SAND2011-2201C

Pond Crash Forensics

4/8/2011
Algae Platform Peer Review

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Goal Statement

- The goal of this project is to develop tools and methods that will be used to identify the etiological agents of pond crashes through the forensic analysis crash samples. Once identified, we will confirm by reproducing crashes in laboratory experiments.
- Diagnosing the root causes of pond crashes will be critical to informing the development of inexpensive screening and monitoring tools for early crash detection, as well as engineering and biological countermeasures that will enhance pond stability and increase long-term productivity.
- This work will support the Algae Platform goal of developing sustainable production processes/systems to increase yield and lower cost.
- Ultimately this work will support the OBP strategic goal to develop sustainable, cost-competitive biomass technologies to enable the production of biofuels.

Quad Chart Overview

Timeline

- Project start date: 10/01/2010
- Project end date: 9/31/2012
- Percent complete: 25%

Barriers

- **Sustainable Production**
  - cost
  - productivity
  - sustainability

Budget

Total project funding: $800K

- DOE share: $800K
- Contractor share $0

FY2010 Funding: $800K

Partners

Interactions/collaborations:

NAABB TAMU
PNL/Univ AZ
Carbon Capture Corp
NRC Institute for Marine Biosciences

Project management:

Project Lead: Todd Lane
Sandia National Labs, Livermore

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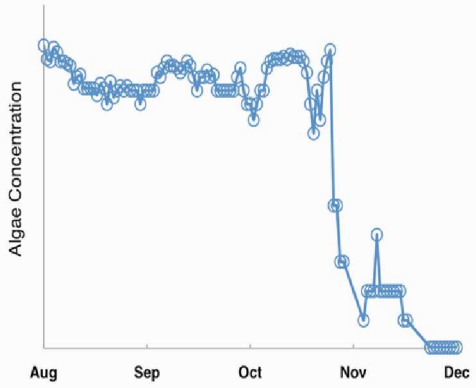
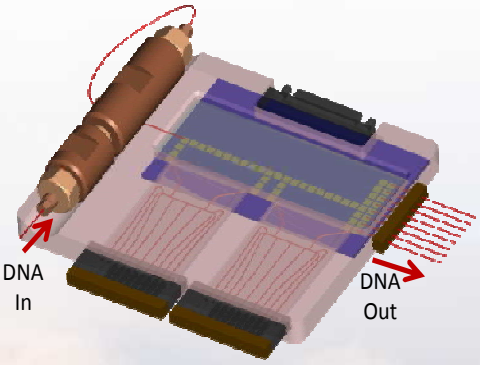
Project Overview

- **Context:** "Perhaps the most worrisome component of the large-scale algal cultivation enterprise is the fact that algal predators and pathogens are both pervasive and little understood." DOE Algal Biofuels Technology Roadmap (2009)



- **Goal:** Develop diagnostic tools and methods to identify the root causes of pond instabilities through the forensic analysi of samples taken from raceways and PBRs post-crash.

- **Leverage:** Internal Sandia $12M investment in Biodefense to develop microfluidic technology which enables ultra high throughput sequencing to rapidly and cheaply identify an etiologigical agent without the need for isolation. Utilize Sandia's hyperspectral imaging capabilities.



Approach

Develop methods to enrich for nucleic acids that are likely to derive from the etiological agent of the crash.

Utilize ultra high throughput sequencing to identify agents.

Create quantitative assays that facilitate the detection of agents at low concentration.

Recreate the crash under laboratory conditions.

Utilize advance spectroscopic methods to detect early hallmarks of algal stress.

- **Management approach**
  - We are using specific milestones to measure our progression from relatively simple defined samples to more complex field samples
- **Technical metrics of progress**
  - Speed of analysis
  - Sensitivity of detection
  - Efficiency of the analysis
- **Unique aspects:** The use of nucleic acid target enrichment followed by ultra high throughput sequencing as a strategy for agent identification.

Project Objectives/ Technical accomplishments.

