Guidelines for the Bioremediation of Freshwater Wetlands

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Topics to be discussed

- Wetland environment
- Summary of St. Lawrence River Study
- Bioremediation on water
- Guidance for implementation of bioremediation
  - Decision tree
    - Pretreatment assessment
    - Bioremediation planning
    - Implementation, assessment, termination
  - Conclusions

Wetland Environment

- Freshwater oil spills most likely to affect marshes and wetlands
- Only research data available is ORD-funded study in Quebec on St. Lawrence River
    - Multiple plots studying effect of ammonium and nitrate addition with and without plants

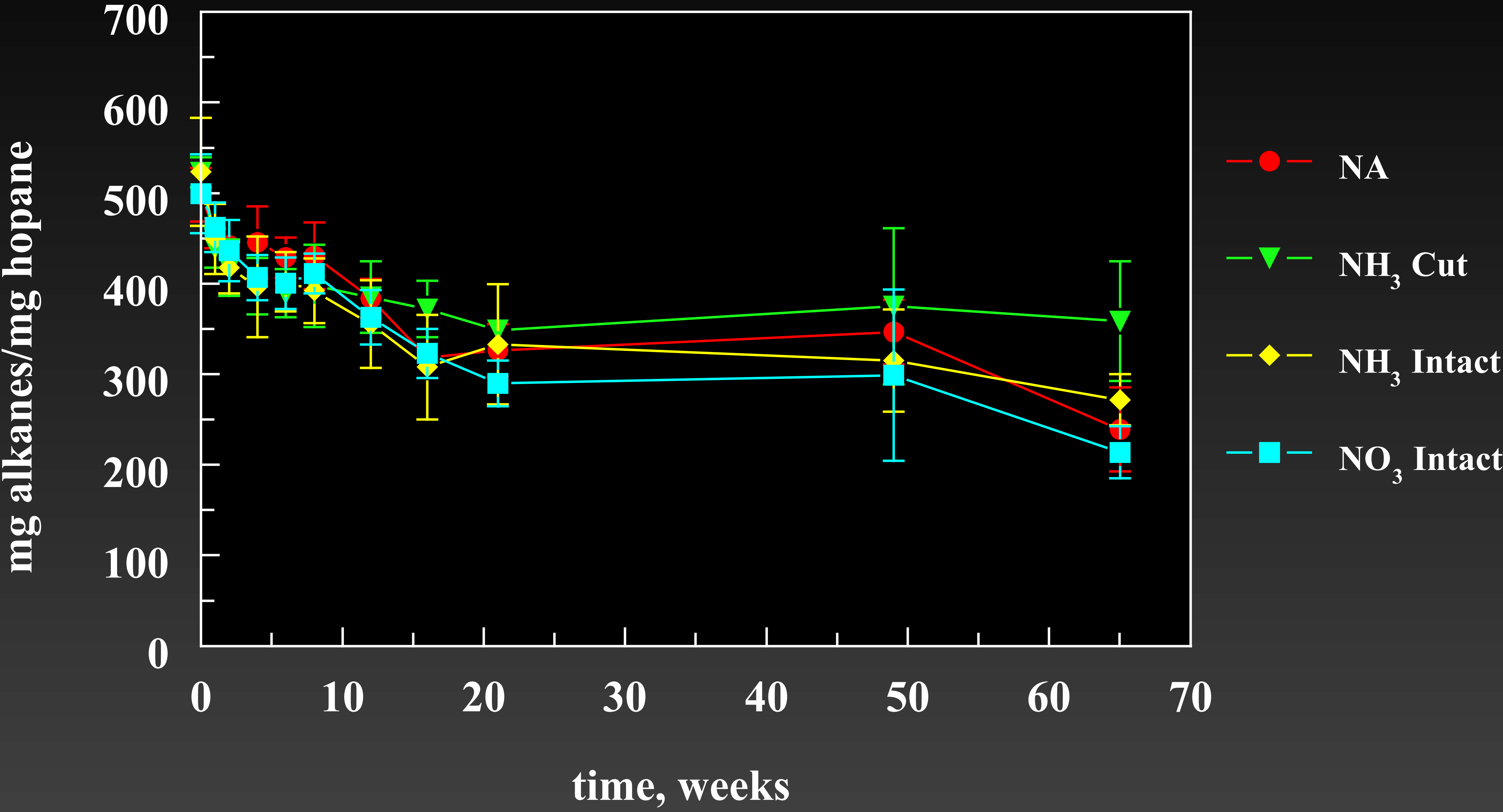
St. Lawrence River Study

- Oil penetration very low due to wet, clayey soil (typical of all wetlands)
  - Oil raked into top 3 cm to assure penetration
- Oxygen became limiting a few mm below ground surface
- Very quiescent, very little wave action
- Tidal effects

