Golden Alga (*Prymnesium parvum*) Workshop
Summary Report

Photo by Carmelo Tomas

October 24-25, 2003
Holiday Inn – North
Fort Worth, Texas

Texas Parks and Wildlife Department

Liz Singhurst and David Sager, editors

ACTS-2004-001

PWD RP T3200-1203

Golden Alga Workshop Preface & Agenda

The Golden Alga Workshop was organized to bring together stakeholders and regional, national, and international experts to discuss the state of knowledge on this toxic alga, the information lacking for understanding the alga, and important actions or projects needed to develop management options for this alga. This information will be used by the Texas Parks and Wildlife Department in decisions for implementing and funding projects to develop management strategies for Texas. The Texas Parks and Wildlife Department Golden Alga Task Force developed, organized, and coordinated the workshop. The workshop took place at the Holiday Inn – North in Fort Worth, Texas on October 24-25, 2003. The workshop was facilitated by Group Solutions, Inc. Funding for the workshop came from several sources. The U.S. Fish and Wildlife Service provided federal aid funding for projects relating to this workshop, mainly through a State Wildlife Grant (T-14-P). The Brazos River Authority, Lower Colorado River Authority, and Texas Chapter of the American Fisheries Society sponsored this workshop through funding or services provided. Copies of the workshop report (on CD or hardcopy) can be obtained by requesting the document ACTS-2004-001 from:

Aquatic Conservation Branch
Resource Protection Division
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, Texas 78744

Golden Alga Workshop Agenda
October 24 & 25, 2003

Friday, October 24, 2003

9:00 AM Larry McKinney - Welcome and a perspective on Texas algal blooms

9:30 AM Joan Glass - Historical review of golden alga (*Prymnesium parvum*) problems in Texas

9:45 AM Greg Southard - Overview of Texas hatchery management of golden alga, *Prymnesium parvum*

10:00 AM Break

10:30 AM Bente Edvardsen and Aud Larsen- Phylogeny, life history, autecology and toxicity of *Prymnesium parvum*

11:00 AM Carmelo R. Tomas - *Prymnesium parvum* - an overview and questions

11:30 AM Richard L. Kiesling - Analysis of *Prymnesium parvum* blooms in Lake Whitney, Texas

12:00 PM Lunch

1:15 PM Edna Graneli - Kill your enemies and eat them: the role of *Prymnesium* toxins

1:45 PM Paul Kugrens - *Prymnesium parvum* laboratory studies: structure, reproduction, salinity tolerance and bioassay

2:15 PM Linda Medlin, Gundula Ellers, Kerstin Toebe, and Katja Kerkmann - Rapid tests for the detection of *Prymnesium parvum* and its toxins

3:00 PM Break

3:30 PM Donald M. Anderson and Mario R. Sengco - Bloom control strategies for harmful algal blooms

4:00 PM Panel Session with Invited Speakers

4:45 PM Summary of 1st Day's Recommendations and Review of 2nd Day's Objectives

Saturday, October 25, 2003

9:00 AM Welcome and Review of Goals and Agenda

9:30 AM Jan Landsberg - A review of fish-killing microalgae: causes, impacts, and management with emphasis on *Prymnesium*

10:00 AM Karen A. Steidinger - How to use the past to plan for the future

10:30 AM Break

11:00 AM Facilitated Question/Answer Session and Overview Discussion on Workshop Presentations;

Facilitated Discussion on Afternoon Deliverables

12:00 PM* Lunch

1:00 PM Facilitated Discussions:

- Identification of key research needs and goals concerning the biology of the golden alga
- Research needs and goals for management of the golden alga
- Prioritize the research needs and goals identified

Facilitated Discussion on an Agenda to Support a Research Program, Assumptions, and Potential Roles, Responsibilities, and Milestones

Facilitated Discussion and Development of a Communiqué for the Workshop

3:45 PM Review, Wrap-up, and Discuss Where TPWD is Going from Here

4:00 PM Adjourn

***** The times for lunch and breaks during the facilitated discussion sessions may vary, as the facilitators deem appropriate.

Golden Alga Workshop Table of Contents

2. **List of Golden Alga Workshop Attendees**
3. **List of Golden Alga Vendors**
4. **Additional Information Provided on the Golden Alga**
   Sean Watson - Literature Review of the Microalga *Prymnesium parvum* and its Associated Toxicity

Golden Alga Workshop Background Information

Since the early 1980s, the occurrence of toxic golden alga (*Prymnesium parvum*) blooms have increased in Texas in number and range. These toxic alga blooms have resulted in large fish kills in five major rivers in Texas (Pecos River, Colorado River, Brazos River, Wichita River, and Canadian River). In recent years, toxic blooms have impacted major reservoirs managed for recreational fisheries that provide important recreational and tourism opportunities for the public. State fish hatcheries associated with the Brazos and Red rivers have also been impacted by this toxic alga. The resulting economic impacts to Texas Parks and Wildlife Department (TPWD) fisheries management efforts, state parks, and local businesses have been significant. During this time, the golden alga has been confirmed in seven other states: Arkansas, Alabama, Georgia, New Mexico, North Carolina, South Carolina, and Wyoming.

TPWD held a workshop in July 2001 at Possum Kingdom, Texas, with state, regional, and local agencies; government leaders; university researchers; and representatives of the public. The workshop discussed the problem and potential actions. One action from the workshop was for the Harmful Algal Bloom Subcommittee (of the state interagency Toxic Substances Coordinating Committee) to work with member agencies and university researchers to draft a report to the legislature that included potential actions and research with rough cost estimates.

Using information from this report and other sources, the state legislature authorized TPWD to spend $600,000 in each year of the 2004-2005 biennium ($1.2 million total) to address the golden alga problem. TPWD decided that in order to use these funds wisely and efficiently, the priority needs for developing management options for the golden alga had to be determined. The TPWD Golden Alga Task Force was organized to manage the departmental response to golden alga blooms and the problems they cause. The department held a scientific Golden Alga Workshop in October 2003 to generate and prioritize research and other needs for Texas. The workshop invited state, national, and international experts on the golden alga and other harmful algae to meet with stakeholders from nearby states, Texas state agencies, federal agencies, river authorities, university researchers, private industry, and the public. Invited speakers gave presentations discussing the situation in Texas, international research and understanding about the golden alga, and possible options to address the problem. The presenters and the stakeholders then interacted in facilitated sessions to develop lists of actions for a management plan for the golden alga.

TPWD will use the results of this workshop in determining priority actions and projects that can be undertaken with the funding available. Through a federally funded state wildlife grant, funding from other sponsors (Brazos River Authority and Lower Colorado River Authority), and cooperative efforts with the Texas Chapter of the American Fisheries Society, this workshop was conducted without expending any of the legislative authorized funds. Efforts will continue to try to increase the funds available for these activities through additional funding and cooperative efforts.

Golden Alga Workshop
October 24 / 25 2003

International Experts
National Experts
Academia
Resource Managers
Business Representatives

The TPWD "GOLD" Team

TEXAS PARKS & WILDLIFE

Technical Support:

David Sager - RP
Loraine Fries - IF
David Buzan - RP
Joan Glass - RP
Kip Portis - RP
Jack Ralph - RP
Greg Southard - IF
Aaron Barkoh - IF
Bob Betsill - IF
John Prentice - IF

Administrative Support

Dee Halliburton - RP
Paula Hawkins - IF
Liz Singhurst - RP
Julia Gregory - RP
Toni Oldfather - RP

From Phil Durocher
And Larry McKinney:

THANK YOU

Responding to Golden Alga Has Been A Partnership:

TEXAS PARKS & WILDLIFE

LCRA ENERGY • WATER • COMMUNITY SERVICES

Brazos River Authority

U.S. FISH & WILDLIFE SERVICE

State Wildlife Grants

TCAFS

Texas Chapter of the American Fisheries Society

SPORT FISH RESTORATION

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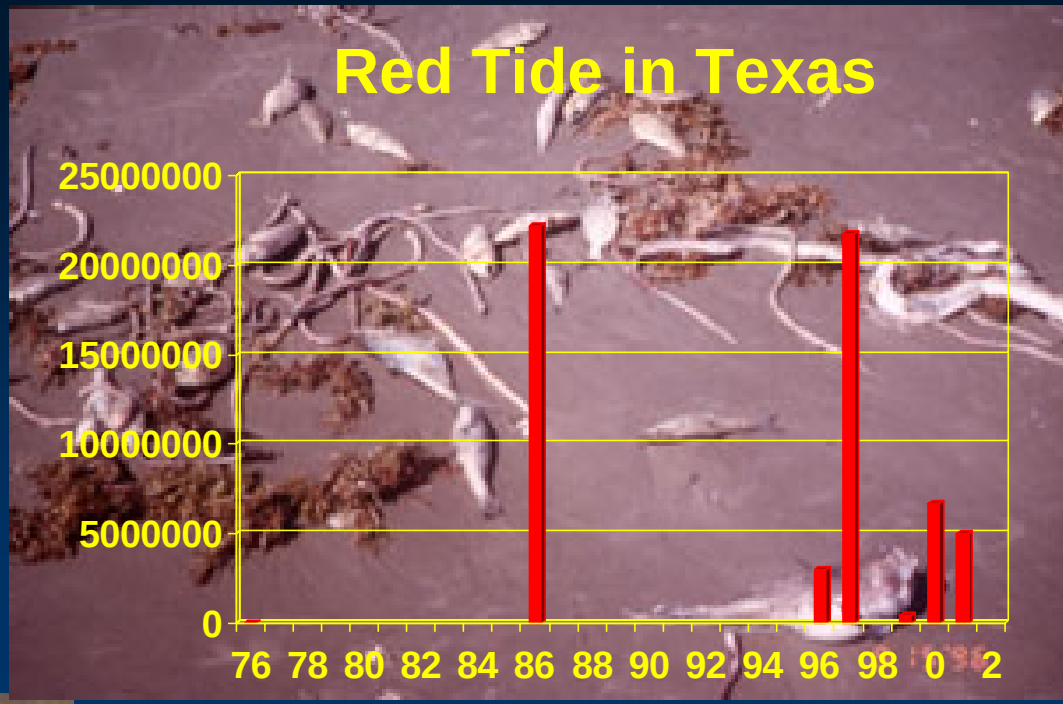
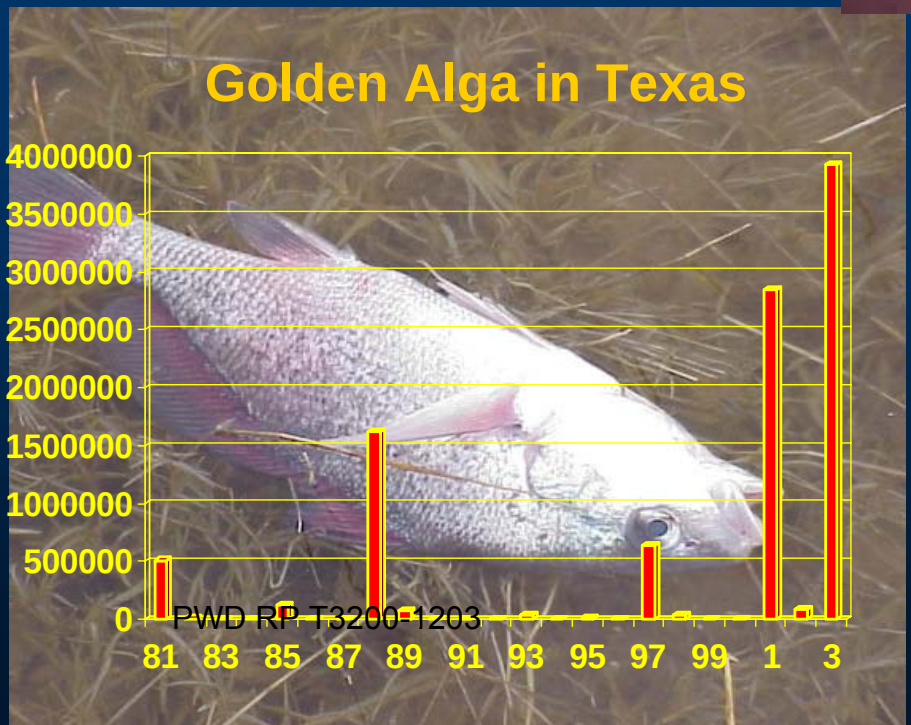
While There Is Much We Do Not Know About Harmful Algal Blooms (HABs) In Texas . . .

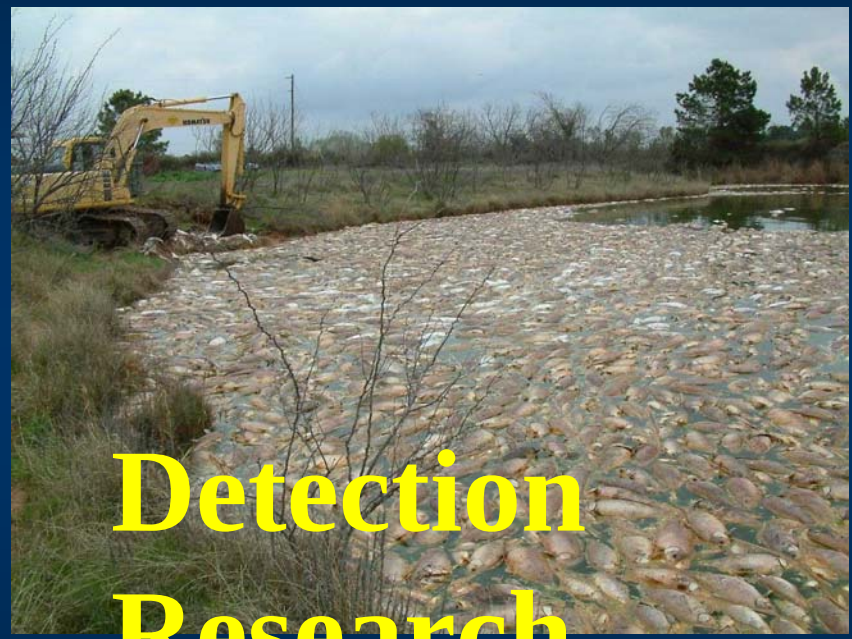




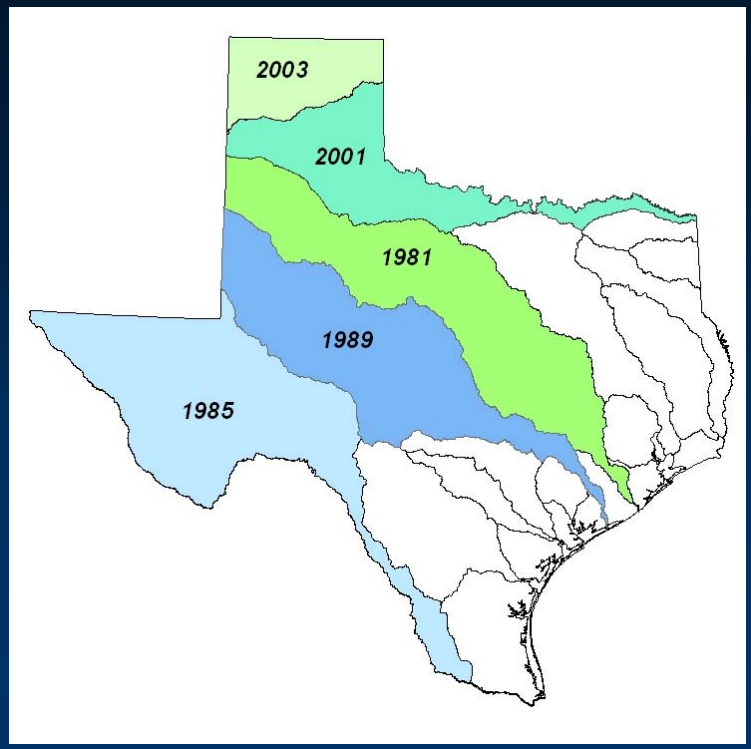
We Do Know They Are Occurring More Frequently And Causing Increased Economic Distress

What Can We Do About *P. parvum* And the Harmful Algal Blooms It Causes?

(Besides cleanup the mess)



Detection
Research
Management
Education



Detection

Early

Simple

Basic Research

Autecological

Biological

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Communications

Professional

Public

Management

Hatcheries

Ponds

Reservoirs

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Historical Review of Golden Alga (*Prymnesium parvum*) Problems in Texas

Joan Glass

Resource Protection Division, Texas Parks & Wildlife Department, 1601 E. Crest Dr., Waco, Texas 76705 USA

Abstract.--Since 1985, blooms of the golden alga *Prymnesium parvum* have been documented as a major cause of fish kills in Texas river basins. Over 17.5 x 10^6 fish with an estimated value of 6.5 million dollars have been killed by *P. parvum*. The majority of the golden alga kills occur during the winter months, with the number and magnitude increasing. In 2003 the fish kills within five river basins (19 lakes) culminated in over 6.4 x 10^6 fish killed with a value in excess of 2 million dollars. The Texas river basins with confirmed golden alga fish kills are Canadian, Wichita (Red), Brazos, Pecos (Rio Grande) and Colorado.

View the presentation

Historical Review of Golden Alga (*Prymnesium parvum*) Problems in Texas

Golden Alga Workshop Oct. 24-25, 2003

Prymnesium parvum
Photo by Dr. Carmelo Tomas

This workshop was partially funded by the federal State Wildlife Grant No. T-14-P

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SPORT FISH RESTORATION

Joan Glass
Texas Parks & Wildlife Department

Prymnesium parvum

Cyst

- Chrysophyte
- Cell size: 8-12 µm
- 2 long flagella - 1 haptonema
- C-shaped chloroplast
- Round to oblong in shape
- Mixotrophic
- Found in brackish water
- Resting cyst stage
- Characteristic swimming motion

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Prymnesium parvum Phase Contrast
by Dr. John La Claire

Where Has *Prymnesium parvum* Been Found?

- 1st identified 1930's in Holland & Denmark
- Fish kills reported in brackish coastal waters of Israel, China, England, Norway, United States, Australia, Morocco, Scotland, Germany, Spain, Bulgaria, and South Africa
- Inland United States: Confirmed in Texas, New Mexico, Colorado, Wyoming, North Carolina, South Carolina, Georgia, Arkansas & Alabama
- Suspected in Oklahoma and Nebraska

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Shad kill at Granbury Reservoir 2003
Photo by Joan Glass

Prymnesium parvum in Texas:

- 28 events - 1981 to 2003
- First documented kill - Pecos River, 1985
- Golden alga first confirmed - Pecos River, 1987-88
- 17.5 million fish killed using conservative estimates
- Value of fish killed over $7 million

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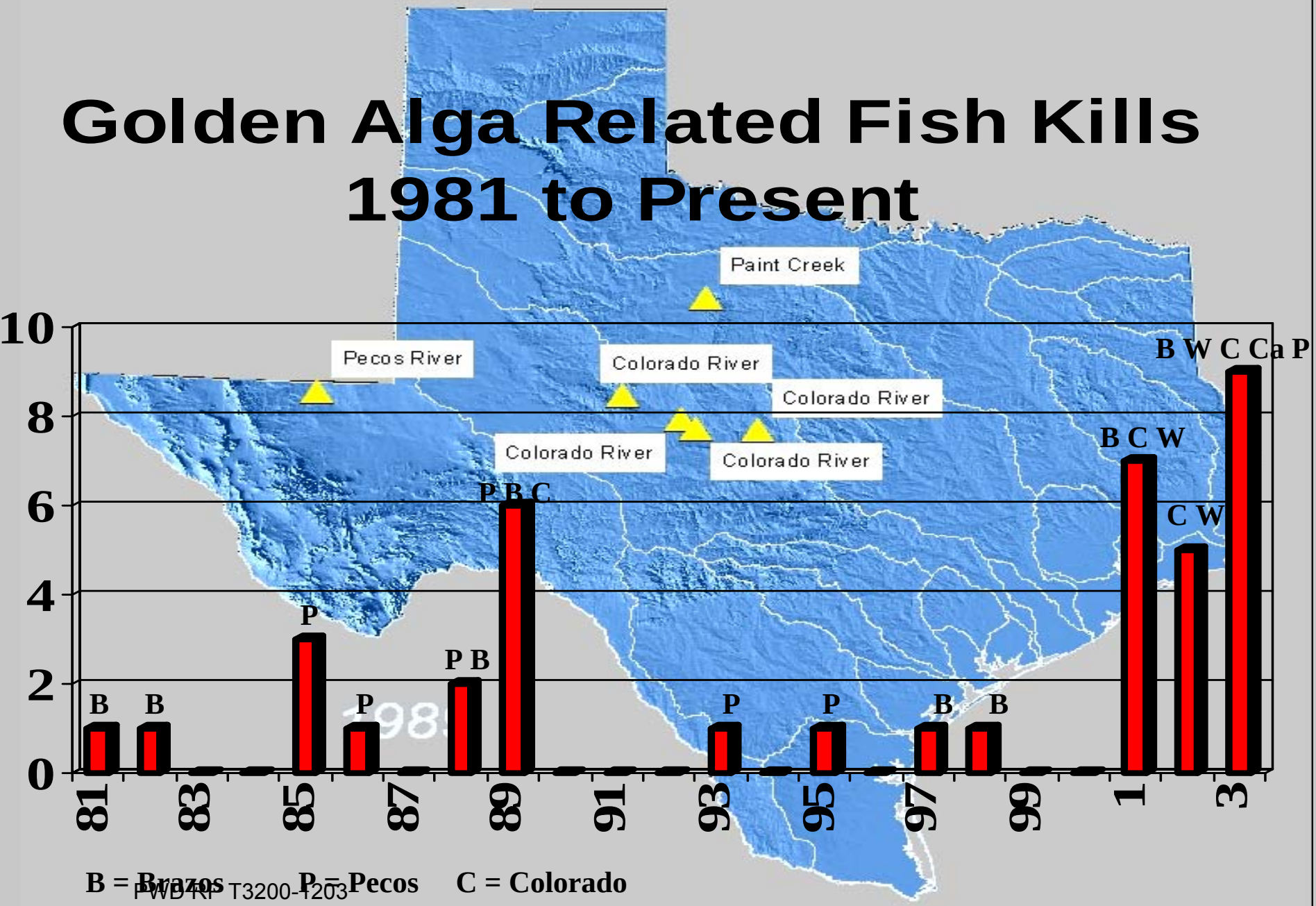
Jackson Bend, Lake Granbury 2003
Photo by Joan Glass

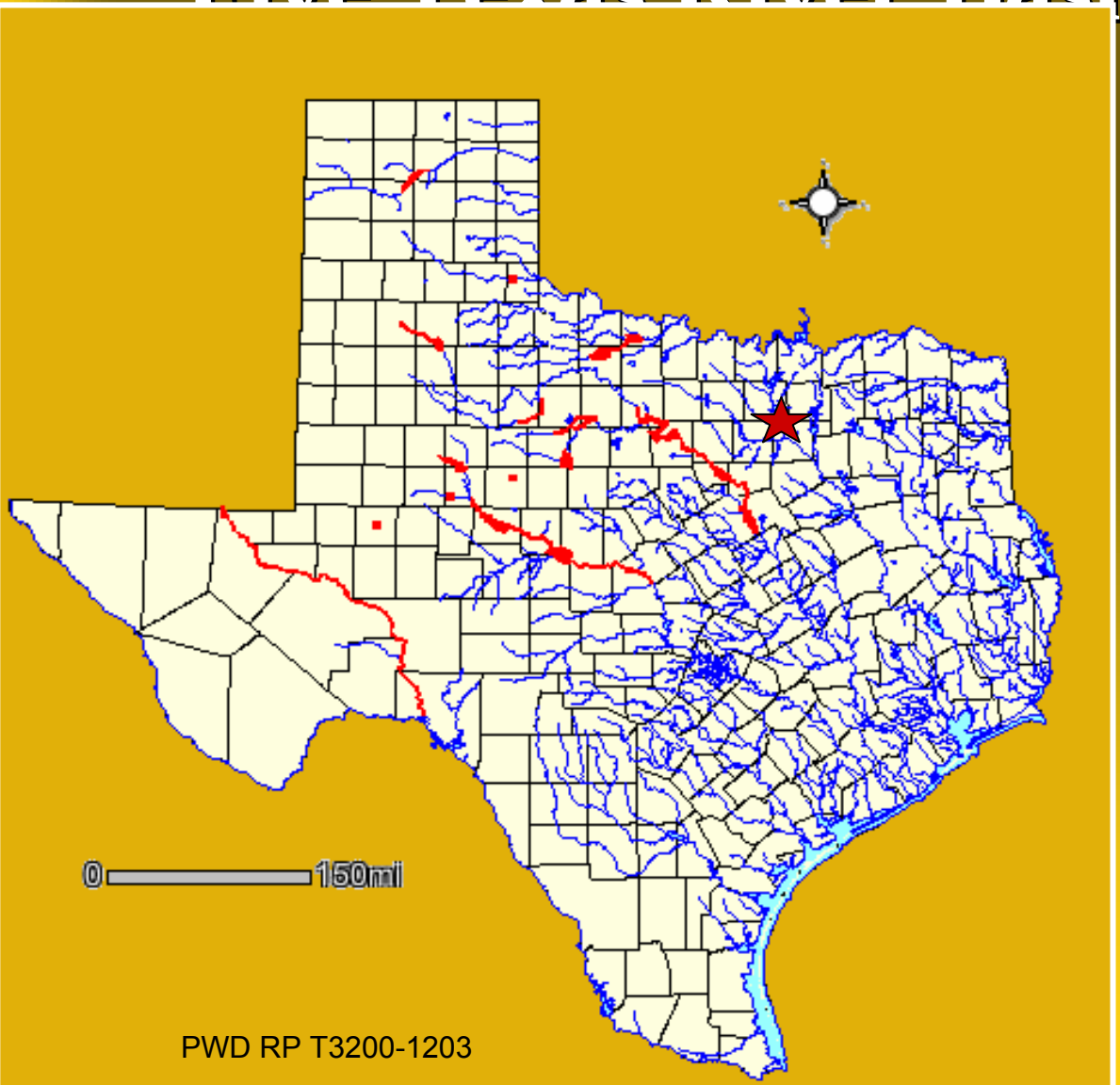
- Unknown indirect losses to local tourism, sport fishing and state revenues
- Reports of fish kills back to 1960's in the Pecos, Brazos and Wichita rivers are suspected to be caused by *P. parvum*

Golden Alga Related Fish Kills 1981 to Present



B = Brazos P = Pecos C = Colorado
W = Wichita Ca = Canadian

Five Texas River Basins Impacted



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- Canadian
- Red (Wichita)
- Brazos
- Colorado
- Rio Grande (Pecos)

Other River Basins:

- ☆Bloom confirmed in Sulphur Basin (Cooper Lake) - no fish kill
- ☆Chrysophyte cells also found in Trinity Basin

23 Texas Reservoirs Impacted

Severe	Moderate	Slight to No
Stilling Basin of Lake Meredith	Baylor	O.H. Ivie
6 Lubbock City Lakes	Diversion	Amistad
Buffalo Springs	Kemp	Meredith
E.V. Spence	Sweetwater	Cooper
Colorado City		
Moss Creek		
Possum Kingdom		
Granbury		
Whitney		
Red Bluff		

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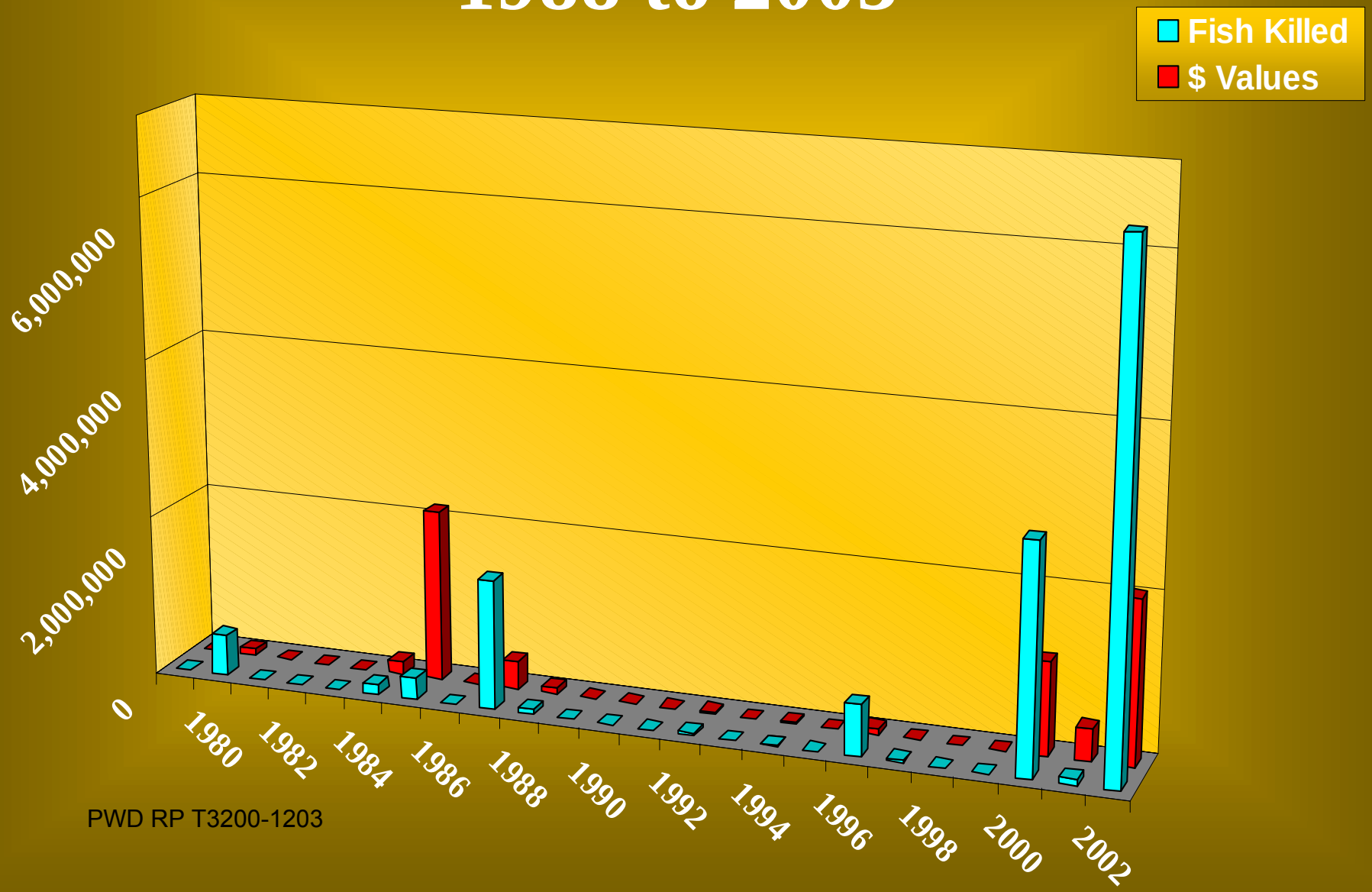
Dying shad at Granbury Dam 2003
Photo by Joan Glass

Number and Value of Fish Killed 1988 to 2003



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Fish Losses by *Prymnesium parvum*

Pecos River
Fish killed: 2,006,500
Value: $2,680,200*

Brazos River
Fish killed: 8,289,600
Value: $2,933,800

Canadian River
Fish killed: 48
Value: $1,400

Red River
Fish killed: 9,500
Value: $53,000

Colorado River
Fish killed: 2,355,900
Value: $863,600

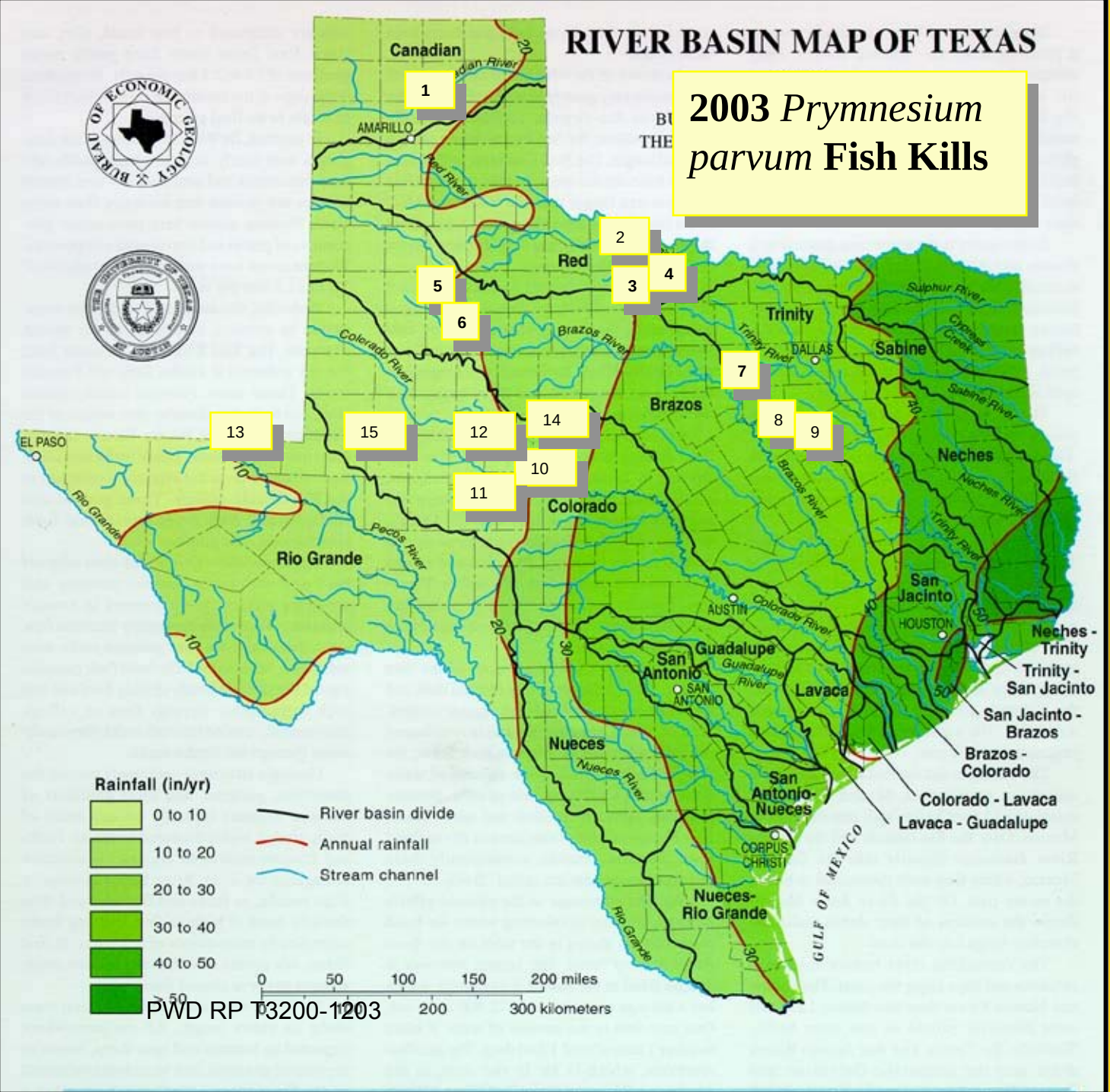
Dundee 5,000,000 production fish 2001
Value $430,000

Total Losses of fish
17,661,500

Total Value of fish killed
$6,962,000*

* Includes values for threatened species - Rio Grande Darter and Blue Sucker

Freshwater Drum dying in Lake Diversion Wichita River 2001
Photo by Joan Glass



Impacted Resv.

1. Meredith
2. Baylor
3. Kemp
4. Diversion
5. Lubbock City Lakes 1-6
6. Buffalo Springs
7. Possum Kingdom
8. Granbury
9. Whitney
10. Spence
11. Moss Creek
12. Colorado City
13. Red Bluff
14. Sweetwater
15. Wadley Barron Pond in Midland

2003
6.3 million
Fish Killed

Value over
$2 million

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What We Know

- Majority have occurred in winter
- Summer kills occur when water stops flowing
- All kills are west of I-35
- Frequencies and locations of fish kills are increasing

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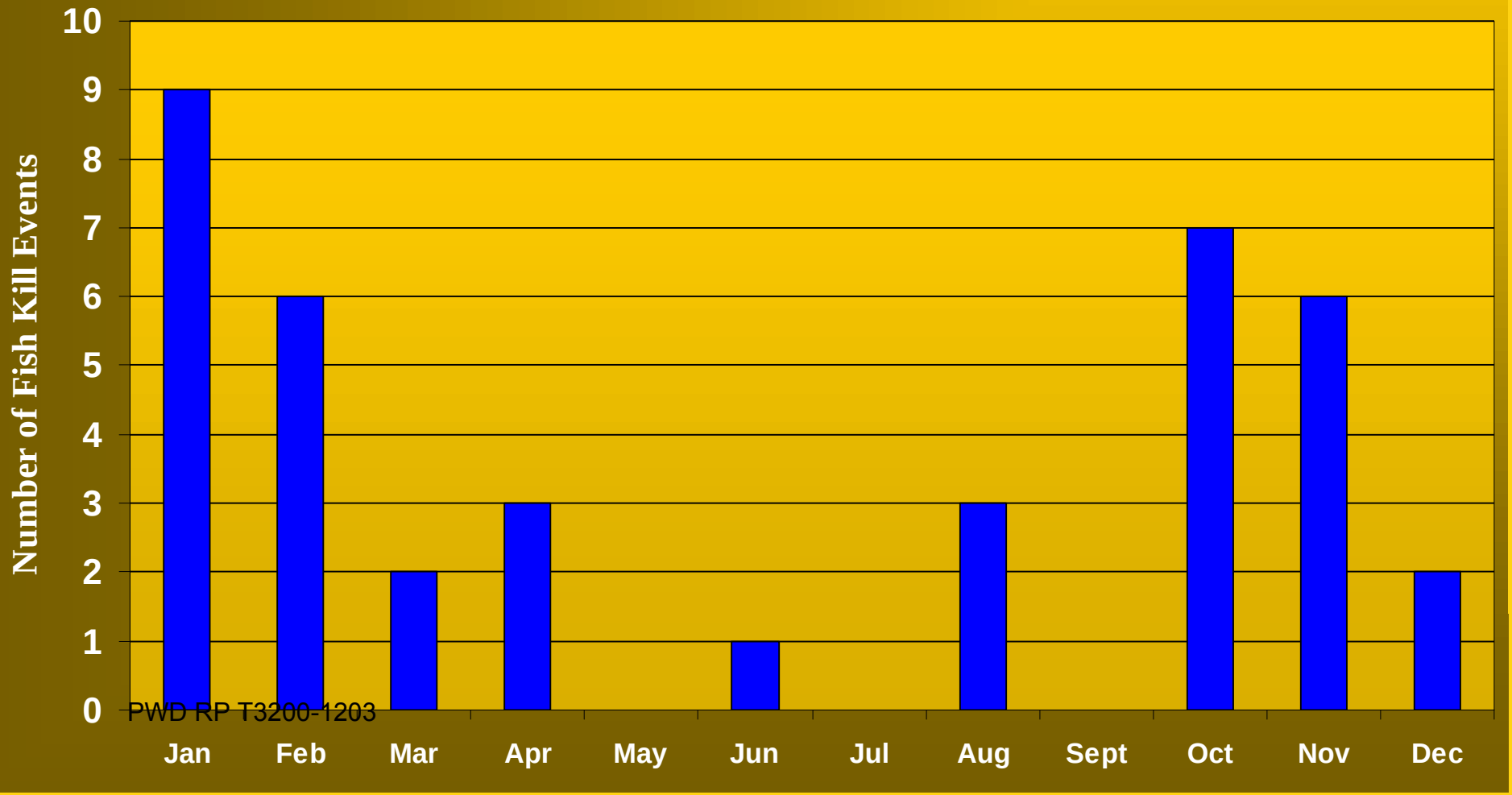
David Moduline, TPWD Game Warden
Possum Kingdom Reservoir 2001
Photo by Dave Buzan

Month Golden Alga Fish Kills Began 1981 to 2003



What We Know :
Conditions During Blooms

- Temperature extremes (usually colder)
- Low flows with increased salinity
- pH above 7
- Decreased competition from normal algal community due to environmental conditions

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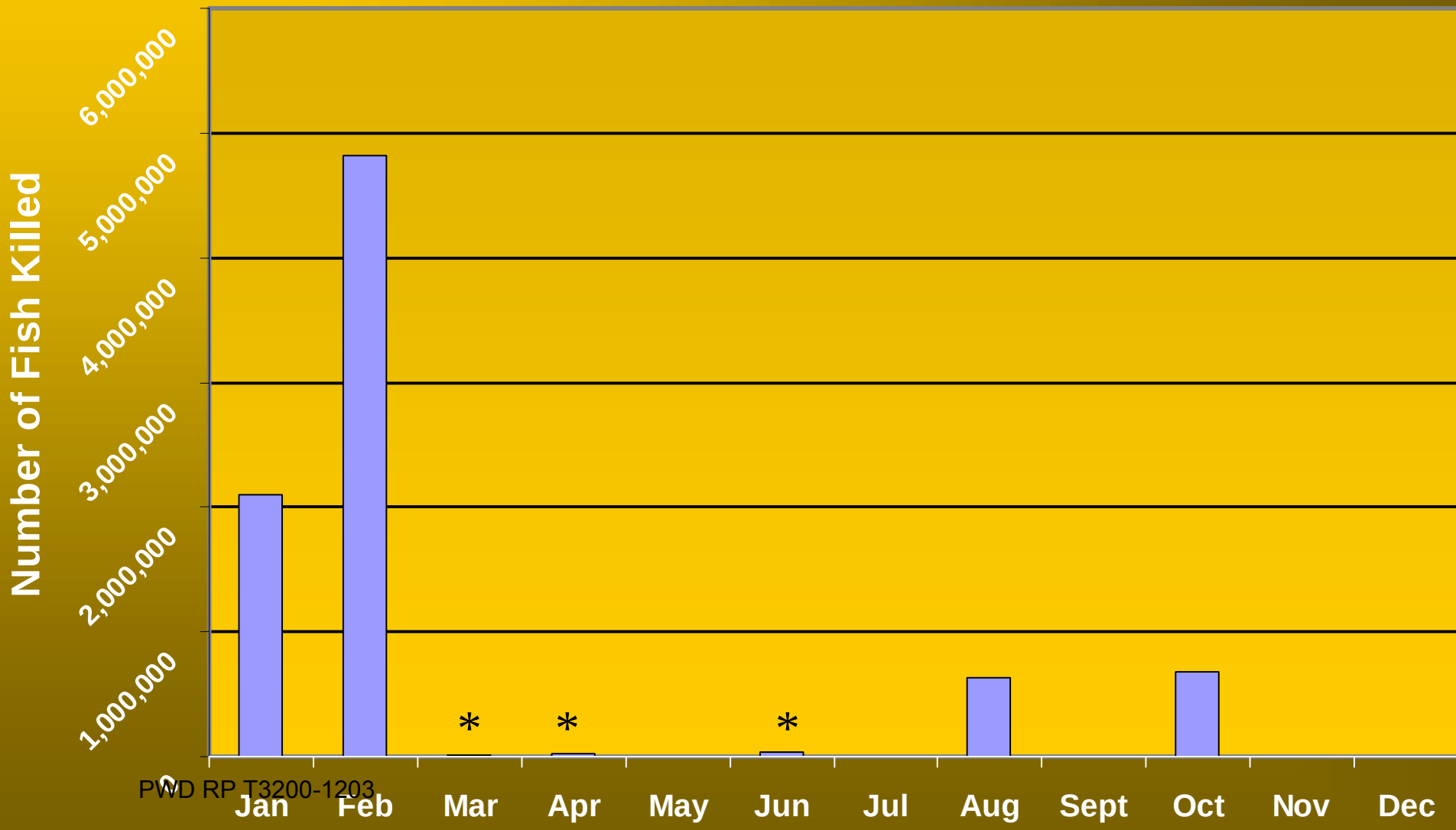
Possum Kingdom Resv.
2003 Foaming at airport
Photo by Joan Glass

Brazos Basin Losses 1981 to 2003 by Beginning Month



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We Want to Reduce the Impacts

- Prediction and prevention of toxic blooms
- Bloom control tactics for natural systems
- Prevent release of toxin and control toxins in water when bloom occurs

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Possum Kingdom Resv. 2001
Prymnesium parvum
Photo by Dave Buzan

We Want to Solve the Problem

Factors contributing to golden algal blooms:

- Factors enhancing source sites and causes of cyst emergence
- Fuels for blooms and triggers for toxicity
- Causes of senescence and pathogens

Gizzard Shad with hemorrhaging from gills mixed with heavy mucus.