- **Genetically identify unknown etiological agents of pond crashes without the need for agent isolation.**
  - Developed a test system with model algae and contaminants.
  - Developed collaborations and obtained samples from groups running pilot scale open ponds
  - Tested sample preparation and analysis trained on laboratory and field samples
  - Developed host and background subtraction reagents
- **Isolate and propagate several previously unknown agents of pond crashes.**
  - Developed agent isolation and propagation methods
- **Reproduce pond crashes on demand at various scales and culture conditions to understand the environmental parameters that modulate the effect of the etiological agent or the natural resistance of the algae.**
- **Demonstrate efficacy of the combined metagenomic and spectroscopic diagnostic system in the field with industrial and academic collaborators to monitor pond health and detection of pathogens.**

FY11 Milestones

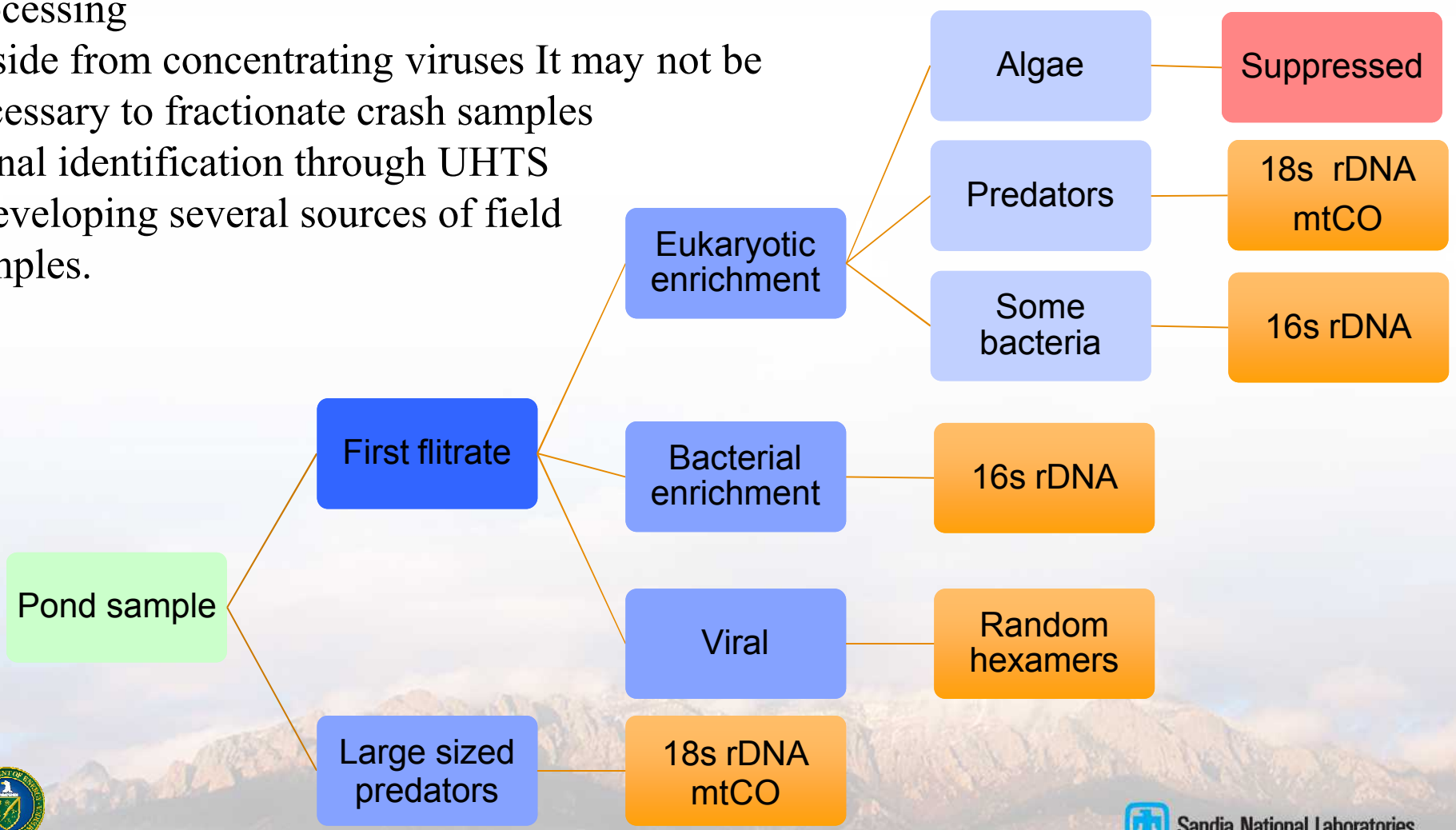
Milestone	Title	Due date	Status
Task 1	**Metagenomic analysis**		
1.1	Develop nucleic acid extraction methods for algal pond samples	12/30/10	Complete
1.2	Demonstrate background removal in pond samples	3/30/11	Complete
1.3	Acquire and process crash samples	6/30/11	On track
1.4	Demonstrate identification of unknown agents	9/30/11	On track
Task 2	**Agent Isolation**		
2.1	Develop viral and predator isolation methods	12/30/10	Complete
2.2	Deliver isolated agents to Task 3	9/30/11	On track
Task 3	**Advanced Spectroscopic Analysis**		
3.1	Compete design pathogen/algae experiments	6/30/11	On track
3.2	Completed pathogen/algae experiments with existing agents	9/30/11	On track