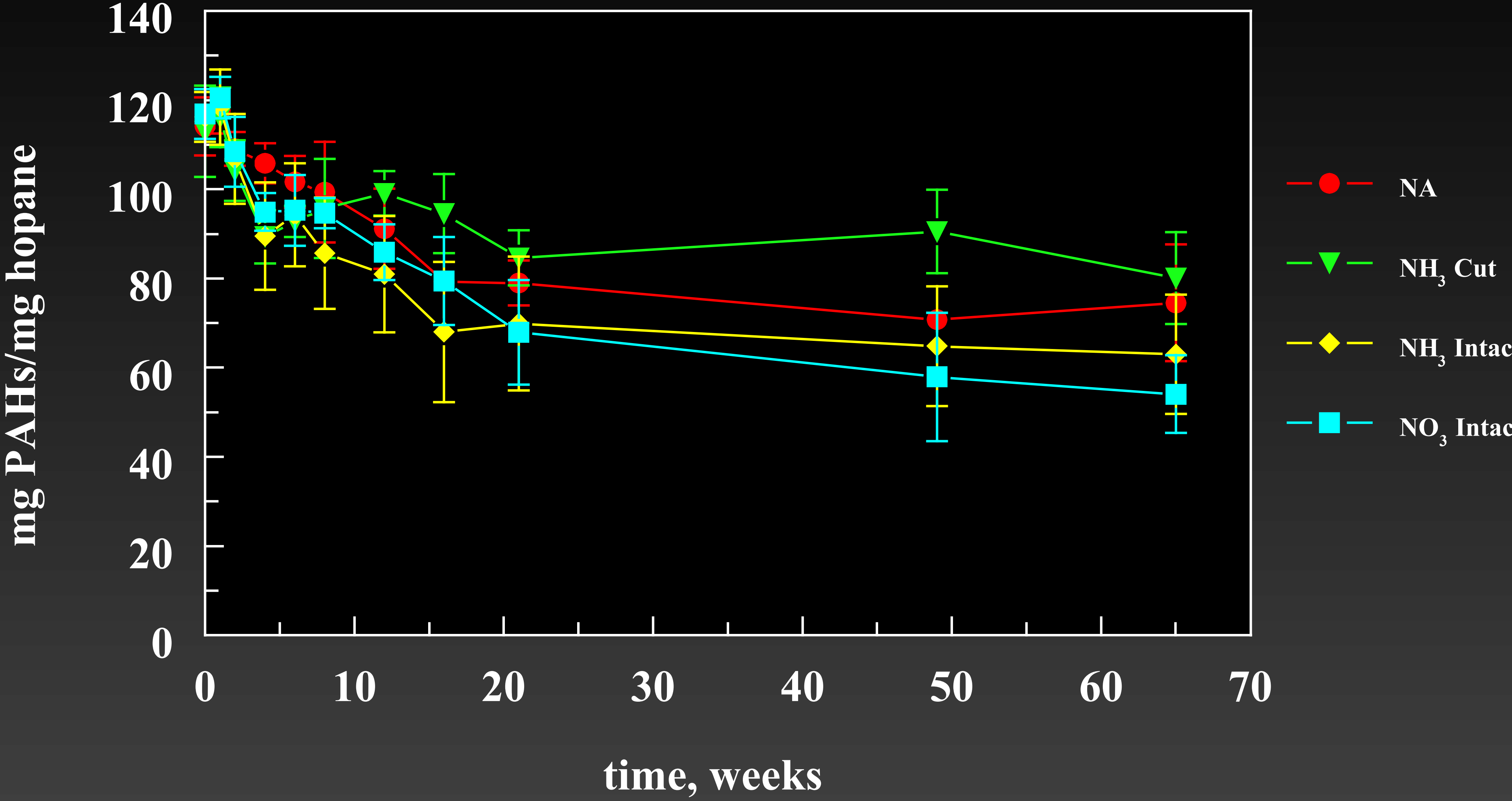
Treatments Studied

- Natural attenuation (no amendments)
- Ammonia addition with plants cut back to suppress growth
- Ammonia addition with plants intact
- Nitrate addition with plants intact

Change in Total Alkanes Normalized to Hopane



Change in Total PAHs Normalized to Hopane



Summary St. Lawrence Findings

- No treatment differences noted for biodegradation of total alkanes and PAHs except for plots with plants cut
  - Highly suggestive that oxygen was limiting
  - Presence of healthy plant roots may be important for biodegradation to take place