Possum Kingdom 2001 Photo by Dave Buzan

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Possum Kingdom 2001
Prymnesium parvum
bloom along shoreline
Photo by Dave Buzan

What We Have Done

- Identified the fish kill cause
- Searched the literature
- Contacted toxic algae researchers
- Provided water from toxic blooms
- TPWD developed acceptable method of golden algae bloom control in hatchery ponds
- Created a library of cell lines at UTEX Algal Collection

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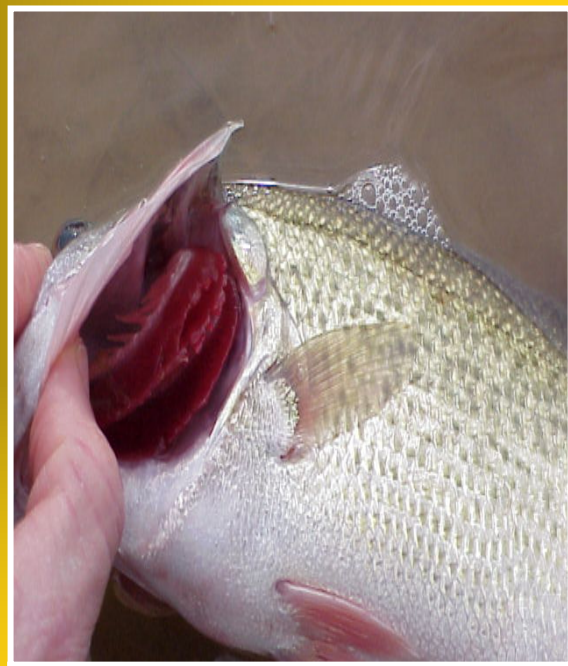
Freshwater drum showing hemorrhaging in fins. PK Resv. 2001
Photo by Joan Glass

Paint Creek 1988
Photo by Joan Glass

What We Have Done - continued

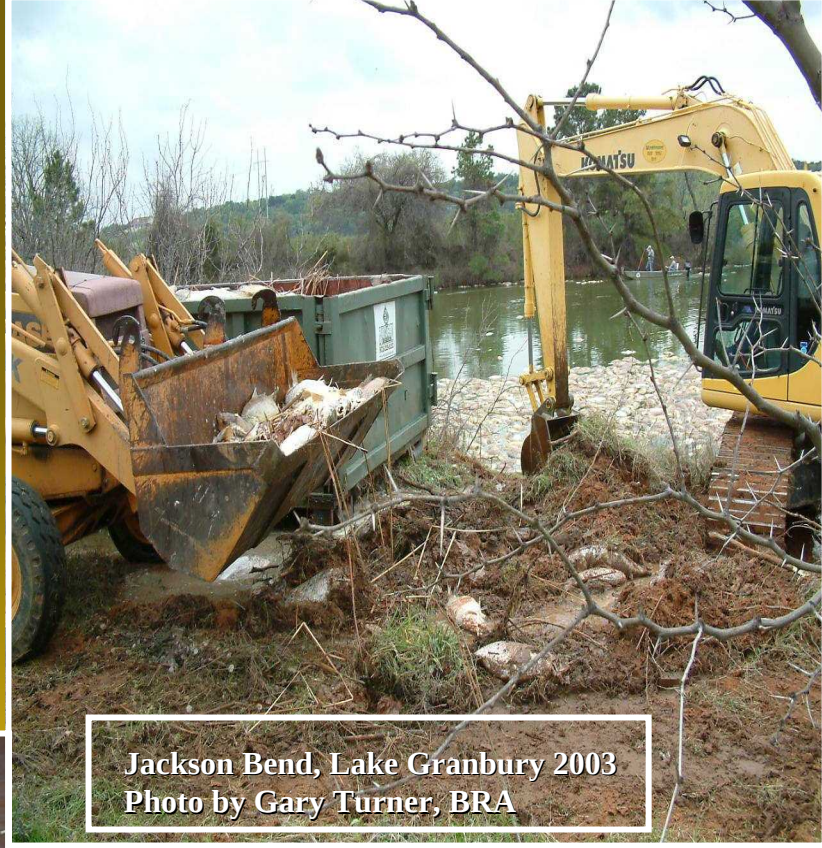
- Reported to Texas Legislature documenting losses
- U.S. Fish & Wildlife Service State Wildlife Grant awarded:
  - ☆ golden alga workshop
  - ☆ assess Texas historical events
  - ☆ website for public access
  - ☆ statewide survey in Texas
  - ☆ genomic study of the Texas golden alga strains



Largemouth bass with hemorrhaging from gills and fins PK Resv. 2001
Photo by Dave Buzan

Problem Summary

- >17.5 million fish killed in Texas by golden alga since 1981
- >6.3 million in 2003 alone
- Additional information on golden alga needed



Jackson Bend, Lake Granbury 2003
Photo by Gary Turner, BRA

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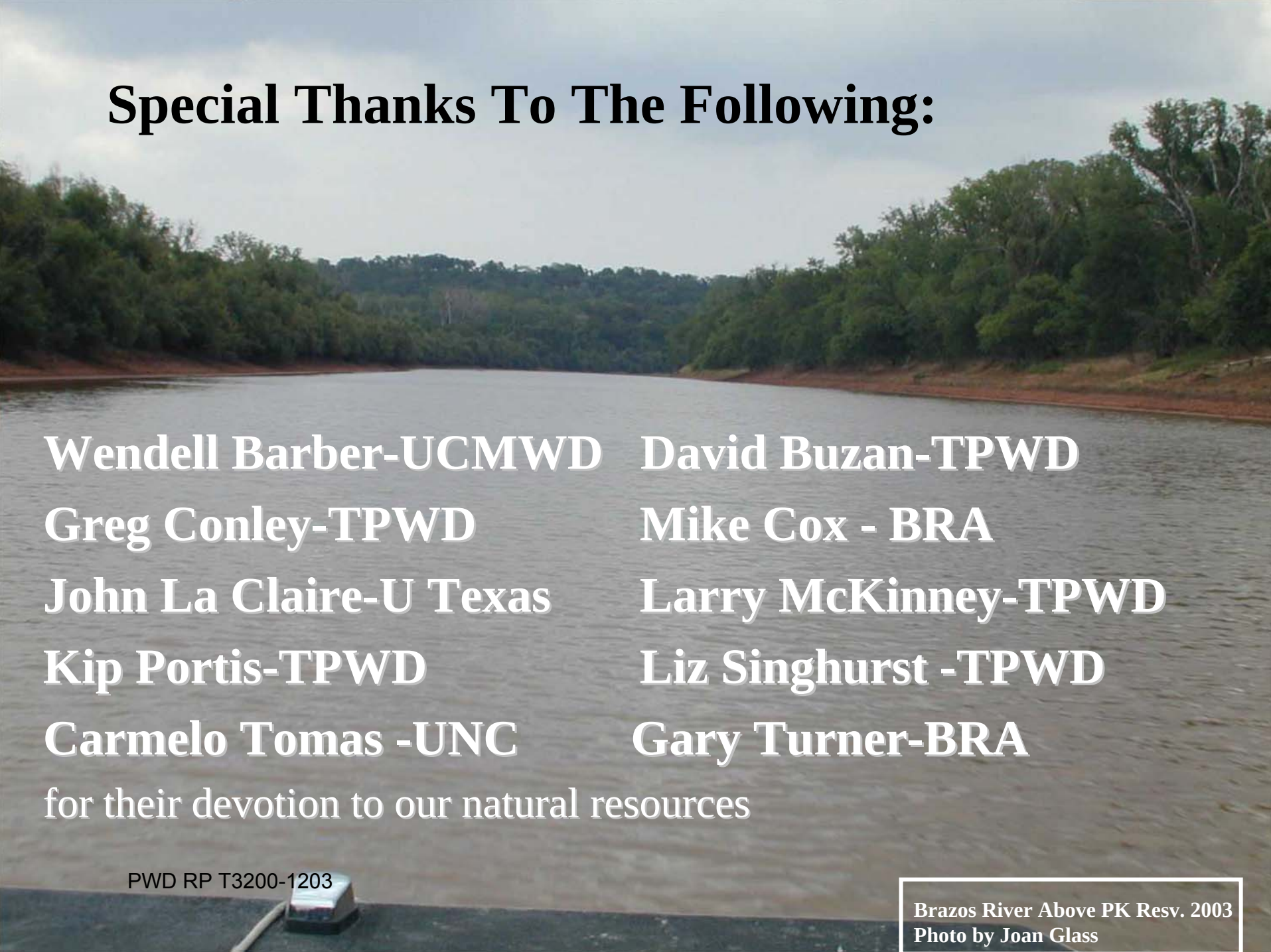
E.V. Spence 2003
Photo by Kip

- Total economic impacts and long term effects are unknown
- Texas distribution unknown

Special Thanks To The Following:



Wendell Barber-UCMWD
David Buzan-TPWD
Greg Conley-TPWD
Mike Cox - BRA
John La Claire-U Texas
Larry McKinney-TPWD
Kip Portis-TPWD
Liz Singhurst -TPWD
Carmelo Tomas -UNC
Gary Turner-BRA

for their devotion to our natural resources

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Brazos River Above PK Resv. 2003
Photo by Joan Glass

Overview of Texas Hatchery Management of Golden Alga, *Prymnesium parvum*

Greg Southard

A. E. Wood Fish Hatchery, Inland Fisheries Division, Texas Parks and Wildlife Department, 507 Staples Road, San Marcos, Texas 78666 USA

Abstract.--During the spring of 2001, the toxin-producing alga *Prymnesium parvum* was identified as the cause of widespread fish mortality at the Texas Parks and Wildlife Department (TPWD) Inland Fisheries Dundee State Fish Hatchery, resulting in complete loss of the striped bass *Morone saxatilis* and hybrid striped bass (*M. saxatilis* x *M. chrysops*) production for that year. Also greatly impacted were largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* brood fish and future brood fish, as well as rainbow trout *Oncorhynchus mykiss*, channel catfish *Ictalurus punctatus*, and koi carp *Cyprinus carpio* production. This alga was also identified as responsible for a large fish kill at Possum Kingdom Reservoir, which is the source water for another TPWD fish hatchery. The TPWD formed the *P. parvum* task force, with the goal of developing strategies to effectively control this alga in order to produce fish. A basic management plan was created, including methods to identify and quantify *P. parvum*, monitor toxicity levels, and investigate physical and chemical control methods to counter blooms and toxic events. Identification and enumeration of *P. parvum* are accomplished using a light microscope, hemacytometer, and trained personnel. A standard bioassay, adapted from Israeli fish culturists, is used to monitor the ichthyotoxin at sub-lethal concentrations in order to warn biologists of impending toxicity. A wide variety of physical and chemical control methods were evaluated for their effectiveness to destroy *P. parvum* cells or mitigate toxicity. Applications that were deemed effective include UV sterilization of hauling tank water, the use of potassium permanganate to mitigate toxicity, and ammonium sulfate and copper sulfate to destroy this toxin-producing alga. Although these methods have enabled TPWD to produce fish at these *P. parvum*-afflicted facilities, some challenges remain. Chemical treatments are temporary. Current *P. parvum* management practices are time consuming and labor intensive. More sensitive and efficient methods are needed.

View the presentation

Overview of Texas Hatchery Management of Golden Alga, *Prymnesium parvum*

Greg Southard

TPWD Inland Fisheries

Fish Health and Genetics Lab

A. E. Wood Fish Hatchery

San Marcos, Texas

PWD RP T3200-1203

Dundee State Fish Hatchery

- Near Wichita Falls, TX
- Lake Diversion
- Water quality
  - Salinity 2-3 ppt
  - Chlorides 1899 mg/L
  - Calcium 296 mg/L
  - Magnesium 63 mg/L
- Fish produced
  - striped bass, hybrid striped bass, smallmouth bass, largemouth bass, channel catfish, koi carp, rainbow trout

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Dundee State Fish Hatchery

- February 2001
- Mortality begins
  - Rainbow trout
  - Cause unknown

Dundee State Fish Hatchery

- March 2001
- Mortality continued
  - Smallmouth bass broodstock
  - Northern largemouth bass broodstock

Dundee State Fish Hatchery

- Cause of fish mortality?
  - *P. parvum*
    - Dave Buzan (TPWD-RP) confirmed on 3/15/01

Image by Carmelo Tomas

- April 2001
- Complete mortality
  - Striped bass fry
  - Hybrid striped bass fry

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TPWD Hatchery Water Sources Affected by *P. parvum*

- **Lake Diversion**

Dundee State Fish Hatchery

- **Possum Kingdom Reservoir**

Possum Kingdom State Fish Hatchery

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TPWD Inland Fisheries *P. parvum* Task Force

- **Goal**
  - **To develop strategies to effectively control *P. parvum* to ensure fish production**

TPWD Inland Fisheries *P. parvum* Task Force

- **Process**
  - **Understanding *P. parvum***
    - **Literature review and contact experts**
      - **How do blooms occur?**
      - **What causes production and release of toxins?**
      - **What are the best control strategies?**
  - **Research**
  - **Develop hatchery management plan**

TPWD Inland Fisheries *P. parvum* Task Force

- **Hatchery *P. parvum* Management Plan**
  - **Identification**
  - **Monitor densities**
  - **Monitor toxin levels**
  - **Pond treatment**
  - **Prevent dispersal**

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Identifying *P. parvum*

- **Compound light microscope** (≥ 400X)
  - **8 - 12 µm**
  - **2 flagella**
  - **1 haptonema**
  - **C-shape or saddle-shape chloroplast**
  - **Characteristic swimming patterns**

Image by Carmelo Tomas

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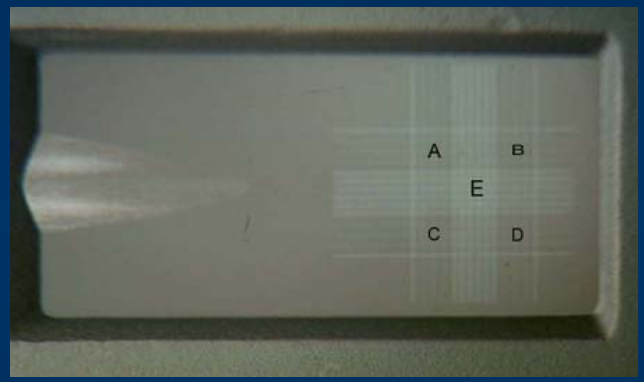
Monitoring *P. parvum* densities

- **Cell density**
  - **Hemacytometer**
  - **Fixation with Lugol's solution**



  - **Count # of *P. parvum* cells per large square on hemacytometer grid**
  - **[(A+B+C+D+E) ÷ 5] x10^4 = # cells/mL**

Monitoring Toxin Levels

- **Bioassay***
  - **Water sample**
  - **Cofactor (pH 9.0)**
    - **0.02 M TRIS**
    - **0.003 M 3,3'-iminobis-propylamine**
  - **Test organism**
    - ***Pimephales promelas*** **(fathead minnow) juveniles**
  - **28°C for 2 hours**

***Dr. Isaac Bejerano - Central Fish Health Lab, Israel**

Monitoring Toxin Levels

- **Bioassay**
  - **Mortality determines treatment**
    - **sample + cofactor = 1 ITU***
      - **Low toxicity → no treatment, monitor cell density**
    - **1/5 diluted sample + cofactor = 5 ITU**
      - **Moderate toxicity → immediate treatment**
    - **sample = 25 ITU**
      - **Water is toxic to fish**

***ITU = Ichthyotoxic unit (1/25th the lethal dose to fish)**

Cell Counts vs. Bioassay Toxicity

- **Problems with current applications**
  - **Toxicity variable of cell concentration**
  - **Both methods are:**
    - **time consuming**
    - **labor intensive**
    - **not always reliable as a measure of impending bloom or toxic event**

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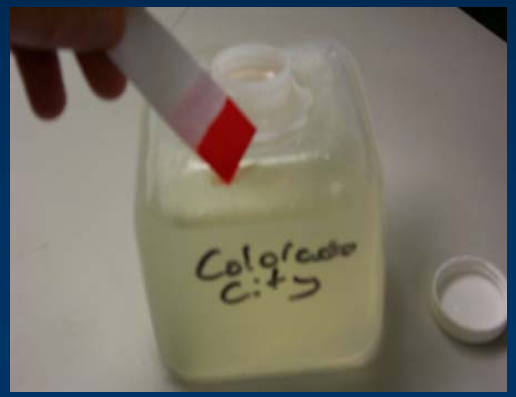
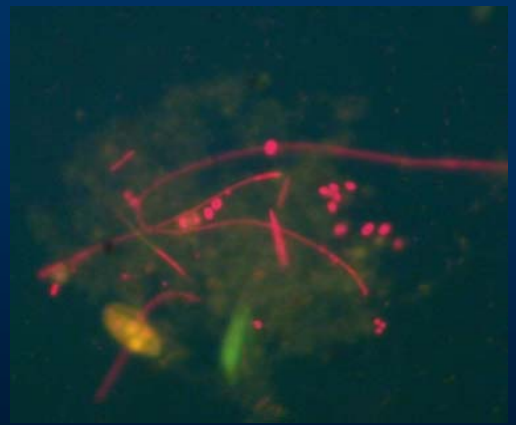
Monitoring of *P. parvum*

- **Needs**
  - **Simpler, quicker, and more accurate method(s) for:**
    - **Identification**
    - **Estimating concentration**
    - **Monitoring for impending toxicity**



"Dip-test" similar to a litmus test



Epifluorescence image of mixed algal community

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Treatments

- **Physical methods**
  - **cause lysis**
- **Chemical methods**
  - **lysis and/or detoxify**

Image by Carmelo Tomas

Physical Treatments

X **Sonication (i.e., Aquasonic)**

? **Ozonation**

? **Bio-control agents**

✓ **Ultraviolet Sterilization**

Treatments - Physical

- **Ultraviolet sterilization**
  - **Treatment**
    - **Mean dose of 210 mJ/cm^2**
    - **Intensity of 91.5 mW/cm^2**
  - **Results/Conclusions**
    - **All cells were destroyed; toxicity reduced**
    - **Not suitable for large scale water treatment**
    - **UV-sterilized water is option for hauling unit tank water**

TPWD fish hauling units

TEXAS PARKS & WILDLIFE

Chemical Treatments

- X Hydrogen peroxide
- X Acids (HCl and H_2SO_4)
- ? Nitrogen : Phosphorous ratio
- ✓ Potassium permanganate
- ✓ Ammonium sulfate
- ✓ Copper sulfate & Cutrine® Plus

Treatments – Chemical

- Potassium permanganate - $KMnO_4$
  - Detoxifying agent
  - Treatment rate = 2 mg/L $KMnO_4$ above the oxidative demand
    - e.g., oxidative demand (4 mg/L), then treatment rate would be at 6 mg/L $KMnO_4$
  - $KMnO_4$ suitable for treating toxic water, but ineffective in *P. parvum* cell lysis

Treatments – Chemical

- Ammonium sulfate - $(NH_4)_2SO_4$
  - Most commonly used method by TPWD
  - Effective at 15°C to 28°C
  - Prophylactic treatment maintaining a minimum effective level of un-ionized ammonia (NH_3) = 0.2 mg/L
  - Not recommended for low temperatures, low pH, high ambient NH_3, or sensitive fish species or delicate life stages

Treatments – Chemical

- Copper sulfate – $CuSO_4$
  - Effective at temperatures <18°C
  - Effective rate dependent upon organic load and alkalinity concentrations
  - Some species and life stages of fish are very sensitive to copper ion in water
  - Not a preferred method
    - Harmful to primary and secondary production
    - Corrosive to aluminum screens in ponds

Treatments – Chemical

- **Cutrine® Plus**
  - **Chelated form of copper**
  - **Research indicated to be safe for rainbow trout and effective at lysing *P. parvum***
    - **Used when temperature ≤15 °C**
    - **Effective treatment = 0.2 mg/L total copper**
    - **Treatments ≥ 0.4 mg/L caused significant fish mortality**

Prevent Dispersal

- **Hazard Analysis and Critical Control Point (HACCP) Plan for *P. parvum***
  - ***P. parvum*-free water (UV-treated or well) water used to fill hauling unit tanks**
  - **Production fish rinsed 2X in *P. parvum*-free water**
  - **Water samples from unit checked for alga**
  - **Tank is flushed with (UV-treated or well) water if *P. parvum* detected**

Achievements

- Development of hatchery strategies
- Effective control methods
- Fish production returned to normal

Challenges

- **Needs**
  - **Efficient and sensitive method(s) to**
    - **Identify *P. parvum***
    - **Estimate density**
    - **Monitor toxin levels in water**
  - **Better algal control methods**
  - **Time-release products that allow long-lasting treatments**

TEXAS PARKS & WILDLIFE

TPWD Inland Fisheries
P. parvum Task Force

- Aaron Barkoh
- Tom Dorzab
- Jason Vajnar
- Loraine Fries
- John Paret
- Dennis Smith
- Gerald Kurten
- Joe Warren
- Jake Isaac
- Greg Southard

Phylogeny, Life History and Autecology of *Prymnesium parvum*

Bente Edvardsen[1,2] and Aud Larsen[3]

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Abstract.--The flagellate *Prymnesium parvum* has a worldwide distribution in the temperate region of both the Southern and Northern hemispheres. Most records are from low salinity ponds, lakes and river systems, and from coastal and in-shore waters, but it has also been recorded in oceanic localities. Harmful blooms of *P. parvum* are recurrent in many parts of the world (Asia, Europe, North America, North Africa and Australia) and may result in fish kills causing great economic losses. *Prymnesium parvum* belongs to the algal division Haptophyta. It is unicellular and has oblong cells with the length 8-16 µm and width 4-10 µm. The cells have two flagella for motility and a third appendage, a haptonema that can be used for attachment to a substrate. The cells have two golden brown chloroplasts containing the photosynthetic apparatus that convert inorganic carbon into organic. Two layers of plate-like, organic scales cover the cells. The scale morphology, which can be seen in the electron microscopy only, is considered to be species-specific. About ten *Prymnesium* species are presently known. All *Prymnesium* species sequenced to date group together in the ssu rRNA gene tree, but included in this group are also some species in the genera *Chrysochromulina* and *Platychrysis*, indicating that a revision of the taxonomy is needed. *Prymnesium nemamethecum* and *Chrysochromulina polylepis* are the closest relatives to *P. parvum* in this phylogeny. *Prymnesium parvum* and *P. patelliferum* were previously considered as two separate species, but DNA sequencing and ploidy analyses revealed that they most probably are stages in the life cycle of one and the same species. *Prymnesium parvum* thus consists of two forms, *P. parvum* f. *parvum* and *P. parvum* f. *patelliferum* that differ slightly in scale morphology. *Prymnesium parvum* is believed to have a sexual haplo-diploid life cycle embracing four morphologically distinct stages: two-flagellated haploid cell types, one-flagellated diploid cell type and a non-motile cyst. The non-flagellated cysts are considered to be a resting stage. Autecology is the relationship between one organism and its environment. *P. parvum* is extremely euryhaline and eurytherm and may grow at salinities between 1-100 psu. The optimal salinity and temperature for growth as well as maximal growth rate varies among the different strains that have been studied, but in general the highest growth rates have been achieved in the salinity range 10-20 psu and at temperatures 20-26 ˚C. In addition to perform photosynthesis, *P. parvum* has the ability to utilize various dissolved organic nutritional resources as well as bacteria and other small prey and is thus mixotrophic. Despite its very euryhaline nature, *P. parvum* bloom formation has been restricted to waters of low salinities, between 1-12 psu. Usually, the blooms develop at water temperatures above 10 ˚C, but blooms have also been recorded at a lower temperature. Fish kills have usually occurred only at very high algal concentrations (>50-100 million cells per liter). Considerable amounts of nutrients such as nitrogen and phosphorus are needed to build up *Prymnesium* populations of this size. Many of the affected waters are also clearly eutrophic due to cultivation of fish, discharge of sewage, or runoff from agricultural land. *Prymnesium parvum* may bloom in almost any low-salinity, nutrient-rich area in the temperate region of both the Northern and Southern hemispheres. To reduce the risk for harmful *Prymnesium* blooms it can be recommended to reduce the levels and discharges of dissolved inorganic phosphorus and nitrogen as well as dissolved organic material to these water bodies, and when possible to keep the salinity below 1 psu.

View the presentation

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Phylogeny, life history, autecology and toxicity of *Prymnesium parvum*

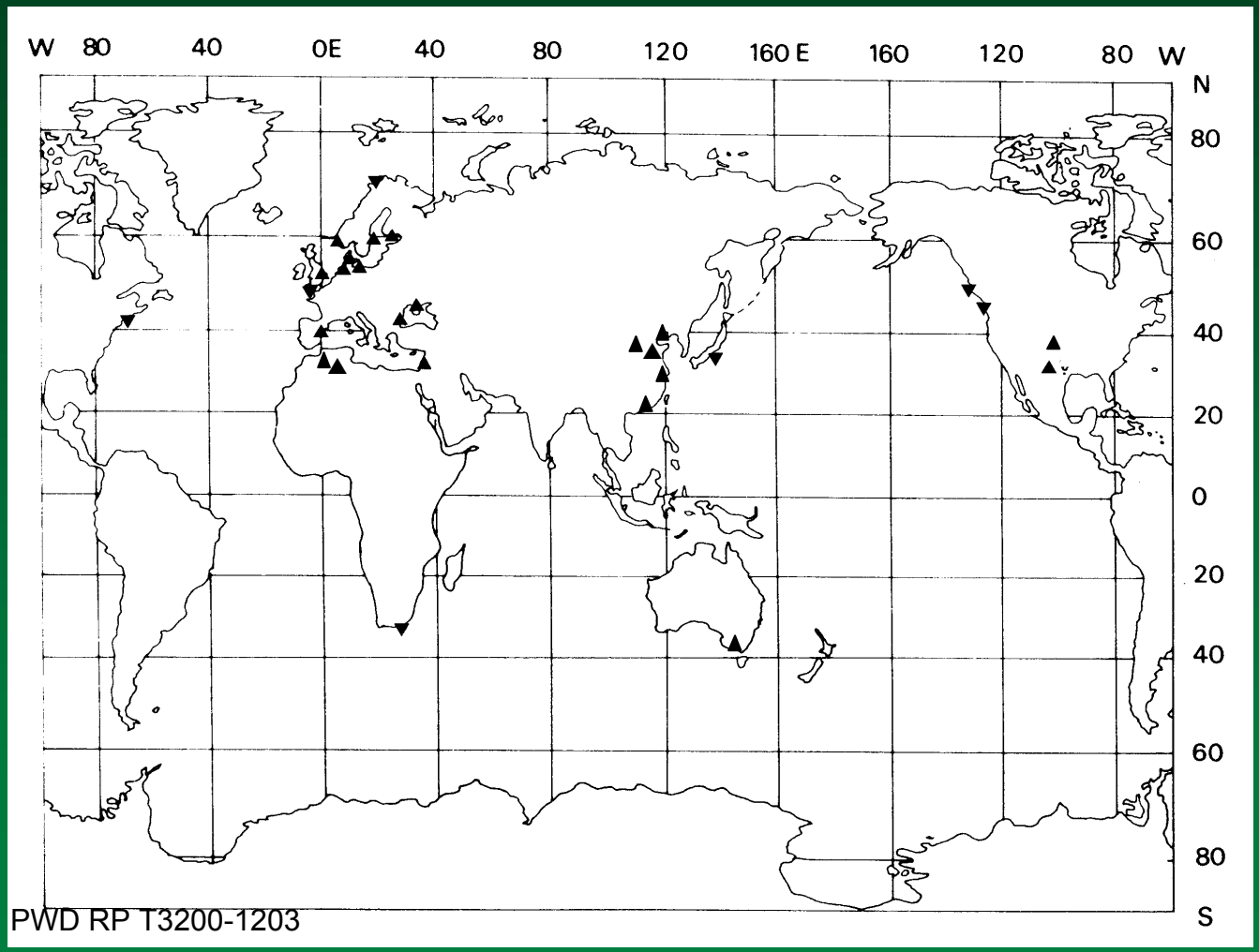
Bente Edvardsen[1,2] and Aud Larsen[3]

1 University of Oslo, Norway, 2 NIVA, Norway

3 University of Bergen, Norway

PWD RP T3200-1203

Distribution of *Prymnesium parvum*

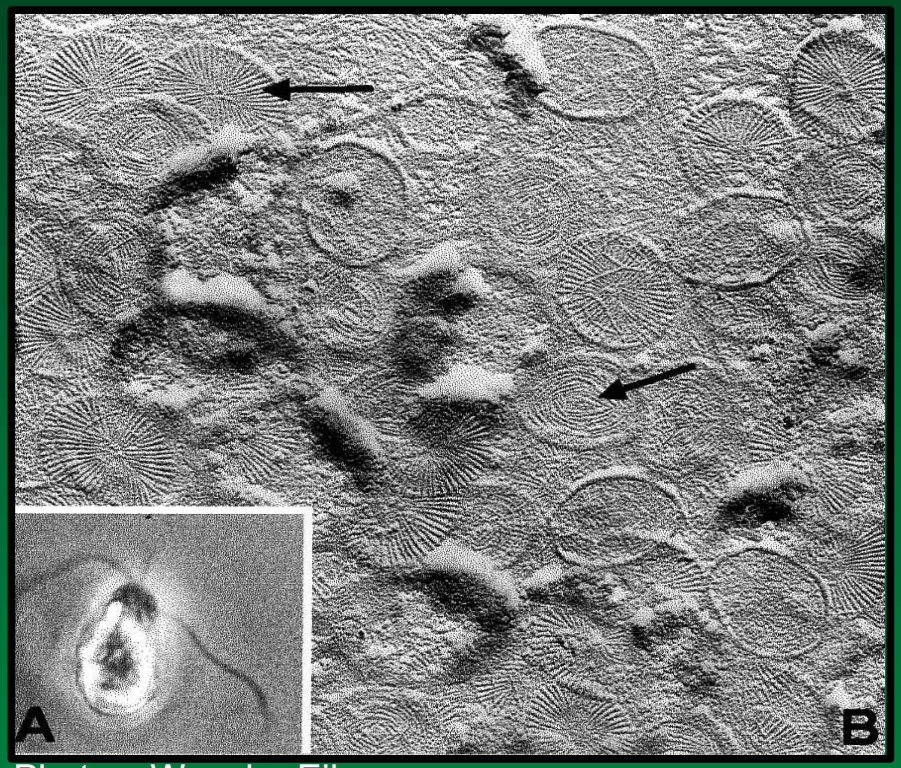


Overview

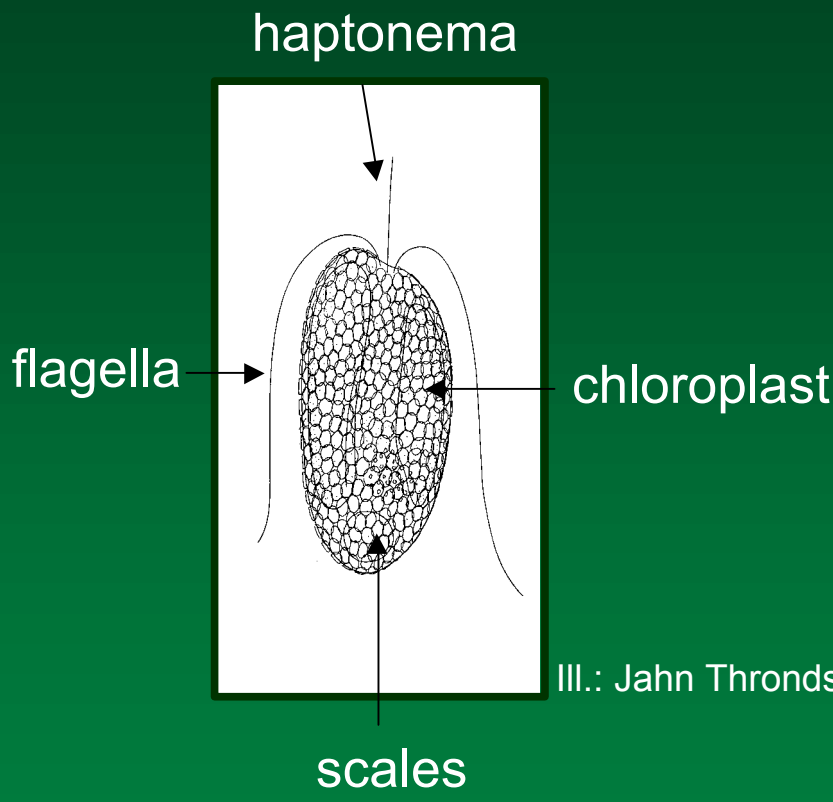
- morphology - what it looks like
- phylogeny - how is *P. parvum* related to other organisms
- life cycle – with alternating cell types
- physiology - nutrition and toxicity
- autecology - growth as a function of environmental factors
- occurrence of *P. parvum* - interpreting environmental conditions that cause blooms
- how can we reduce the risk for harmful blooms?

Division: **Haptophyta**
Class: **Prymnesiophyceae**
Species: ***Prymnesium parvum***
forms: **f. *parvum* and f. *patelliferum***

Morphology of *P. parvum*



Photos: Wenche Eikrem



Ill.: Jahn Thronds

A Light micrograph of cell

B Electron micrograph of scales

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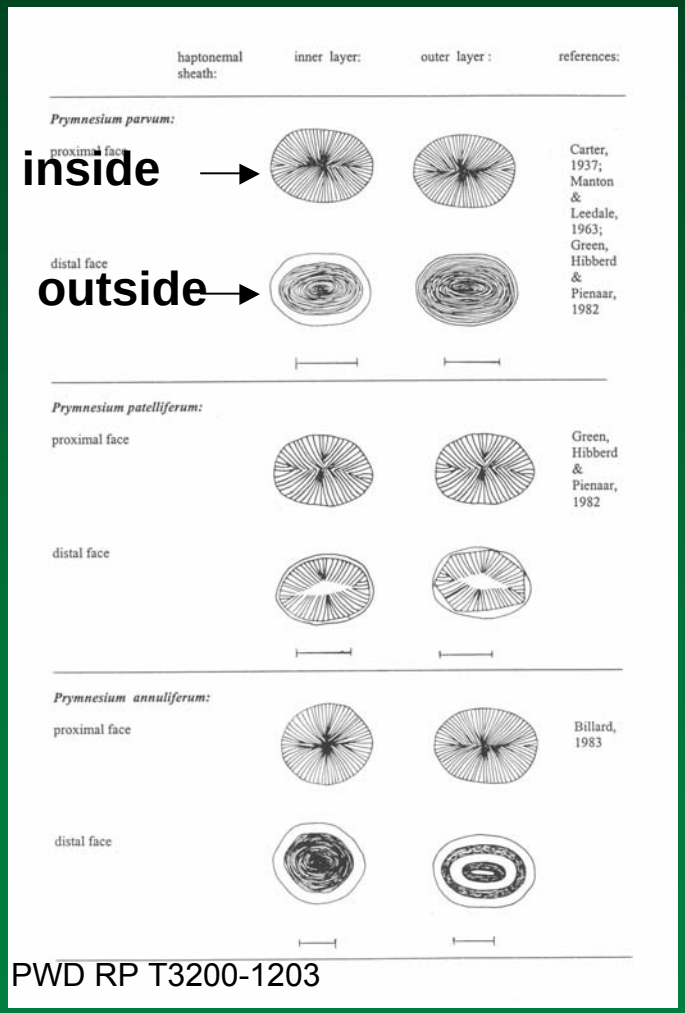
Organic scales covering the cells

- character for species identification



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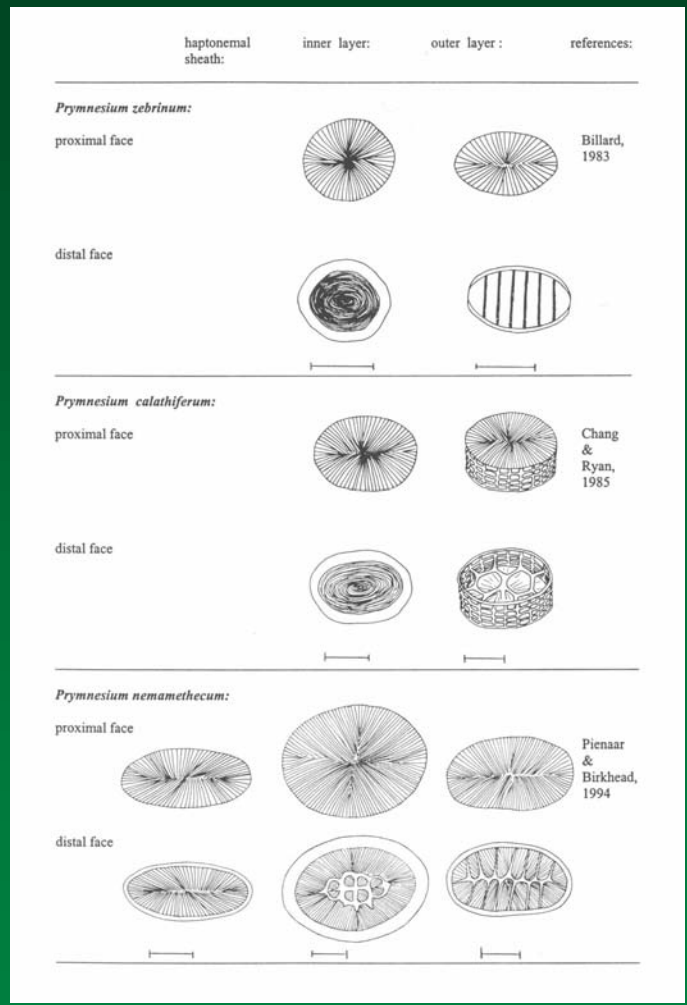
(Larsen 1998)



Prymnesium species

Species	Habitat	Distribution	Toxic
P. parvum	brackish	worldwide, temperate zone	yes
P. annuliferum	marine	France (Med. Sea)	unknown
P. calathiferum	marine	New Zealand	yes
P. faveolatum	marine	France, Spain	yes
P. nemamethecum	marine	S Africa, Australia	unknown
P. zebrinum	marine	France (Med. Sea)	unknown

P. czosnowskii, P. gladiociliatum, P. minutum, P.papillarum and *P. saltans* have uncertain status

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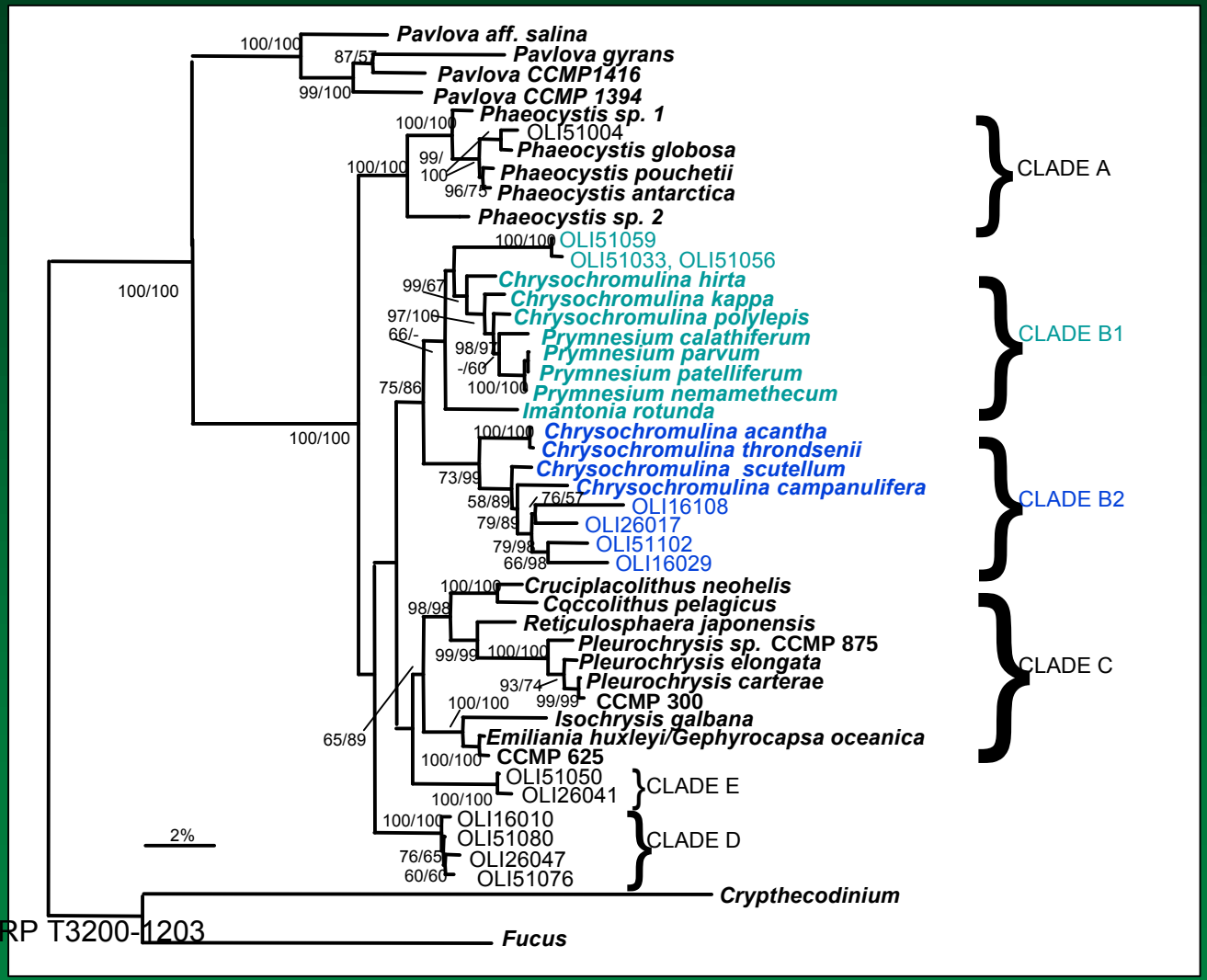
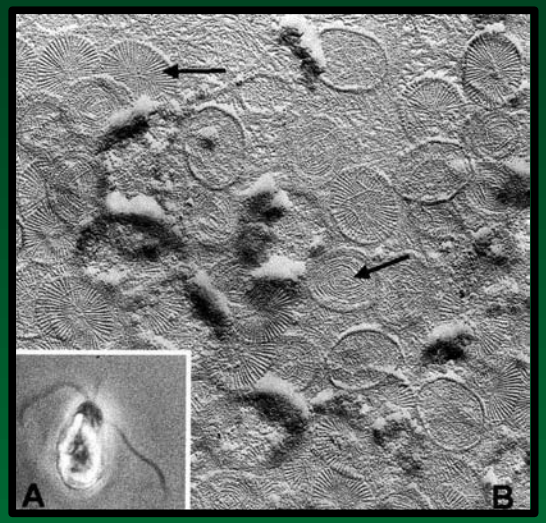
Haptophyte phylogeny



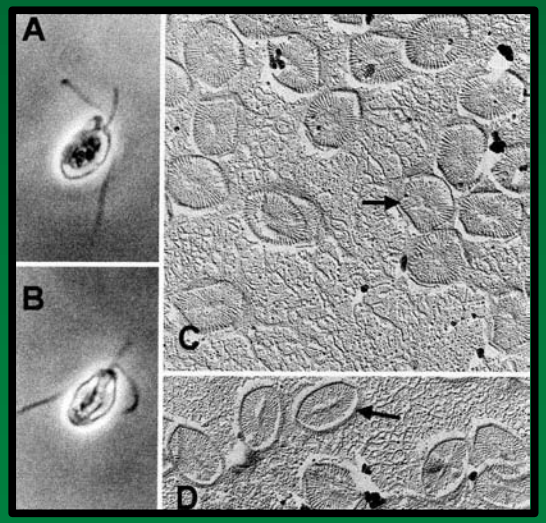
(Edvardsen et al. 2000)

Haplo-diploid life cycle

P. parvum f. *parvum*



P. parvum f. *patelliferum*



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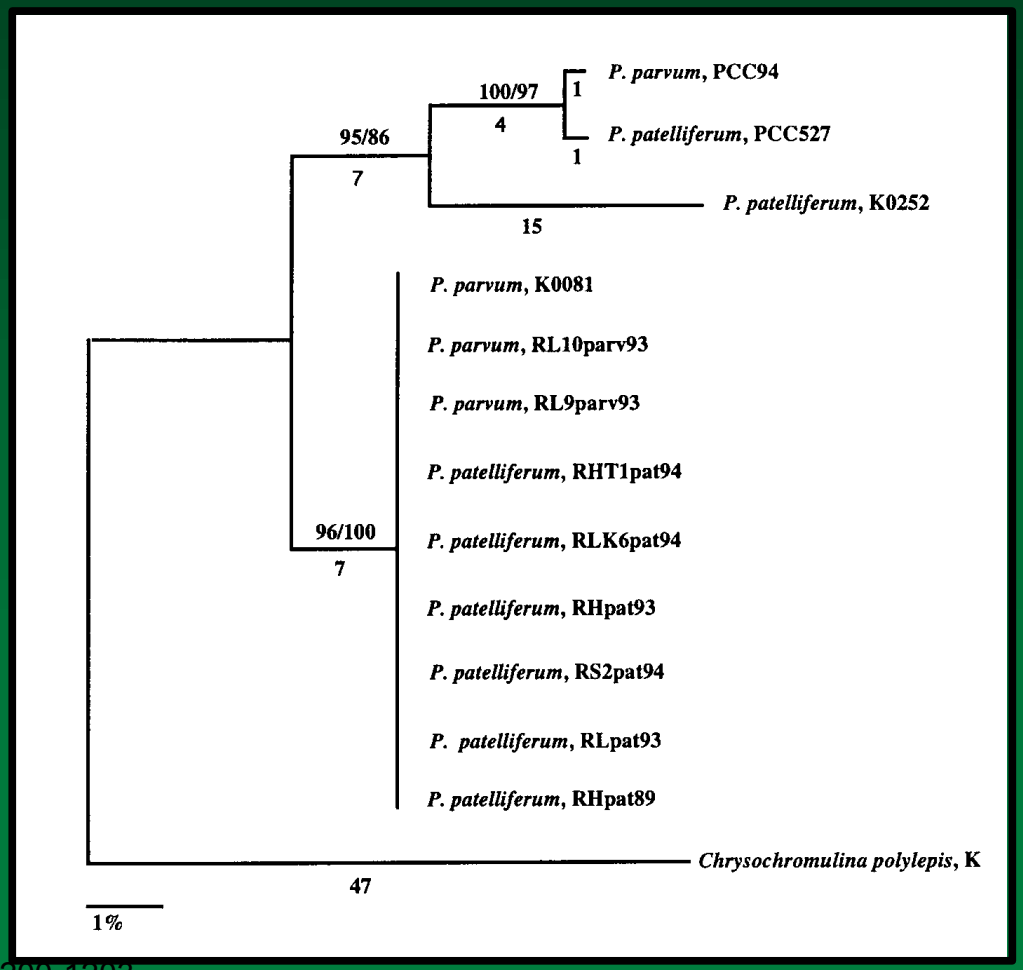
Photos: Wenche Eikrem

ITS rDNA phylogeny of *P. parvum* strains



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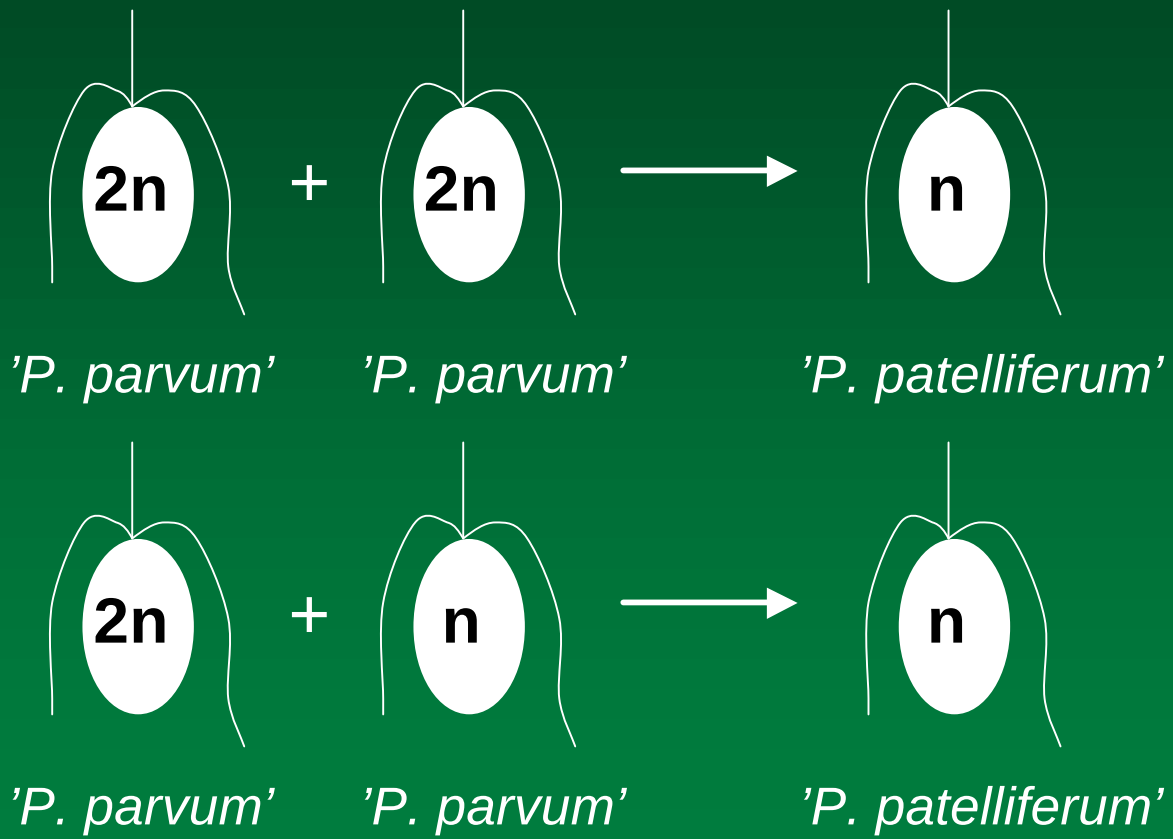
(Larsen & Medlin 1997)