FY12 Milestones (all on track)

Milestone	Title	Due date
Task 1	**Metagenomic analysis**	
1.5	Identify and develop assays for additional agents.	12/30/11
1.6	Deliver nucleic acid signatures and assays	3/30/12
1.7	Complete out metagenomic analysis of field samples	9/30/12
1.8	Deliver final metagenomic data on field demonstrations	9/30/12
Task 2	**Agent Isolation**	
2.3	Acquire and isolate additional agents	12/30/11
2.4	Deliver test agents for field Demonstration (Task 4)	3/30/12
Task 3	**Advanced spectroscopic analysis**	
3.3	Identify key factors modulating susceptibility to collapse.	6/30/12
3.4	Identify spectral signatures of early indications of crashes	6/30/12
Task 4	**Field trial**	
4.1	Complete metagenomic analysis and agent detection assays of pond crashes	8/30/12
4.2	**Complete demonstration of prototypical technologies**	9/30/12

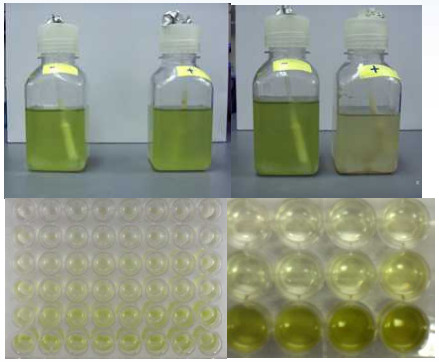
We have developed a sample analysis scheme

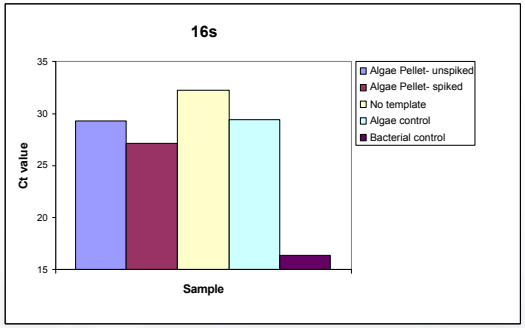
- The final goal is to limit the extent of pre-processing
- Aside from concentrating viruses It may not be necessary to fractionate crash samples
- Final identification through UHTS
- Developing several sources of field samples.