  - More physical loss of oil from plots with plants cut back

Conclusions from St. Lawrence Study

- **Biostimulation may not be appropriate for rapidly degrading oil in a contaminated freshwater wetland *if significant oil penetration has taken place***

- **Lack of oxygen is the most likely cause for the retarded biodegradation in a wetland where oil has penetrated to any significant depth**

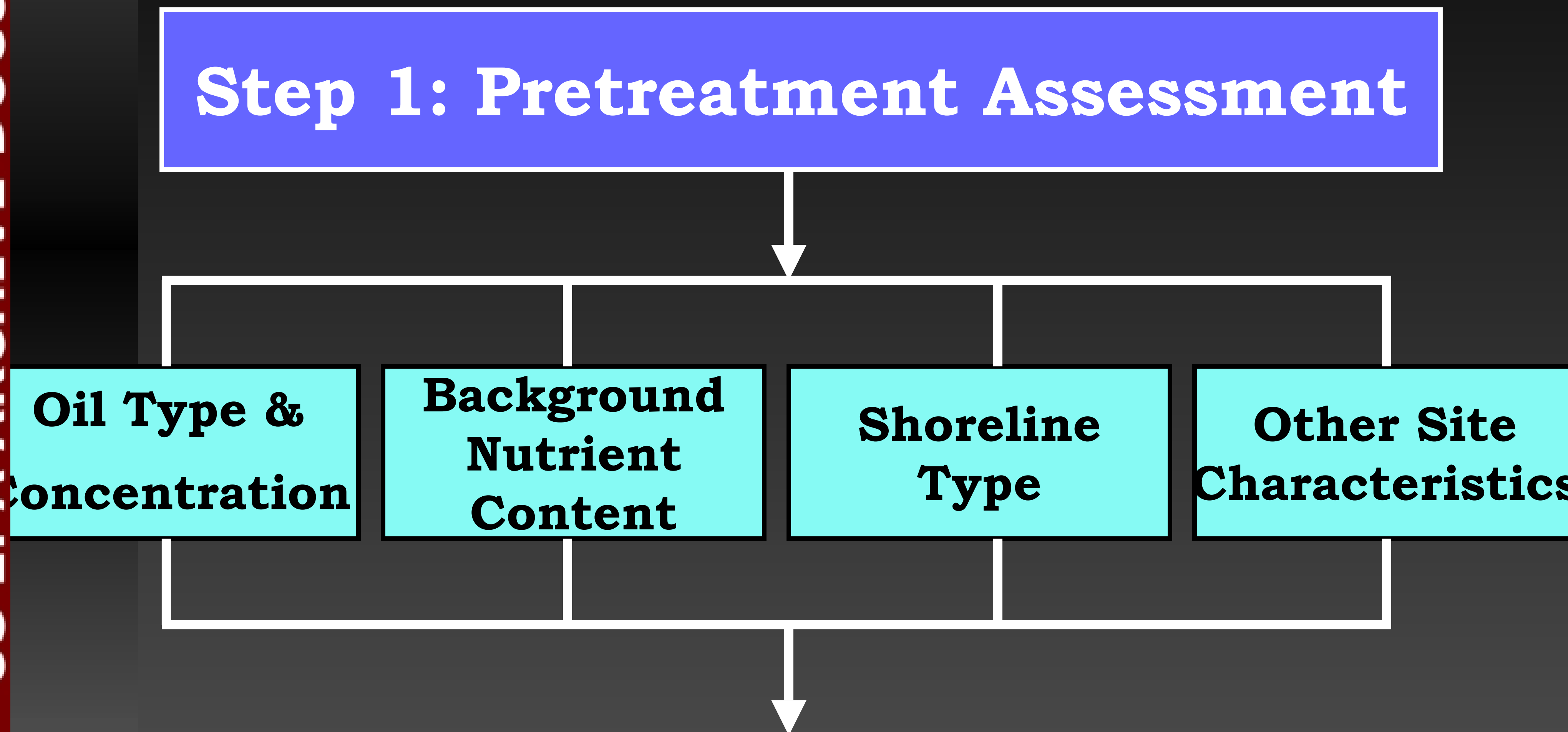
- **If restoration is the primary goal, fertilizer addition might be appropriate**