Life cycle: mating experiment

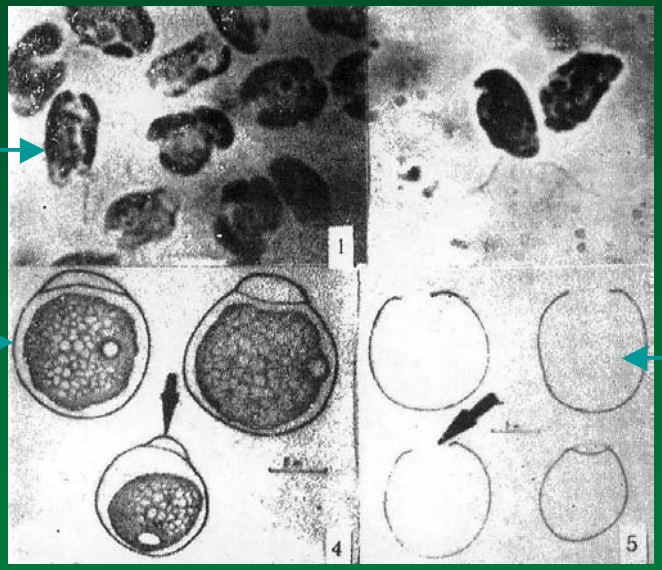


PWD RP T3200-1203
(Larsen & Edvardsen 1998)

Cyst: a possible resting stage

motile cells

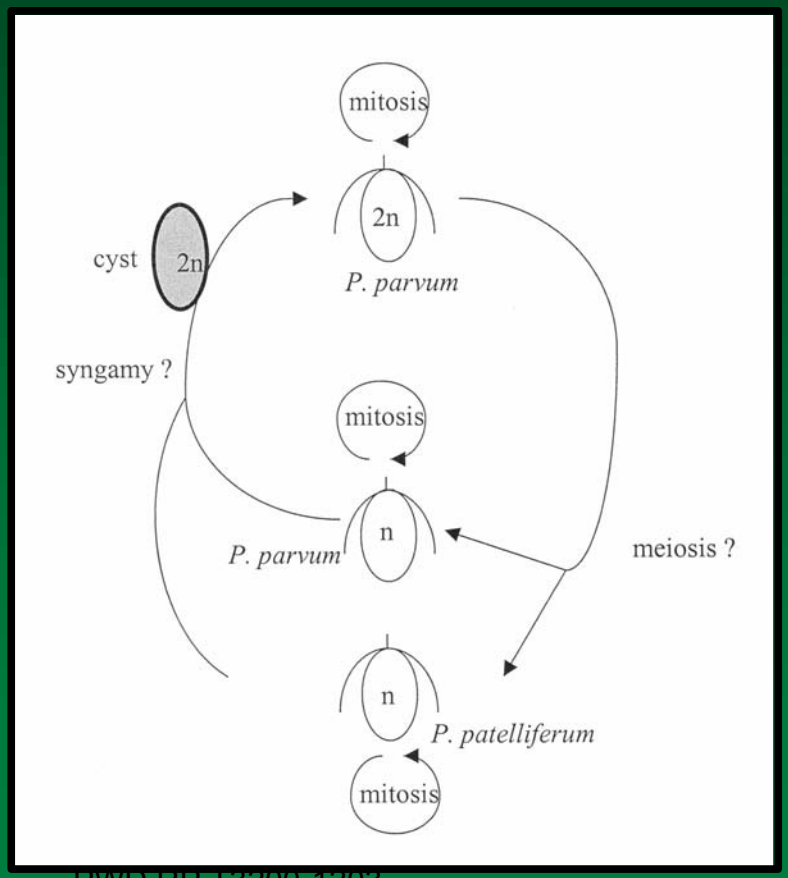
cyst



empty cyst

P. saltans (Wang & Wang 1992)

Possible life cycle for *P. parvum*

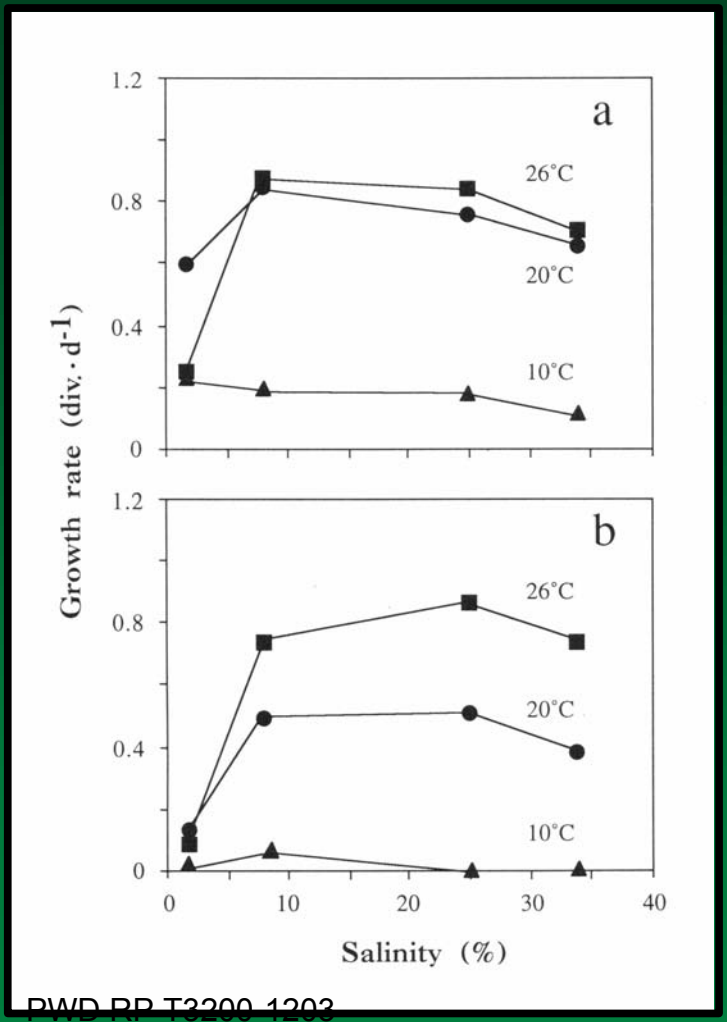


PWD RP T3200-1203
(Larsen & Edvardsen 1998)

Haplo-diploid life cycle

- 4 cell types:
  - f. *parvum* 2n
  - f. *parvum* n
  - f. *patelliferum* n
  - cyst
- all flagellates can grow vegetatively
- meiosis or syngamy have not been seen

Growth optimum and tolerance



PWD RP T3200-1203

(Larsen et al. 1993)

a *P. parvum* f. *patelliferum* Norway

b *P. parvum* f. *parvum* Norway

- No differences in growth pattern between the two forms from Norway

Optimum and tolerance for growth

Parameter	Optimum	Tolerance
Temperature (°C)	21-26 (15-30)	5 - 30
Salinity	10-20 (3-50)	0.8 - 45 (100)
Irradiance ($\mu mol\ m^{-2}s^{-1}$)	200	<25 - >500

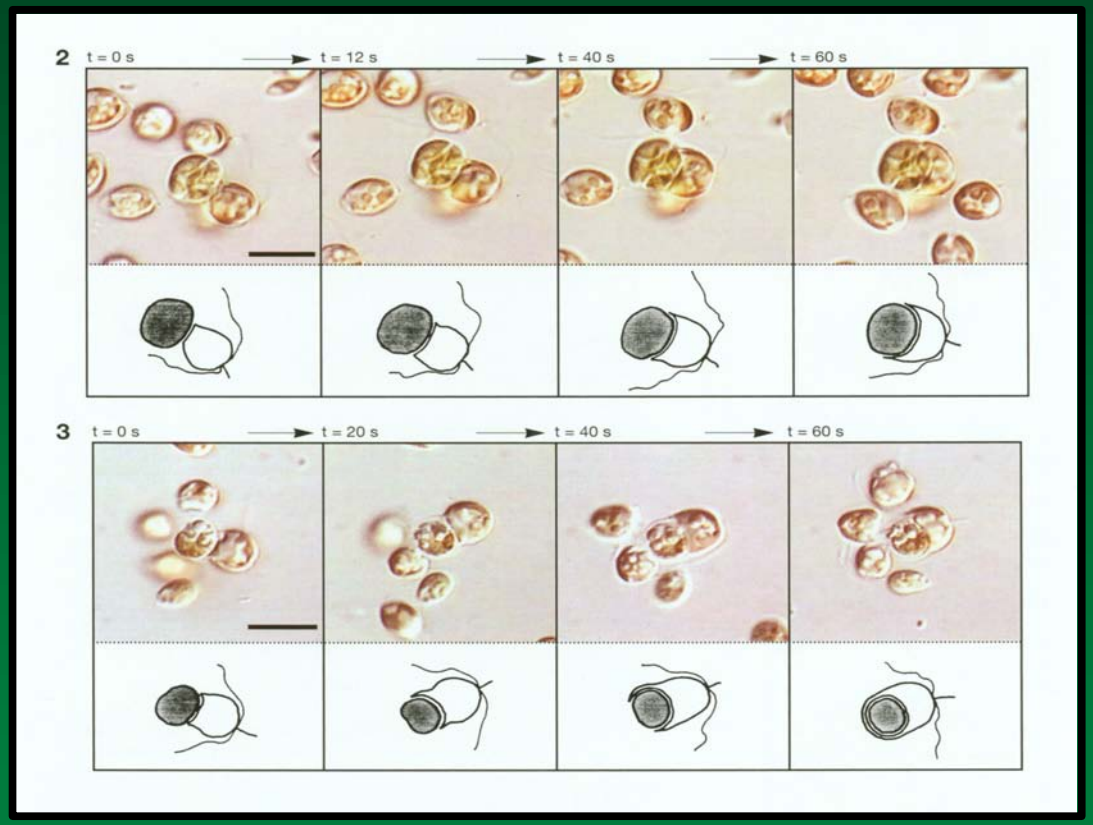
- *P. parvum* is extremely euryhaline and very eurytherm
- Maximum growth rate 0.3-1.4 divisions per day
- Large strain differences

Nutrition

- Autotrophic organisms need only inorganic nutrients (N, P and trace elements), CO_2 and light for growth
- Auxotrophic: *P. parvum* also needs vitamin B_{12} and B_1
- It can utilise organic nutrients in darkness
- Phagotrophic: it can ingest particles

P. parvum is mixotrophic

Mixotrophy



***P. parvum* f. *patelliferum* ingesting green algae**

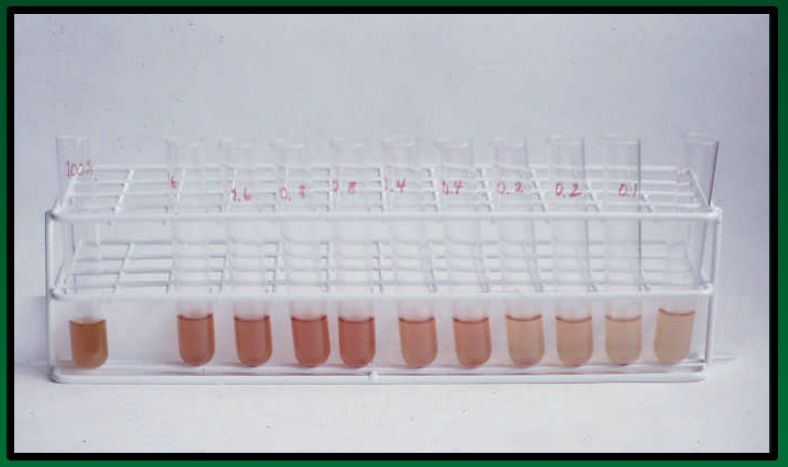
(Tillmann 1998)

- *P. parvum* can ingest and assimilate food particles such as microalgae and bacteria
- Toxins may be used to immobilise or kill the prey

Toxicity

Toxic effects:

- ichthyotoxic
- cytotoxic
- hemolytic
- hepatotoxic
- neurotoxic
- antibacterial
- allelopatic

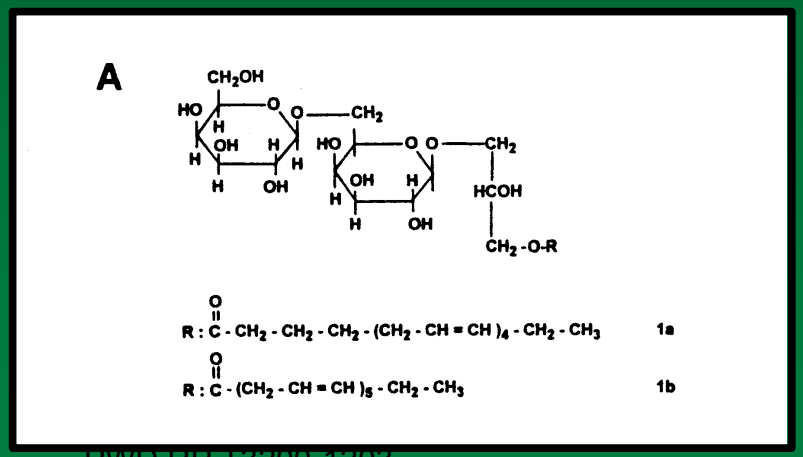


Mode of action:

- act on cell membranes
- loss of selective permeability
- disrupt ion regulation in gills

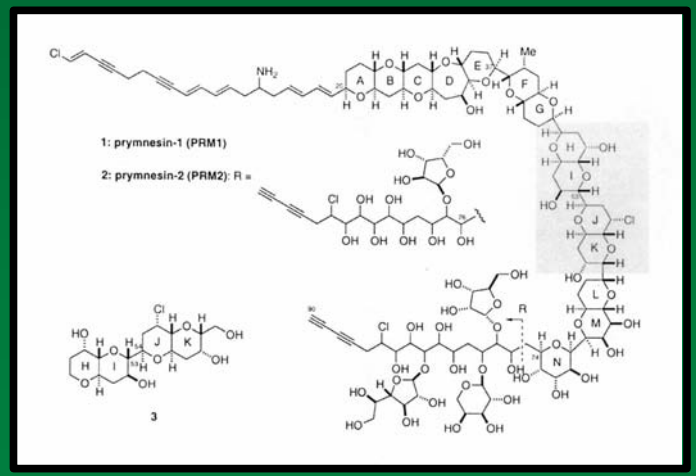
Toxins

- proteolipids (Ulizur & Shilo 1970)
- lipopoly-saccharide (Paster 1968)
- galactoglycerolipid (Kozakai et al. 1982)
- polyene polyethers (Igarashi et a. 1995)



PWD RP T3200-1203

hemolysin (Kozakai et al. 1982)



prymnesin-1 and -2

Toxicity varies with growth conditions

- P- and N-deficiency
- cationic substances
- pH
- aeration
- growth phase
- salinity
- temperature

- There are also strain differences

Occurrence of harmful *Prymnesium* blooms

Typical habitat:

- Low salinity : 1-12
- Limited in area: ponds, lakes, river systems, fjords, lagoons
- Nutrient rich (high N and P levels)
- Moderate to high temperature: 10-25°C

Conclusions from previous blooms

- Fish kills usually only occur at algal concentrations >50-100 million cells per L
- Considerable amounts of nutrients are usually needed
- Many of the affected waters are clearly eutrophic due to cultivation of fish, discharge of sewage or run-off from agricultural land

What can be done?

- Establish a monitoring programme for water quality including measurements on:
  - physics (T, S, conductivity, light, $O_{2,}$)
  - chemistry (dissolved and particulate nutrients)
  - phytoplankton (composition and concentrations)
- Sampling of affected and non-affected localities at least every month
- It can possibly be recommended to reduce **both** N and P, and when possible the salinity
- Avoid conditions which increase toxicity

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Prymnesium parvum: An overview and challenges to our understanding.
Major Questions left after 55 years of problems

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5600 Marvin K. Moss Lane, Wilmington, NC 28409 USA

The ichthyotoxic flagellate *Prymnesium parvum* has historically been a persistent problem in lakes, ponds, rivers and estuaries. In 1937, N. Carter described this species from a brackish pool on the Isle of Wight, England. Within the first decade of its description, this species was identified as causing extensive fish kills in Lake Kinneret (Sea of Galilee), Israel, and in aquaculture ponds. From 1947 through the 1960's, Israeli scientists performed extensive studies to determine methods of controlling the blooms. The hemolytic nature of the toxin, a general understanding of how the toxin was formed, and the reaction to the fish were described, a standard bioassay was established, and mitigation efforts involving liquid ammonia were tested and offered as a means to control the blooms. This effort, notwithstanding, the blooms of *P. parvum* persisted and spread elsewhere, and now this species is identified from 14 different countries, spanning Scandinavia, Europe, Africa, Asia, New Zealand, and North and South America.

One of the first challenges in dealing with *P. parvum* is its identification. As a small (> 10 μm) highly motile cell, detection of its flagella and haptonema can be accomplished with brightfield microscopy, but the diagnostic body scales and other features require the use of electron microscopy (TEM and SEM). This is time consuming and requires specialized equipment not practical for routine observations. The blooms of this organism are episodic, appearing overnight and either persisting or disappearing rapidly. Maximum densities often exceed 10^8 cells/liter and once established, *P. parvum* becomes a persistent feature of the phytoplankton. This last feature suggests a survival stage that needs to be described and detected.

Conflicting evidence for conditions accompanying blooms are noted. They occur at low (<1 PSU) and high (>35 PSU) salinities. This species is photosynthetic but extremely tolerant to low light intensities and can survive in the dark with organic carbon sources. It requires a dark cycle for the production of toxin and has been reported to produce toxins at low (<10) as well as high (>30 °C) temperatures. Nutrients, discussed by others, give an equally confusing picture.

Great headway has been accomplished as to the toxins, but it is clear that they are complex, difficult to characterize and detect, and consist of multiple toxins having hemolytic, ichthyotoxic, neurotoxic, reactive oxygen, and other components. Linkages between environmental variables and toxin production are very complicated to discern.

Our present knowledge of *P. parvum* blooms requires a series of efforts to be able to help in understanding their dynamics. Some of the major problems to be confronted are:

- Easy, rapid and accurate identification of the *P. parvum* in natural waters particularly at low cell concentrations.
- Detection and distributional mapping of *P. parvum* resting stages (cysts) for identifying potential bloom initiation points.
- Accurate and easy detection of its toxins (kinds and levels) to diagnose problems and to evaluate remediation efforts to relieve bloom effects.
- Development of a series of bioassays to guide efforts in defining the fate and effects of the *P. parvum* toxins (bioassay guided fractionation and mitigation to determine toxin ½ life and potential beneficial uses).
- Mitigation and control of the blooms in defined situations via particle removal, cell lysis or other effective agents causing minimal damage to the environment.

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Prymnesium parvum – An overview and Questions

Carmelo R. Tomas

Center for Marine Science

University of North Carolina at Wilmington

PWD RP T3200-1203

Presently recognized Prymnesium species:

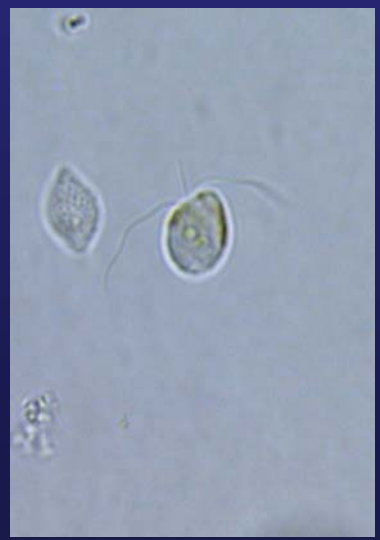
Prymnesium parvum – first described by Carter 1937

P. patelliferum – now considered a form of P. parvum

P. saltans – described by Massart and Conrad 1926

P. calathiferum – Chang 1987

P. zebrinum – Billard 1983



Size : < 20 µm

Grow rapidly : > 1 div/day

Maximum densities:

> 100 million cells/liter

Cell Characteristics

- Cells less than 10 µm
- Highly motile
- 2 flagella with haptonema
- 2 chloroplasts
- body scales (EM)

Bloom History

• Within a decade of its initial description, P. parvum was identified as causing massive fish kills in Israel's Lake Kenneret (Sea of Galilee) and in aquaculture ponds.

• Prymnesium species were identified from

- •German lakes 1920
- •Holland 1920
- • Denmark – 1938

Presently Prymnesium species are now identified from 14 different countries from Scandinavia, Europe, Asia, New Zealand, North and South America.

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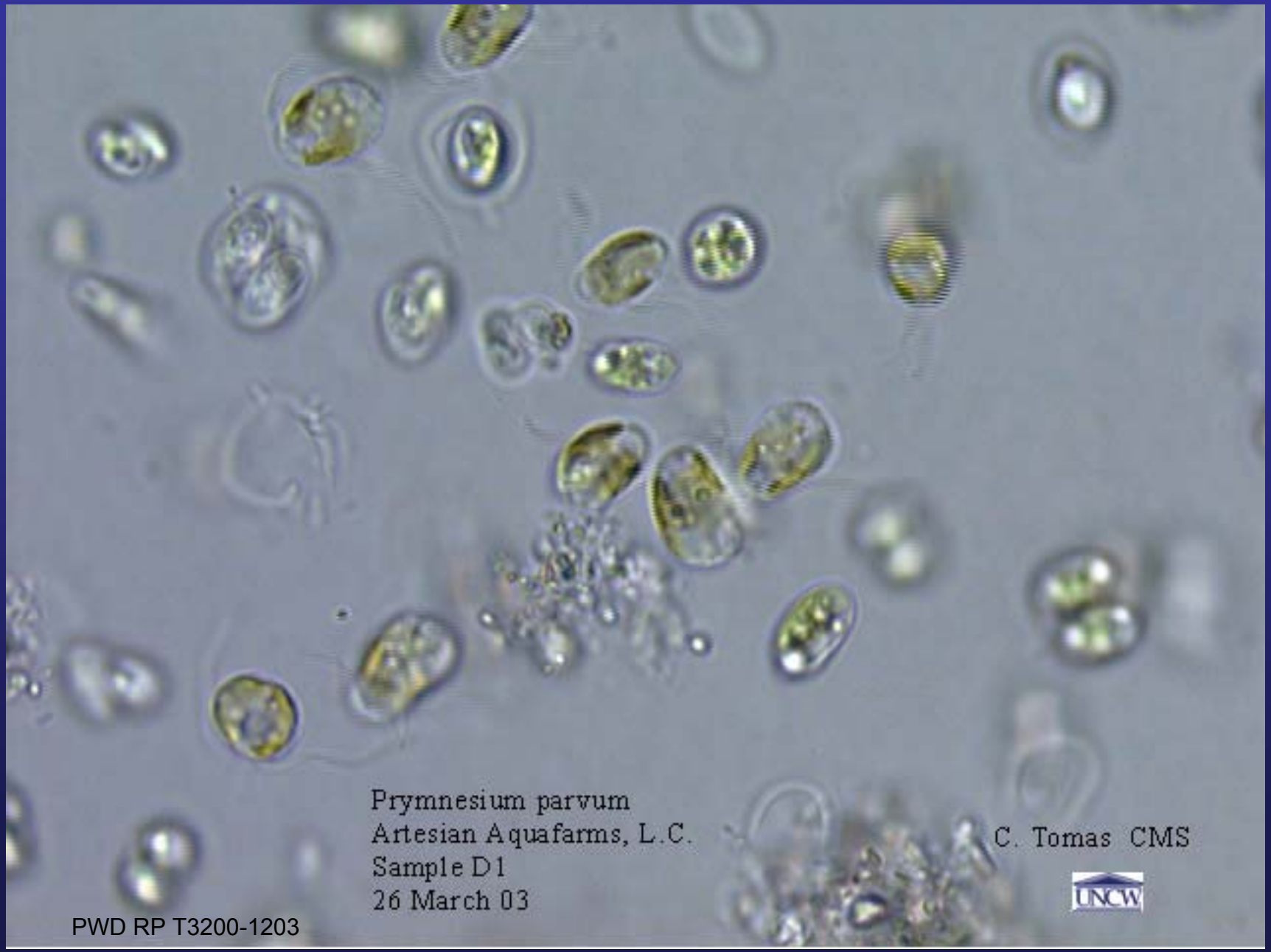
P. Parvum bloom in Possum Kingdom Lake, Texas 2001 – courtesy of TPWD



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Blooms were episodic:

- Appearing and developing rapidly
- Reaching maximum densities of >100 million cells/liter
- Once established became permanent
- Indicates a survival stage functioning in a similar was as do cysts in dinoflagellates

Bloom conditions:

- Wide salinity range (1 to > 35 PSU)
- grow in highly enriched waters (aquaculture ponds, eutrophic coastal embayments, lakes, ponds and rivers)
- Photosynthetic – mixotrophic, auxotrophic, phagotrophic
- Allelopathic

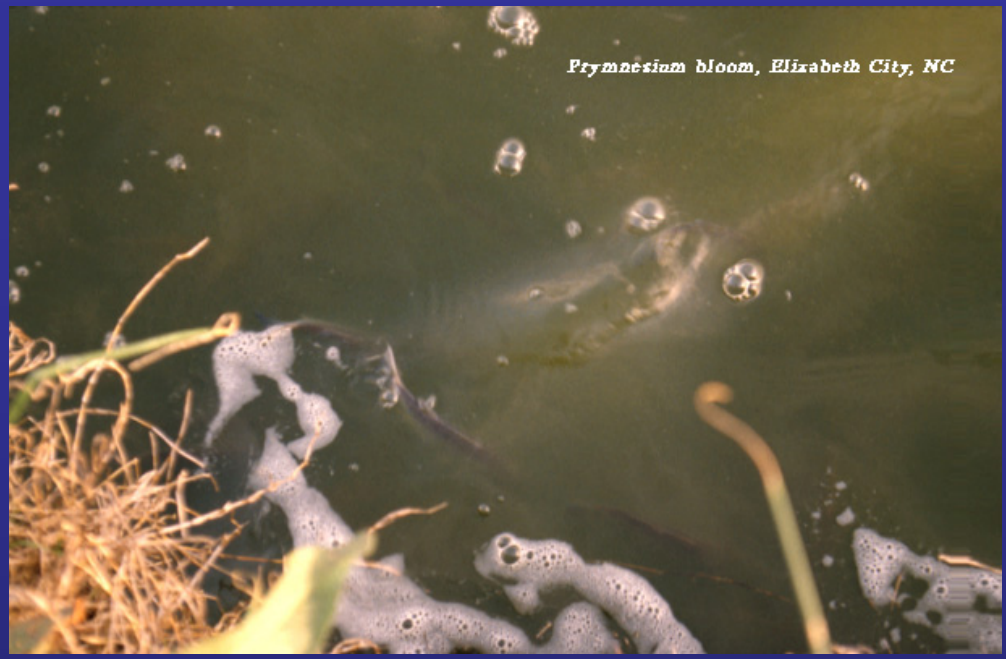
Dying Shad – Texas - courtesy of C. Contraras

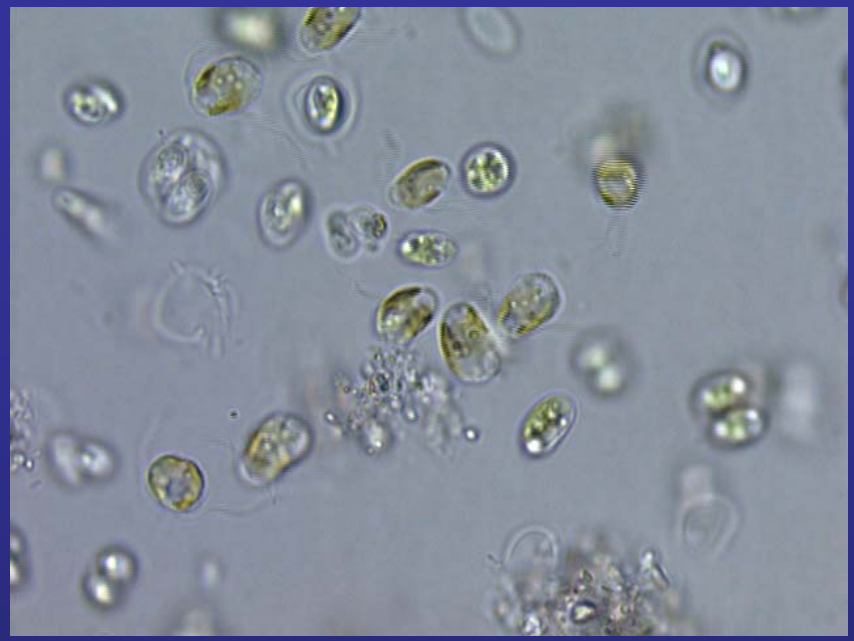
PWD RP T3200-1203

Elizabeth City, NC May 2002

Prymnesium parvum bloom

Toxin - prymnesin





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Possum Kingdom Lake, Texas - courtesy of the Texas Parks and Wildlife

Dead fish at a dam site in Texas - courtesy of C. Contraras

Hemolysis symptoms

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Fish showing hemmoragic areas from exposure to Prymnesium parvum toxins


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Toxins: (Lethal Cocktail)

There is presently evidence for the presence of more than one toxin from P. parvum.

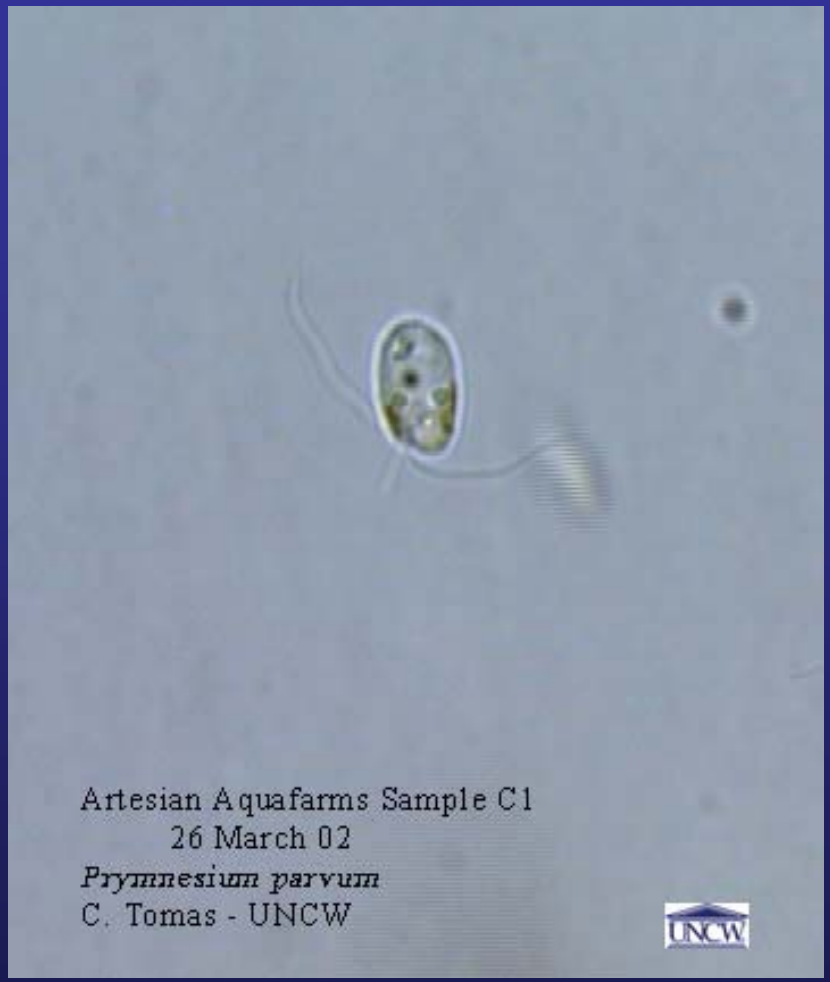
They include:

- **Hemolysins**
- **Neurotoxins**
- **Fast Acting Ichthyotoxins (Cyclo amines)**
- **Reactive oxygen species (ROS) H_2O_2, O_2^- and OH^-**
- **DMSP**
- **Toxic fatty acids**

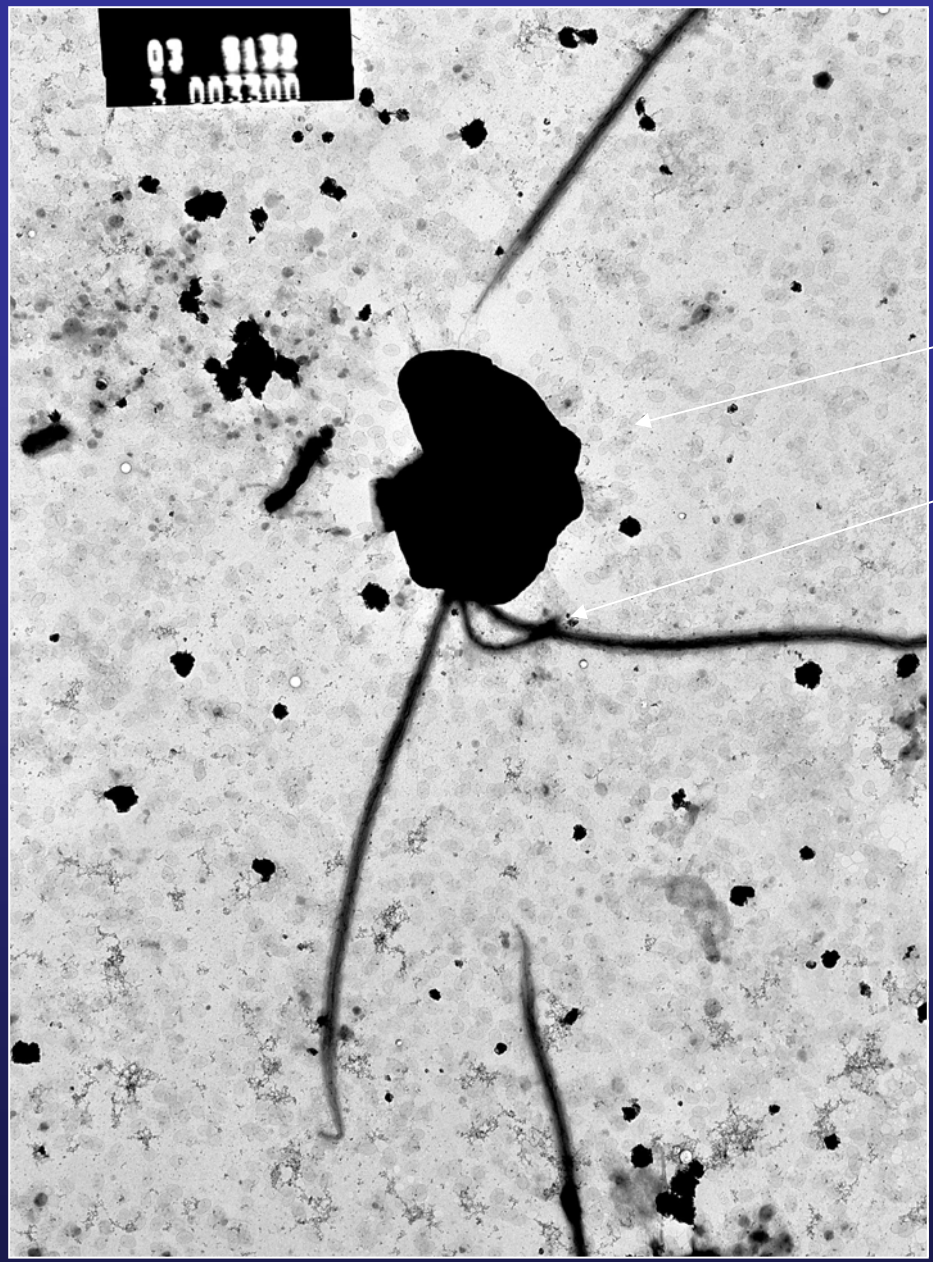
Problem in identifying what regulates toxins:

Conflicting evidence:

- **Toxin mixtures make it difficult to extract what is influence what toxin component**
- **Detection of toxins (except for hymolysins) difficult**
- **Structures of toxins (prymnesins 1 & 2, difficult to resolve)**
- **Conditions for toxin production also confusing**
  - **obligate need for a dark cycle**
  - **nutrients and their interactions**
  - **conflicting temperature/salinity evidence**
  - **fish stimulated production**



How easily can they be identified from field samples?



Body Scales

Haptonema

Flagellum

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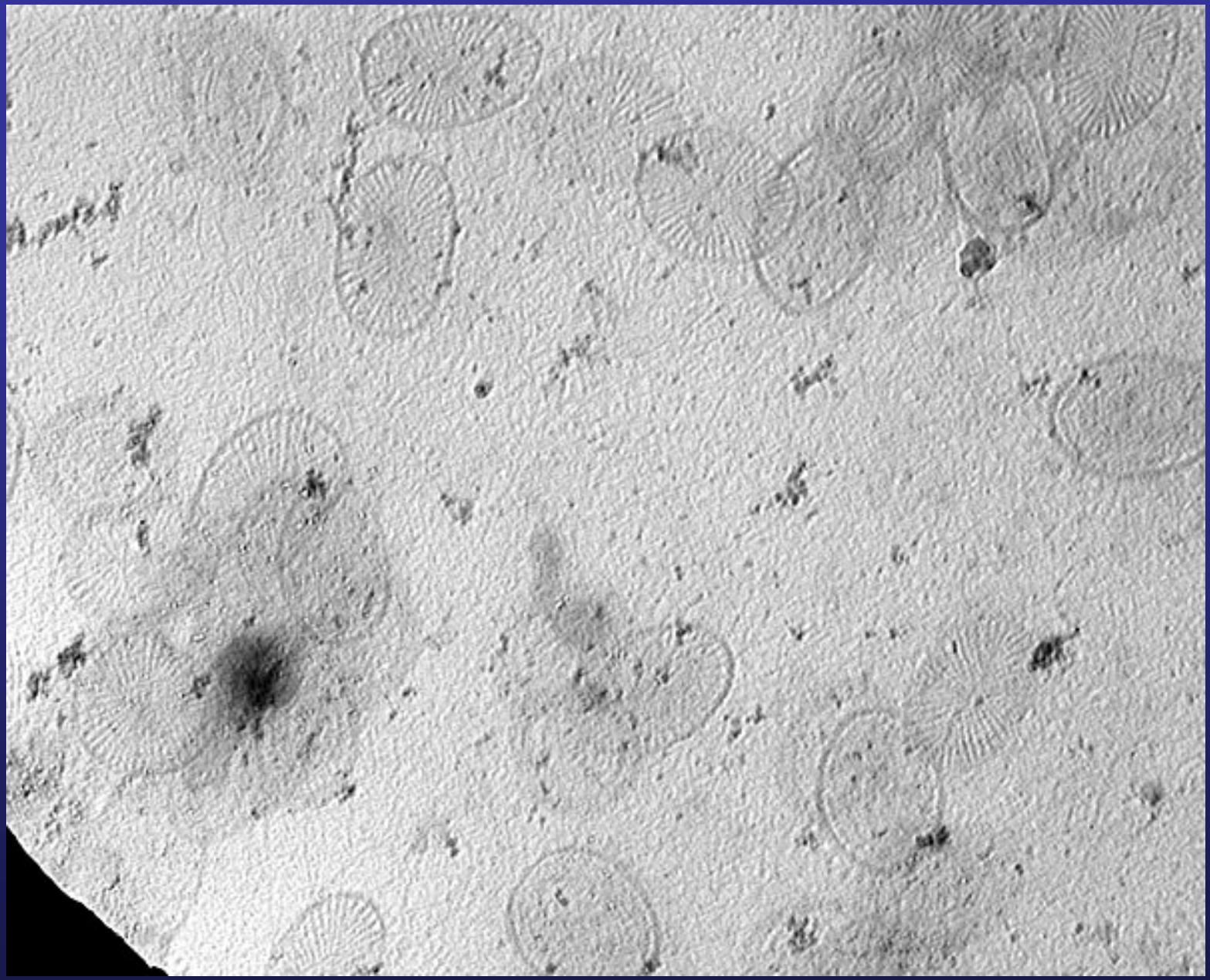

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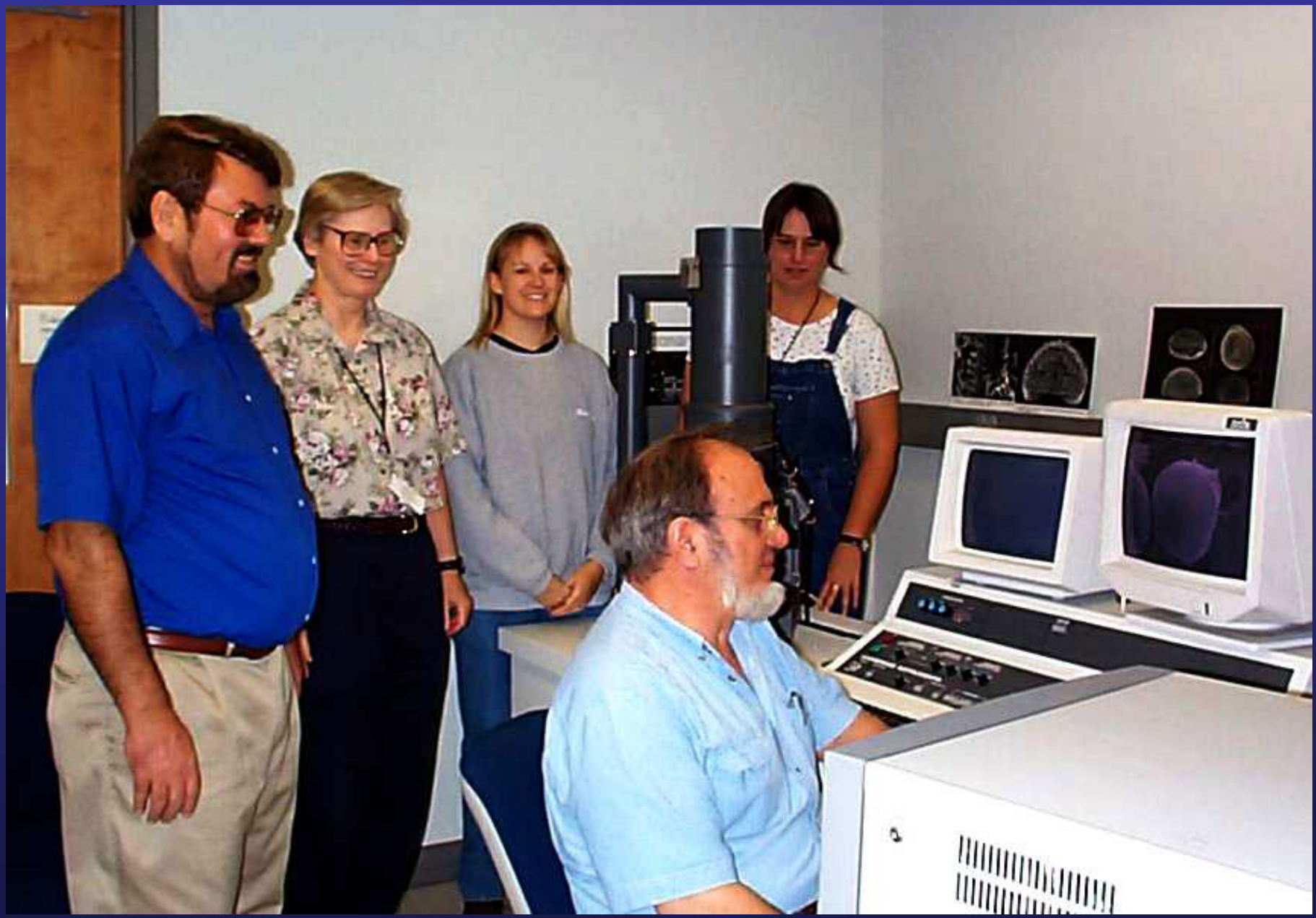
Body Scales of P. parvum



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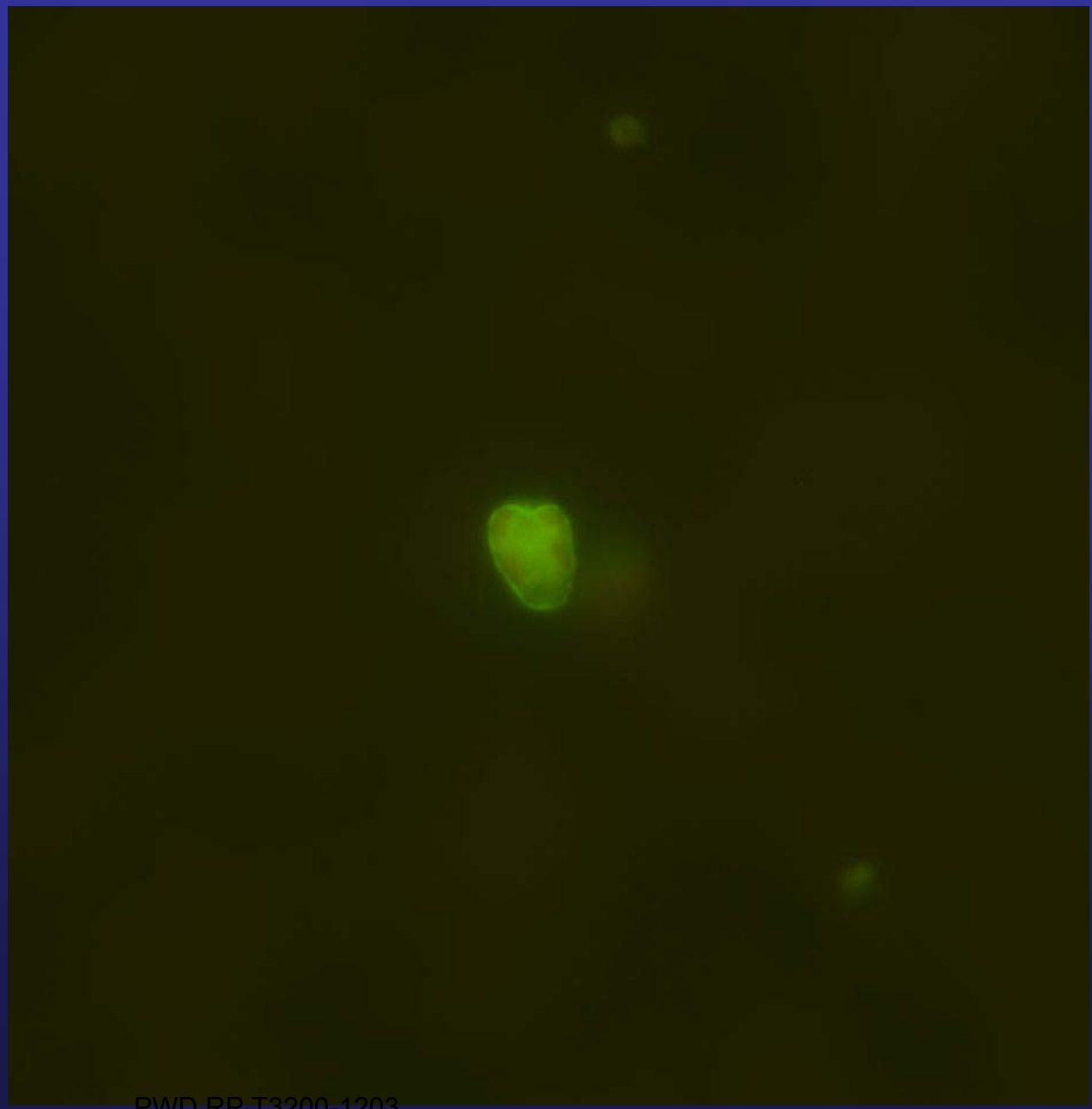
SEM facility at FMRI, St. Petersburg, FL



Fluorescent labeled

P. parvum cell

Surface recognition

Probes

Can be used in conjunction with flow cytometry for ID, isolation and counting

Courtesy of Nyree West

Mitigation and Control:

- **Accurate and rapid detection of P. parvum in natural waters**.
  - Confirms species presence prior to blooms or fish-kills
  - Can be indicative of distribution of the species
  - Detecting resting stages and mapping their distribution

- Detection of toxins – various components at low ambient levels
  - Guides mitigation efforts for destruction of toxins via chemical means
  - Determines the level of risk for cultured fish

- Mechanical removal of P. parvum and neutralization of toxins

Priorities:

Cells:

- accurate and rapid detection, identification, quantification
- detecting and mapping resistant (dorment) stages

Toxins:

- detection and quantification of different toxin components
- factors regulating those toxin elements
- understanding the synthesis of these toxin elements

Mitigation:

- development of means for cell removal (including lysis)
- using toxin and cell detection guided methods for mitigation
- developing agents against the specific toxins

Indirect Gradient Analysis of *Prymnesium parvum* Blooms in Lake Whitney, Texas: Population Responses to *in situ* Environmental Gradients and Experimental Nutrient-Enrichment Gradients

Richard L. Kiesling

Environmental Science Institute, University of Texas and USGS, Austin, Texas USA

Abstract.--Within the past three years, several major fish kills attributable to toxins released by *Prymnesium parvum* blooms have occurred in large surface-water reservoirs in the Brazos River basin. Between January and May of 2001, 2002, and 2003, Possum Kingdom Lake, Lake Granbury, and Lake Whitney, Texas, all experienced significant fish kills associated with *P. parvum* blooms. The factors controlling the appearance of *P. parvum* blooms in these reservoirs have yet to be determined, but the pattern of fish kills raises several questions about the physical, chemical, and biological characteristics that trigger the build-up of *P. parvum* populations leading to toxin production. We investigated *P. parvum* bloom dynamics in Lake Whitney by measuring the phytoplankton community response to in-reservoir environmental gradients and to experimental nutrient enrichment gradients. Indirect gradient analysis of Lake Whitney *P. parvum* densities during the spring 2003 bloom suggest a relationship between water chemistry (e.g., conductivity) and algal abundance. Experimental nutrient-enrichment gradients provide evidence of nitrate-nitrogen limitation during the *P. parvum* bloom. Nitrate additions produced *P. parvum* growth rates that were significantly elevated over control rates. Ammonium-nitrate additions produced significant negative *P. parvum* growth rates, and phosphorus and silica additions produced no net growth effects. Preliminary results from a nutrient-dilution bioassay suggest that micro-flagellate mortality is a function of *P. parvum* densities. Additional experiments are planned that simultaneously investigate *P. parvum* nutrient limitation, toxin production, and micro-flagellate growth rates along a *P. parvum* density gradient.

