DETERMINING TOXICOLOGICAL EFFECTS OF INORGANIC PHOSPHATE ON CORAL REEF SPECIES

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Background

• Measured porewater phosphorus (P) concentrations at 8 out of 12 sites at St. Croix, U.S. Virgin Islands ranged from 0.051 – 0.367 mg/L, which were well above the EPA total phosphorus water quality criteria of <0.050 mg/L. In addition, Acropora palmata, a critically threatened coral species, was shown to have exceedingly low reproductive effort at 5 out of 9 locations sampled at St. Croix.

• Do nutrients play a role in coral reef decline?
  • Although high nutrient input from N and P is known to cause hypoxic conditions phosphorus may also directly impact the susceptible, early life stages of reef organisms.

Phosphorus impacts on early life stages of coral

• Decreased fertilization rates at phosphate concentrations of 0.095 mg/L (0.03 mg/L P) and greater in Acropora longicyathus.[1]
• Abnormal development in Goniatrea aspera embryos at 0.048 to 0.095 mg/L and greater of phosphate (0.016 - 0.03 mg/L P)[1]
• Smaller eggs produced by adult Acropora longicyathus and Acropora aspera after chronic exposure to phosphorus in situ[2]

Phosphorus impacts on early life stages of sea urchins

• Greater abnormal development and arrested development relative to controls when Lytechinus variegatus was exposed to 0.8 to 3.2 mg/L of inorganic phosphate (0.21 to 0.83 mg/L P)[1]
• Feeding, fecal production, nutrient absorption and allocation, growth, and righting behavior in L. variegatus were inhibited by 0.8 to 3.2 mg/L (0.21 to 0.83 mg/L P) inorganic and 1 to 1,000 mg/L (0.17 to 170 mg/L P) organic phosphate[2]

Speculations for physiological mechanisms

• Inorganic and organic phosphate inhibits the enzyme acetylcholinesterase in sea urchins which would cause many malfunctions with muscular function and development[3]
• Phosphates inhibit amino acid uptake which would compromise metabolic activity necessary for development[4]

Hypothesis & Objective

Hypothesis

Exposure to phosphate is detrimental to the reproduction and early life stages of Scleractinia (stony coral) and Echinodermata (sea urchin) reef species.

Objective

Determine the effect of phosphate on fertilization and/or the survival of the early life stages of coral reef species to discern, whether or not nutrient input may be contributing to coral’s low reproductive output.

Methods

Coral fertilization and survival with exposure to potassium phosphate (KH₂PO₄)

Acropora palmata and Orbicella faveolata

Collection: Gametes were from Horseshoe and Elbow Reefs in Key Largo, FL.

Fertilization: Gametes (sperm and eggs) were pooled from multiple genotypes to ensure successful fertilization. Eggs and sperm were added separately into dosing vials containing 5 mL of solution (Table 1). Exposure time: A. palmata 6 h exposure at 29°C. O. faveolata 4 h exposure at 29°C

Survival: Embryos fertilized in seawater were added to 5 mL of solution. Exposure time: 48 h exposure with static renewal for A. palmata and static for O. faveolata; both sp. were exposed to 29°C and 31°C.

Sea urchin development with exposure to potassium phosphate (KH₂PO₄)

Lytechinus variegatus

1. Spawning by injecting KCl into coelomic cavity
2. Gamete collection: Eggs (Figure 3); Sperm was collected dry from genital plates
3. Sperm (in varying dilutions) and eggs were added to 5 mL of artificial seawater
4. Fertilization was assessed after 30 min and best fertilization rate was used (>90% fertilization)
5. A small volume containing roughly 250 fertilized eggs was added to 5 mL of solution and incubated for 48 h at 25°C
6. Development was assessed

Phosphate Microplate Assay: Colorimetric method adapted from EPA ascorbic acid method using Hanna Checker phosphate reagents and measured on a spectrophotometer

Results

Table 1: KH₂PO₄ concentrations used for dosing and amount of phosphate and phosphorus in KH₂PO₄ for reference

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>PO₄ (mg/L)</th>
<th>Inorganic P (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (ASW Control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.045</td>
<td>0.011</td>
</tr>
<tr>
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<td>0.15</td>
<td>0.034</td>
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<td>5.0</td>
<td>0.84</td>
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</tr>
<tr>
<td>10.0</td>
<td>1.68</td>
<td>0.291</td>
</tr>
</tbody>
</table>

Figure 1. A. palmata embryos: Blue circle (fertilized); Red circle (unfertilized)

Figure 4. Glass vials (20 mL) used in experiments

Figure 5. Fertilized and unfertilized sea urchin eggs[5]

Figure 6. L. variegatus planktonic stage. Development is categorized as normal (a.), underdeveloped (b.), and malformed (c.)

Figure 7. Phosphate colorimetric assay measuring orthophosphate in blue.

Conclusions

• Higher concentrations will need to be used to find LC₅₀
• While A. palmata had lower survival at 31°C compared to 29°C, phosphorus may only be a confounding factor when present at higher concentrations
• Acclimation to their eutrophic environment could have caused these gametes to be more resistant

Significance

Coral, the world’s fundamental reef species which provide protection and economic stability, are in a state of decline. This study should provide some guidance on nutrient criteria necessary to protect the crucial life stages of reproduction and development.

Future Research

• Examine the health of the adults by using endpoints of wound healing and physio scores
• Measure the photosynthetic efficiency of the dinoflagellates
• Examine the toxicity of other forms of phosphate, such as condensed phosphates and organic phosphates, or examine the toxicity of another contaminant found in high concentrations in St. Croix

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References