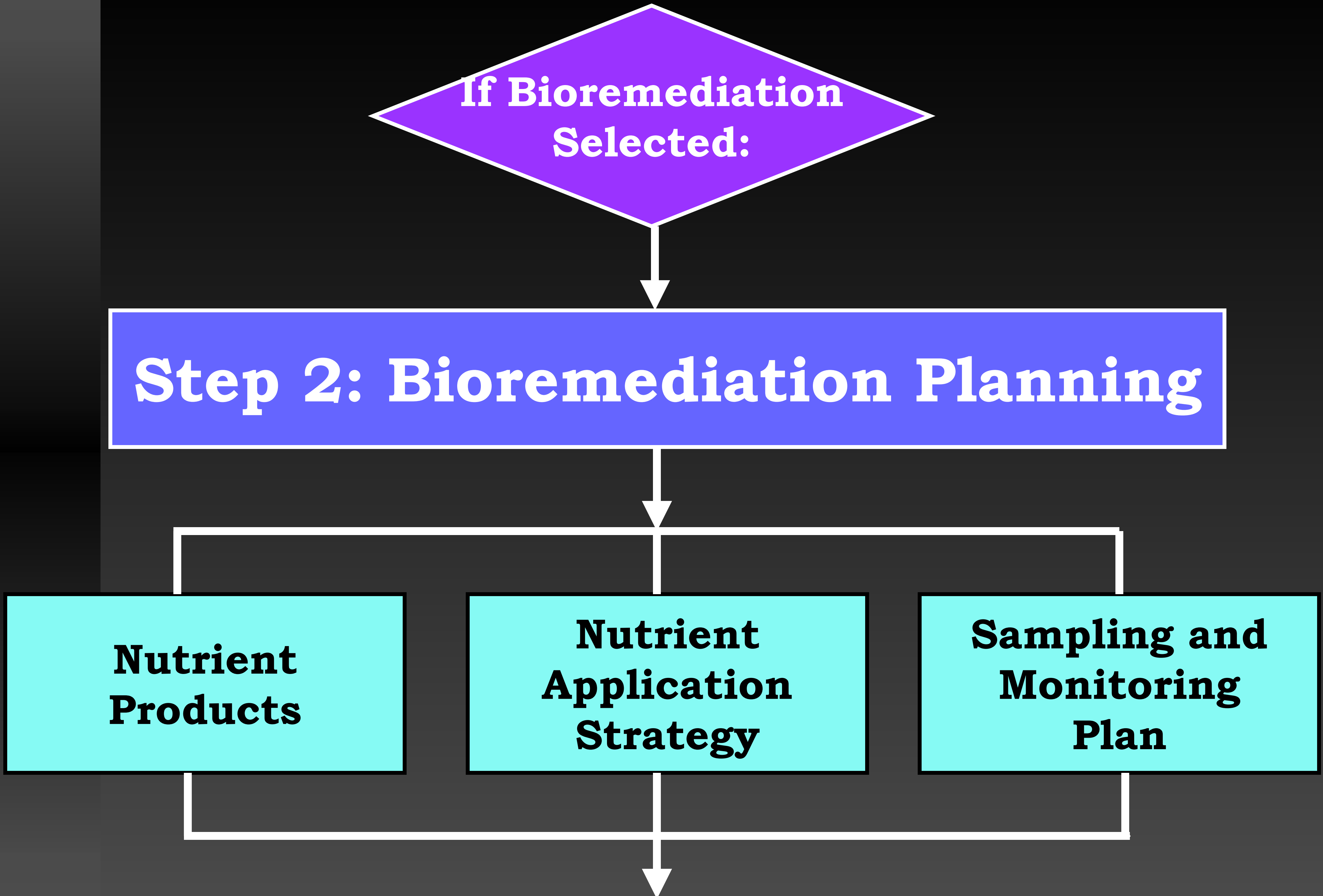
Bioremediation on Water

- To be successful, all amendments must stay with the slick and not disperse
  - This is extremely unlikely, even with oleophilic fertilizers
  - Therefore, bioremediation on water not considered viable

Guidance for Implementation of Bioremediation in the Field

Decision Tree for Selection and Application of Bioremediation




If Bioremediation Selected:
Step 2: Bioremediation Planning
Nutrient Products
Nutrient Application Strategy
Sampling and Monitoring Plan

Step 3: Implementation, Assessment, and Termination

Analysis of Biodegradation and Physical Loss

Toxicological and Ecological Analysis

Step 1: Pretreatment Assessment

- **Oil type**
  - **Higher API gravity (> 30°) oils easier to degrade**
  - **Order of sensitivity: *n*-alkanes>branched alkanes>low MW PAHs>cyclic alkanes>high MW PAHs>resins/asphaltenes**

Step 1: Pretreatment Assessment

- **Oil concentration**
  - **Low** (10s to 100s of mg/kg): less likely to be limited by N and P; thus, natural attenuation may be appropriate
  - **Intermediate** (~1-80 g/kg): likely to be limited by N and P, may or may not need nutrient addition
  - **High** (> 80 g/kg or higher): may be inhibitory or toxic

Step 1: Pretreatment Assessment

- **Background nutrient content**
  - Determine background concentration of N, P
  - Determine historical range of N, P at the spill site
    - If low, biostimulation likely to be effective
    - If high, consider natural attenuation