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Analysis of *Prymnesium parvum* blooms in Lake Whitney, Texas

Richard L. Kiesling[12]

[1]United States Geological Survey, Water Resource Division, Texas District, 8027 Exchange Drive, Austin, TX, 78754

[2]Dept. Chemistry & Geosciences, Tarleton State University, Stephenville, TX, 76402

Overview

- Problems:
  - Winter-Spring blooms of *Prymnesium parvum* in numerous Texas reservoirs (PK, Granbury, Whitney)
  - Frequent blooms of *P. parvum* in the upper Colorado River
  - Associated fish kills in reservoirs, rivers, and fish hatcheries

Overview

- Questions Identified by Texas HAB Team:
  - What factors control the development of *P. parvum* blooms in Texas reservoirs?
  - What physical, chemical, and biological characteristics trigger the build-up of *P. parvum* populations?
  - What causes toxin production in *P. parvum*?

Overview

- What factors contribute to *P. parvum* blooms?
  - Is there a relationship between environmental conditions and development of blooms?
  - Is this relationship mediated through algal population dynamics or trophic-level interactions?

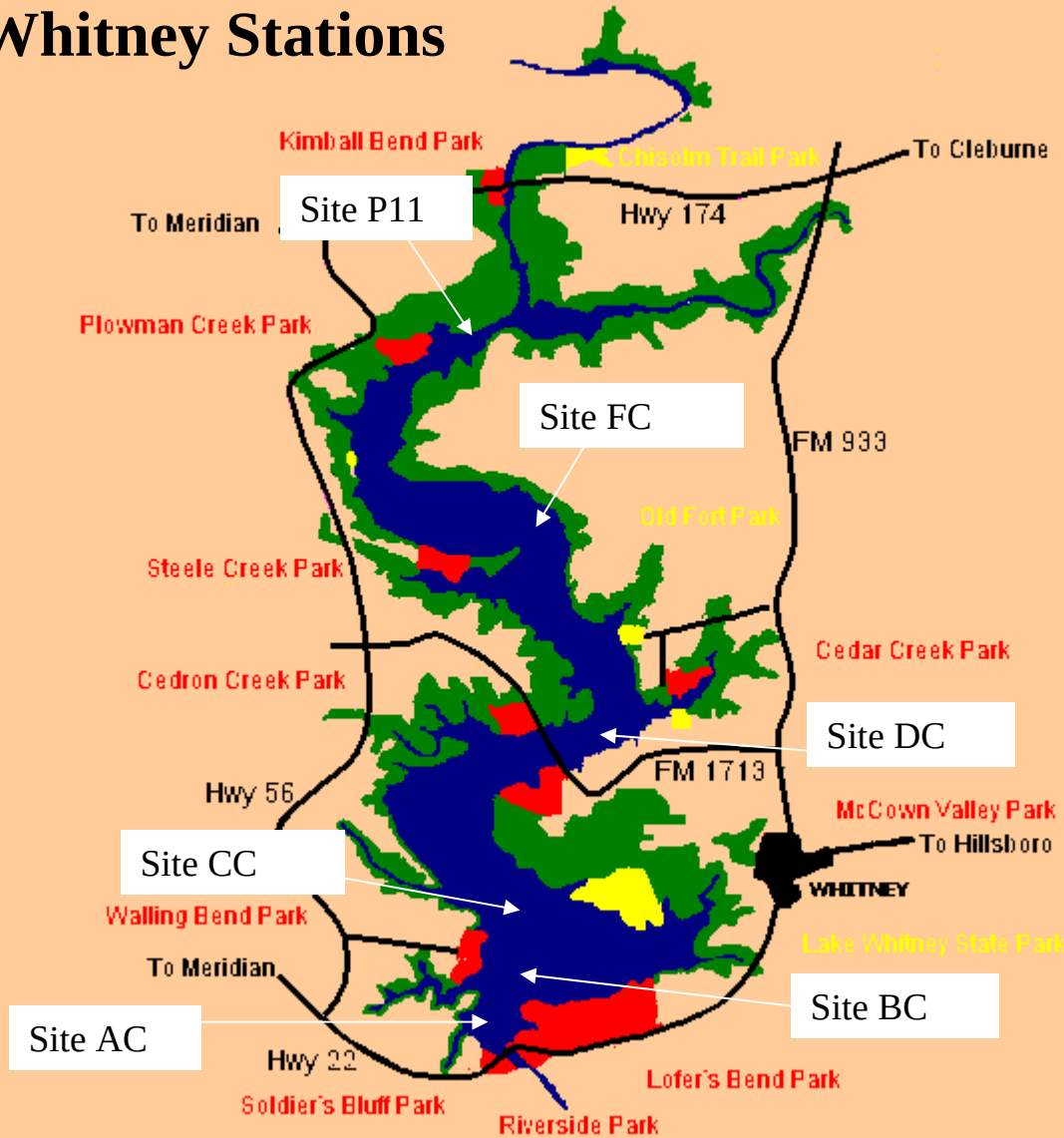
Approach - proposed studies

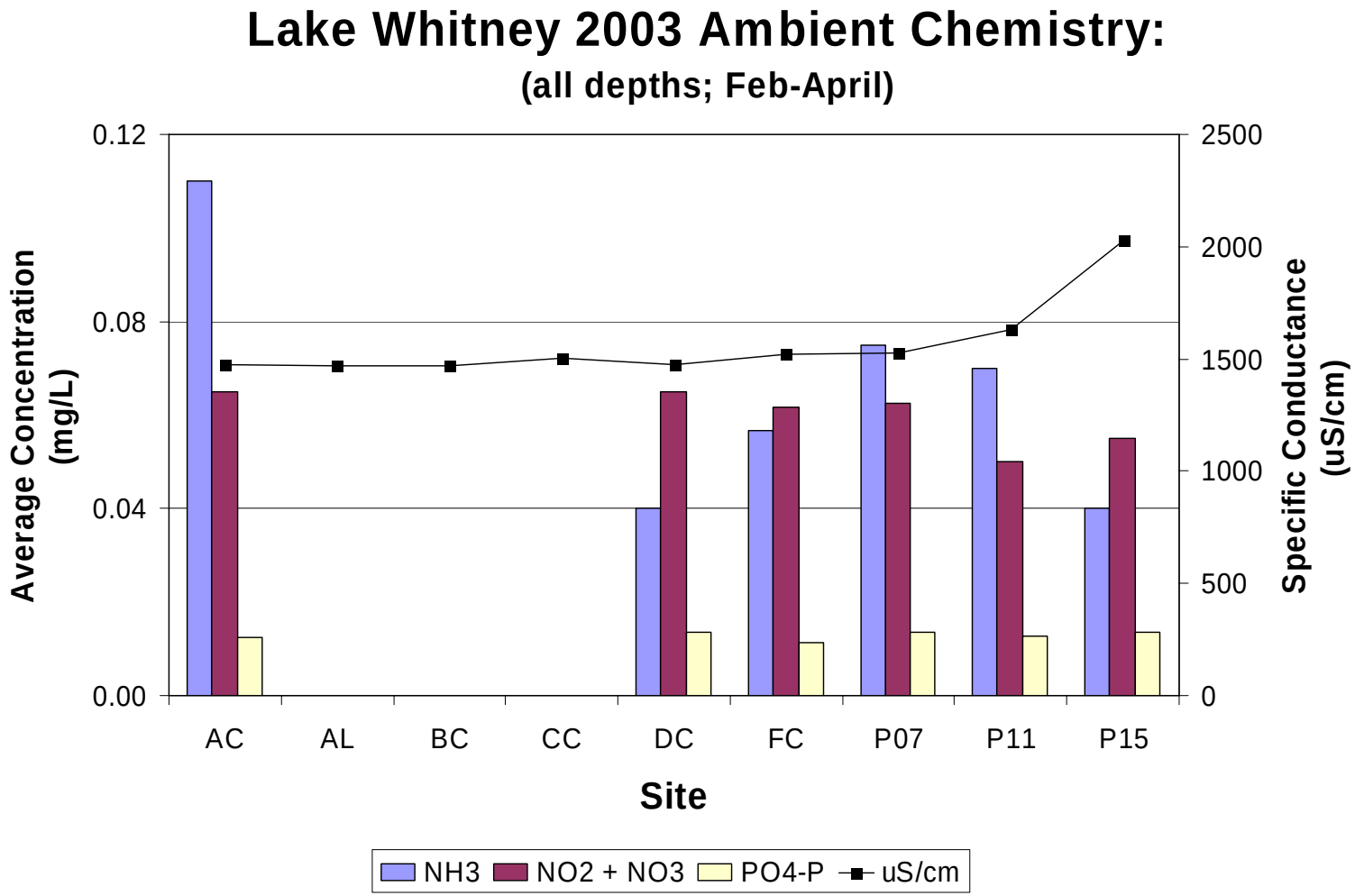
- Synoptic sampling of paired reservoirs during *P. parvum* blooms
  - Simultaneously assess population densities and the physical and chemical environmental gradients
  - Correlate biological responses to environmental gradients
- Experimental manipulation of important gradients
  - Nutrient enrichment experiments to ID potential limitation
  - Dilution bioassays to estimate importance of grazing
  - Functional response to limiting factors

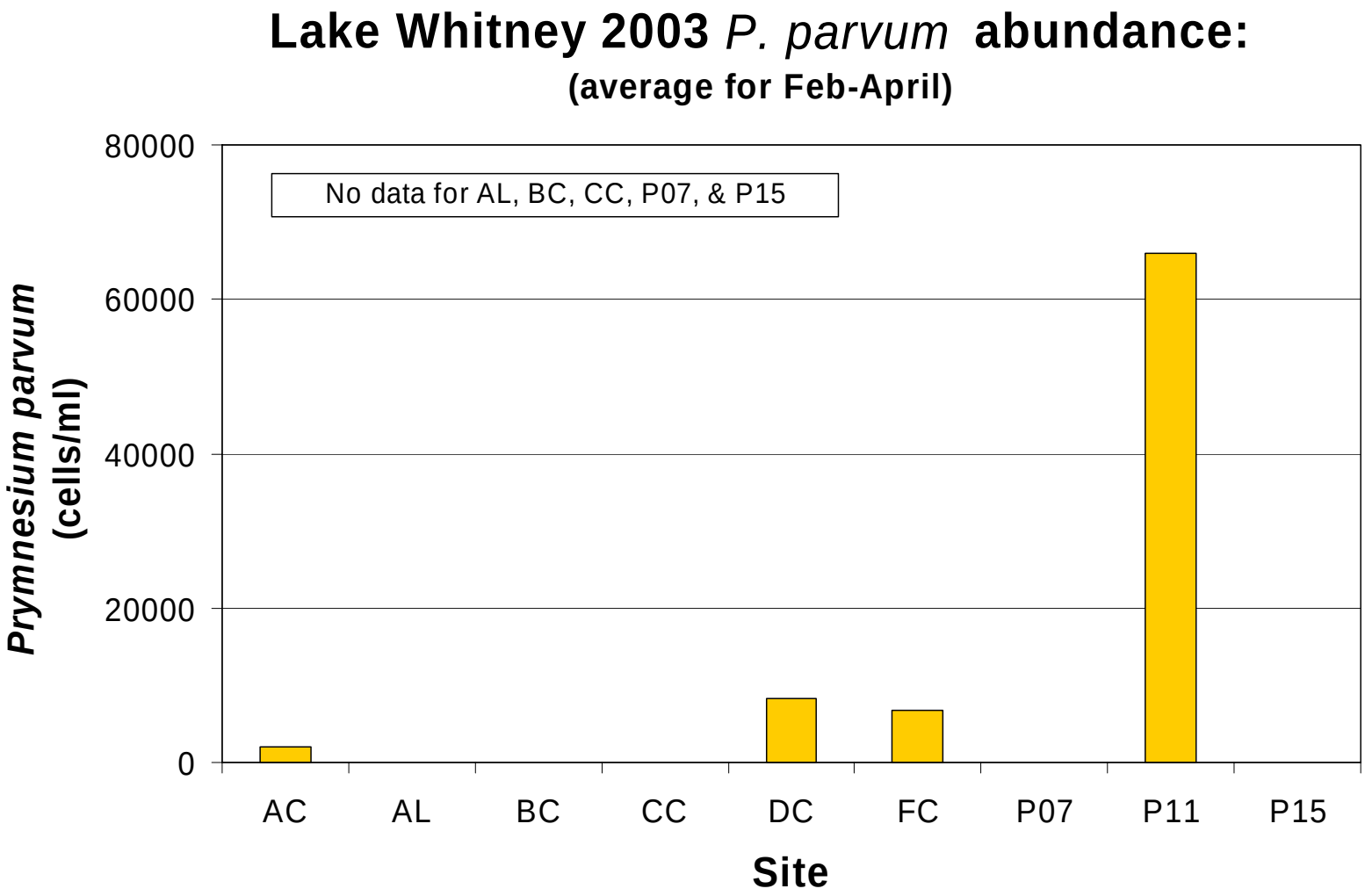
Pilot Study on Lake Whitney

- Assessment of biological responses to gradients in Lake Whitney
  - Synoptic cruises during the *P. parvum* bloom to document physical and chemical gradient
  - Experimental nutrient enrichment gradients to assess potential for nutrient limitation of *P. parvum* populations
  - Experimental grazing gradients to assess loss rates (dilution and addition)

Lake Whitney Stations




Lake Whitney 2003 Ambient Chemistry:
(all depths; Feb-April)
Average Concentration (mg/L)
0.12
0.08
0.04
0.00
2500
2000
1500
1000
500
0
Specific Conductance (uS/cm)
AC
AL
BC
CC
DC
FC
P07
P11
P15
Site
NH3
NO2 + NO3
PO4-P
uS/cm



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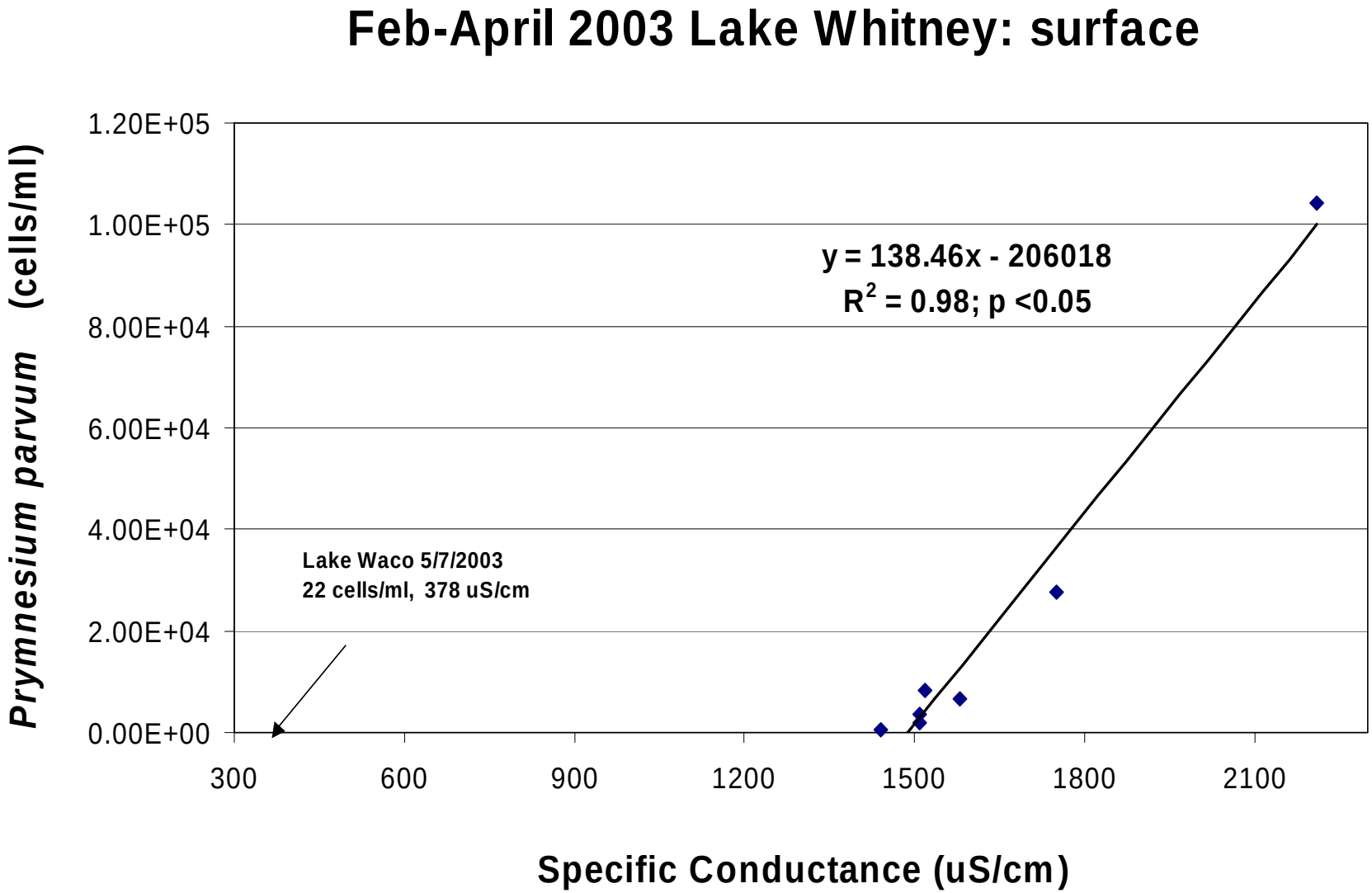
Correlations between *P. parvum* densities and selected variables

Lake Whitney *P. parvum* Surface Densities, Composite Nutrient Data					
	Cond.	NH_3-N	NO_2+NO_3	PO_4-P	CELLS/ML
Cond.	1.00	-0.49	-0.78	0.36	0.98
NH_3-N		1.00	-0.01	-0.46	-0.51
NO_2+NO_3			1.00	0.16	-0.72
PO_4-P				1.00	0.50
CELLS/ML					1.00



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Step-wise Multiple-Regression Summary: CELLS/ML Surface Densities, Composite Nutrient Data						
	Beta	Beta SE	B	B SE	t(1)	p-level
Intercpt			3907	13932	0.28	0.83
COND	0.52	0.03	73	4	18.53	0.03
NH_3-N	-0.12	0.01	-113936	12177	-9.36	0.07
NO_2+NO_3	-0.37	0.02	-1869286	121535	-15.38	0.04
PO_4-P	0.32	0.01	1876156	71168	26.36	0.02
R= .99 Adjusted R^2= .99						
F(4,1)=5297.0 p<.01030 Std.Error of estimate: 615.29						

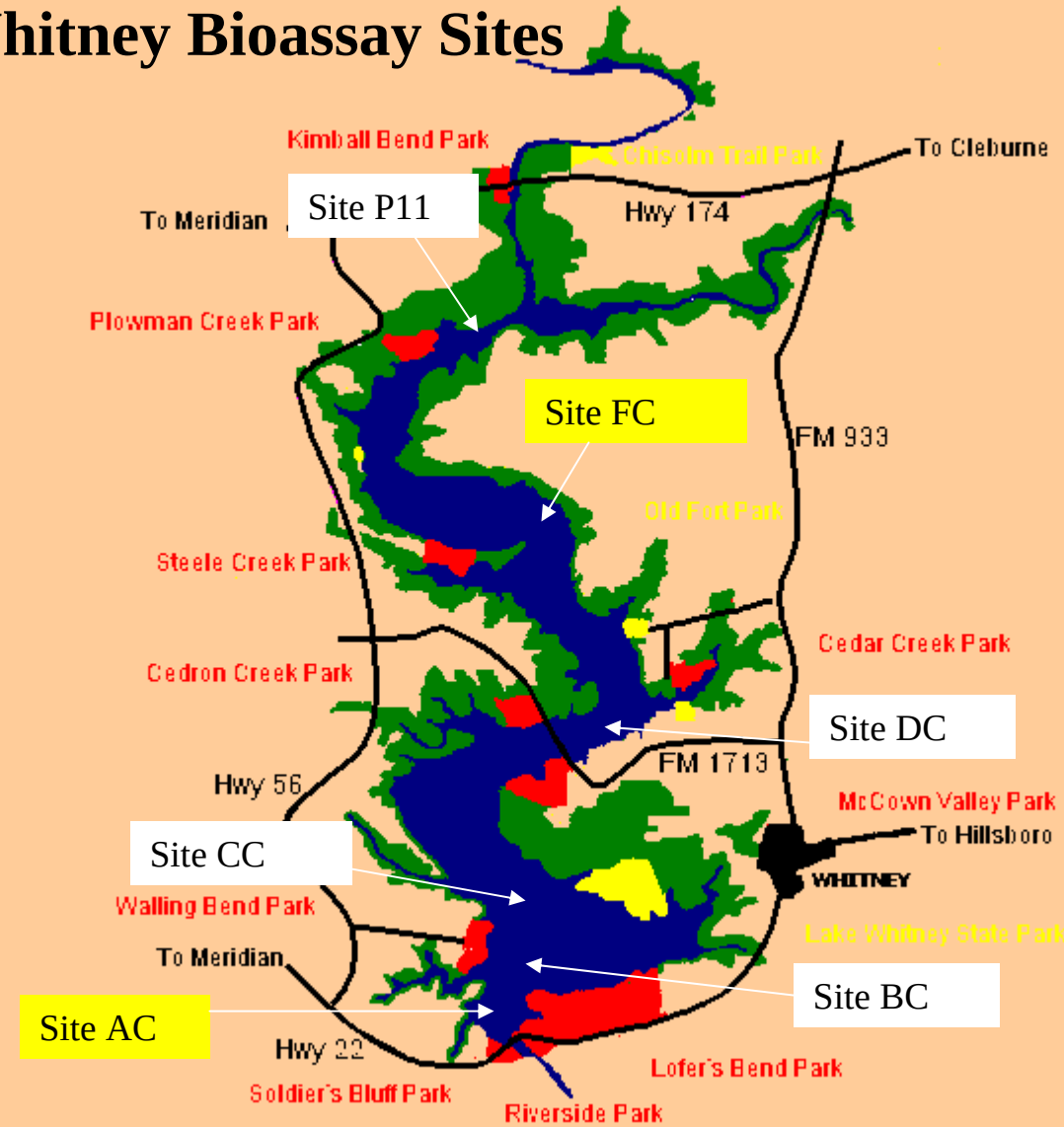
Lake Bioassay Methods

- Acclimated growth-rate method using IVF followed by cell counts to estimate daily growth (*r*)
- Treatments included N, P, and Si additions to ambient lake water
- Laboratory incubations at ambient temperature and light lasted 8 days

Lake Bioassay Methods (cont.)

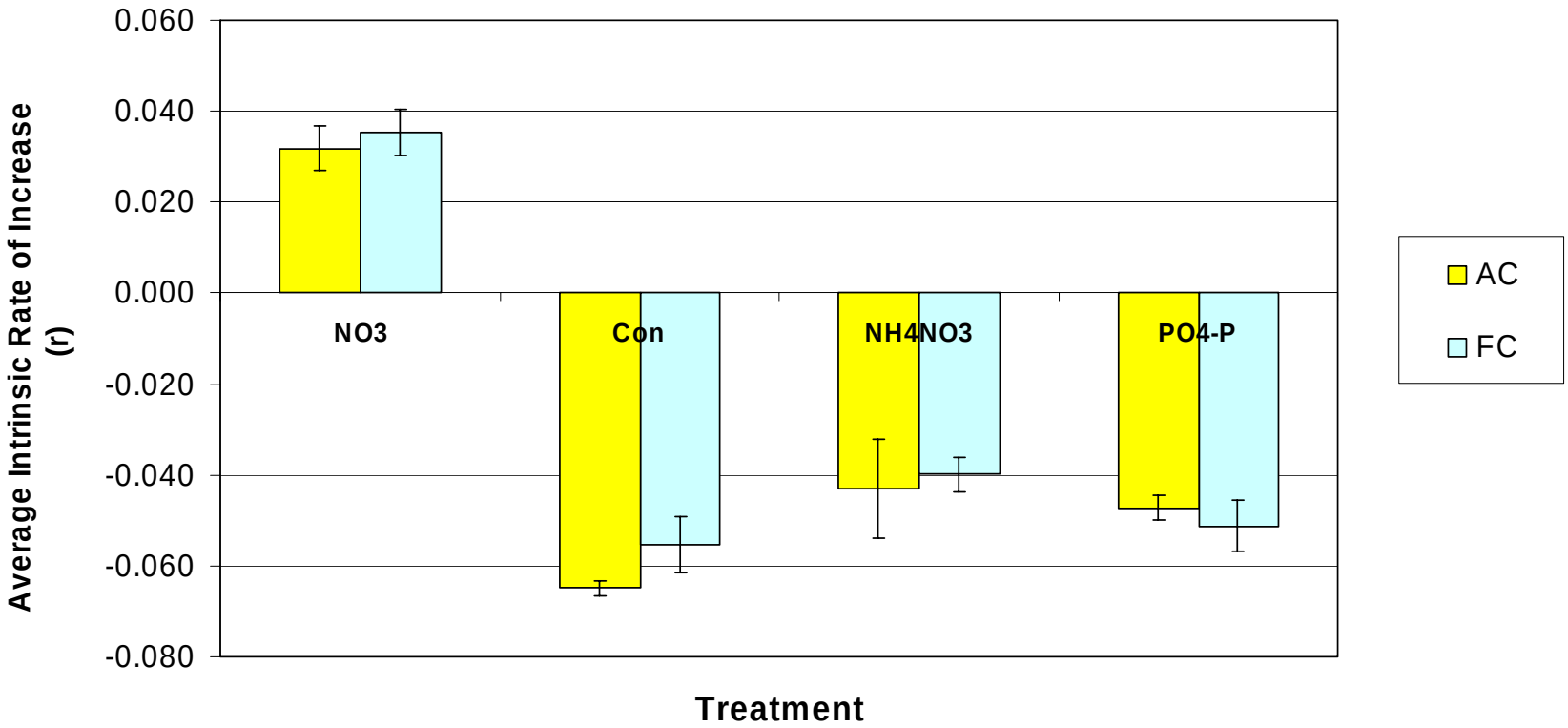
- Five or six replicates per treatment
- Zooplankton removed using 153μm Nitex
- Results recorded using *in vivo* fluorescence
- Growth responses to treatments were calculated using an exponential growth model ($N_t = N_o e^{rt}$)
- Replicate estimates of *(r)* for each treatment

Lake Whitney Bioassay Sites



Lake Whitney Nutrient Bioassay 4/29/03				
Site	Treatment	Mean r	SDEV	95% CI
AC	NO_3	0.032	0.006	0.005
AC	Con	-0.065	0.002	0.002
AC	NH_4NO_3	-0.043	0.014	0.011
AC	PO_4-P	-0.047	0.003	0.003
FC	NO_3	0.035	0.006	0.005
FC	Con	-0.055	0.007	0.006
FC	NH_4NO_3	-0.040	0.004	0.004
FC	PO_4-P	-0.051	0.007	0.006

Lake Whitney Nutrient Bioassay 4/29/03



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Lake Whitney Nutrient Bioassay 4/29/03: large volume, un-replicated design

Dilution Bioassasy			
Site	Treatment	r	r_{uf}
FC	50%D NO_3	0.033	0.039
FC	50% D Con	-0.042	-0.037
FC	NO_3	0.035	0.023
FC	Con	-0.055	-0.063
FC	NH_4NO_3	-0.040	
FC	PO_4-P	-0.051	

Original Questions

- What factors contribute to *P. parvum* blooms?
  - Is there a relationship between environmental conditions and development of blooms?
  - Is this relationship mediated through algal population dynamics or trophic-level interactions?

Results to date – April 2003 survey of Lake Whitney

- *P. parvum* densities follow in-lake environmental gradient
- *P. parvum* growth is stimulated by nitrate addition during latter part of bloom
- *P. parvum* net growth rates may be sensitive to species interactions

Next steps?

- Continue paired-reservoir assessment between Lake Whitney and Lake Waco - add others
- Continue to assess importance of grazing and nutrient limitation for *P. parvum* populations
- Assess toxin levels as a function of P. parvum density, conductivity, and nutrient limitation
- Explore options for Lake Whitney sediment core analysis using *P. parvum* biomarkers

Acknowledgements

- Texas HAB Team, TPWD
- Joan Glass, TPWD
- Dan Roelke, TAMU
- US Army Corps of Engineers
- USGS
- Texas Commission on Environmental Quality

DY III Media Stock Addtions

Nutrient	Final Concentration
Na_2HPO_4	8 mg/L
NH_4NO_3	5 mg/L
$NaNO_3$	20mg/L
Na_2SiO_3-$9H_2O$	30 mg/L

Kill Your Enemies and Eat Them: The Role of *Prymnesium* Toxins

Edna Graneli

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Abstract.--The haptophyte *Prymnesium parvum* is known to produce a set of highly potent exotoxins commonly called prymnesins. These toxins have been shown to have several biological effects, including ichthyotoxic, neurotoxic, cytotoxic, hepatotoxic and hemolytic activity towards a range of marine organisms. Toxic incidents of the haptophyte *Prymnesium parvum* have been known since the end of the 19th century. Since then, toxic blooms have been reported from brackish water localities in Europe, the Middle East, Ukraine, China and U.S.A. These blooms have affected coastal marine ecosystems heavily, and caused economic problems for commercial aquaculture. Therefore, it is important to understand the selective forces leading to bloom formation of this species. The ability of a specific phytoplankton species to become dominant and form blooms in natural environments is, apart from its competitive ability, also dependent on mortality losses. Grazing by herbivorous zooplankton is considered a major loss factor, preventing the development of phytoplankton blooms. Adaptations of algae to escape grazing would therefore directly favor the ecological success of that particular species. Several studies have shown that *Prymnesium*-species are able to diminish or completely avoid grazing by excretion of toxins into the water. Another important aspect in bloom formation is the ability to out-compete co-occurring algal species for nutrients. Over the last few years strong evidence has accumulated that *Prymnesium* spp. are able to kill not only their grazers but also other algal species, a process called allelopathy. Killing the nutrient-competing phytoplankton species enables *Prymnesium* to freely utilize limiting resources. Mixotrophy, i. e., the capability to ingest bacteria, other algae and even potential grazers, also contributes to the bloom-forming ability of *Prymnesium* spp. Allelopathy, mixotrophy and grazer deterrence increase dramatically when *Prymnesium* spp. cells are grown under N or P deficiency, and so does toxicity. On the other hand, if cells are grown in a medium with high amounts of N and P in balanced proportions, allelopathy, mixotrophy, grazer deterrence and toxicity decrease in intensity or cease completely. Usually additions of *Prymnesium* filtrates from nutrient deficient cultures have almost the same strong effect on grazers and other plankton cells as *Prymnesium* cells grown together with their target. This suggests that the toxins and the allelopathic/grazer deterring compounds are the same substances. In conclusion, it seems that toxin production in *Prymnesium* spp. works not only as a defense mechanism, but also, by killing competitors, improve the competitive ability of *Prymnesium* under conditions of severe nutrient depletion. Also, it seems that stress in general, rather than solely P- or N-limitation, is the cause for an increase in toxin production. *Prymnesium* toxins are poor in N and P, but have a high C content. Toxin production might be a way to store excess organic carbon, made available in photosynthesis under nutrient stress. This is thus similar to the way e.g. lipids or carbohydrates are produced in excess by most "normal" phytoplankton cells when there is not enough N or P available to build up material for cell division (DNA, proteins, etc).

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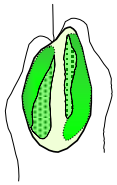

Kill your enemies and eat them: the role of *Prymnesium* toxins

Edna Graneli

Marine Sciences Department, University of Kalmar, SE-39182 Kalmar, Sweden

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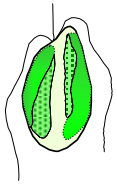

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I wish to thank Urban Tillmann, Catherine Legrand, Giovana Salomon, Per-Juel Hansen, Alf Skovgaard, for kindly providing their published and unpublished results.

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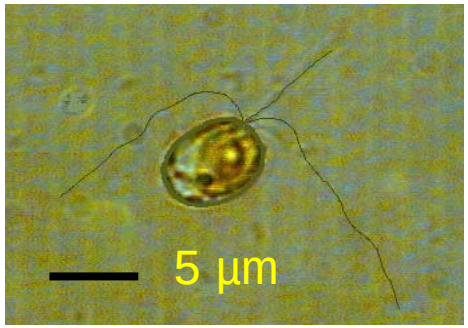

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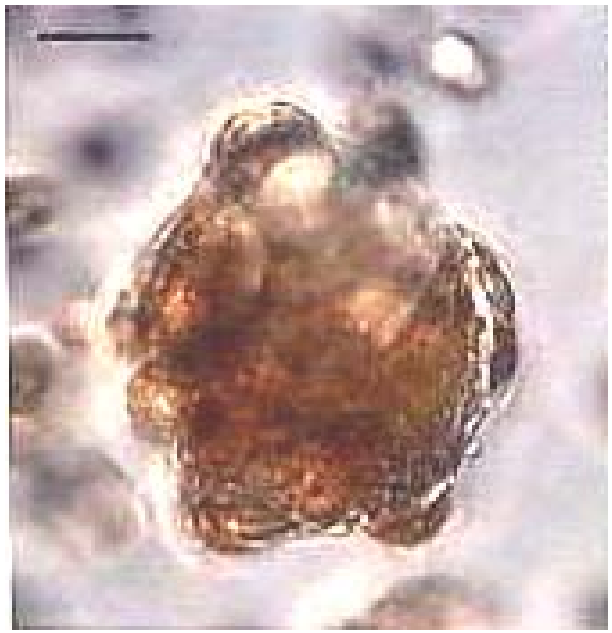
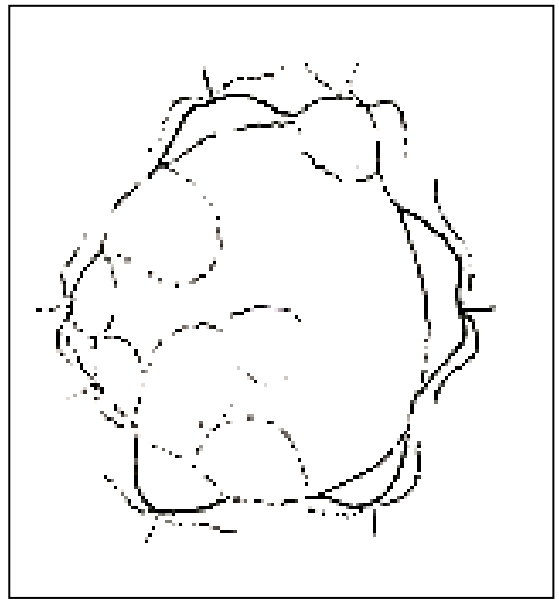
Killed salmons in aquaculture pens by *Chrysochromulina polylepis*

Prymnesium phagotrophy on algae





Photos by Urban Tillmann

TIllmann, U. (1998) AME 14: 155-160

Vardagar 4:50
Söndagar 5:–
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040-93 44 00

SYDSVENSKA DAGBLADET

SNÄLLPOSTEN.

GRUNDAD 1848

VÄDRET
Växlande molnighet och möjligen någn regnskur. Högst 19 gr. Sydväst 5 m/s.

Nr 241.. Måndagen den 5 september 1988 • Vecka 36

DEL 1

NÄR FLYTER DEN FÖRSTE DÖDE BAD-GÄSTEN ILAND?

When will the first dead bathing guest float ashore?

10 000 tågade för rent Öresund

10 000 helsingborgare gick man ur huse för att protestera mot miljöförstöringen i Öresund.

Ett mäktigt demonstrationståg gick genom stan ner till Strandvägen, och utanför fylldes Sundet av småbåtar. Det var tre kvinnor lyst från demonstrationen, men samtidigt riktade den ett tydligt budskap till politikerna två veckor före valet:

– De politiker som inte är beredda att lägga all kraft på att hejda miljöförstöringen har inte vårt förtroende, hette det i den avslutande resolutionen.

10 000 marched for a clean Öresund

PWD RP T3200-1203

Sydsvenska Dagbladet September 5, 1988

100-tals protesterade vid Nordiska rådets miljömöte

Fiskare kämpar för havet

Fishermen fights for the sea

Minister: you might forget I will NOT !

Sea assassination

HELSINGÖR-S

Vem kunde ana miljöminister Birgi Dahl skulle få applå av många hundra bittrade fiskare som på tisdagen demonstrerade utanför Nordiska rådets mötesslokaler i Helsingör?

De hade kommit i egna båtar från hela Danmark och från Halland för att demonstrera mot en miljöpolitik som gör dem arbetslösa. Havsdöden har utbrett sig skrämmande snabbt och omfattar nu praktiskt taget samtliga inre danska vatten samt Kattegatt söder om en linjen Grenå-Varberg.

– Det finns inget lönsamt fiske i Kattegatt, sa skepparen på Buatrålaren Almy West, Ingemar Torkelsson, till Sydsvenskan innan demonstrationståget avgick från Helsingörs hamn till hotel Marienlyst norr om staden, där de nordiska politikerna samlats.

För första gången på 33 år var han och besättningen därför på väg till Östersjön. Men det är med mycket blandade känslor de lämnar hemmavattnet. Många av de 300 kollegerna i Halland har inte fartyg som är stora nog för långturer. De får bli hemma och se sin näring tvina bort.

"Det råder vrede"

– Det första larmet gick 1980 men inget har hänt, sa Ingemar Torkelsson. Det är en bitter fiskekår du ser. Det råder förstämning och vrede.

På torget ovanför kajen, vid ett uppslaget informationstält, samlades demonstranterna från Sönderborg i sydväst till Låsö och Bua i norr och Rödvig i söder. Plakat som "Mord på havet, stoppa hyckleriet och "Minister, glömmer du, så minns jag", plockades fram.

Utan annat än lågmält inbördes prat drog sedan tåget den korta vägen till Marienlyst.

Ställer krav

Men både danska och svenska fiskare kommer att ställa krav på statsmakterna. Det avslöjade Willy Hansen, dansk fiskeriförenings ordförande, och Wilhelm Alexandersson, representant för Sveriges fiskares riksförbund, när de talade från ett lastbilsläp utanför Marienlyst.

Miljöminister Birgitta Dahl, som sällat sig till åhörarna, fick höra att hallandsfiskarna kräver att få gå skadeslösa ur den katastof, som höstens fiske beskrivs som.

– Det är inte vanligt att fiskare kräver statliga bidrag, men nu tvingas vi till det, sa Wilhelm Alexandersson och fortsatte:

– Ge miljöministrarna bakläxa. Får de bestämma så har vi inget kustfiske om några år.

I en gemensam r ställer därför de bå förbunden krav om snabba radikala åtgärder för att förbättra havsmiljön.

Birgitta Dahl inte bara lyssnade, hon tyckte också hon hade något att säga och återde därför lastbilsflaket var-ifrån hon yttrade sig på att kal-

Fiskarna hade kommit från hela Danmark och från Halland för att demonstrera mot en miljöpolitik som gör dem arbetslösa. Havsdöden har utbrett sig skrämmande snabbt.
FOTO: MAGNUS AF GEIJERSTAM

Ministrar fick bakläxa igen för havsmiljöplan

HELSINGÖR-TT.

Det blev dubbelt underkänt för de nordiska miljöministrarna.

Efter många turer

Den reviderade plan som lades fram av miljöminister Birgitta Dahl och den norska miljöministern Sissel Rönbeck så sent som på tisdagens morgon fann alltså inte nåd inför social- och miljöutskottet.

sade den svenska ledamoten i miljöutskottet Sten Svensson (m) efter det sista, avgörande sammanträdet.

De nordiska miljöministrarnas första förslag till en gemensam havsmiljöplan fick

al- och miljöutskottet.

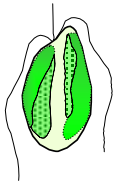
Nu får miljöministrarna skriva ihop en ny och tuffare havsmiljöplan att presentera vid Nordiska rådets ordinarie sammanträde i början av 1989.