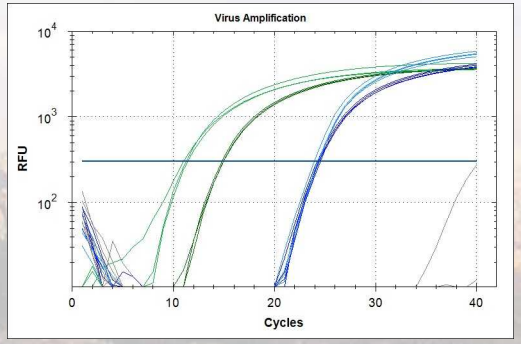


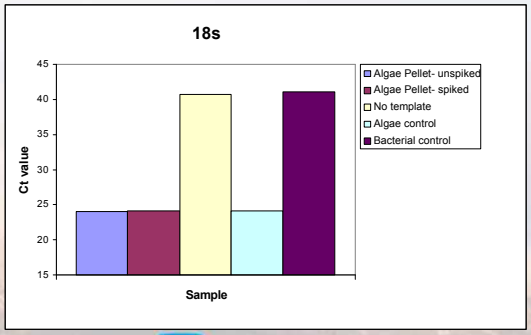
Methods development and validation

- **Agent isolation**
  - Developing methods of isolation by terminal dilution.
  - Developing novel alternative isolation methods
- **PCR assays for model agents**
  - Developed assays to quanititate and amplify nucleic acids from various classes of agents
  - Developed species specific assays
- **Development and validation of fractionation and nucleic acid extraction methods**
  - Utilized species specific and general assays to track how agent "behave" during physical fractionation
  - Allow us to measure extraction and recovery of nucleic acids









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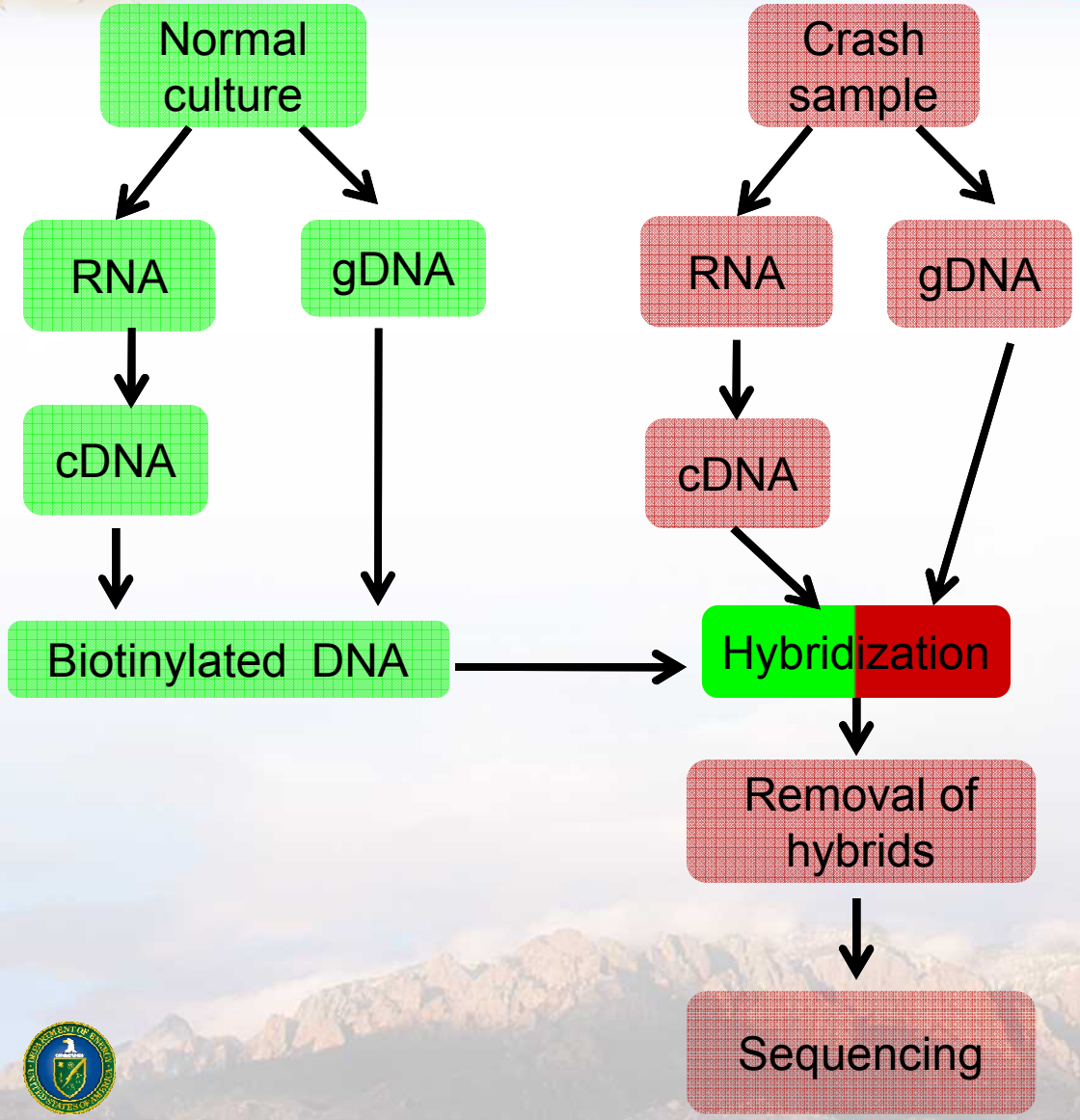
Development of subtraction reagents