Step 1: Pretreatment Assessment

- Types of shorelines
  - High energy not amenable: washout too rapid and waves scour organisms from substrate
  - Low energy favorable for nutrient application, must be aware of possible oxygen deficiency
  - Medium and coarse sandy beaches most favorable
  - Wetlands usually oxygen limited, not nutrient limited

Step 1: Pretreatment Assessment

- **Other Factors**
  - Climate: cold temperatures slow the process
    - Greater viscosity
    - Slower biodegradation due to slower metabolic rates
  - Prior exposure to oil: if none, lag or adaptation period greater

Step 2: Bioremediation Planning

- Treatability studies and considerations
  - Tiered screening protocol for testing products and listing on the NCP Product Schedule
  - Microcosm tests: batch and semi-continuous or continuous flow
  - Nitrate- vs. Ammonium-based fertilizers
  - Human and ecotoxicity impacts
  - Environmental factors
    - Water soluble fertilizers
    - Slow-release fertilizers
    - Oleophilic fertilizers

Step 2: Bioremediation Planning

- Application Strategy
  - Optimal nutrient concentration
  - Frequency of application
  - Methods of application

Step 2: Bioremediation Planning

- Optimal nutrient concentration
  - Microcosm studies
    - Continuous flow with C_{17} on sand: 2.5 mg N/L supported maximal degradation
    - Continuous flow with crude oil on sand: 10 mg N/L supported maximal degradation
    - Tidal flow with crude oil on sand: 25 mg N/L supported maximal degradation