Det socialdemokratiska le-

PWD RP T3200-1203

Sydsvenska Dagbladet November 16, 1988

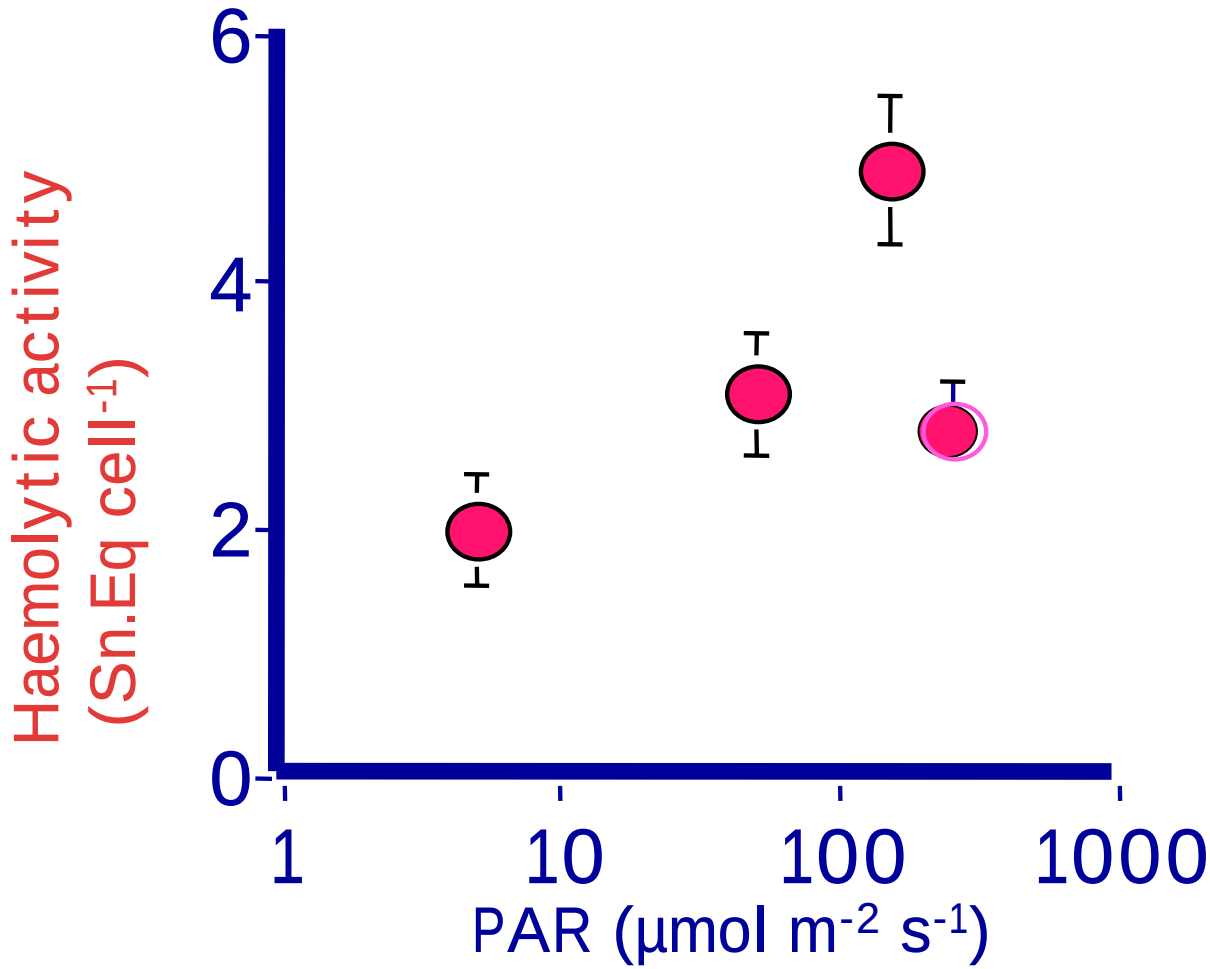
PWD RP T3200-1203



Factors influencing Prymnesium-

1. **Toxin production and degradation**
2. **Allelopathy**
3. **Mixotrophy (phagotrophy)**

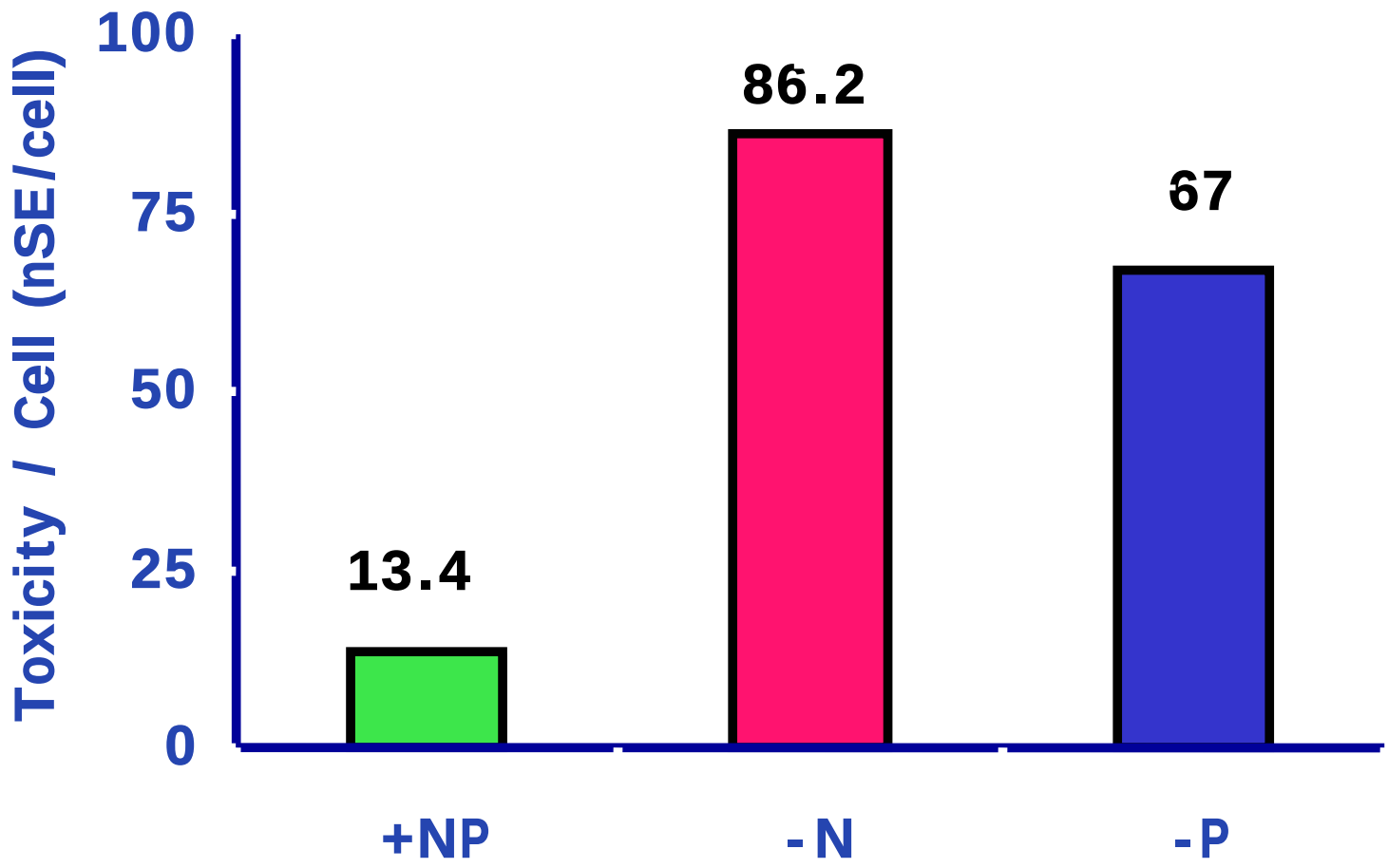
Toxicity in *Chrysochromulina polylepis* at different light conditions



PWD RP T3200-1203

Legrand, Johansson, Johnsen, Borsheim and Granéli, (manuscript)

Toxicity in *Prymnesium parvum* grown under NP sufficient and deficient conditions

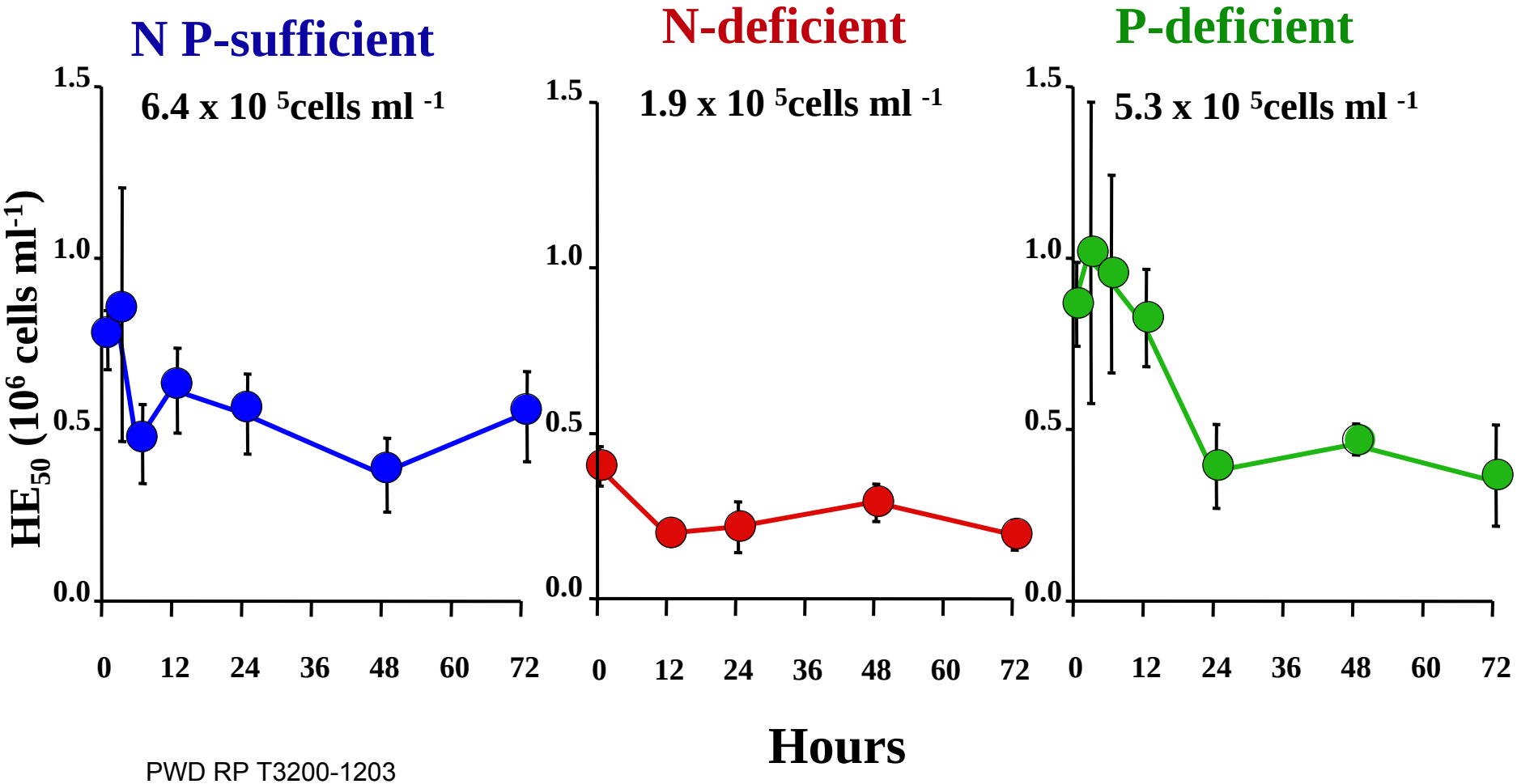


PWD RP T3200-1203

Johansson and Graneli, 1999, JEMBE 239: 243-258

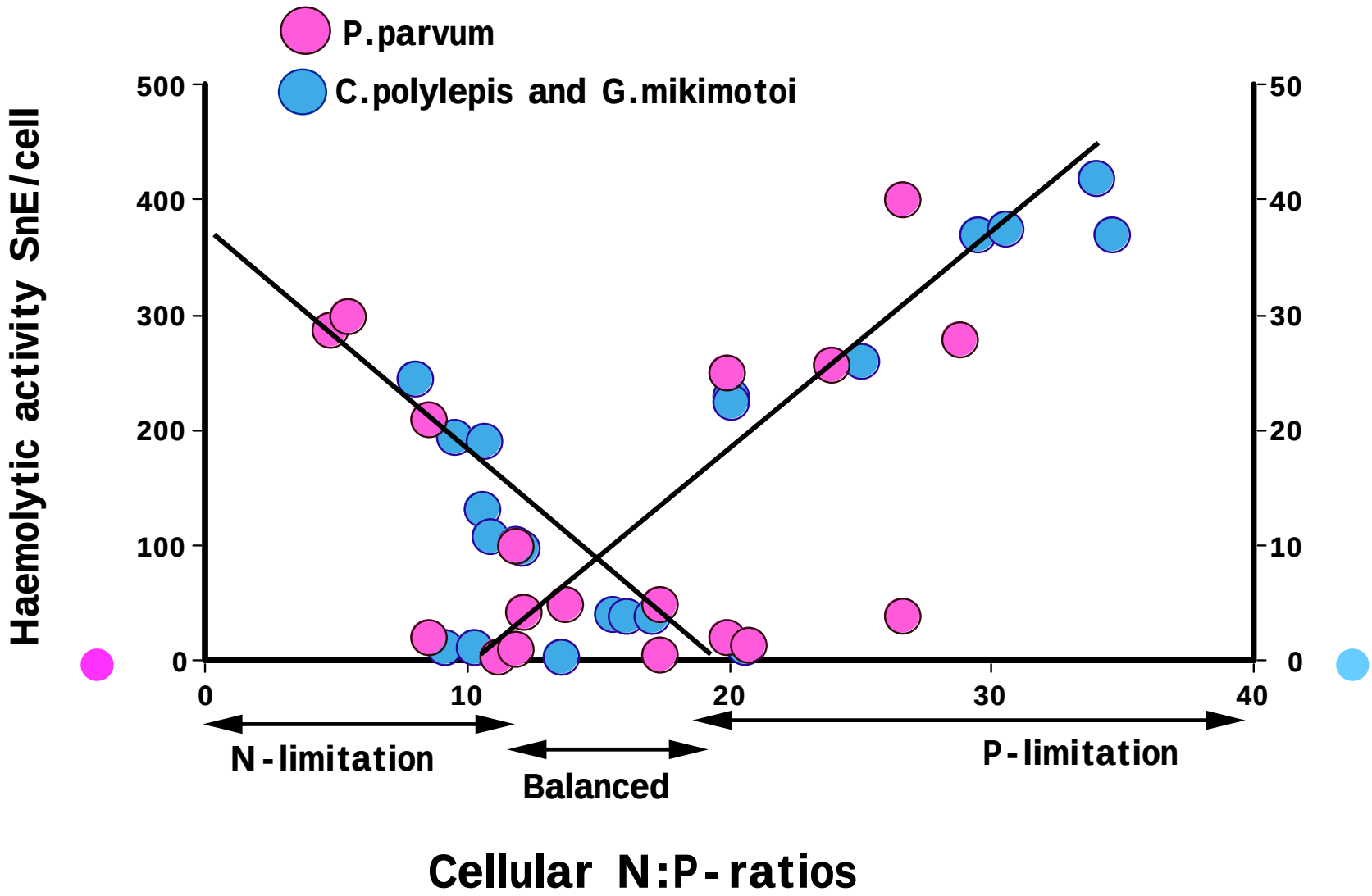
Prymnesium parvum toxicity

(HE_{50} number of *P. parvum* cells necessary to kill an organism
-NOTE that the lower the HE_{50}, the more toxic the cells are)



PWD RP T3200-1203

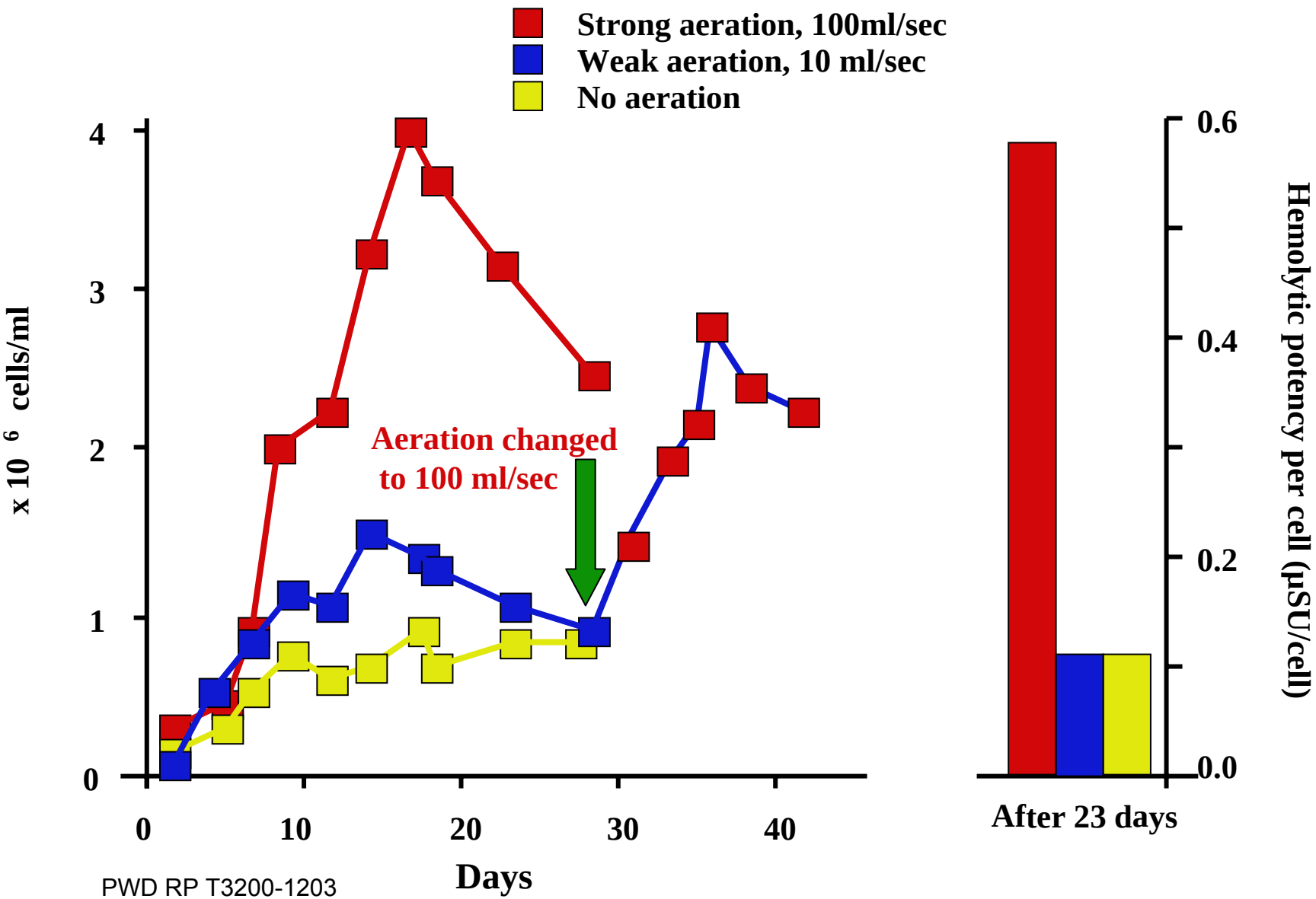
Relation between toxicity and nitrogen/phosphorus



PWD RP T3200-1203

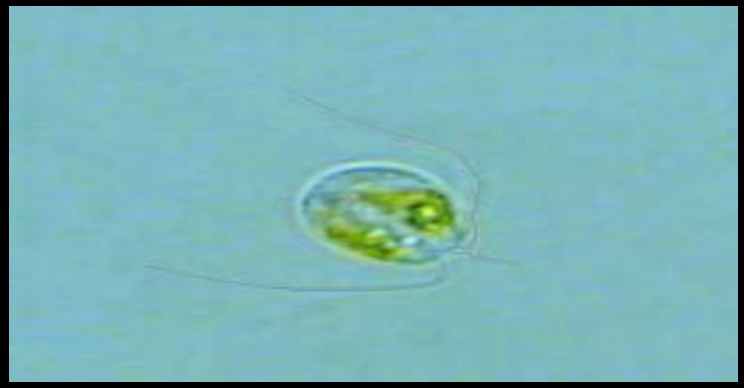
Effect of increasing P-deficent *P. parvum* numbers on flat-fish

Prymnesium parvum

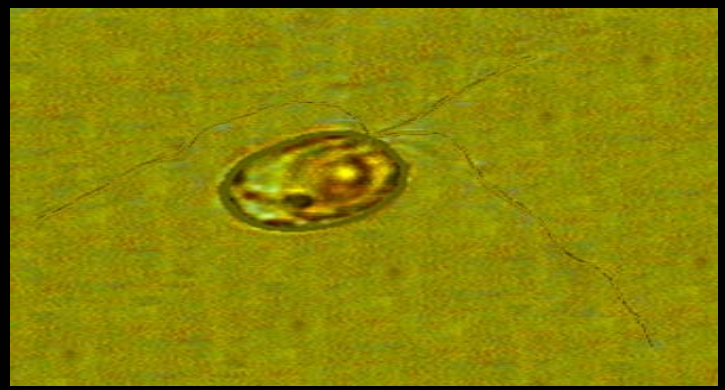


Igarashi, Oshima, Murata and Yasumoto in Harmful Marine Algal Blooms, 1995

Ichthyotoxic species



Prymnesium parvum

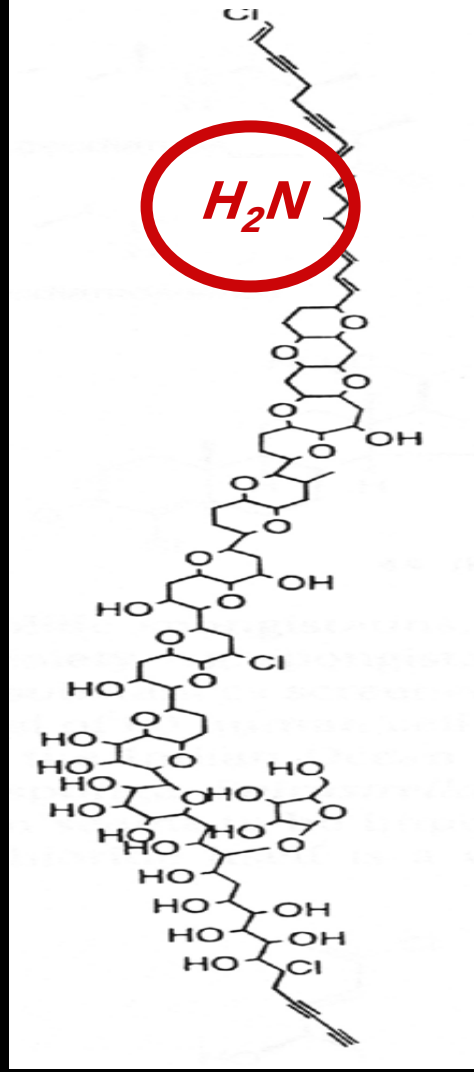


Chrysochromulina polylepis

AND...

***Gymnodinium* spp.,**
Heterosigma akashiwo,
Chatonella* *spp., etc.

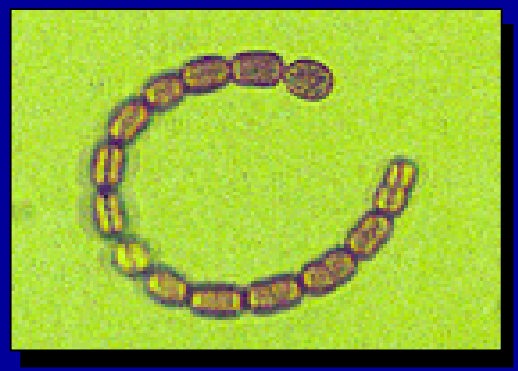
Prymnesin-2

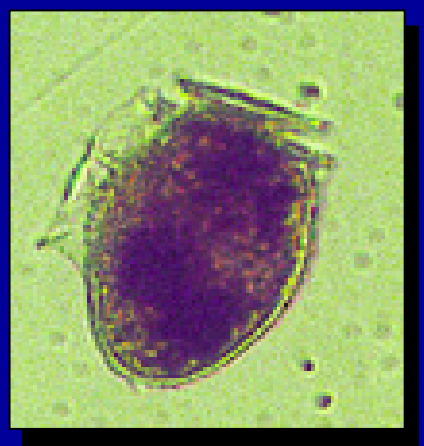


The allelopathic organism, to achieve dominance, release chemical compounds stoping/inhibiting the growth of the competitors

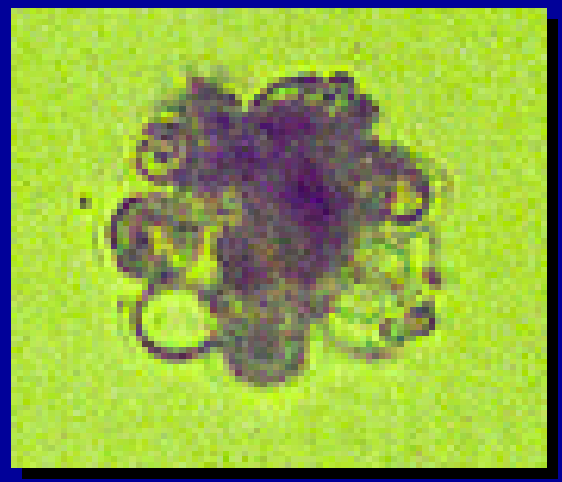
Allelopathic organism:
better competitor for resources

☺ Before

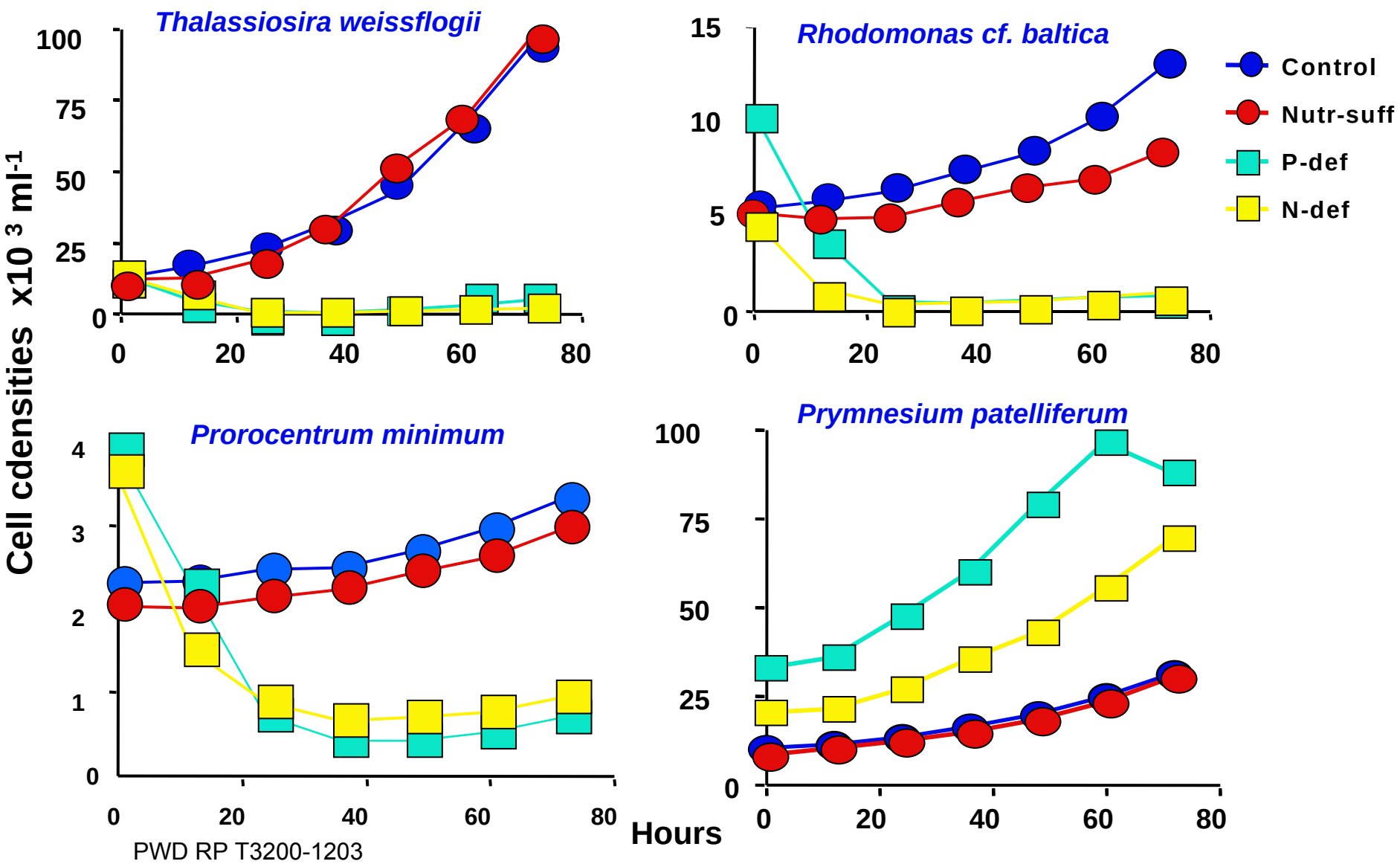




☹ After



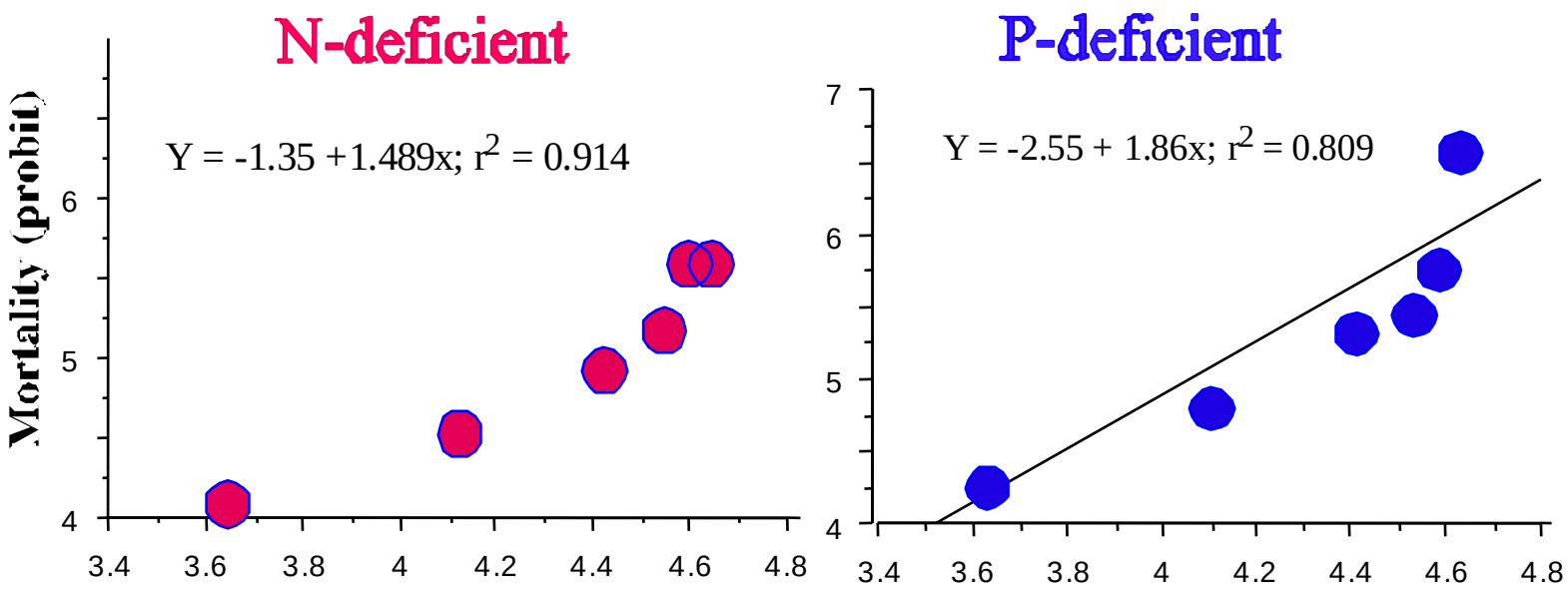
Effect of *Prymnesium parvum* filtrate on different algae



Granéli and Johansson 2003, Harmful Algae 2:135-148

Dose response relationship between *P. parvum* cell-free filtrates on *Artemia salina* nauplia

N and P-deficient (LC50 = 18.4 and 11.5 10^3 cells ml-1 respectively)

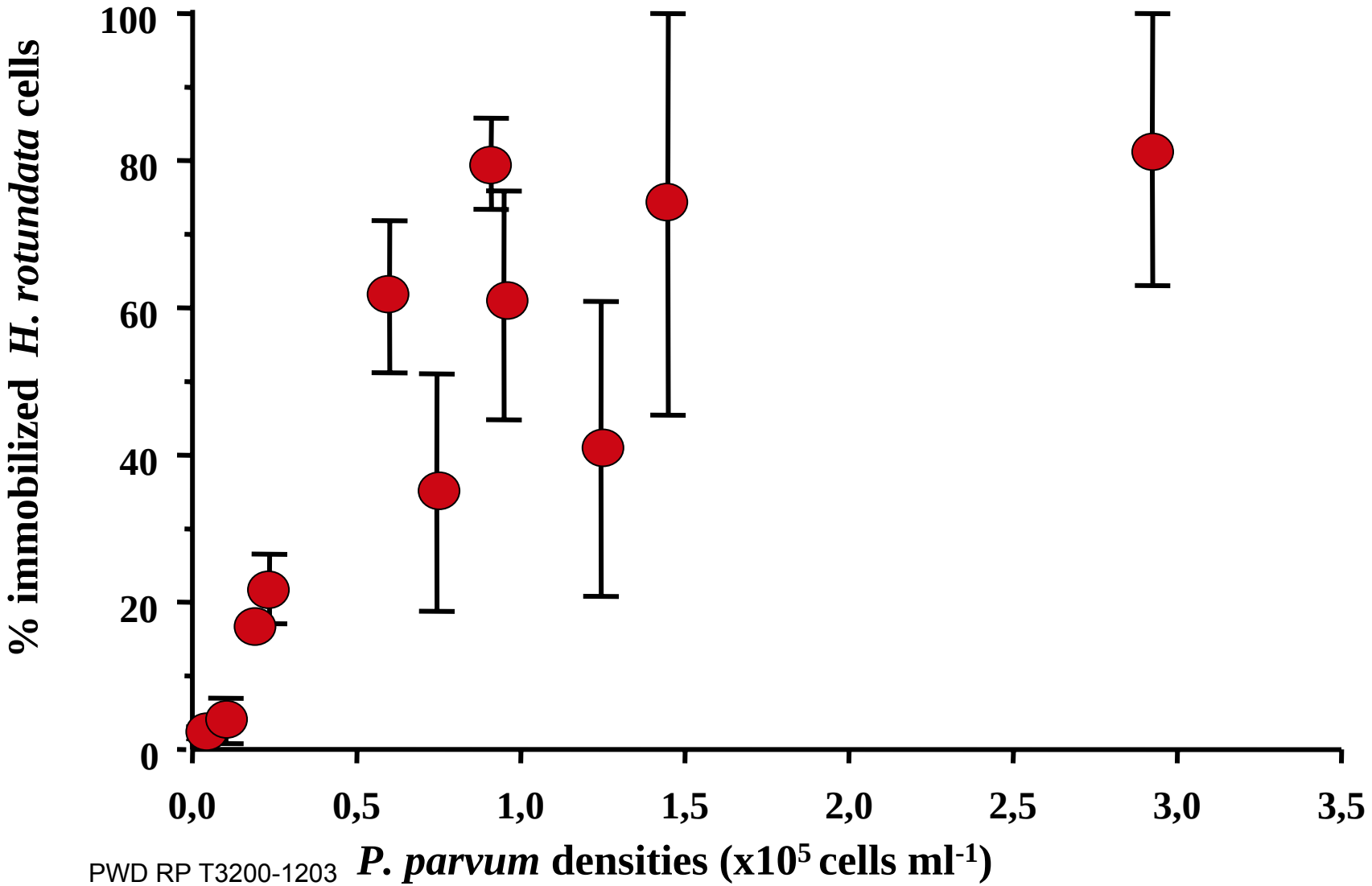


Log cells · ml^{-1}

PWD RP T3200-1203

Graneli and Johansson, 2003, Harmful Algae 2: 135-145

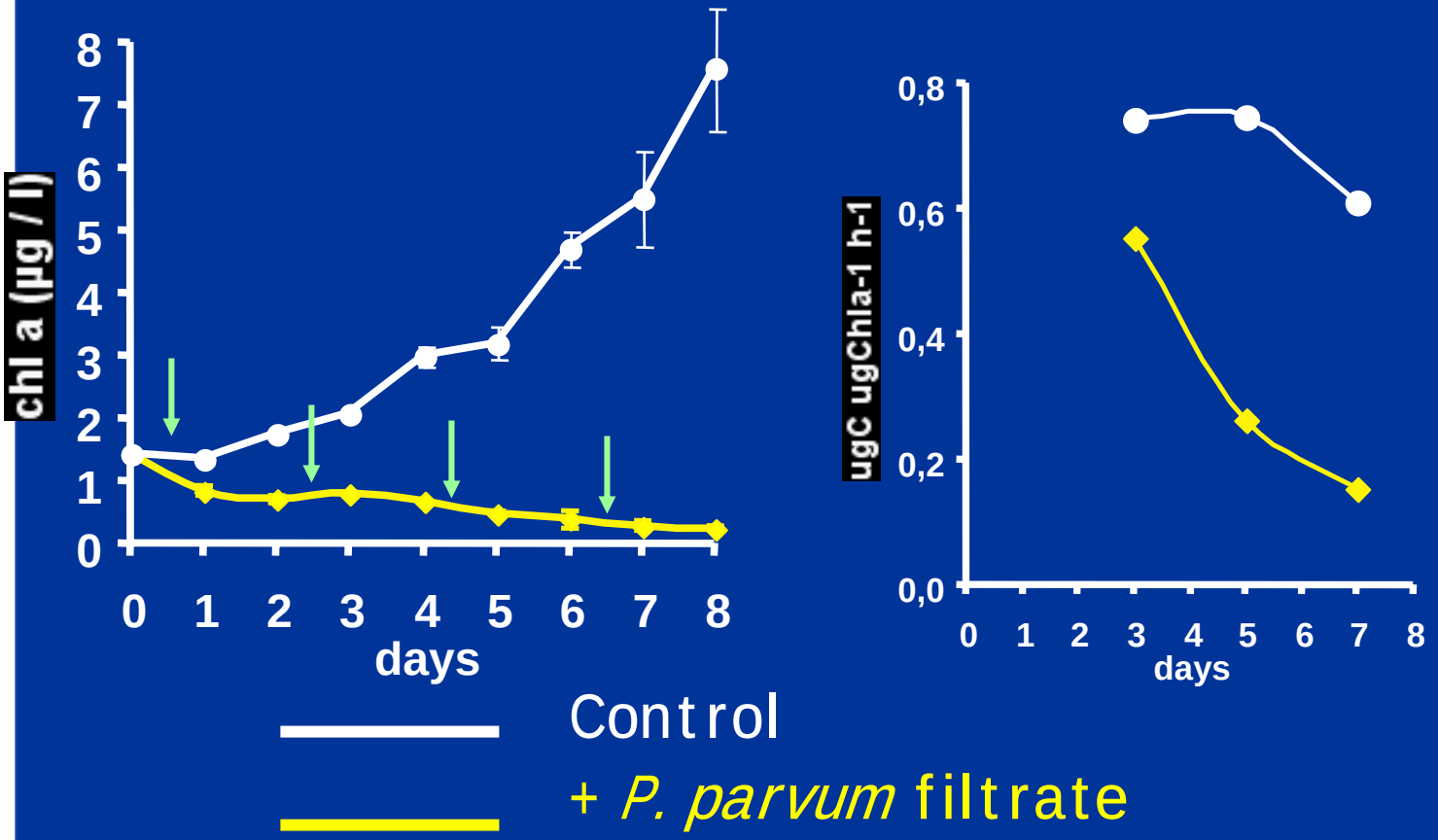
Effect of *P. parvum* filtrates on *Heterocapsa rotundata*



PWD RP T3200-1203

Skovgaard and Hansen, 2003, L & O, 48:1161-1166

Effect of *P. parvum* filtrates on a plankton community



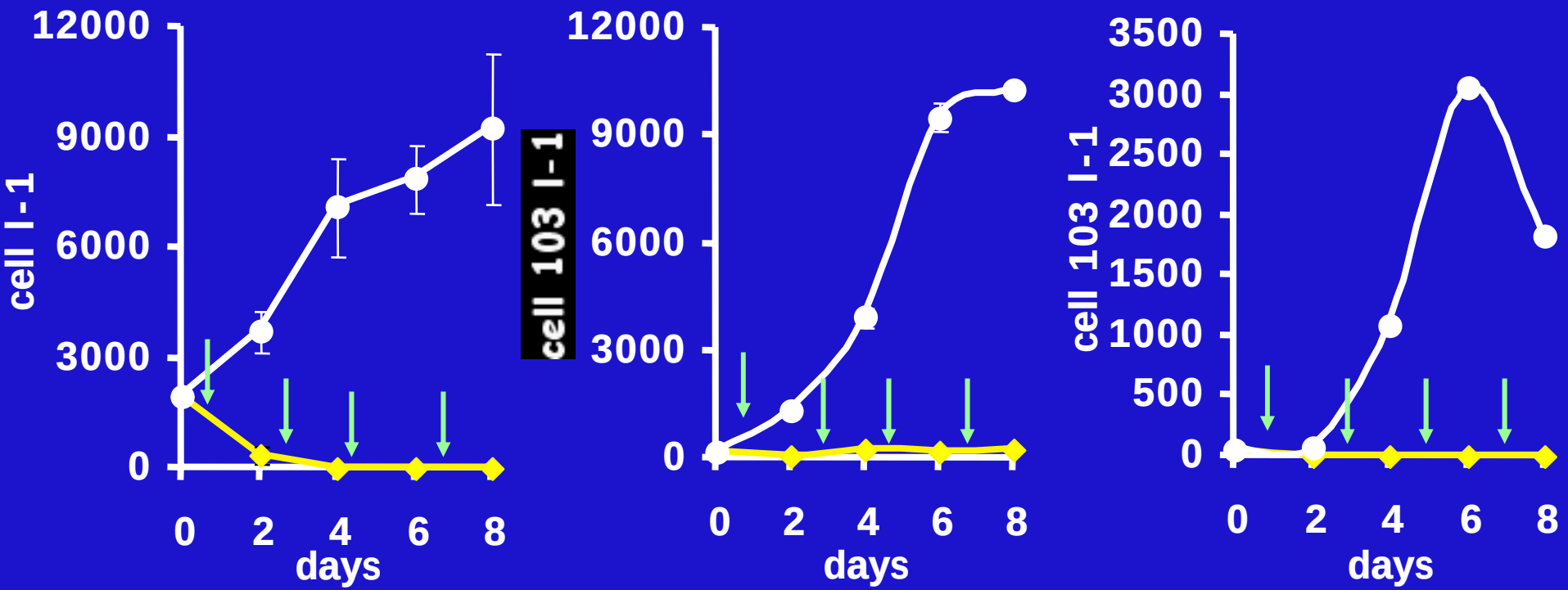
PWD RP T3200-1203

Fistarol, Legrand, Granéli, 2003, MEPS 255: 115-125

Ciliates

Flagellates

Diatoms

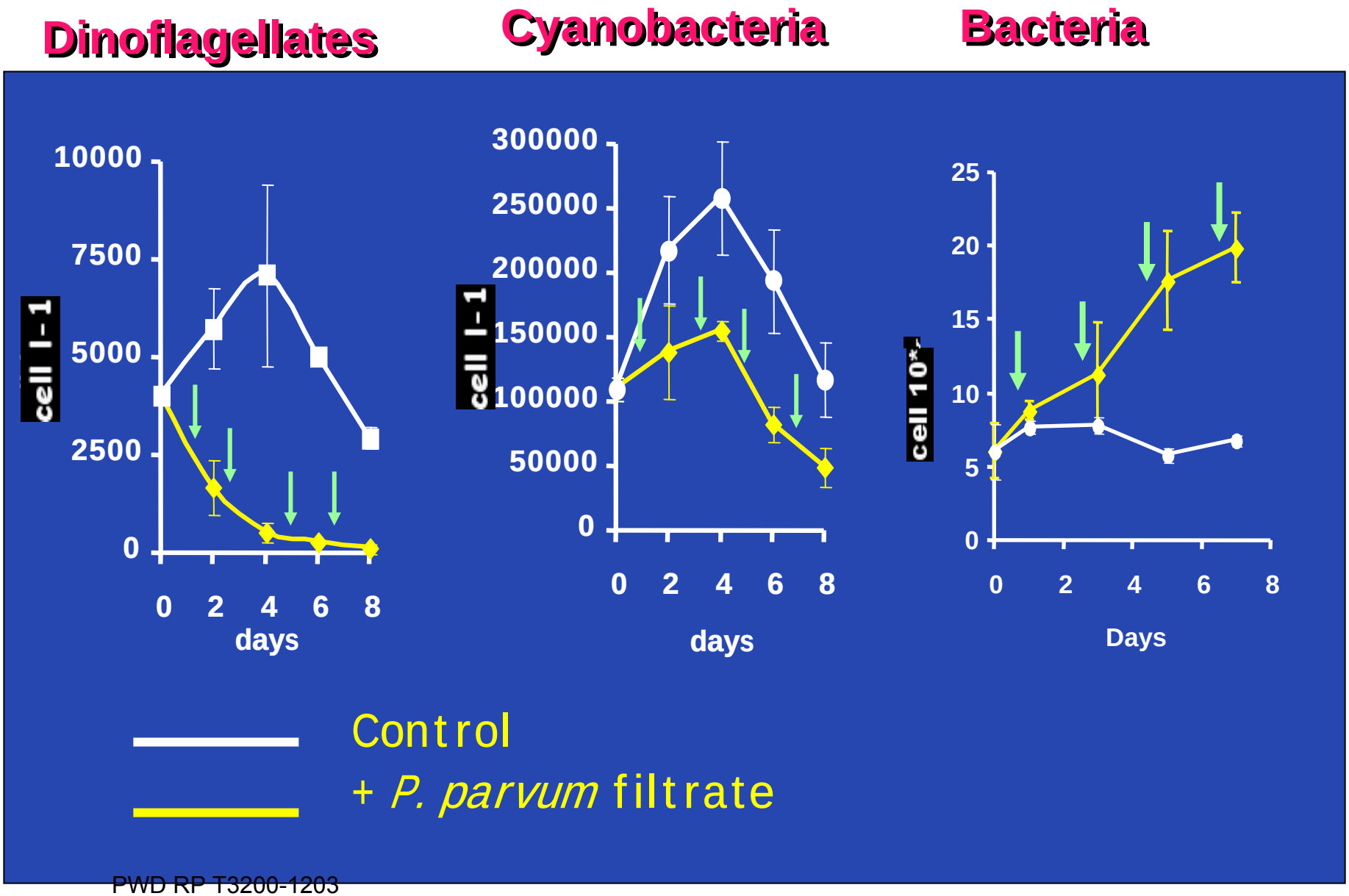


Control

+ *P. parvum* filtrate

Fistarol, Legrand, Granéli, 2003, MEPS 255: 115-125

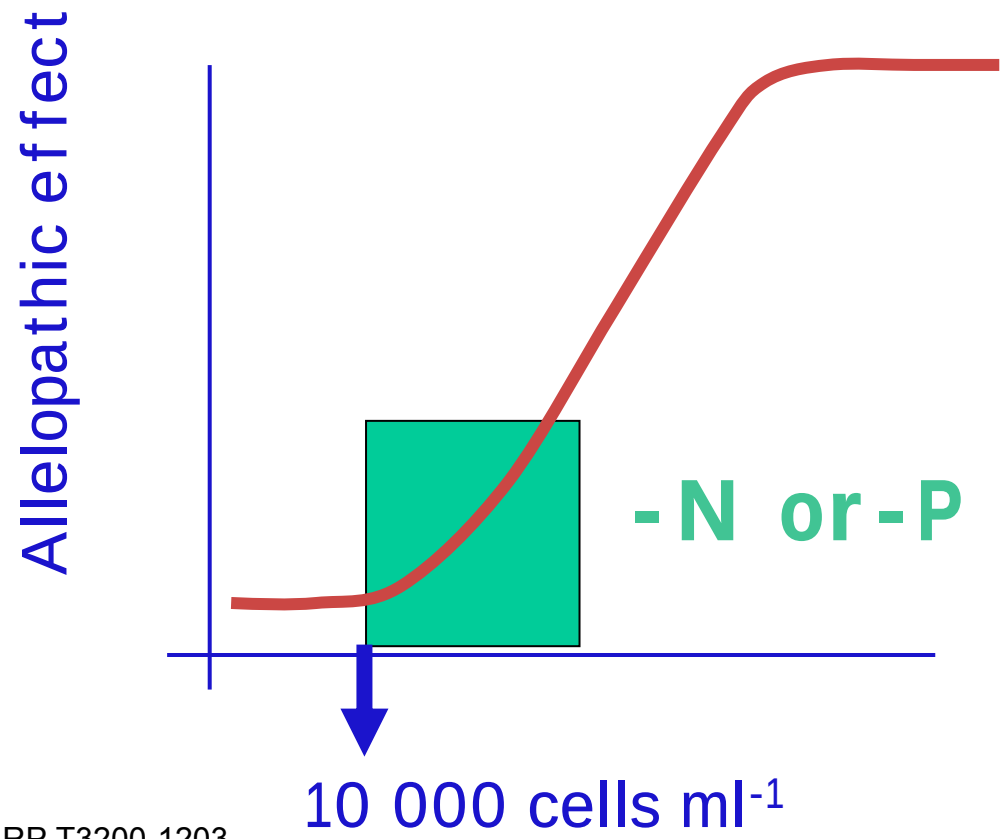
PWD RP T3200-1203



PWD RP T3200-1203

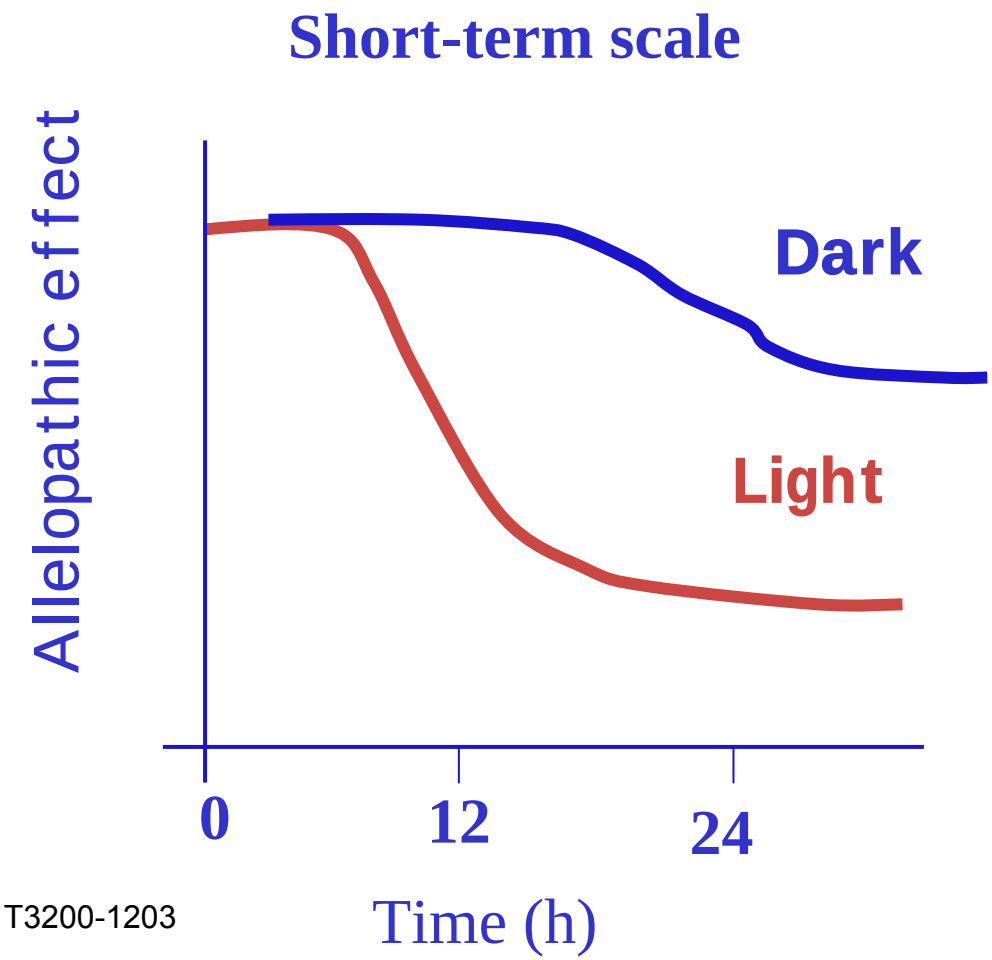
Fistarol, Legrand, Granéli, 2003, MEPS 255: 115-125

