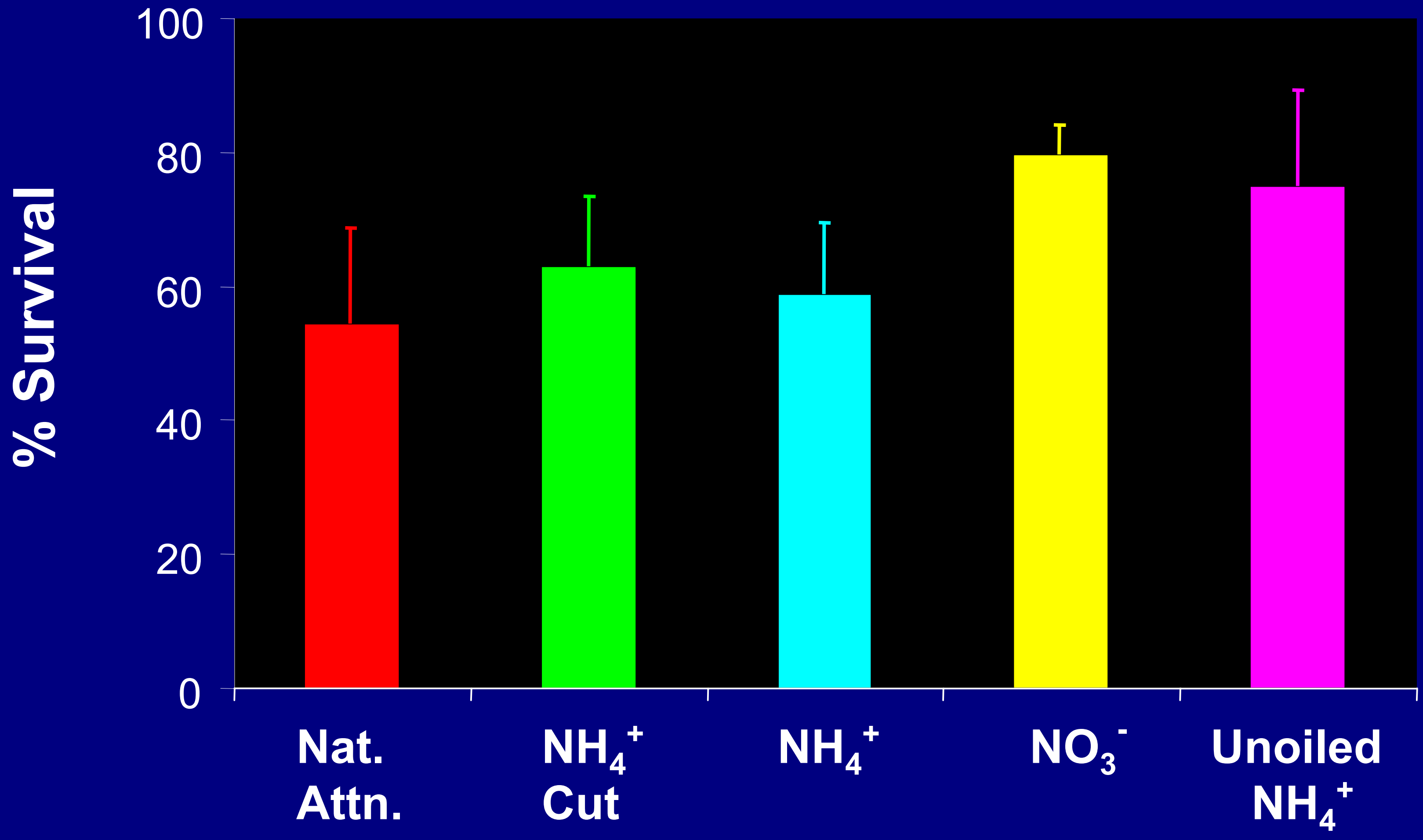
Results - *Amphipod Survival Tests*

- *Hyalella azteca* was highly sensitive to oiled sediments.
- This direct-contact sediment assay demonstrated toxicity reduction by natural attenuation, as well as detrimental treatment effects of elevated ammonia from excess nutrient addition.
- Experimental treatments may offer little advantage on an operational scale.

Viviparus georgianus



% Survival of *Viviparus georgianus* at Week 65



Results - *Gastropod Survival Test*

- The snail, *Viviparus georgianus*, caged *in-situ* for 30 and 60 days, indicated higher toxicity in nutrient amended plots than in natural attenuation plots (except one point: 30-day exposure, intact plants treated with ammonium nitrate).

- Laboratory tests with sediments from Week 65 showed decreased sediment toxicity with reduced residual oil and nutrient concentration.

Conclusion - *Oil Biodegradation*

- Physical and biological processes (natural attenuation) removed a significant fraction of the residual hydrocarbons.
- Periodic application of fertilizer sustained elevated levels of porewater nutrients.
- Experimental treatments caused little or no change in the composition of residual oil.
  - Continuous removal of foliage suppressed biodegradation rates.
  - Oil biodegradation was oxygen limited.

Conclusions - *Habitat Recovery*

- Bacteria and invertebrate bioassays indicated effects ranging from detrimental to enhanced recovery.

  Differences may be attributed to:

  - Intrinsic variation in species sensitivity.
  - Induced tolerance on exposure to contaminant hydrocarbons.
  - Changes in bioavailability of residual oil.
  - Detrimental effects of nutrient formulations.

Conclusions - *Habitat Recovery*

- The dominant plant, *Scirpus pungens*, was tolerant to the oil at experimental concentrations.

- Plant growth was stimulated by nutrient addition.

  - Recommend: fertilizer application for enhancing growth to avoid loss of wetland habitat by erosion.

Conclusions

- Guidelines for the application of bioremediation strategies are needed.
- Biotests should be used as a monitoring tool, since the primary goal of bioremediation is to reduce toxic effects.
- Biotests can help guide the application of bioremediation agents, and reduce the probability of detrimental treatment effects.