Step 2: Bioremediation Planning

- Optimal nutrient concentration
  - Field studies
    - Prince William Sound: rates accelerated by 1.5 mg/L pore water nitrogen
    - Brest France: rates no longer limiting at nitrogen concentrations > 1.4 mg/L
    - Delaware: rates enhanced by maintenance of average 3-6 mg N/L in pore water
  - Thus, to enhance to near maximum rates, maintain 2-10 mg N/L in pore water

Step 2: Bioremediation Planning

- Frequency of nutrient addition
  - Depends on tidal effects
    - Washout high at spring tides and high energy
    - Nutrient persistence longer at neap tides and low energy
- Methods of nutrient addition
  - 4 types of fertilizers:
    - Slow-release briquettes (problematic)
    - Dry, granular (easy and flexible)
    - Liquid oleophilic (easy but expensive)
    - Water-soluble inorganic solutions (complicated equipment)

Step 2: Bioremediation Planning

- Sampling and Monitoring Plan
  - Important variables
    - Interstitial nutrients (very important)
    - Dissolved oxygen
    - Concentration of oil and its constituents (GC/MS)
    - Microbial activity (MPNs)
    - Environmental effects (ecotoxicity)
    - Others (temperature, pH)
  - Samples should cover entire depth of oil penetration
  - Statistical considerations

Step 3: Assessment/Termination

- Analysis of biodegradation vs. physical loss
- Ecosystem function analysis

Step3: Assessment/Termination

- **How To Measure Biodegradation**
  - **Must be able to distinguish between physical vs. biodegradative loss**
  - **Normalize to a conservative internal marker**
  - **Monitor changes in concentrations of individual oil constituents**