Low *Prymnesium parvum* cell numbers can produce enough allelochemicals to stop growth or kill other algae



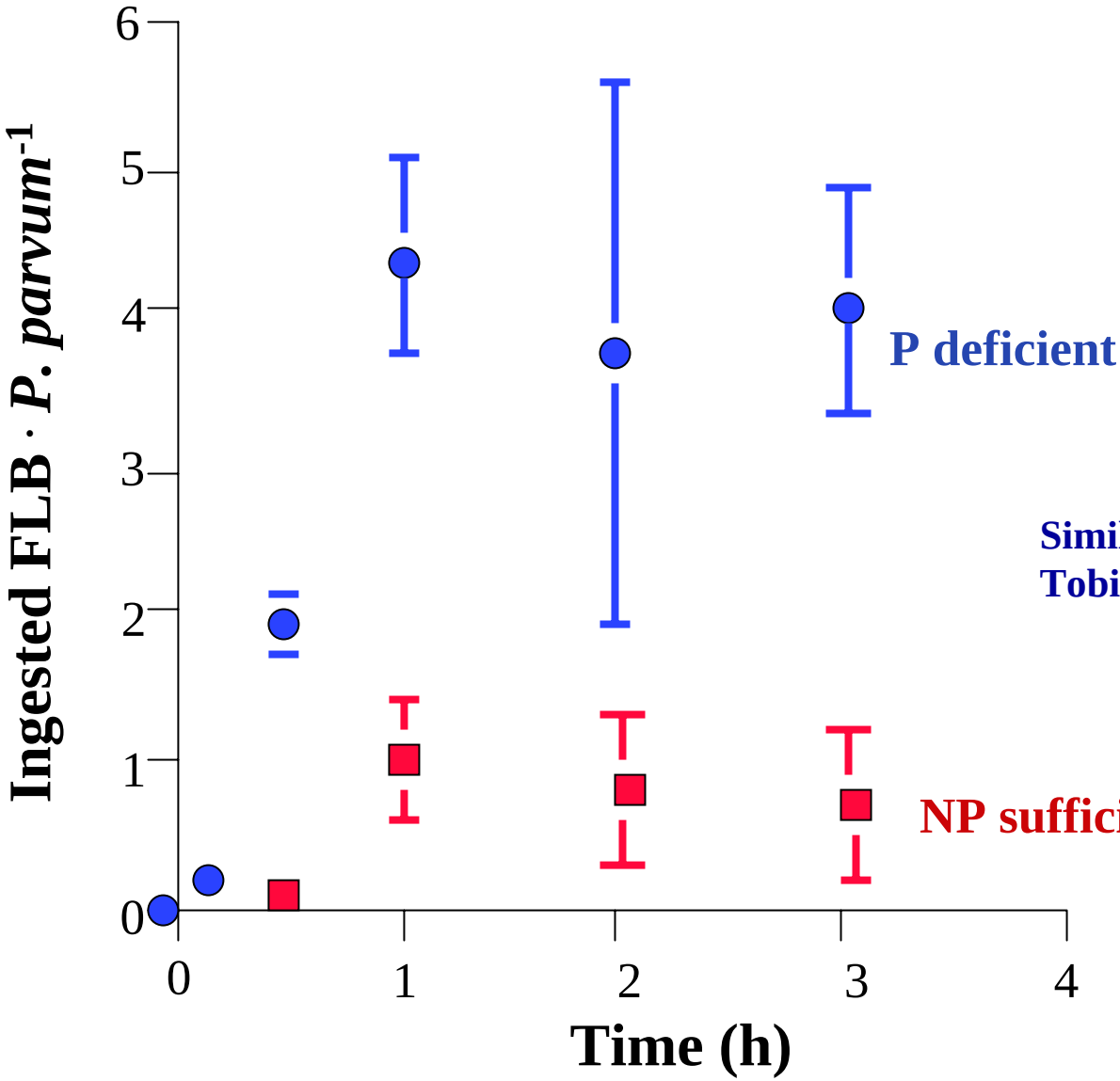
PWD RP T3200-1203

The toxicity of the cell-free filtrate decreases faster in light than in dark



PWD RP T3200-1203

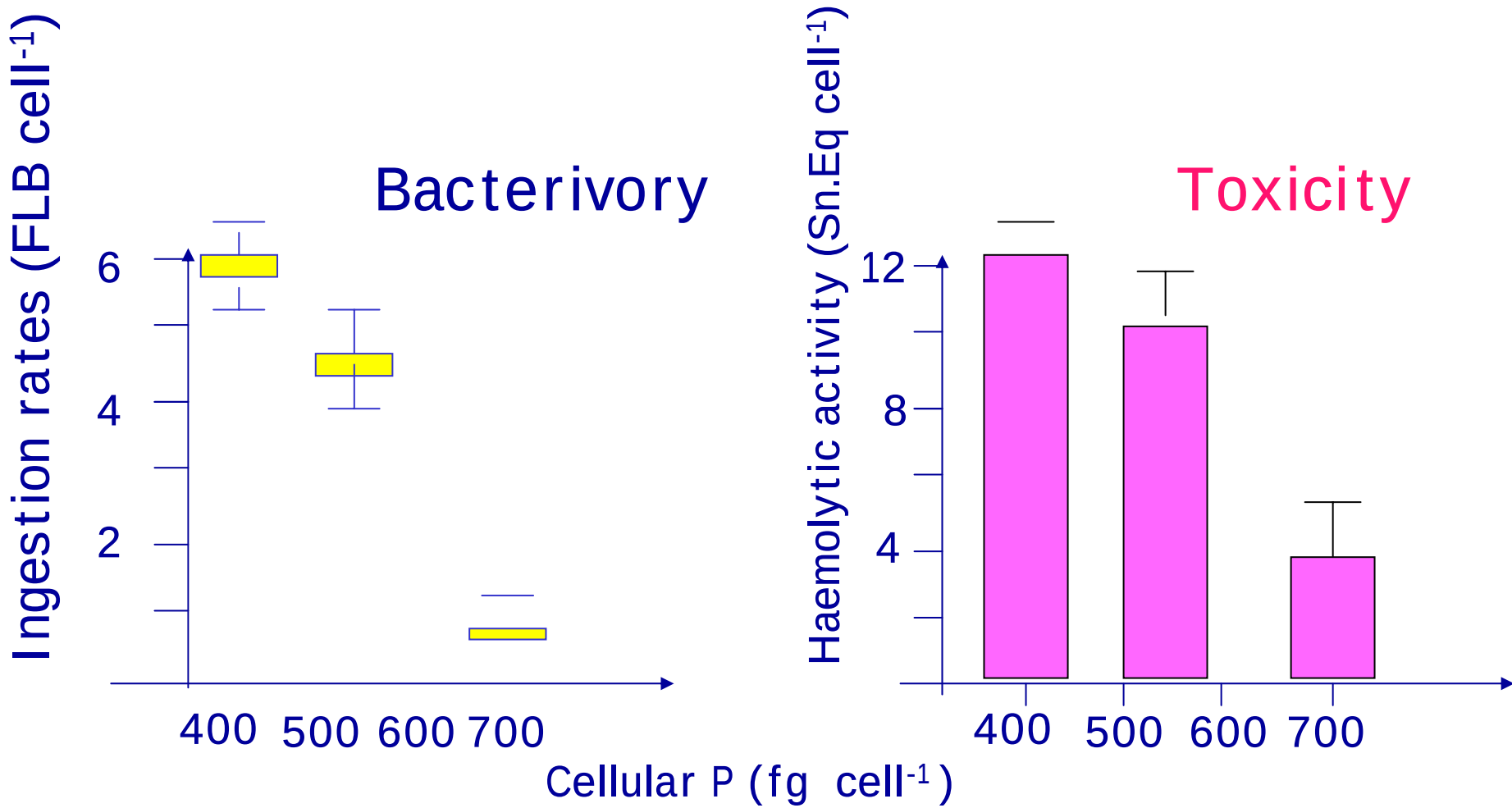
Bacterivory in *P. parvum*



Similar results to Nygaard & Tobiesen 1993, L&O 38:273-279

Legrand, Johansson, Johnsen, Borsheim and Granéli, 2001, L&O 46: 1208-1214

Bacterivory and toxicity in *Prymnesium patelliferum* with different intracellular P content

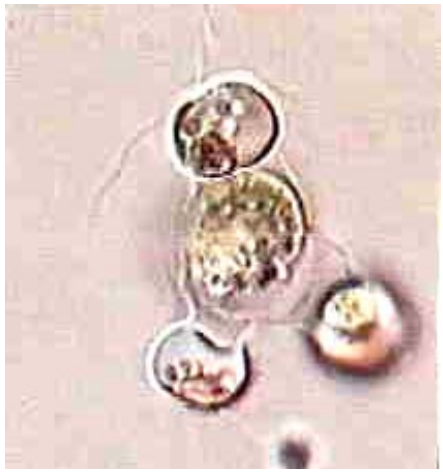


PWD RP T3200-1203

Legrand, Johansson, Johnsen, Borsheim and Granéli, 2001, L&O 46: 1208-1214

P. parvum phagotrophy

(prey ingestion ca. 1 min)



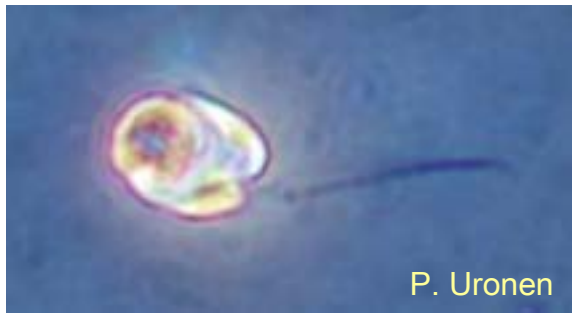
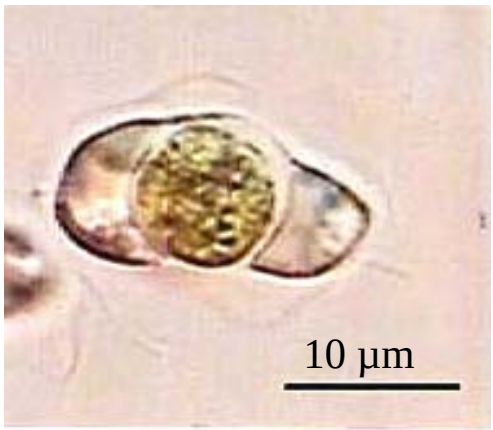
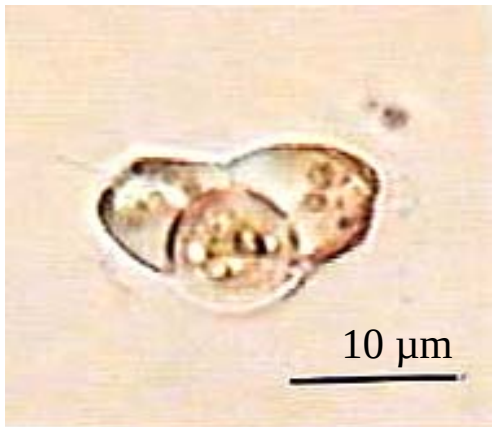
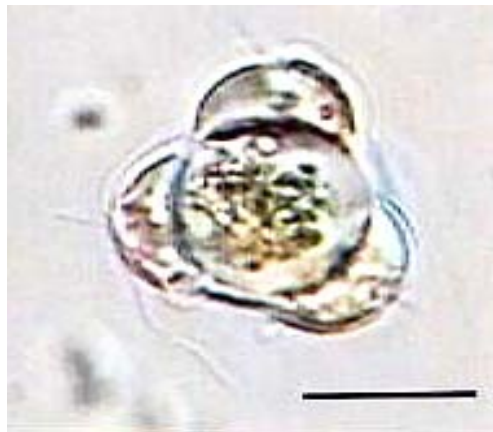


Photo Paulina Uronen



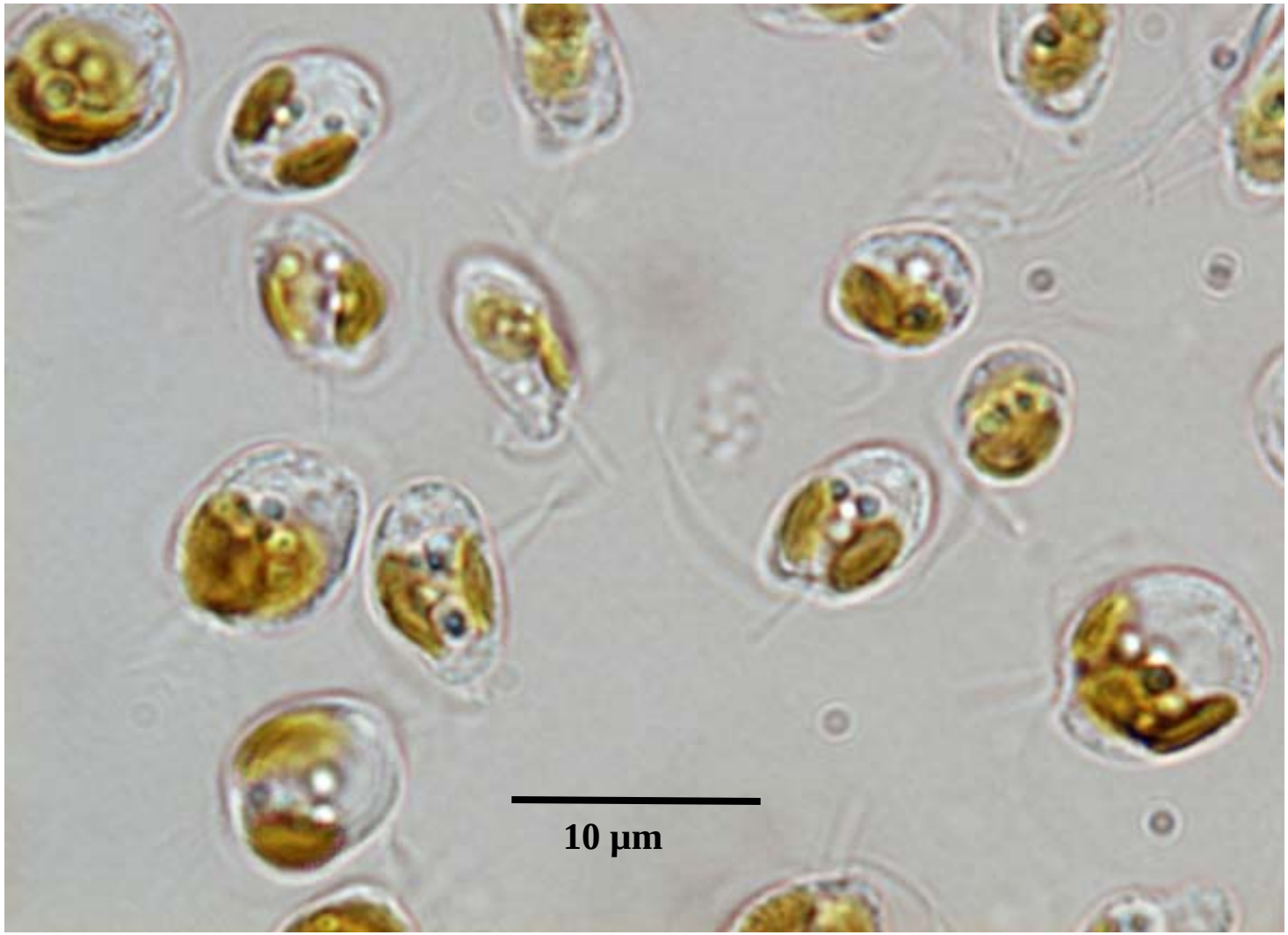




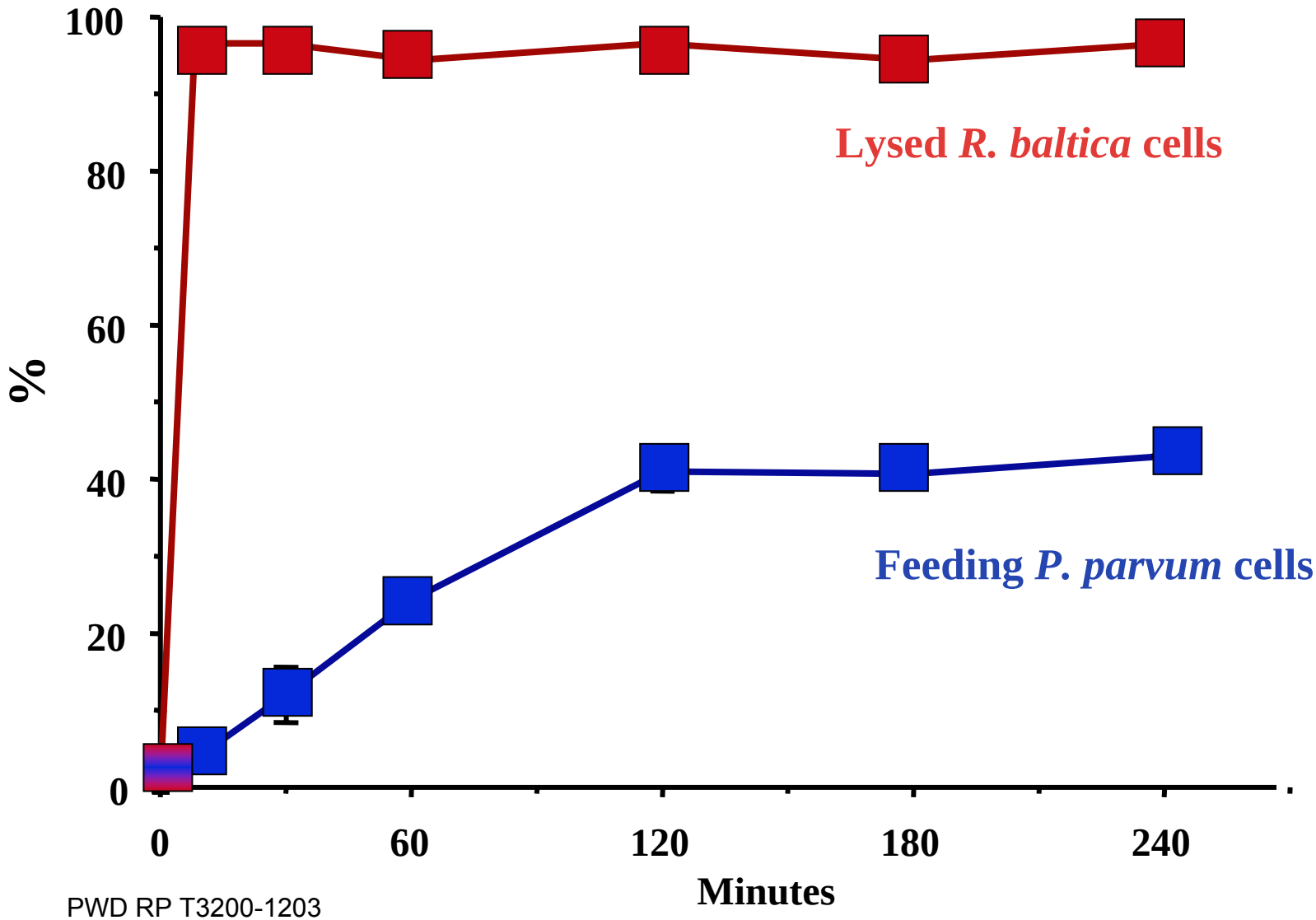
Photos by Urban Tillmann

TIllmann, U. (1998) AME 14: 155-160

Starving cells



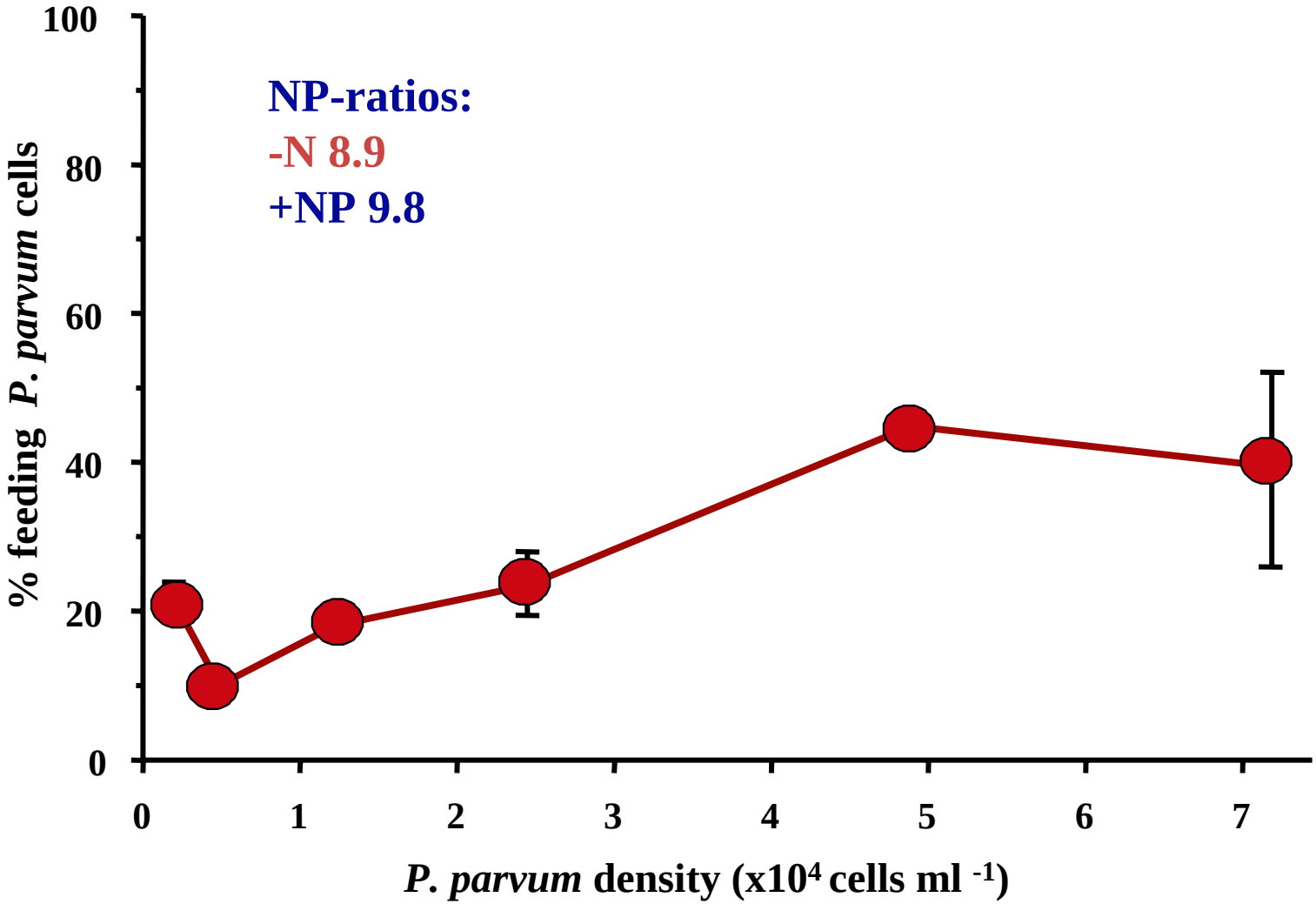
% P. parvum feeding cells and lysed *R. baltica* cells
(-N treatment)



PWD RP T3200-1203

Skovgaard, Legrand, Hansen and Graneli, 2003, AME 31:259-265

P. parvum at different densities feeding on *Rhodomonas baltica*
(-N treatment)

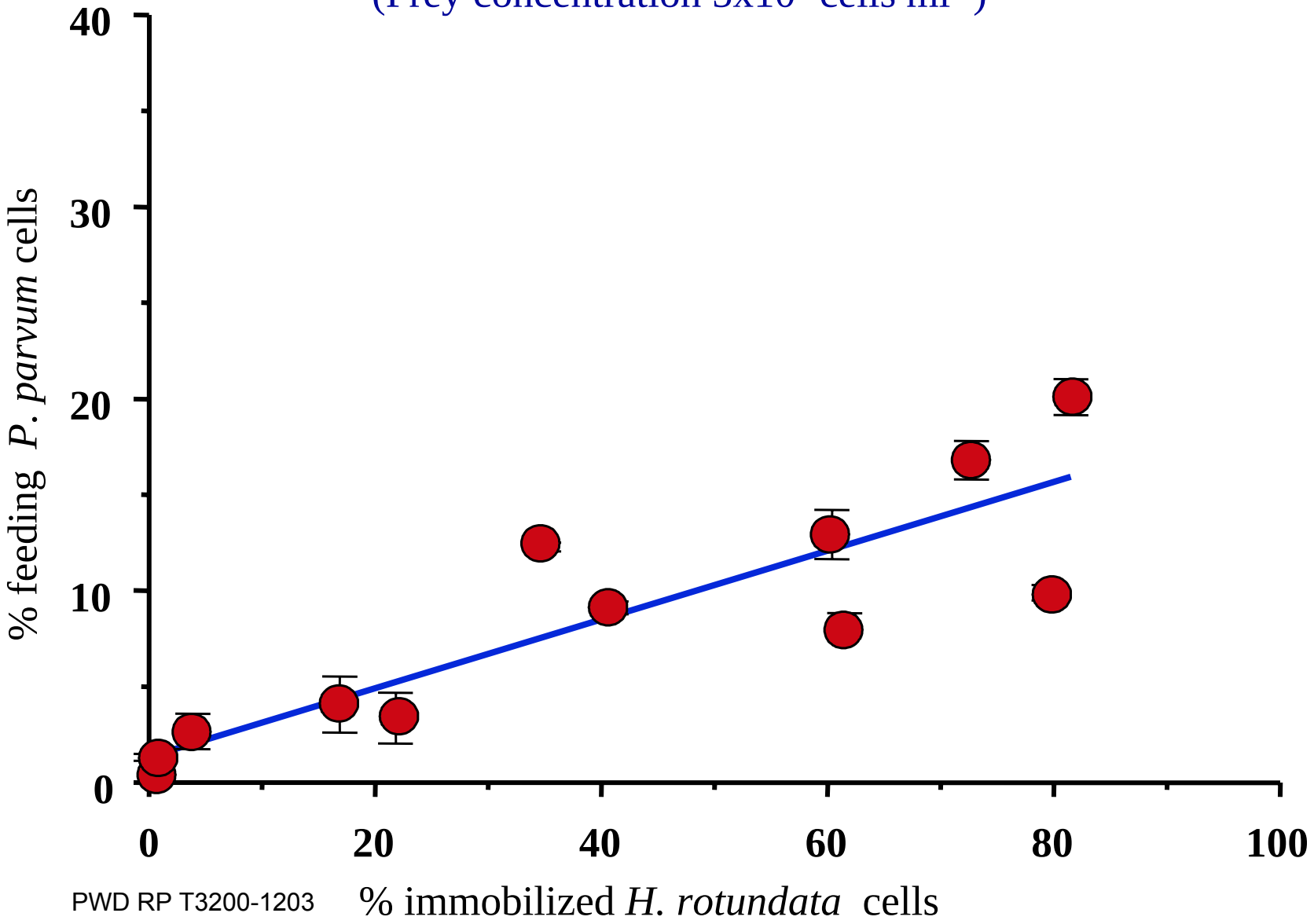


PWD RP T3200-1203

Skovgaard, Legrand, Hansen and Graneli, 2003, AME 31:259-265

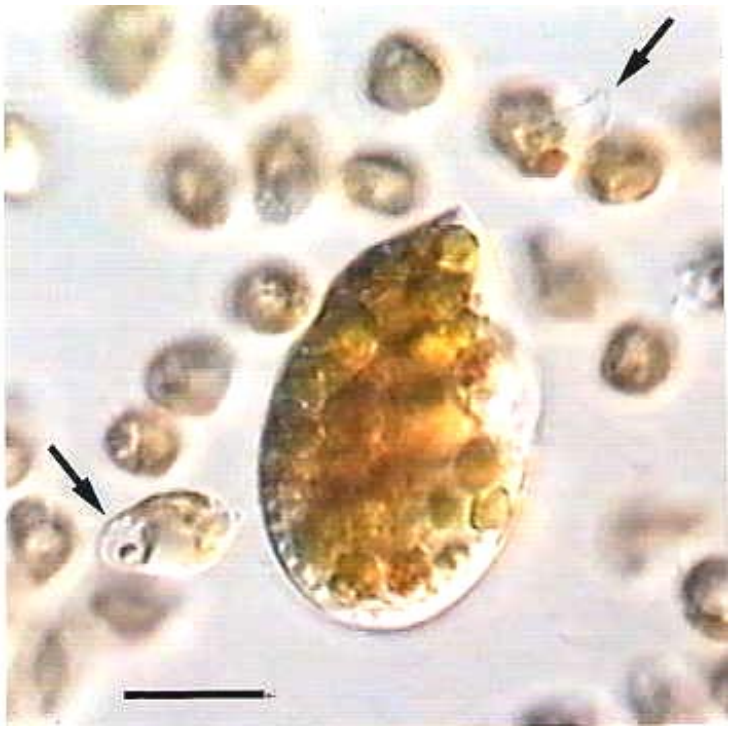
P. parvum feeding cells and toxic effect on *Heterocapsa rotundata*

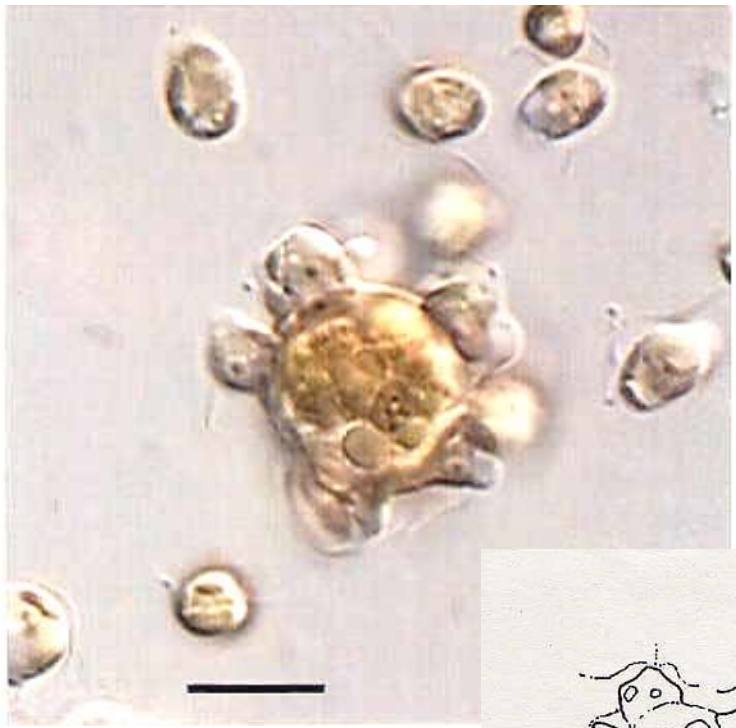
(Prey concentration $5x10^4$ cells ml^{-1})

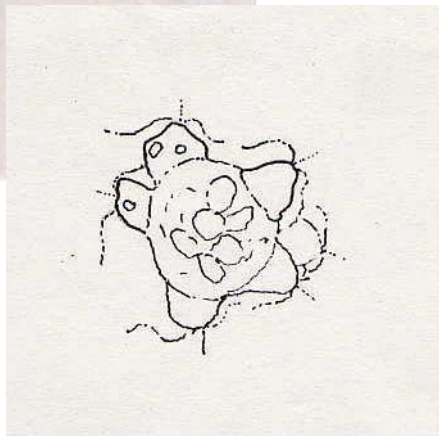


Skovgaard and Hansen, 2003, L & O, 48:1161-1166

Prymnesium phagotrophy on a heterotrophic dinoflagellate

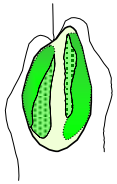




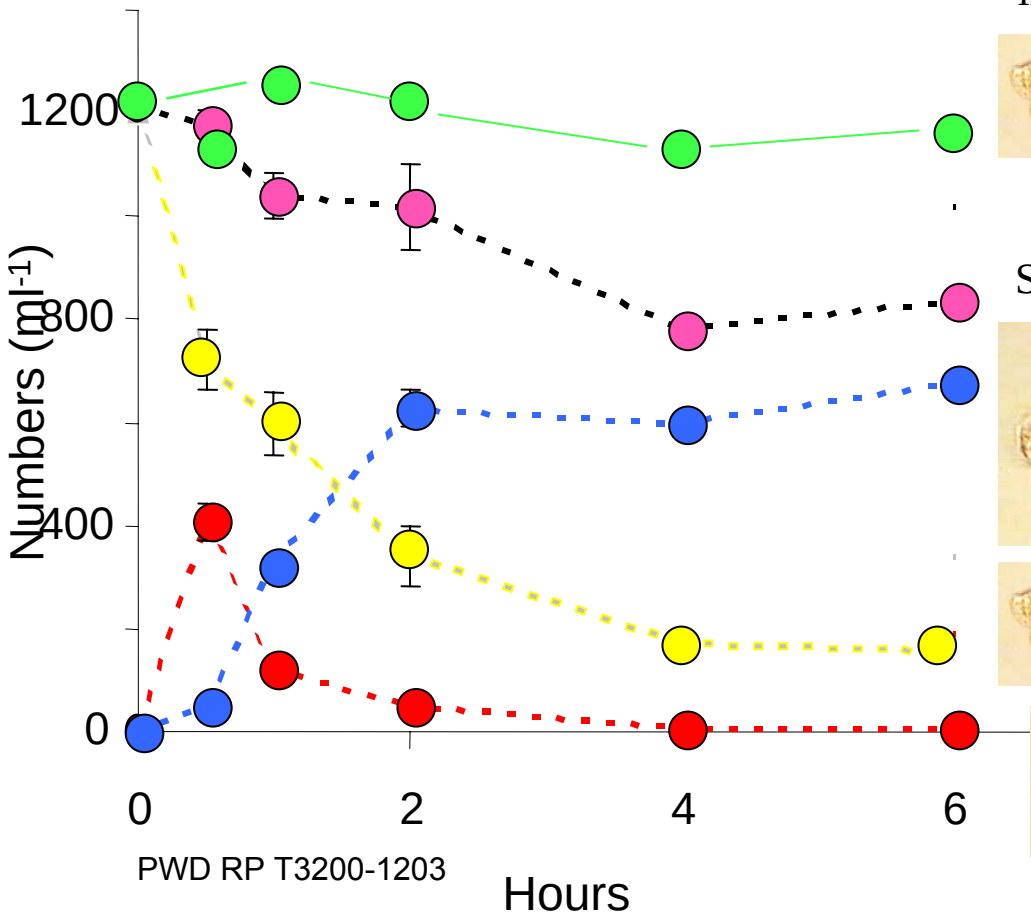


Photos by Urban Tillmann

TIllmann, U. (1998) AME 14: 155-160



Oxyrrhis marina cell lysis in the presence and absence (control) of P- deficient *Prymnesium parvum* cells



intact cells

Control

(No Prymnesium)

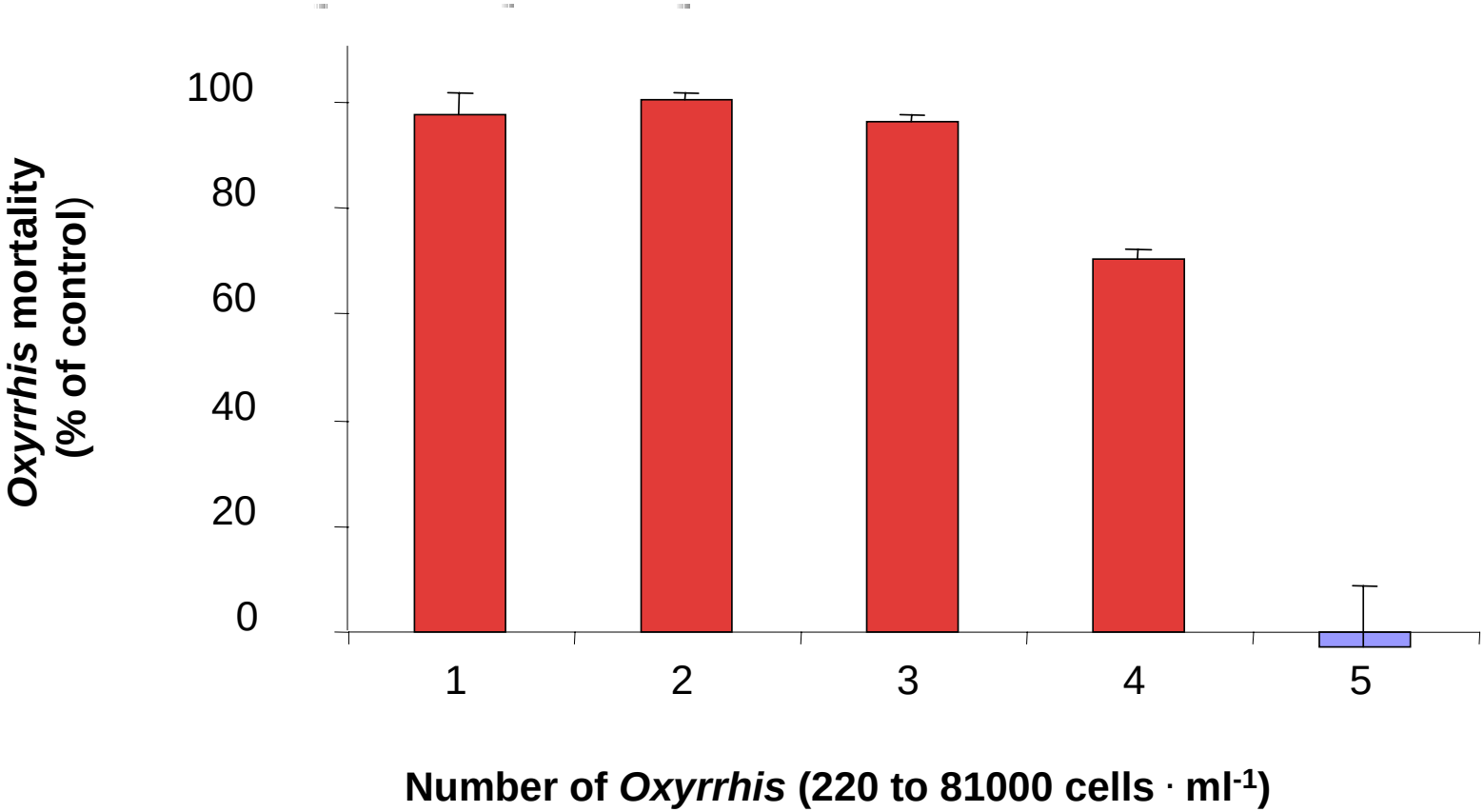
Sum (intact + round cells + feeding aggregates)

feeding aggregates

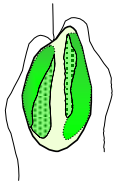
intact cells

round cells (lysed cells)

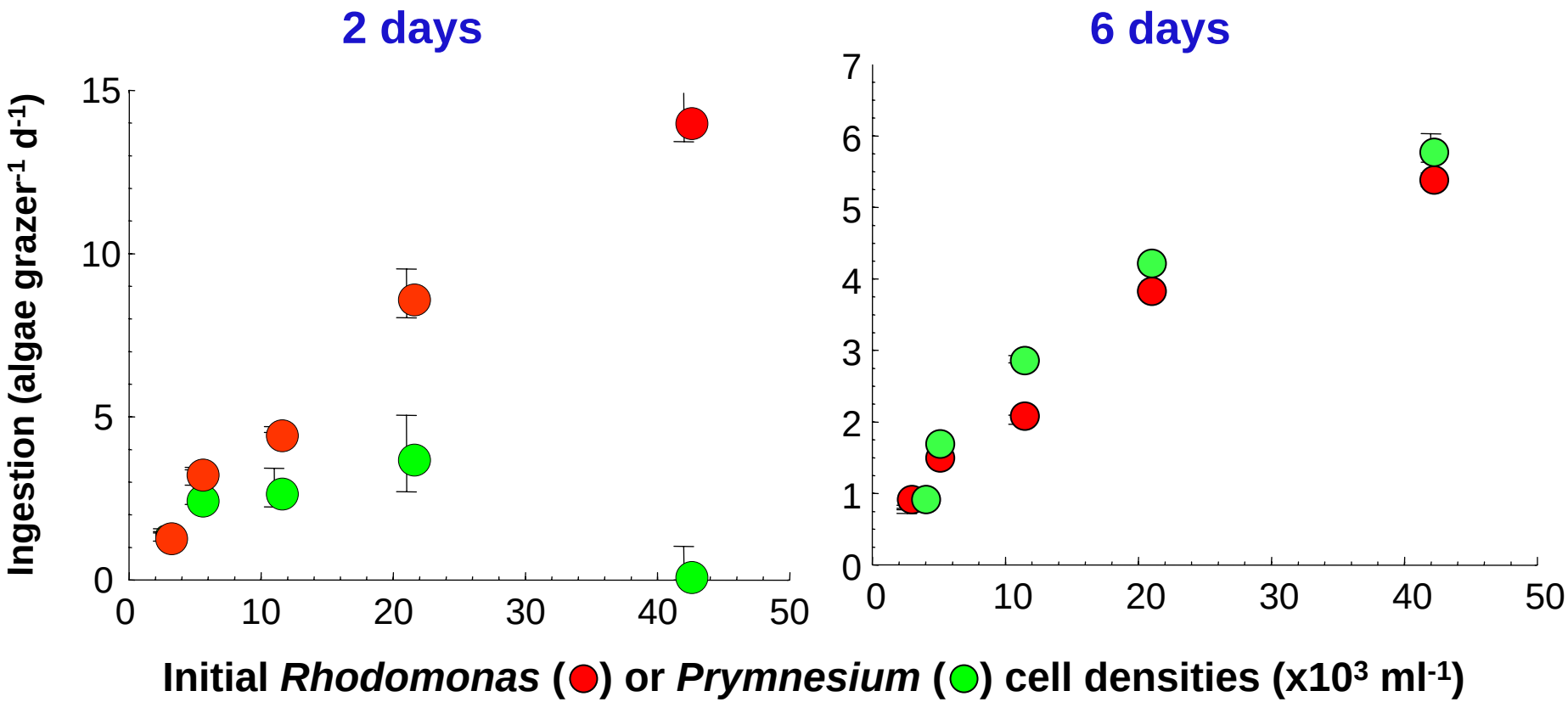
Relation between mortality of *Oxyrrhys* at different cells densities and P- deficient *P. parvum* cells (20000 · ml^{-1})



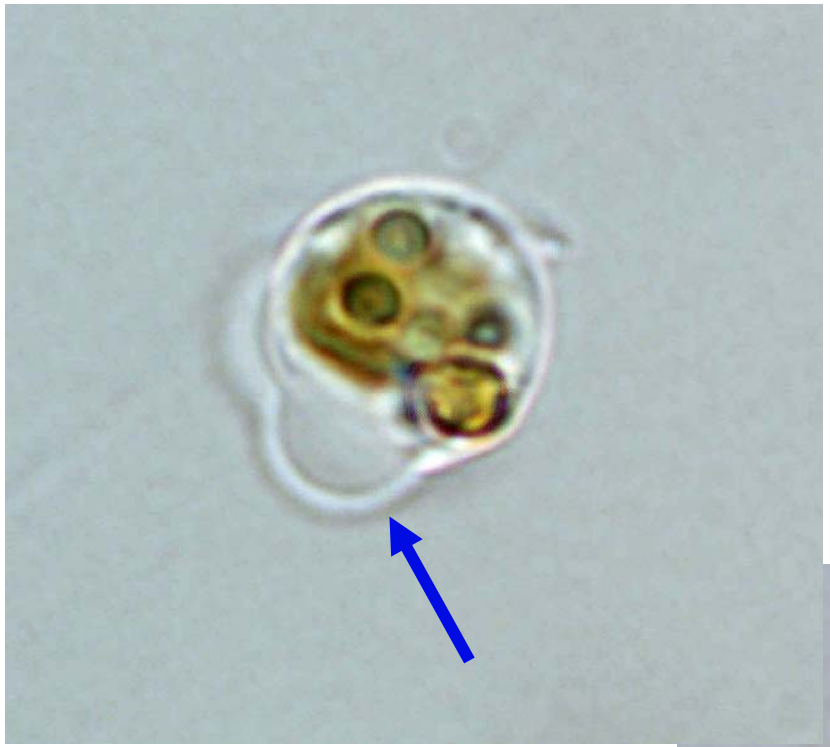
PWD RP T3200-1203



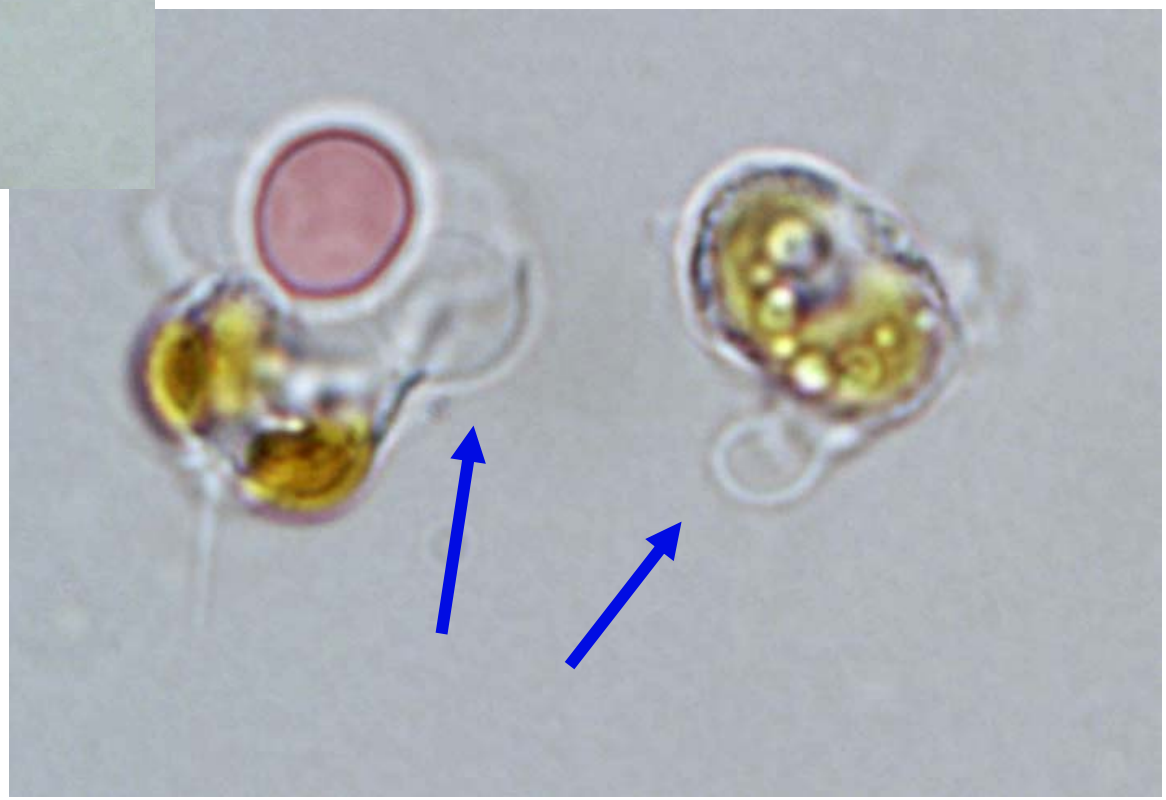
Ingestion of *Oxyrrhis* after 2 and 6 days incubation with increasing *Rhodomas* and *Prymnesium* densities



PWD RP T3200-1203



P. Parvum feeding on horse blood cells



Before capturing a particle *P. parvum* forms vesicles in the posterior end of the cell.