- Anything that is “normal” is depleted from the crash sample
- Enriches for sequences that are likely to be associated with the crash agent
- In the final manifestation these manipulations will be carried out in an automated fashion by RapTOR.

Relevance

MYPP Strategic goal: Develop sustainable, cost-competitive biomass technologies to enable the production of biofuels…

Algae Platform goal: Develop Sustainable Production Processes/Systems to increase yield and lower cost

- Current annualized production is limited by losses due to crashes and etiological agents of these crashes are “pervasive and poorly understood”
- The objectives of the project will contribute to meeting these goals by
  - Creating the ability to identifying, isolate and characterize etiological agents
  - Produce sensitive and rapid assays that allow early detection
  - Enabling the development of a system to test biological and engineering countermeasures
- Application of project results to the algal industry
  - Early detection will enable the “salvage” of biomass prior to crash
  - Will facilitate pond remediation and return to production
  - Will enable the testing of countermeasures against crash agents.
- Project accomplishments to date represent steps on the critical path to developing the final technological capabilities.

Critical Success Factors

- **Efficient and quantitative nucleic acid extraction and recovery**
  - Failure to extract and recover means failure to detect and identify.
  - Efficient extraction and recovery are key to attaining high sensitivity
  - We have developed specific probes to a variety of agents to measure losses incurred in different steps
- **Effective host and background subtraction**
  - The more host and background is removed the less sequencing that is necessary thus controlling cost and complexity of analysis
  - We are strongly leveraging the work of RapTOR that has lead to the development and testing of several subtraction methods
- **Lab confirmation of etiological agent**
  - In some cases it will be desirable to confirm the identity of an agent by isolation and recreating the crash.
  - Effective

Potential Project Challenges

- ***Ability to obtain access to a sufficient diversity of crash samples to demonstrate the robustness of the methodology***
  - *We expend significant effort to generate a network of collaborators*
  - *Early communication of results should facilitate the formation of collaborations.*

- ***Recalcitrant agents:*** *Some types of agent are notoriously difficult to lyse.*
  - *There are several commercial technologies for lysing tough samples that we are evaluating*
  - *We can leverage internal work targeted at lysing bacterial spores*

- ***Ability to effectively remove host and background nucleic acid species***
  - *RapTOR has developed several alternative suppression strategies. We can apply more complex strategies or combinations of strategies.*
  - *Use of group specific primers for amplification and physical separation methods in sample processing should lessen the problem.*

Positive impact on the commercial viability of algal biofuels

Increased annualized production and decreased production costs

- Enable the early and inexpensive detection of low levels of contamination. Identify early indicators of pond stress
  - Enable the salvage harvesting of biomass
  - Facilitate the remediation of the pond and return to production
- Inform biological and engineering mitigation and response strategies.
  - By understanding the characteristics of the agents directed countermeasures can be developed.
- Enable the creation of a system for the testing of countermeasures
  - By creating a “crash on demand” system the speed of countermeasure development will be enhanced

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Future Work

- FY11
  - Complete methods development and validation
  - Demonstrate ability to identify crash agents in samples
  - Key Milestones
    - 9/30/11 Demonstrate identification of unknown agents.
  - Decision points
    - The extent of physical fractionation required
    - Identify the appropriate technology for sample lysis
    - Identify the appropriate method(s) for subtraction
- FY12
  - Characterize the spectral signatures of pond stress
  - Demonstrate efficacy of methods and assays in field trial
  - Key milestones.
    - 6/30/12 Analyze deliberat ponds crashes using metagenomic analysis, and agent detection assays.
    - Identify spectral signatures that are early indicators of pond crashes.
    - 9/30/12 Demonstration of prototypical pond crash analysis technologies