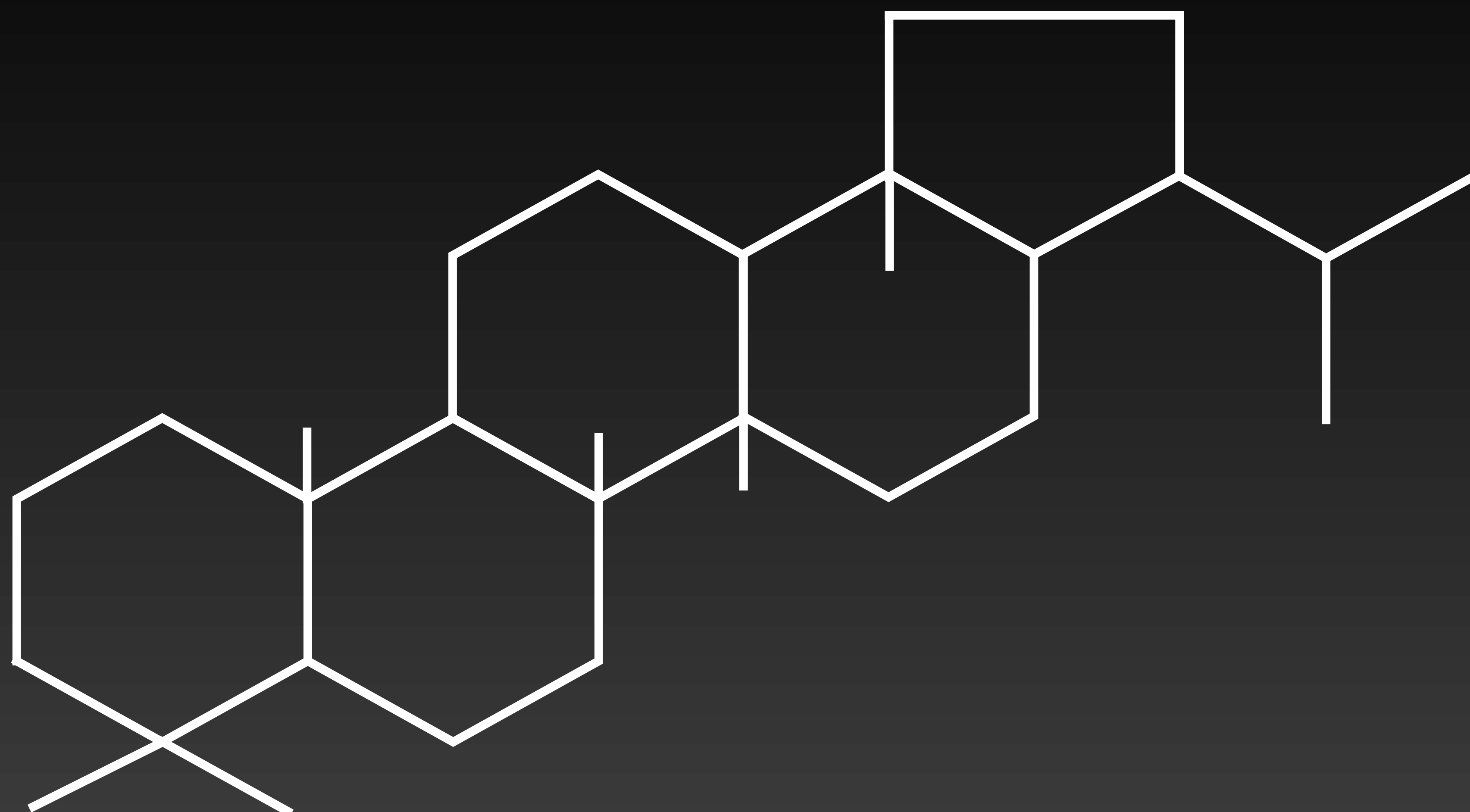
Step 3: Assessment/Termination

- Physical vs. Biodegradative Loss
  - Distinguished by measuring biomarkers
  - Biomarkers (molecular fossils) found in oil are complex organic compounds:
    - Composed mostly of carbon and hydrogen
    - Show little or no change in structure from parent compound in living cells
    - Highly resistant to biodegradation

Step 3: Assessment/Termination

- **Assumptions for an Effective Biomarker**
  - **Must be non-biodegradable**
  - **Must have same or similar volatility and solubility as other oil components**
- **General classes of biomarkers**
  - **Acyclic Diterpanes (pristane and phytane)**
  - **Cyclic Triterpanes (hopanes, steranes)**

Structure of C_{30}-17à(H), 21¬(H)-Hopane ($C_{30}H_{52}$)



Step 3: Assessment/Termination

- Normalize Data to Biomarker
  - Measure concentrations of individual oil components, including hopane
  - Divide the concentrations of each component by the concentration of hopane
  - Losses will be adjusted for physical loss

Step 3: Assessment/Termination

- What If Oil Has No Biomarker?
  - Normalize to a less readily biodegradable constituent, such as C_2-, C_3-, or C_4-chrysene
- Observe the relative rate of decline of alkanes
  - The higher the molecular weight, the slower the biodegradative loss
- Observe rate of decline of parent PAHs to alkylated homologs
  - Alkylated homologs will biodegrade slower

Step 3: Assessment/Termination

- **Ecosystem Function Analysis**
  - Microbial response (MPN)
  - Microtox (solid and liquid phase)
  - Algal solid phase bioassay
  - Daphnia survival
  - Amphipod survival
  - Gastropod (mollusc) survival
  - Fish bioassays

CONCLUSIONS

- Bioremediation a proven technology
- Primarily a polishing step
- Not considered a primary response technology
- Relatively slow process (weeks to months)
- Toxic hydrocarbons destroyed, not just moved to another environment
- Biggest challenge: maintaining nutrients in pore water
  - For wetlands, achieving aerobic conditions
- If background nutrients are high, may not need to use bioremediation for cleanup
  - Could still be considered for ecosystem recovery

CONCLUSIONS

- Bioaugmentation not likely to enhance biodegradation
- If impact area is high energy shoreline, bioremediation less likely to be effective
- Apply nutrients as dry granules at intermittent intervals
- Measure effectiveness by GC/MS, normalize oil components to hopane
- Conduct cadre of ecotoxicological assays to assess endpoints other than hydrocarbon concentrations