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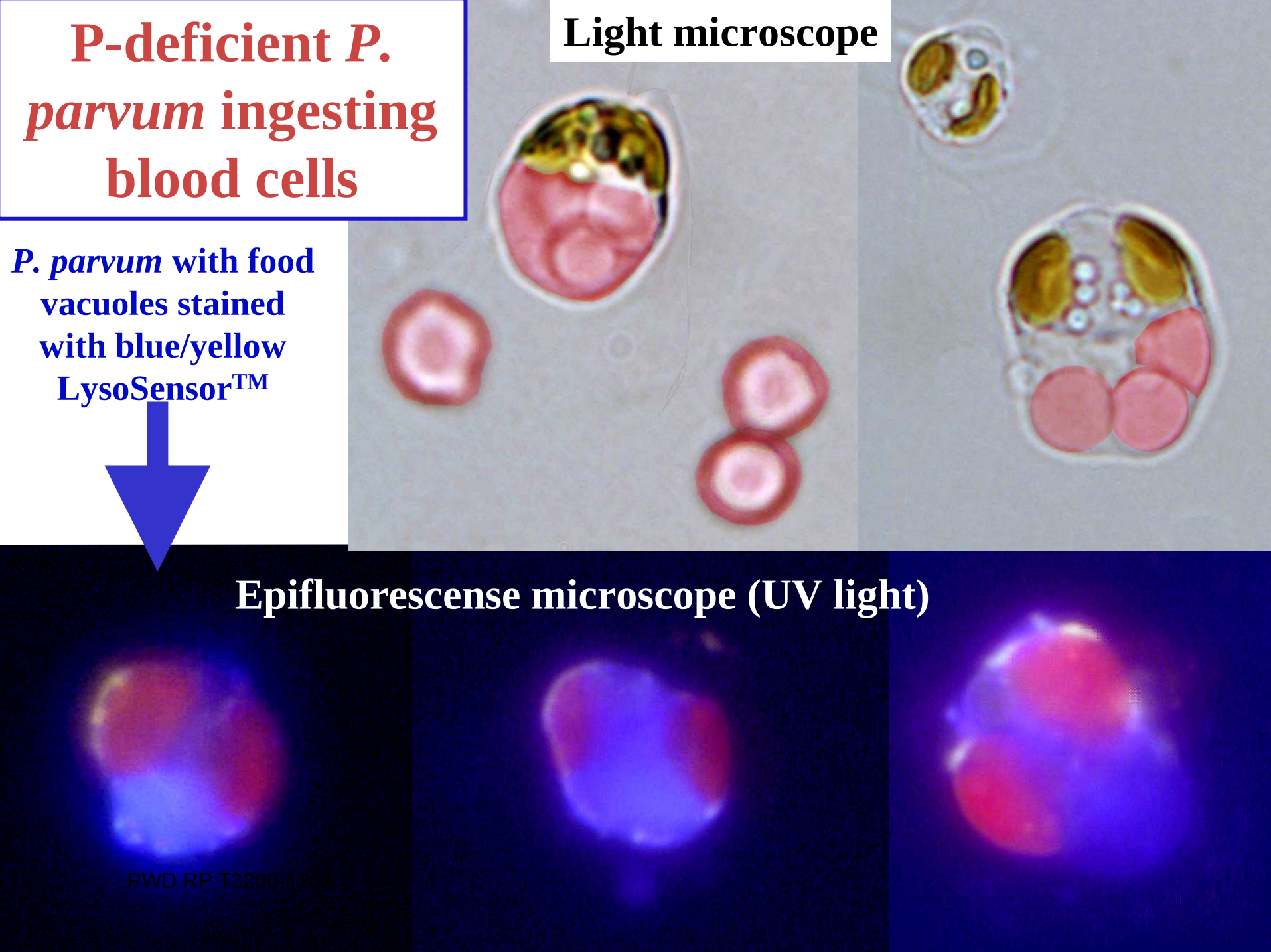
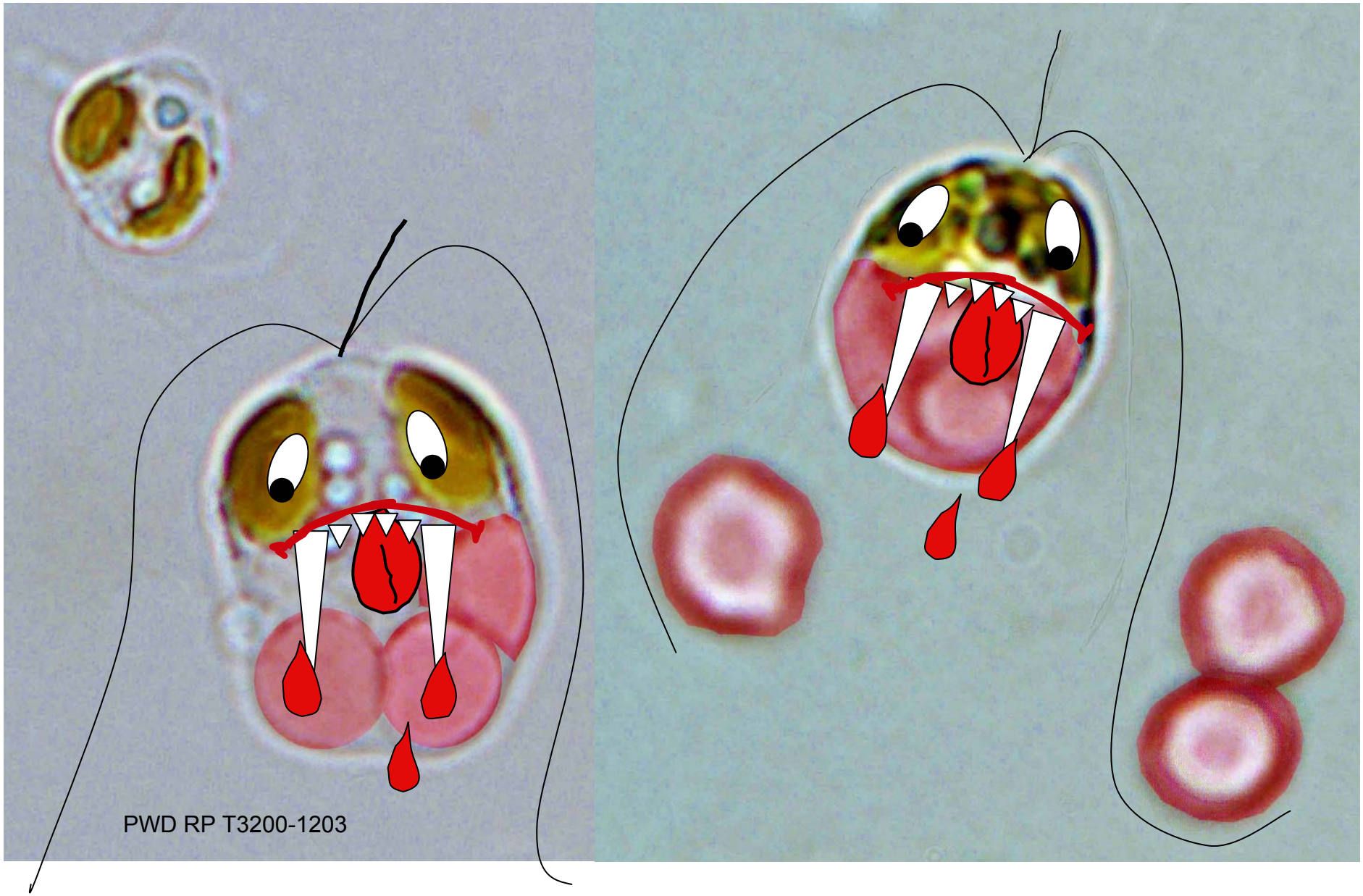
P-deficient *P. parvum* ingesting blood cells

P. parvum **with food vacuoles stained with blue/yellow LysoSensor™**

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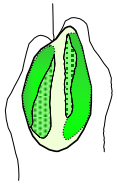


Conclusions

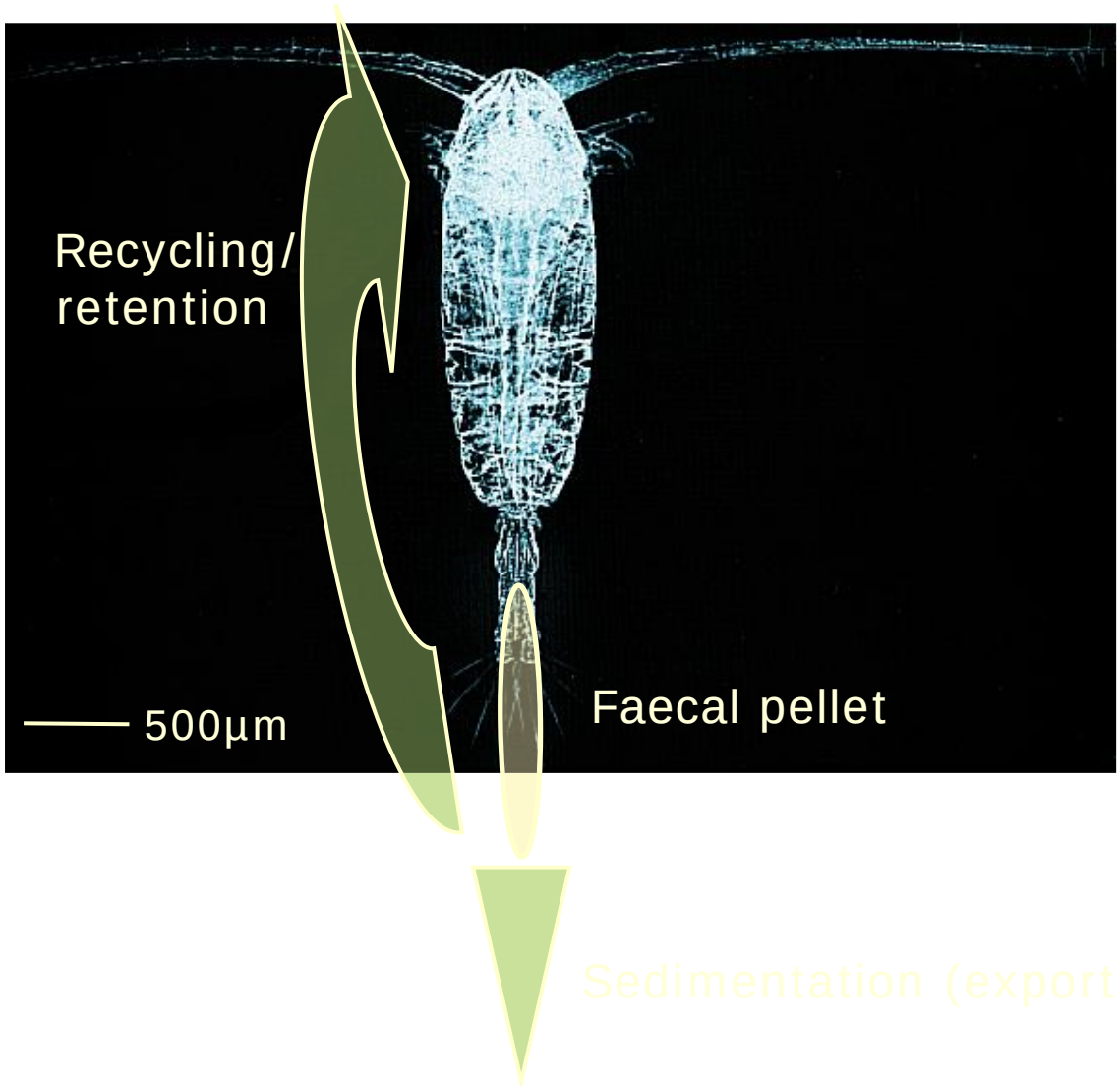
- **Toxicity is the key factor in the success of *Prymnesium* to dominate the plankton food web**

If toxicity is low: *Prymnesium* allelopathic effect is low (or non-existent) and is a suitable prey for grazers

- **At high toxicity levels: (a) most phytoplankton groups are killed, some few species/groups may survive by being more resistent (cyanobacteria, bacteria)**

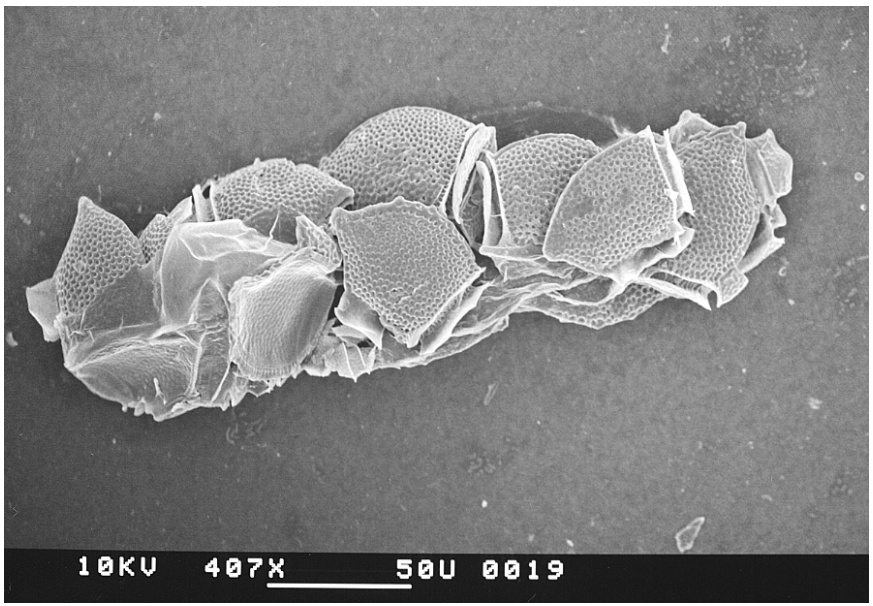
- **At high toxicity levels(b) *grazers* are rapidly killed and ingested by *Prymnesium*, thus reversing the classic grazing pathway between protozoans and algae**

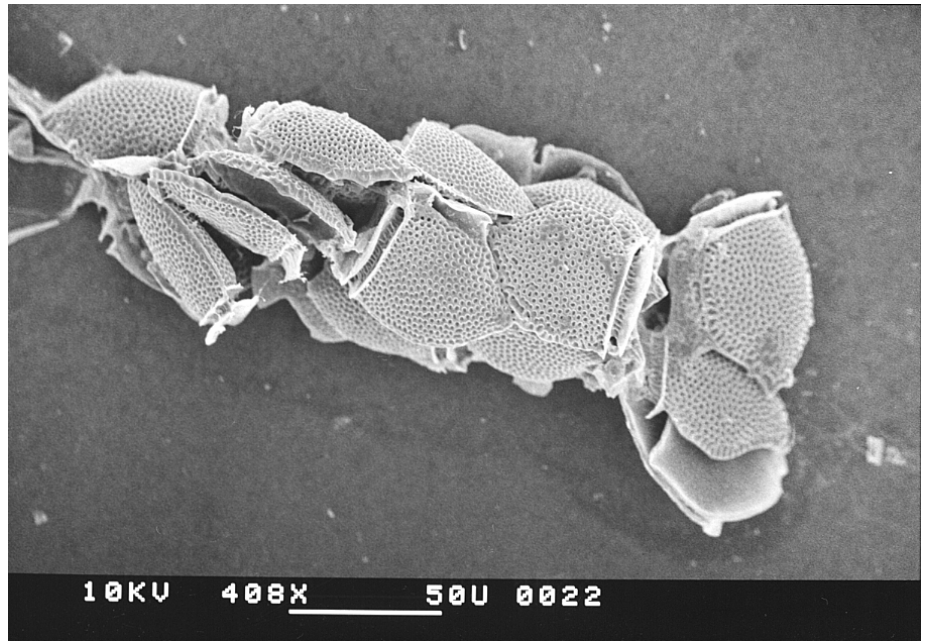
Calanus helgolandicus



C. helgolandicus pellets

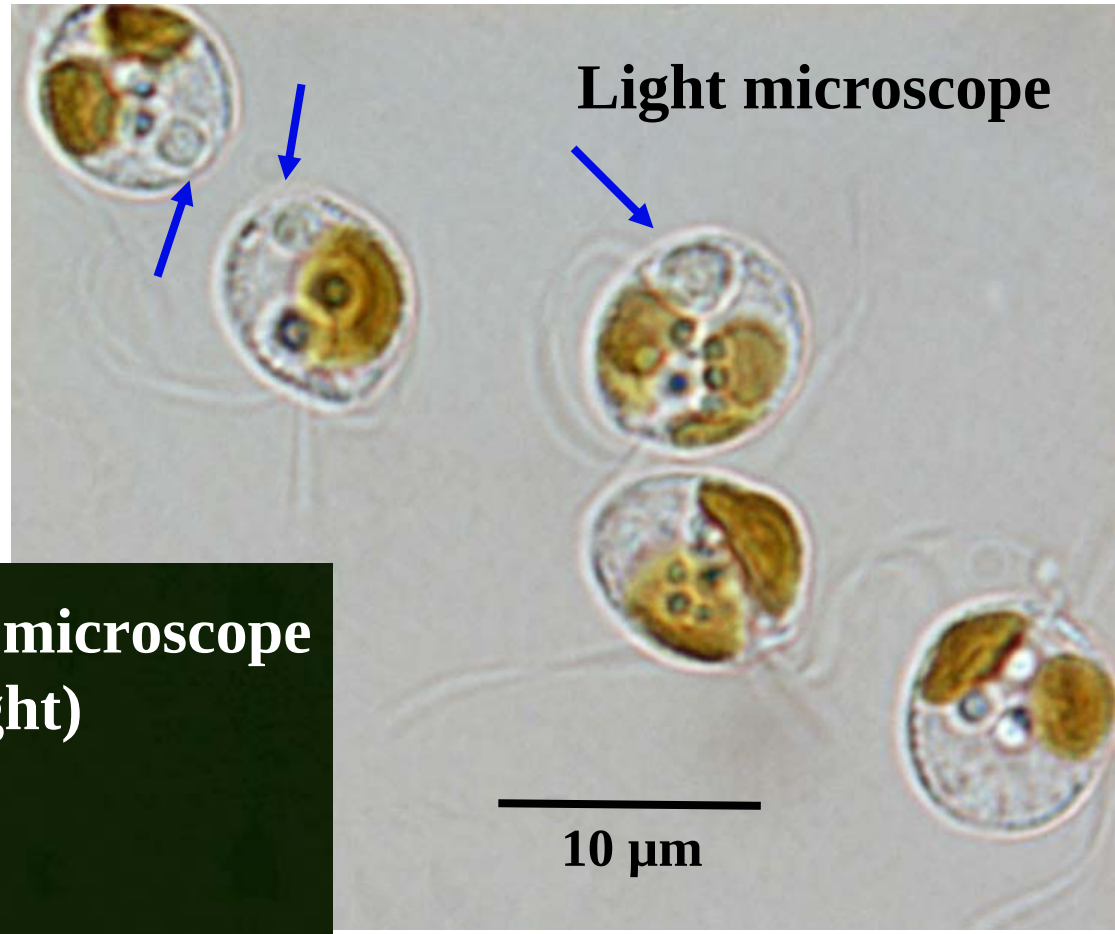
- Pictures taken with SEM
- 400 X magnification





PWD RP T3200-1203

***P. parvum* with ingested particles (arrows)**



Laboratory Studies on *Prymnesium* – Structure, Reproduction, Salinity and Simple Bioassay

Paul Kugrens

Department of Biology, Colorado State University, Ft. Collins, CO 80523 USA

Abstract.--Three isolates of *Prymnesium parvum* from different geographic localities were studied with respect to their structure, reproduction, and range of salinity tolerances. Two isolates were obtained from the UTEX culture collection, which included an isolate from Texas, and the third was isolated from a high plains lake in Wyoming. All strains have a similar cytology and cell morphology, although cell shapes varied within cultures and in different salinities. In culture *Prymnesium* reproduced exclusively by asexual reproduction through cell division. Several media were used in this study. One medium consisted of seawater-based medium, another was an artificial seawater medium, and a third used water from highly alkaline lakes from Wyoming. All strains grew equally well in all seawater media and in the high alkaline water medium from Wyoming lakes, although the marine strain had the lowest growth rates in all of these media. Except for isolate 995 from UTEX, the other two strains grew best at a salinity of 16 ppt NaCl. Induction of spore formation was attempted; however, spore formation was never observed in any treatments, which included nutrient depletion, high or low temperatures, desiccation, and aged cultures, some of which were 6-12 months old. A simple canine blood bioassay technique for testing toxicity was developed since prymnesin is a hemolytic toxin. The blood bioassay consisted of centrifuged and lysed *Prymnesium* cells that were re-suspended in a saline buffer solution. Heparinized canine red blood cells were added to the buffer saline solution that contained the lysate, mixed and examined microscopically. All *Prymnesium* isolates caused 100% hemolysis, whereas all red blood cells remained intact in parallel control experiments that utilized *Chrysochromulina* spp. and *Dunaliella*. Since *Prymnesium* grows best at higher pH, a possible mechanism for its rapid growth will be discussed and is based on the specific membrane arrangement in golden algae cells. Possible biocontrol, using protists belonging to *Kathablepharis*, and the efficacy of using this genus will be presented. Finally, the massive fish kill that occurred in Colorado in May 2002, which was attributed to *Prymnesium*, and the misinformation surrounding this fish kill, will be discussed.

View the presentation

Prymnesium parvum Laboratory Studies: Structure, Reproduction, Salinity Tolerance & Bioassay

Paul Kugrens
Department of Biology
Colorado State University
Fort Collins, Colorado 80523

PWD RP T3200-1203

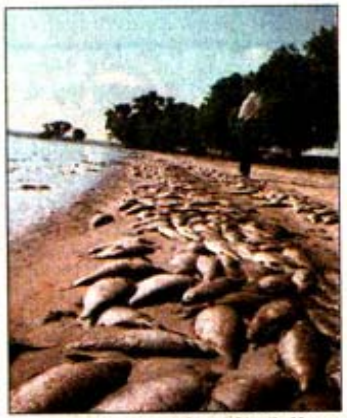
News Release

- **May 28, 2002 - massive fish kill in Prewitt Reservoir in Colorado**
- **500,000+ fish were killed**
- ***Prymnesium* implicated**
  - **Water had a golden brown color**
  - **Identification based on descriptions provided by fisheries personnel in Texas via telephone**

News of Massive Fish Kill at Prewitt Reservoir in Colorado

DENVER AND THE WEST

www.denverpost.com/news • THE DENVER POST / Section B



The Denver Post / Charlie Meyers

Biologist Jay Stafford of the Division of Wildlife surveys the fish kill at Prewitt Reservoir on Monday. The lake northeast of Fort Morgan lost all its game fish to a deadly microbe. No one knows where it came from, or where it might appear next.

Microbe KOs reservoir fish

Golden algae's appearance a mystery, game fish die-off rapid

By Charlie Meyers
Denver Post Outdoor Editor

MERINO — A microscopic organism commonly called golden algae was responsible for the death of nearly every game fish in Prewitt Reservoir last week. Among the more disturbing aspects of the scourge is that wildlife officials don't know where it came from.

And worse, they can't predict where it might go from here.

The only certainty of the sudden die-off that struck the popular impoundment last week is that, save for a few hardy carp, nothing survived.

"It looks like a total loss," Division of Wildlife biologist Jay Stafford declared as he trudged through a seemingly endless pile of carcasses strewn along shore.

Pete Walker, chief pathologist at the DOW Fish Health Lab in Brush, identified the culprit as golden algae, a potent neurotoxin. It quickly enters the bloodstream of fish to cause paralysis and asphyxiation.

First identified in Israel and widely known in the aquarium trade, golden algae made its first appearance in North America approximately two years ago in Texas, where it has become a substantial problem. A golden algae bloom also could be to blame for the loss of thousands of fish in the Pecos River in New Mexico, according to that state's Game and Fish Department. Three fish kills occurred during a two-week period there late last month and an estimated 5,000 fish were found dead. How the organism might have migrated to Colorado and, more specifically, to Prewitt may be forever a matter of speculation.

"Boat bilge, bait buckets, outboard shafts, bird plumage," senior biologist Steve Puttmann ticked off a short list of possible carriers. To help prevent any further transfer, DOW moved quickly to close the reservoir to fishing, wading and boating. The pathogen poses no danger to humans or other warm-blooded creatures.

Wildlife managers worry the pathogen might have arrived among the ample feathers of white pelicans that winter on the Texas coast then migrate north to the reservoirs of the Midwest, including virtually every major impoundment in Colorado. Hundreds of pelicans gathered over the weekend at Prewitt to gorge on dead fish.

Please see **FISH** on 6B

Fish Kill at Prewitt Reservoir

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Catch of the Day

PWD RP T3200-1203

Pelicans putative of transport agents of *Prymnesium* to Colorado

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Golden-Brown Color of the Water

Objectives of this Presentation

- To provide cytological data for *Prymnesium*
- To provide information on cyst formation
- To determine range of salintity tolerances for three strains
- To induce cyst formation and to determine cyst structure

Objectives of this Presentation (Continued)

- To describe a simple hemolytic bioassay technique
- To propose a mechanism for increased growth in high pH conditions
- To explore possible biocontrol for *Prymnesium*
- To determine whether **mixotrophy** occurs
  - Photosynthetic organisms ingesting cells
  - Mixed nutrition

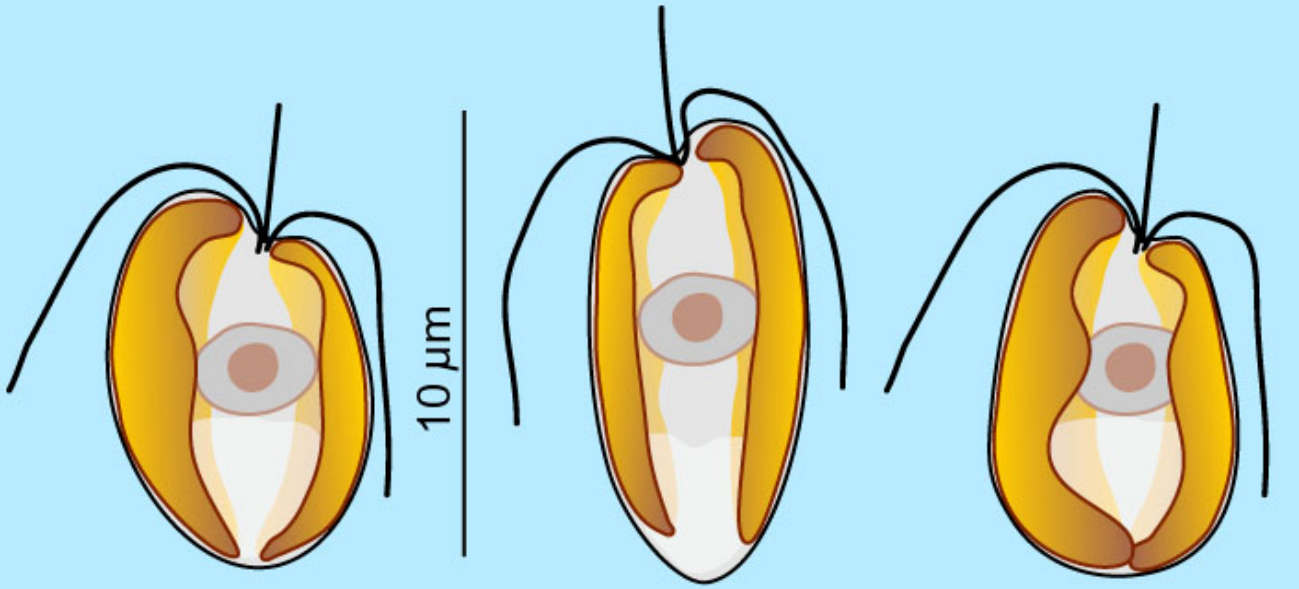
Prymnesium Strains in Culture

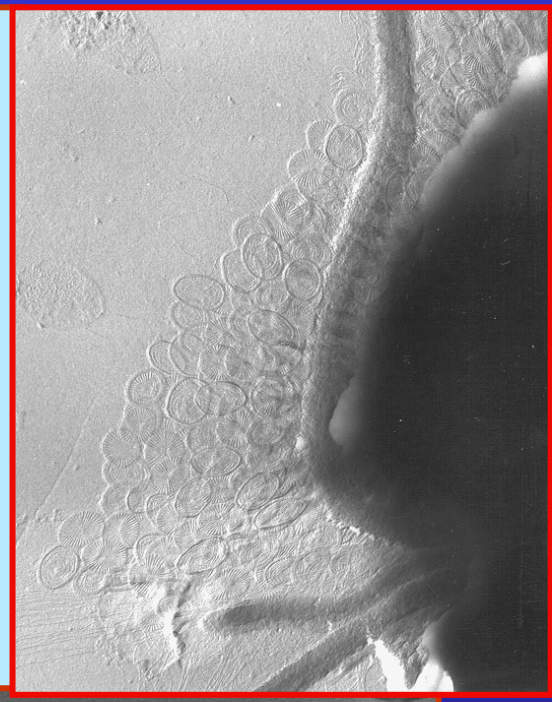
- UTEX - 995 Plymouth, England
- Texas Isolate
- Texas Isolate Lubbock Canyon
- Wyoming Isolate Twin Buttes Lake
- Latvian isolate - Jurmala, Baltic Sea

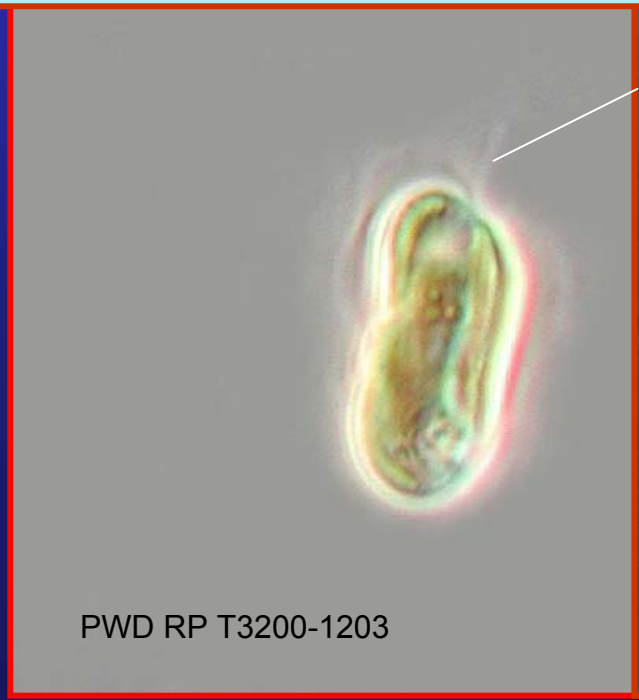
Strains of *Prymnsium parvum* Used in this Study

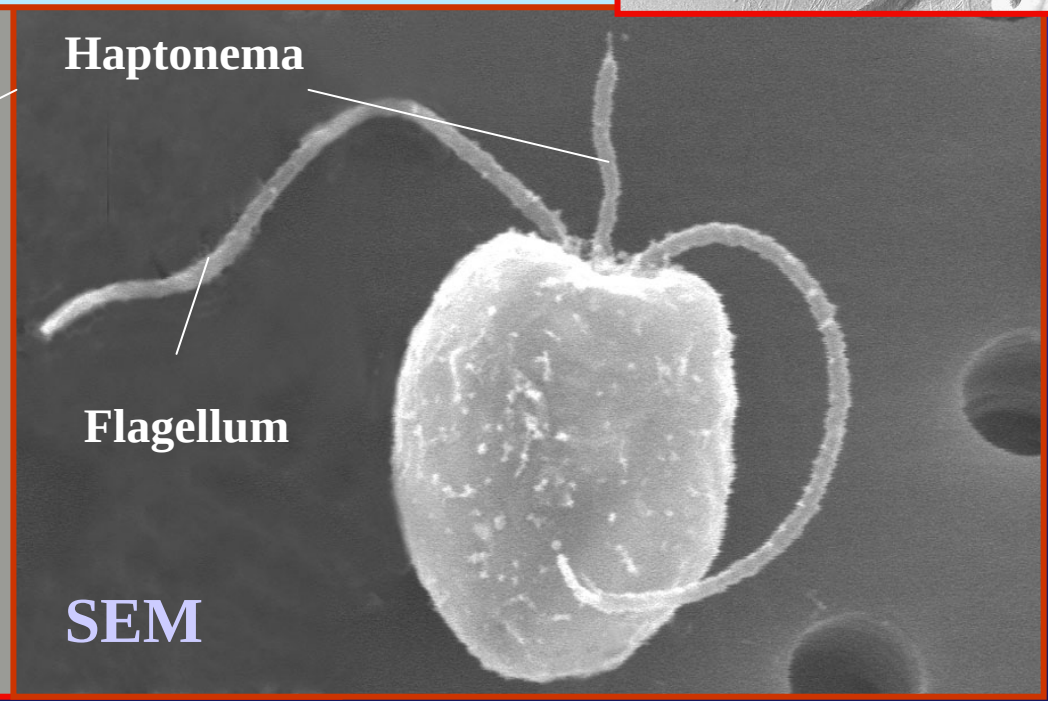
- **UTEX - 995**
- **Texas Isolate**
- **Wyoming Isolate - Twin Buttes Lake**