Summary

- Develop diagnostic tools and methods to identify the agents of pond crashes
- Critical success factors and challenges:
  - Efficient and quantitative nucleic acid extraction and recovery.
  - Effective host and background subtraction
- Technical accomplishments
  - Developed and tested sample preparation and analysis methods
  - Developed host and background subtraction reagents
  - Developed agent isolation methods
  - Algae Platform goal: Develop Sustainable Production Processes/Systems to increase yield and lower cost
  - Early detection will enable the "salvage" of biomass prior to crash
  - Will facilitate pond remediation and return to production
  - Will enable the testing of countermeasures against crash agents.
  - Will increase annualized pond production

Additional Slides

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Definitions

- **Metagenome:** all the genetic material present in an environmental sample, consisting of the genomes of many individual organisms.
- **Suppression:** A general term for the elimination of host and background nucleic acid sequences
- **cDNA:** complementary DNA (cDNA) is DNA synthesized from a mature mRNA template
- **gDNA:** Genomic DNA
- **PCR:** Polymerase chain reaction, at technique to amplify a single or few copies of a piece of DNA across several orders of magnitude
- **UHTS:** Ultra high throughput sequencing, Next generation sequencing

We continue to develop sources of field samples

- **Currently following 16 pilot scale ponds at two geographically distinct sites**
  - 500-1000mL samples on a weekly or biweekly basis
  - Samples of starting materials
  - More frequent samples if pond instabilities become apparent.
  - Rapid physical fractionation followed by archiving of samples
  - Material also used as a “training set”
- **In process of acquiring samples from ponds and PBRs that have undergone a crash**
  - PBRs: Collaborator has already had systems crash and is reproducing the crash to test countermeasures. We ill be analyzing both types of samples
  - Additional outdoor ponds: Collaborator expects “instabilities” and will send us appropriate samples.

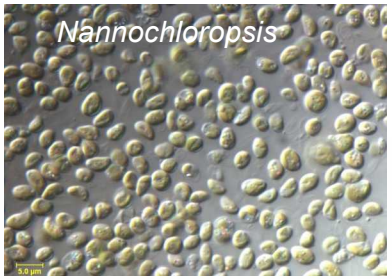
Algae collapses happen

Etiological Agents *Algae* *Collapse*

Virus

Bacteria

+



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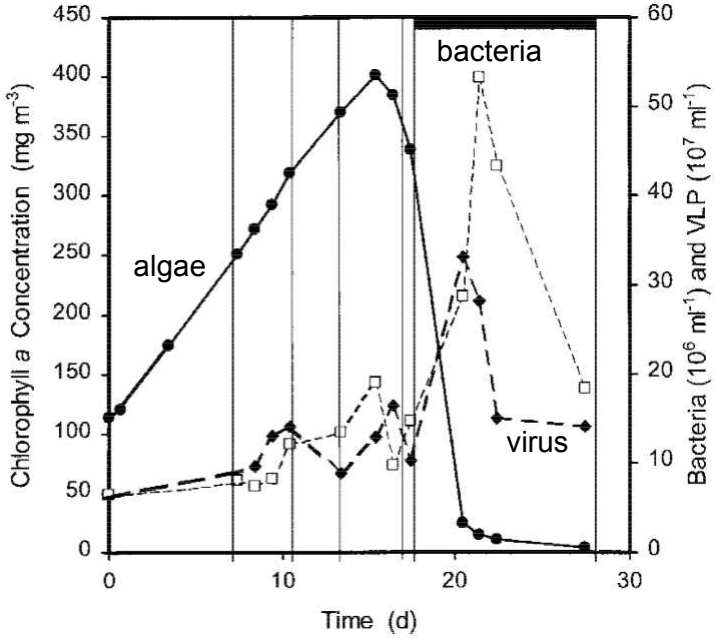
Zoo-plankton

Environment (Temp, salinity, pH, CO2, nutrients)



Herman Gons et al., Antonie van Leeuwenhoek, 81: 319-326, 2002.

"Perhaps the most worrisome component of the large-scale algal cultivation enterprise is the fact that algal predators and pathogens are both pervasive and little understood."

- DOE Draft Algal Biofuels Technology Roadmap (2009)

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