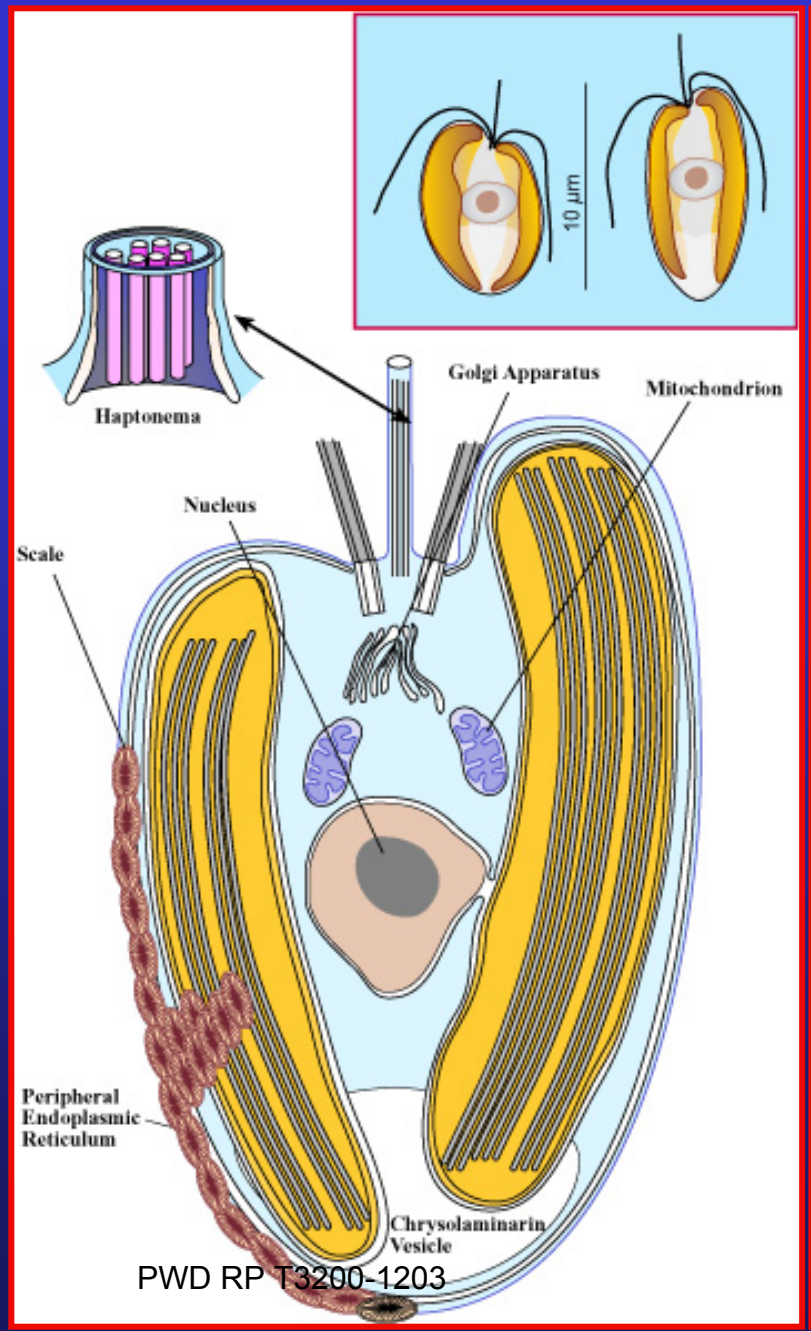
Structure of *Prymnesium*


10 µm




PWD RP T3200-1203


Haptonema
Flagellum
SEM



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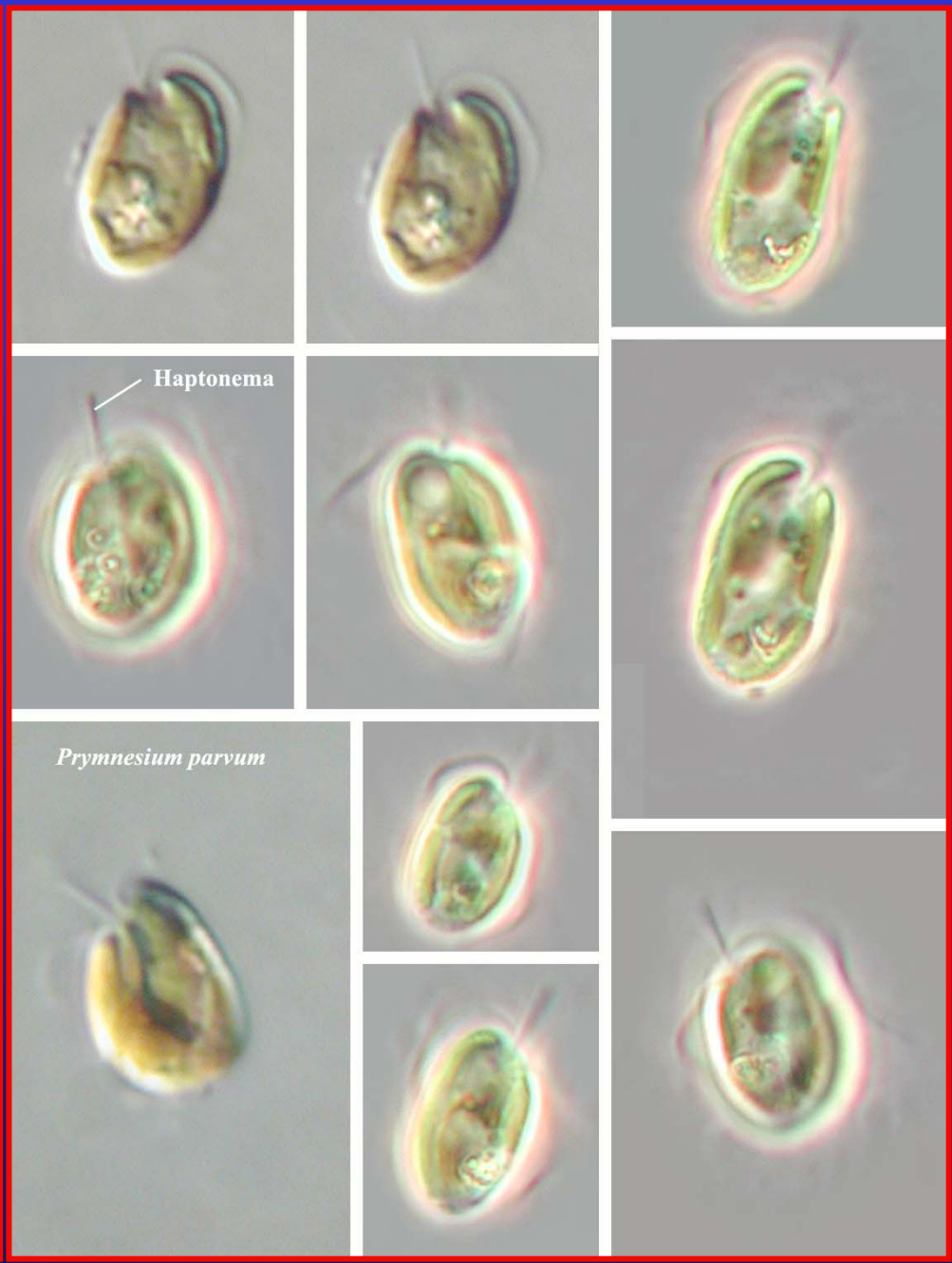
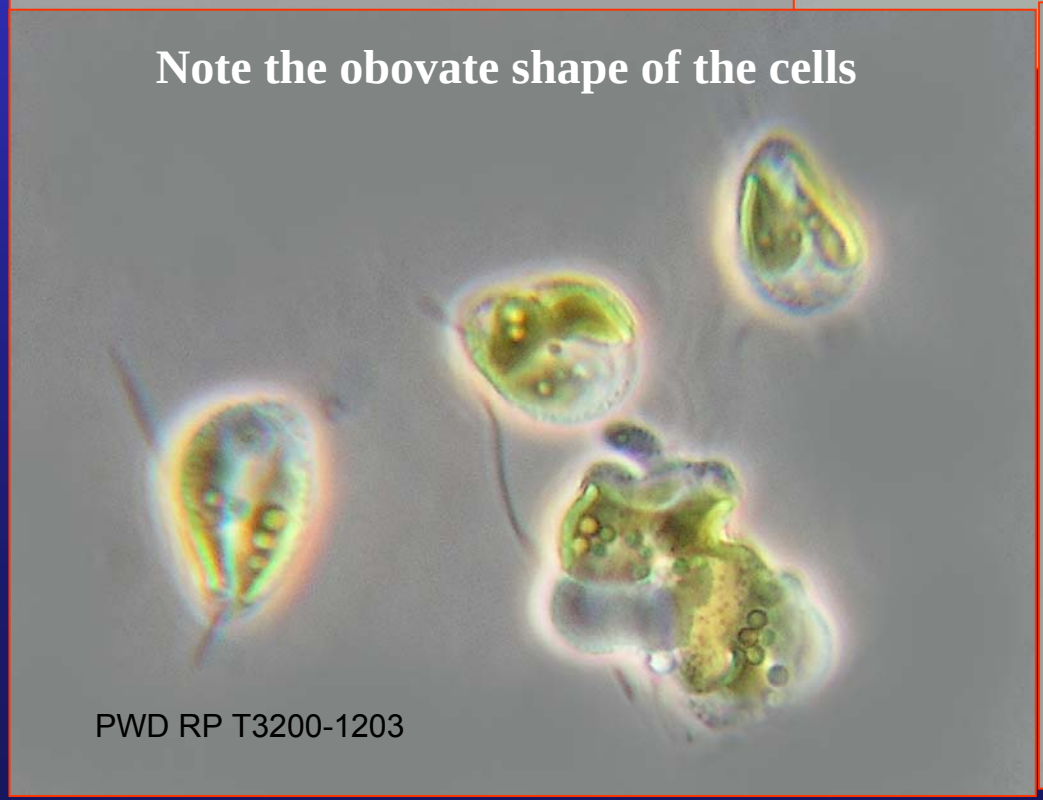
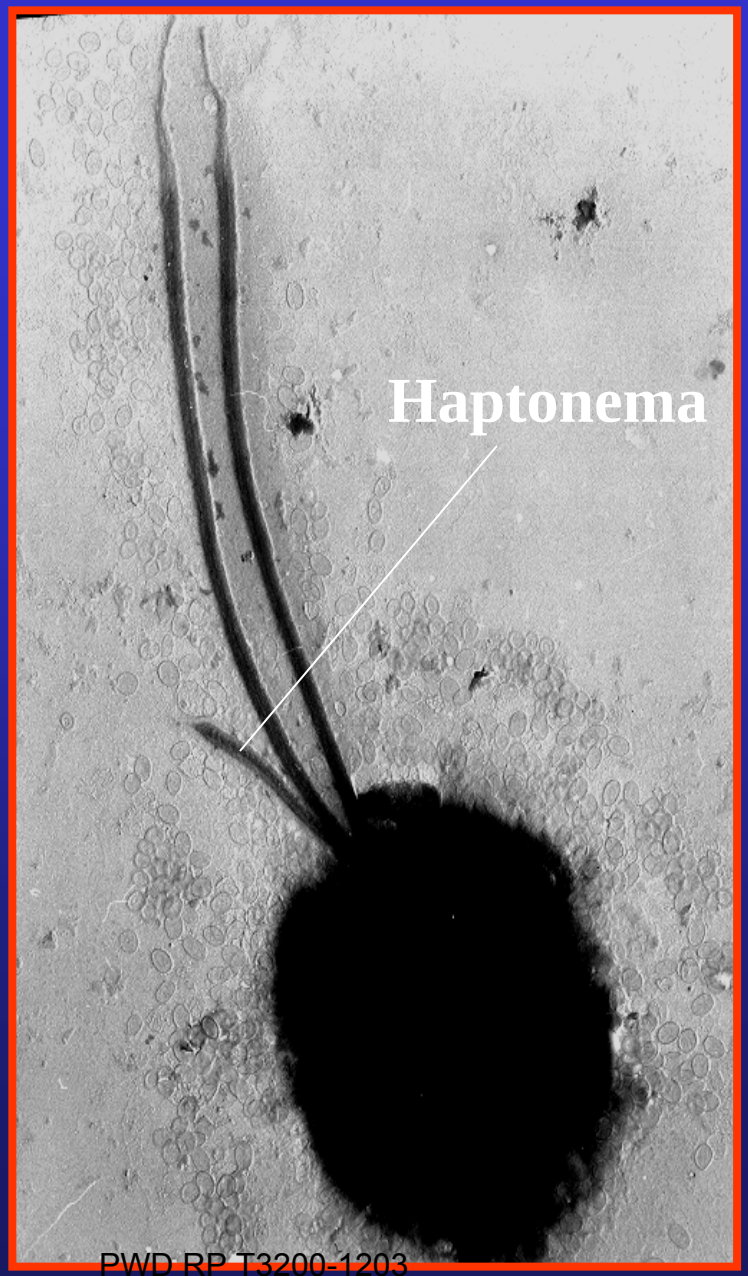
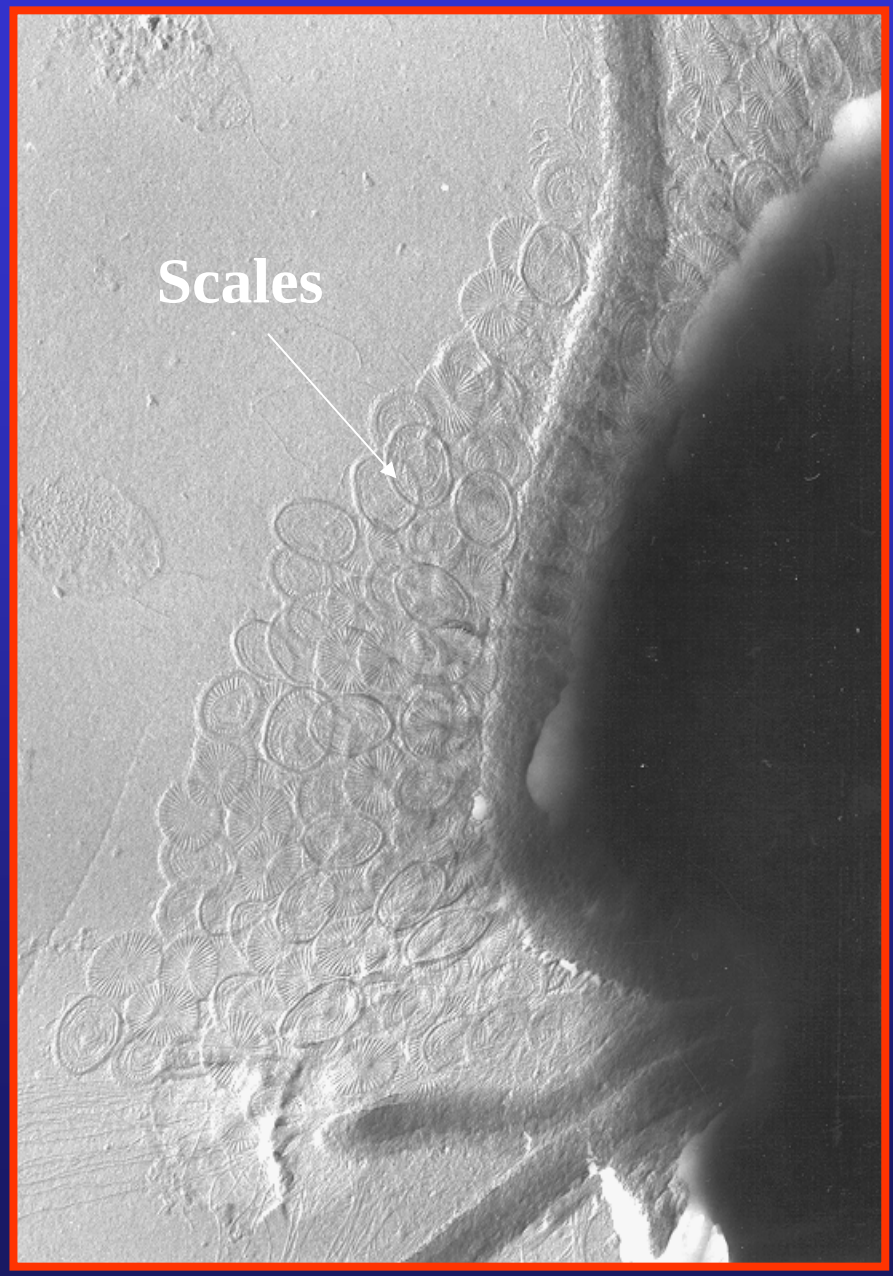


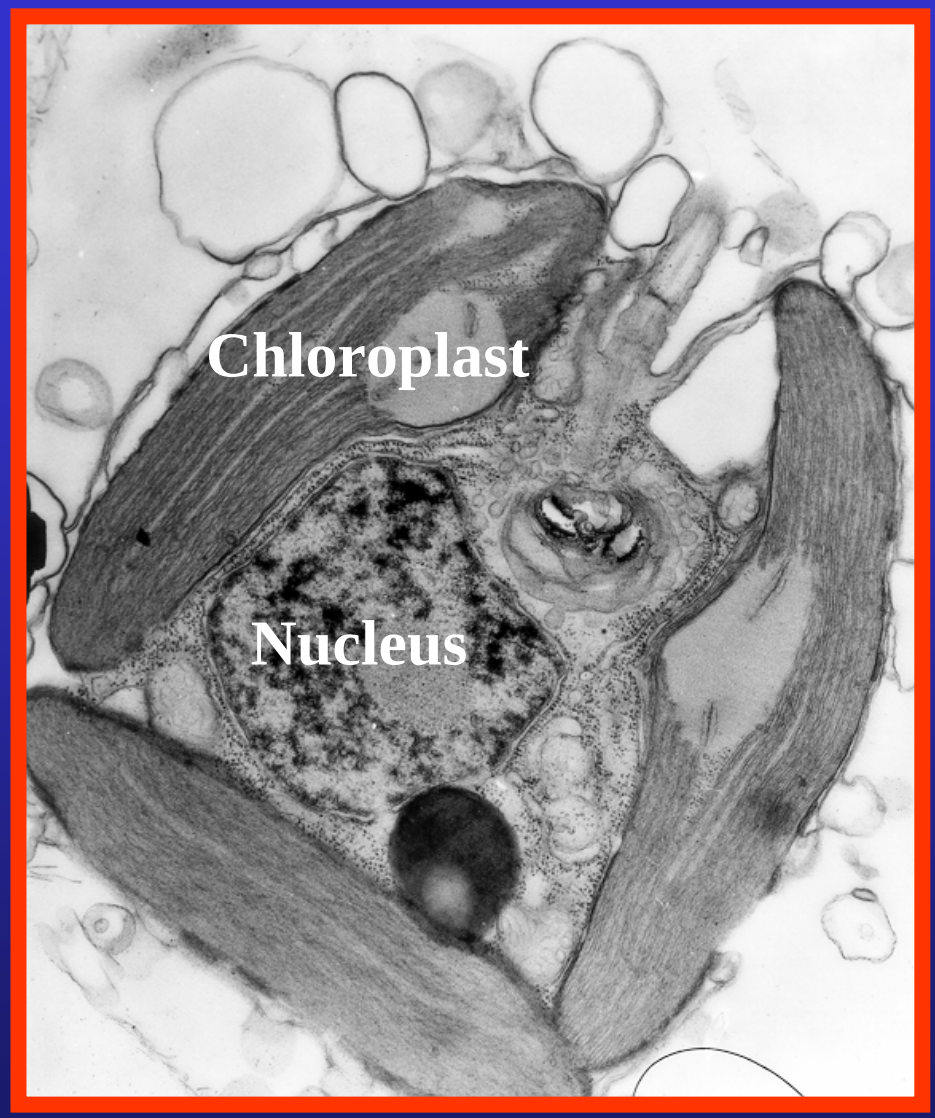
Texas *Prymnesium parvum*







PWD-RP-T3200-1203


Chloroplast
Nucleus

Cyst Formation

- **A variety of conditions failed to induce cyst formation**
- **Cold**
- **Dark**
- **Nutrient depletion periods**
- **Desiccation**
- **Nutrient**

Media Used

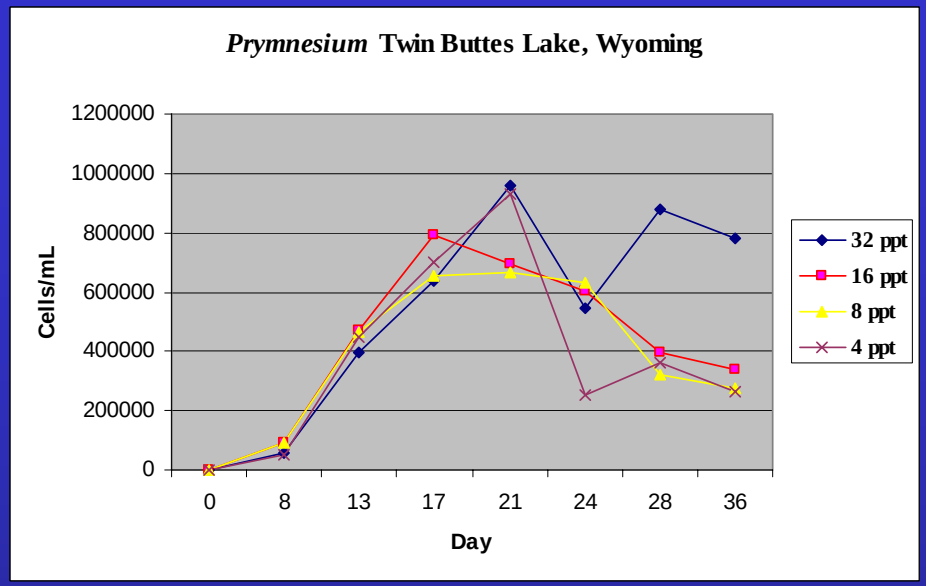
- **Medium Variations**
- **Twin Buttes water (high salinity – primarily calcium sulfate)**
- **Prewitt Reservoir Water (low salinity)**
- **Dowdy Lake water (low salinity)**
- **Seawater**
- **Artificial Seawater Medium**

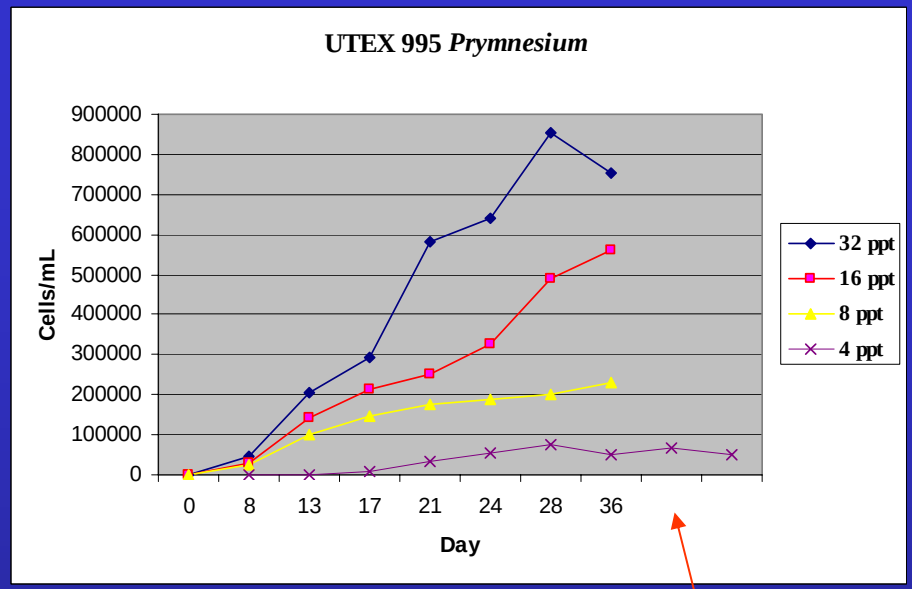
Growth Observations 0n *Prymnesium* strains from Texas & Wyoming

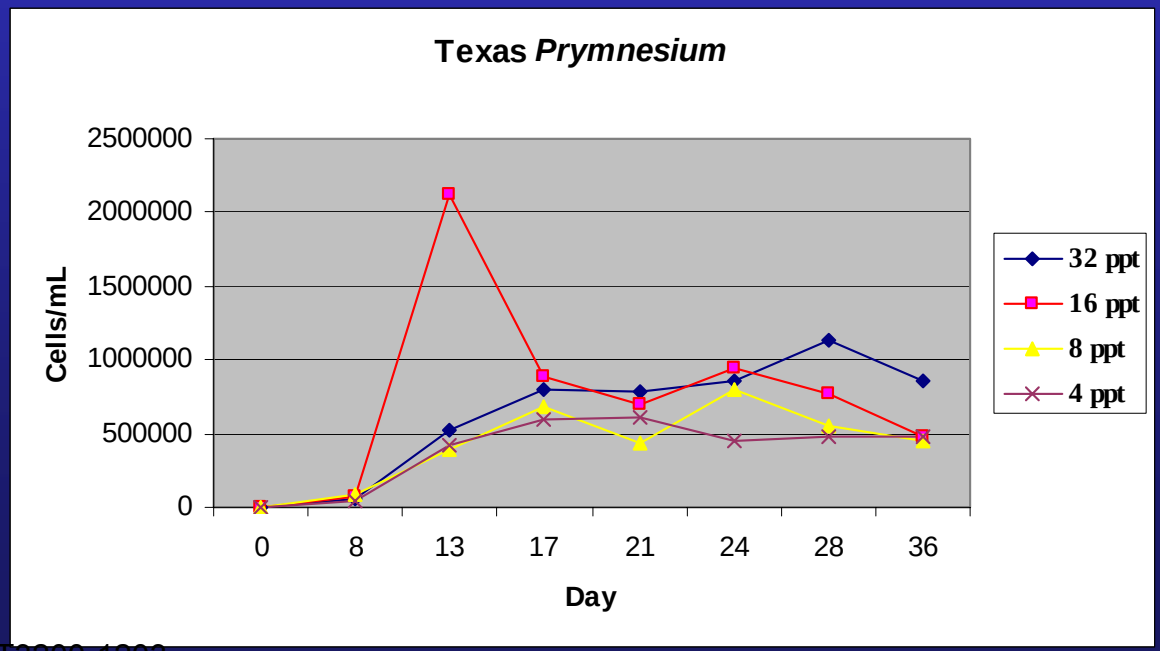
- **Grew well in following media & salt concentrations**
  - **Twin Buttes – 11 ppt**
  - **SW – 32 ppt**
  - **Buffalo Spring Lake – 11 pt**
  - **Lubbock Canyon Lake - 6 ppt**
- **No Growth**
  - **Dowdy Lake Water – >1 ppt**
  - **Prewitt Reservoir – 1 ppt**
  - **Cells hypertrophied & burst within 10 minutes**

Salinity Tolerance

Used Artificial Seawater Medium at Different Salinities – 32, 16, 8, 4, and 1 ppt







Definite Salinity Effect

PWD RP T3200-1203

No growth in 1 ppt for any of the strains

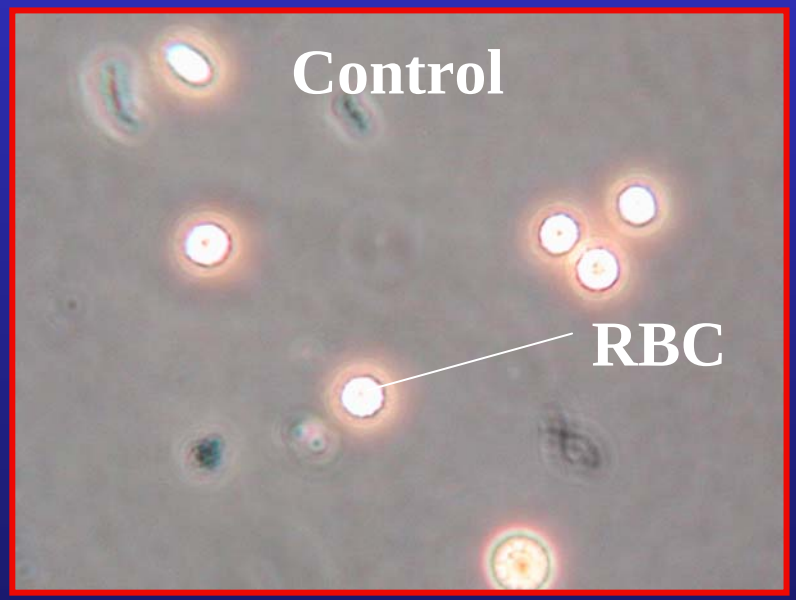
Summary of Salinity Experiments

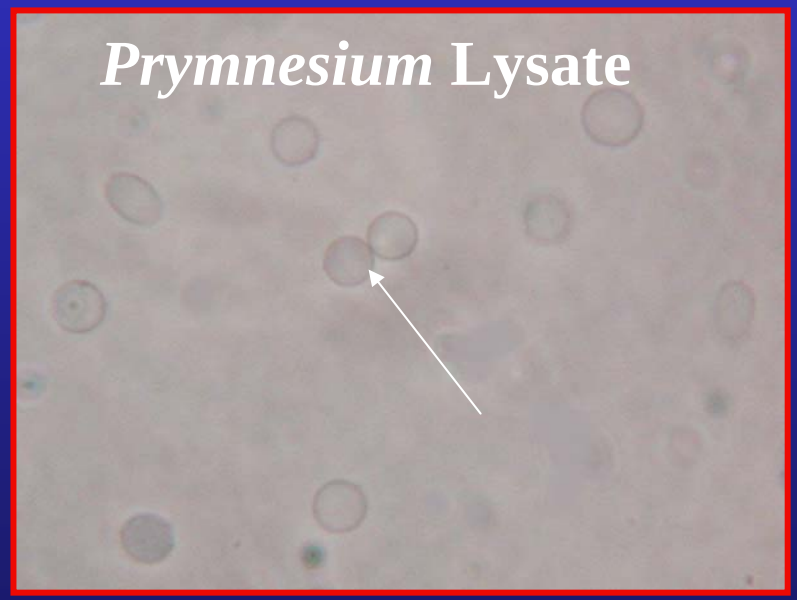
- **All grew well in 32 ppt**
- **Texas *Prymnesium* grew rapidly and had the highest numbers/ml**
  - **Maintained cell numbers between .5 – 1.25 million cells/mL at the end of the experiments**
- **Strain 995 grew best at 32 ppt but growth decreased correspondingly with lower salinities**
- **Texas & Wyoming *Prymnesium* grew well in all salinities tested**

Hemolytic Bioassay Procedures

- 5 ml of cultures placed into culture tubes
- Cultures centrifuged and supernatant discarded
- 1 ml of Ringer's Solution added
- Cells lysed by vortexing with small glass beads & by freezing and thawing
- 3 ml of Ringer's Solution and 1 ml of heparinized canine blood were added & mixed with solution
- Placed on ice for 1 minute
- 10 µl samples were examined microscopically to determine the number of lysed cells/unit area using a Palmer Chamber & Whipple ocular grid

Hemolysis Observations





Hemolysis Bioassay

Alga	*Medium*	*Phase*	*% Lysed Red Blood Cells*
Diacronema	SW/TB	10/20/30 days (Control)	0 %
P. parvum (Tx)	SW	10 Days	3.8 %
P. parvum (Tx)	SW	20 Days	100%
P. parvum (Tx)	SW	30 Days	100%
P. parvum	TB	10 Days	4.2 %
P. parvum	TB	20 Days	100%
P. parvum	TB	30 Days	100%
C. parva RF	DL	10 Days	4.0 %
C. parva RF	DL	20 Days	0 %
C. parva RF	DL	30 Days	0 %
C. parva SDB	DL	10 Days	0 %
C. parva SDB	DL	20 Days	0 %
C. parva SDB	DL	30 Days	8.1 %

Hemolysis Summary

- **Hemolyis of canine red blood cells in *Chrysochromulina & Diacronema* lysates insignificant**
- **100% hemolysis of canine red blood cells occurred with *P. parvum* lysates from Texas & Wyoming**

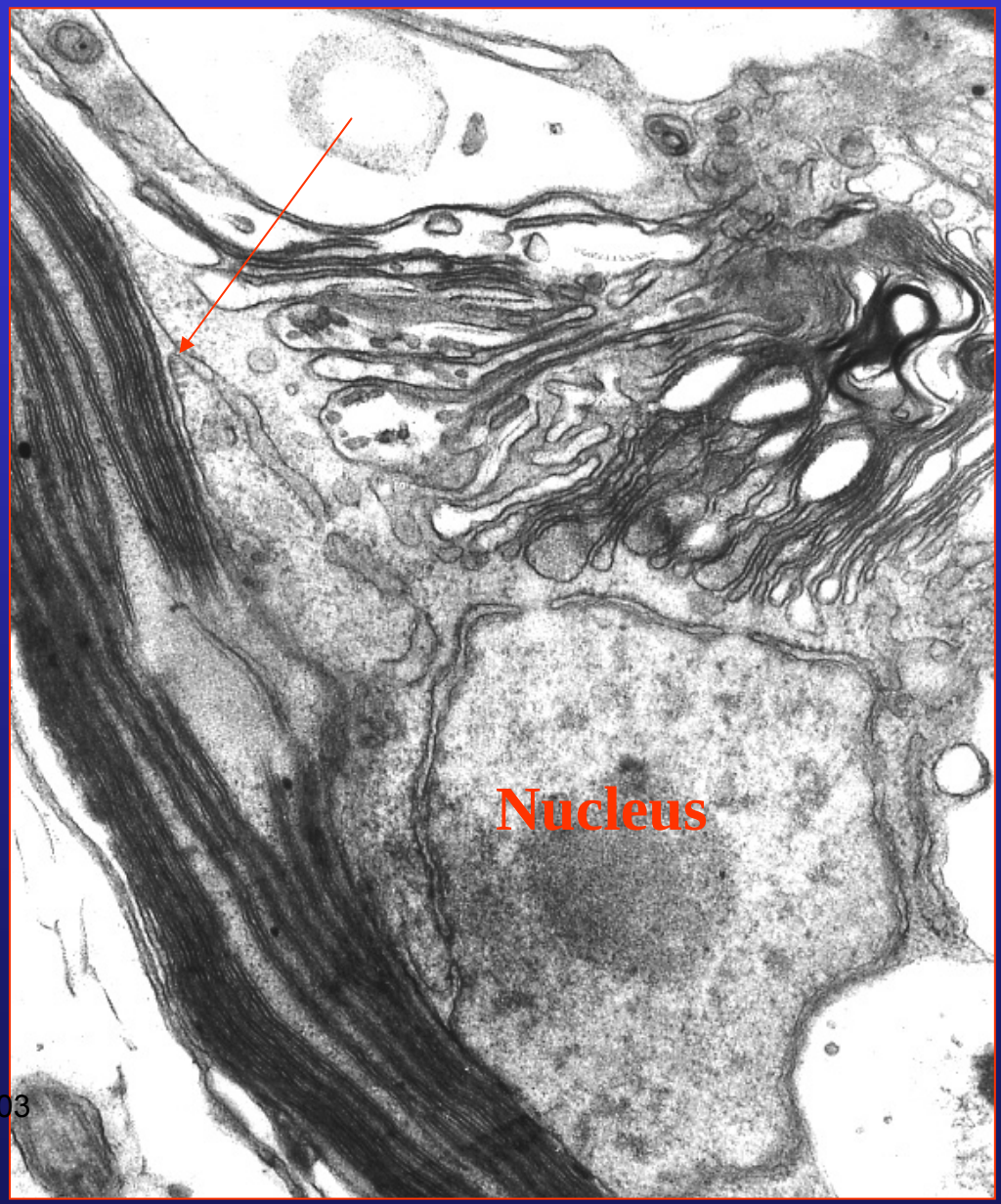
Mixotrophy Studies

- **Several phytoplankton grown with *Prymnesium***
- **Observed daily with inverted light microscopy & transmision electron microscopy (1000's of cells)**
- **No evidence of mixotropy in any of the three strains used in this study**

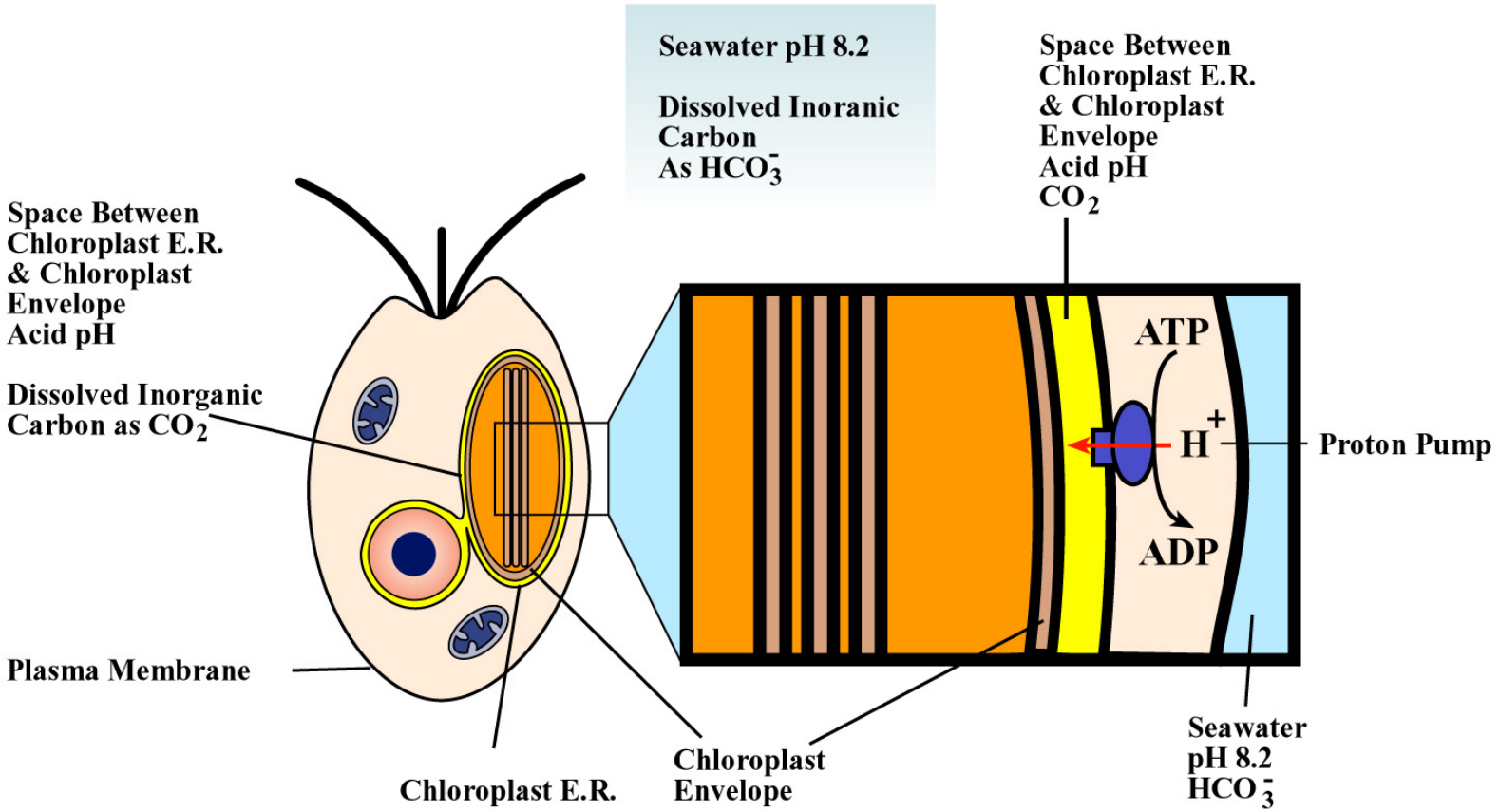
Hypothesis for Rapid Growth of *Prymnesium*

- ***Prymnesium*** **thrives in a wide range of salinities with different salts**
- **Rapid growth at high pHs – e.g. 8.2**
  - **Becomes dominant phytoplankter**
- **Nutrients available to other phytoplankton**
- **Limiting Factor might be carbon dioxide availability**

Chloroplast Endoplasmic Reticulum



Adaptive Advantage of Chloroplast Endoplasmic Reticulum – Carbon Dioxide Utilization?



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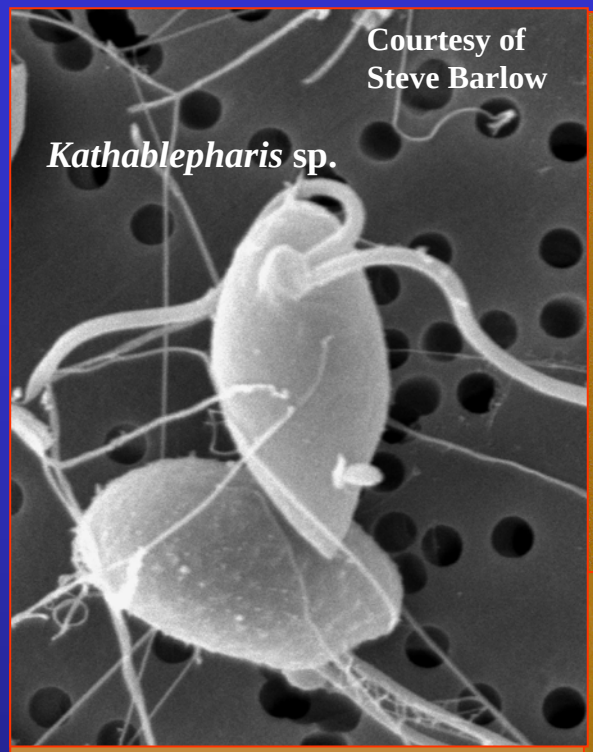
Adaptive Advantage of Chloroplast Endoplasmic Reticulum – Carbon Dioxide Utilization?

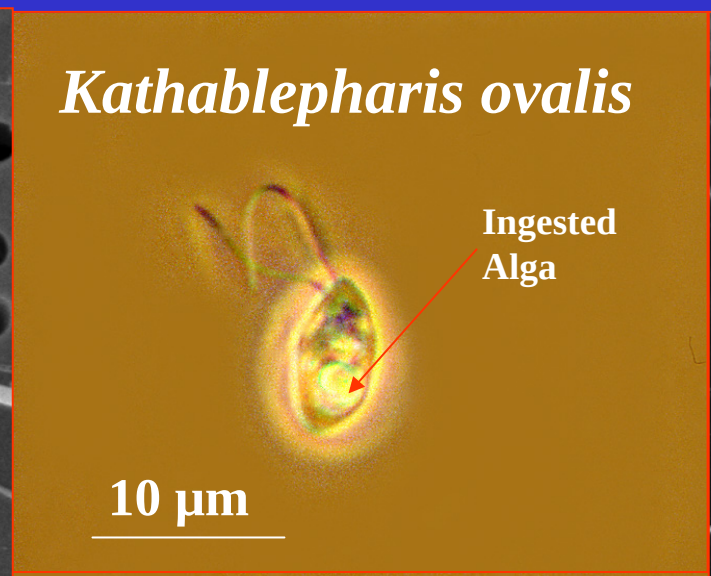
- **Dissolved Inorganic Carbon (DIC) availability might promote rapid growth of *Prymnesium***
- **Chloroplast Endoplasmic Reticulum might provide a ready source of carbon dioxide for the Calvin Cycle**
- **Would impart an adaptive advantage over algae that lack a CER**

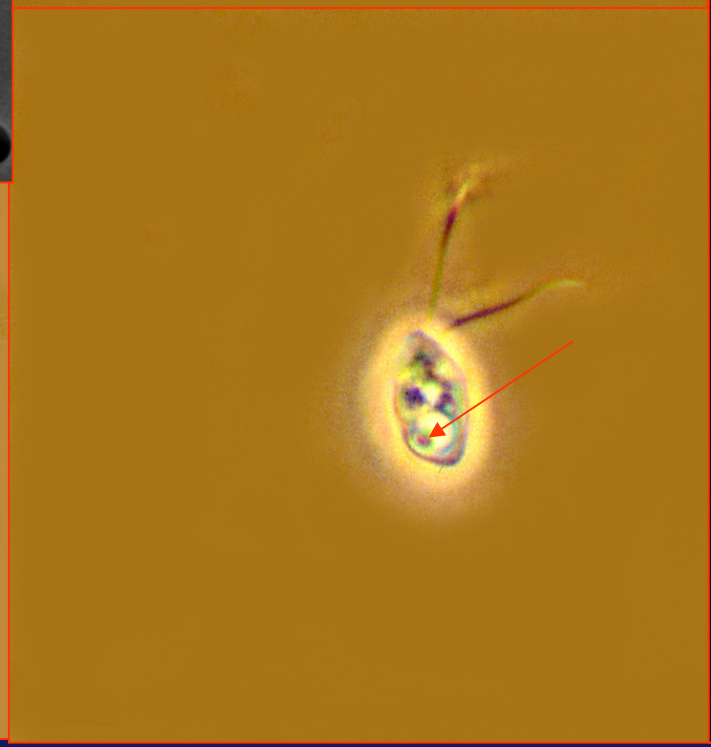
Biocontrol of *Prymnesium*?

- **Organisms that selectively ingest other organisms**
- ***Kathablepharis* – small colorless flagellate that feeds on another Prymnesiophyte, *Chrysochromulina***
- **Can ingest 4-10 cells**
- **Attack in groups of 5-several hundred**


Courtesy of
Steve Barlow
Kathablepharis sp.

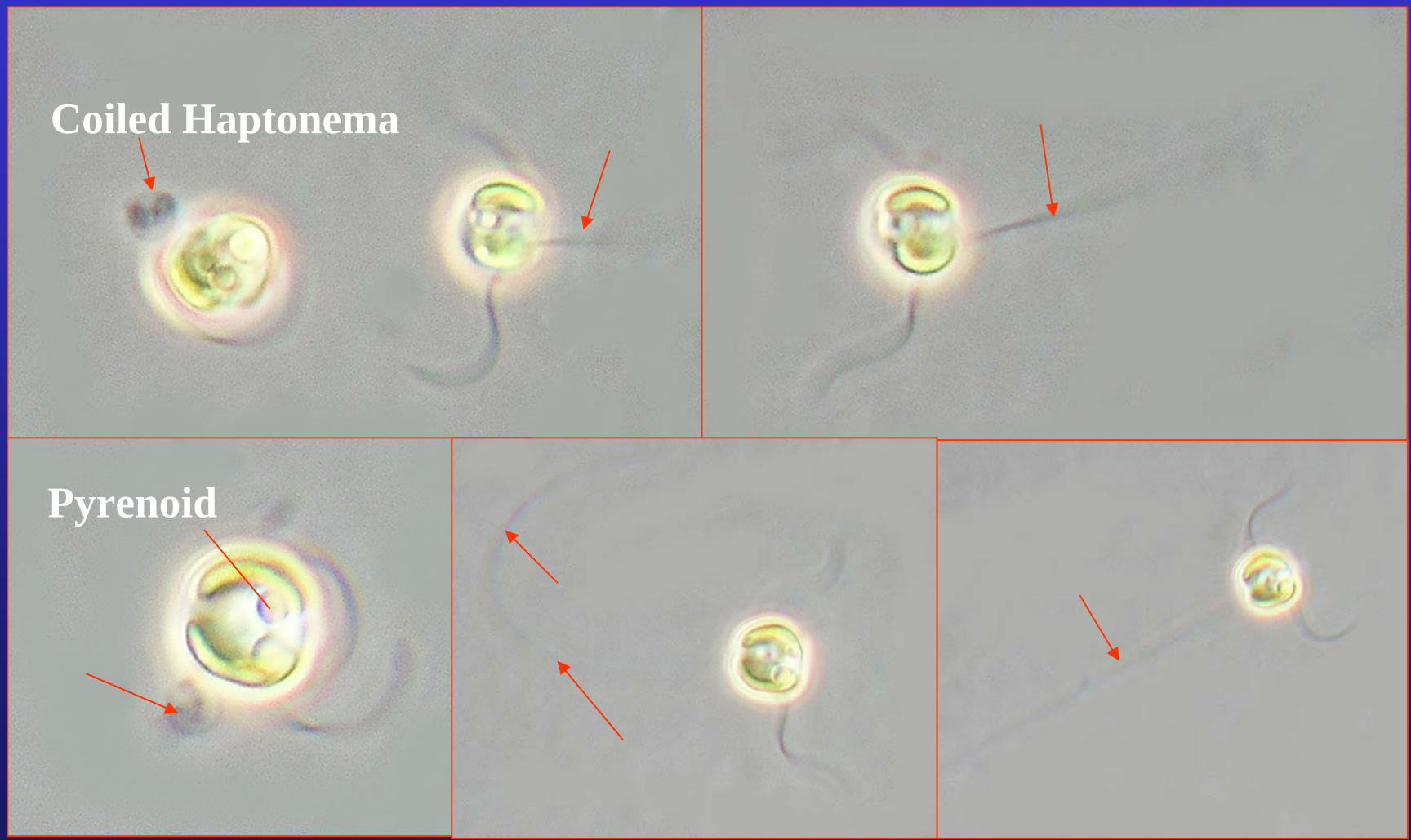

PWD RP T3200-1203


Kathablepharis ovalis
Ingested
Alga
10 µm




10 µm

Chrysochromulina Haptonema



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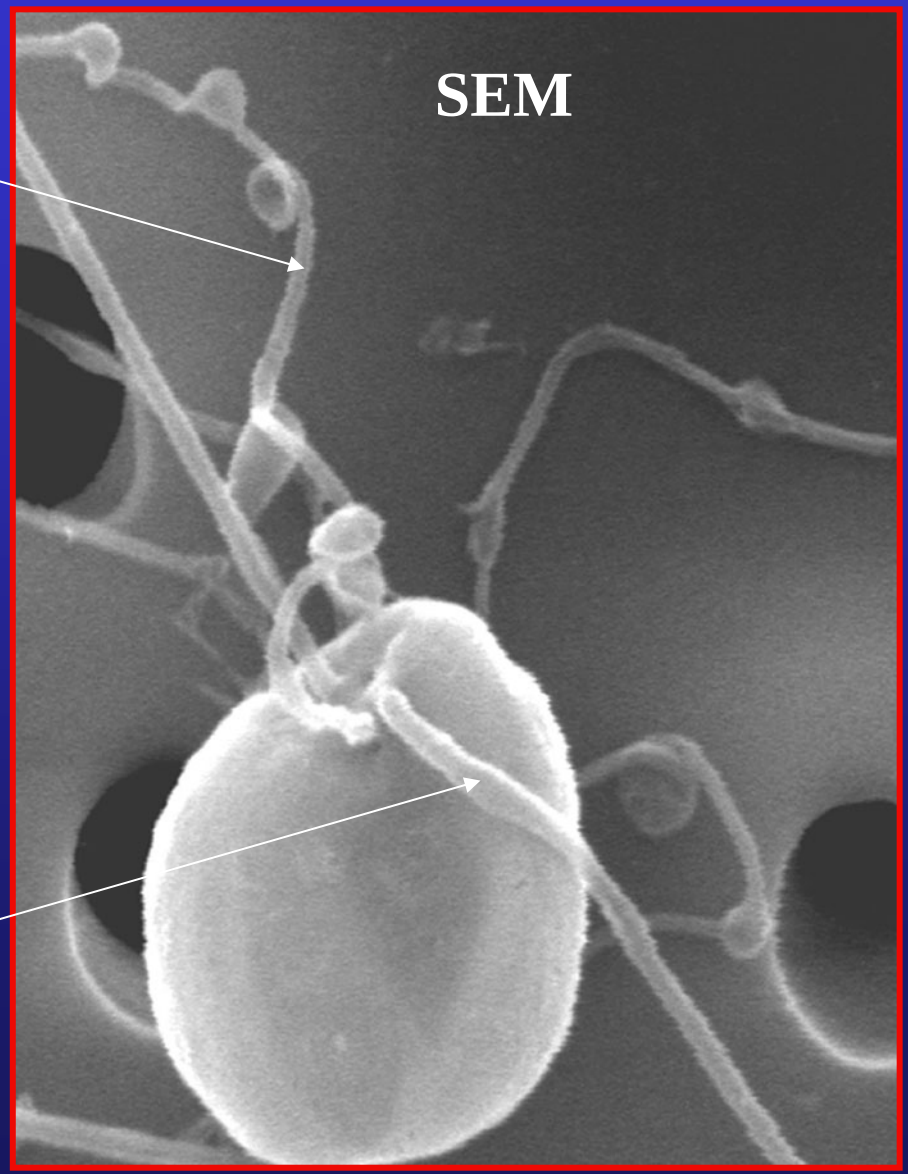
Chrysochromulina parva

Long, uncoiled Haptonema

Flagellum



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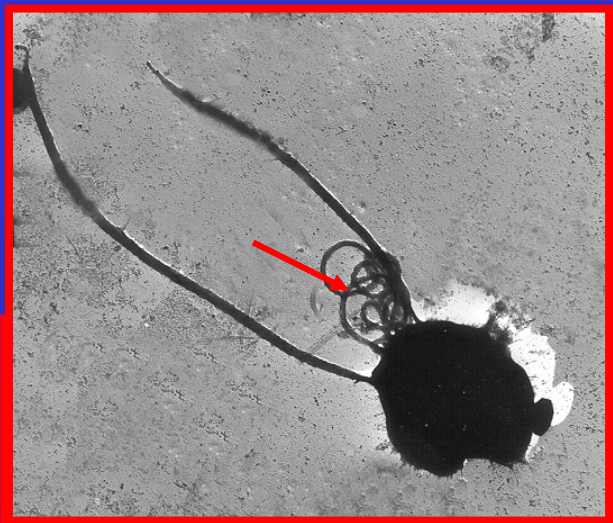
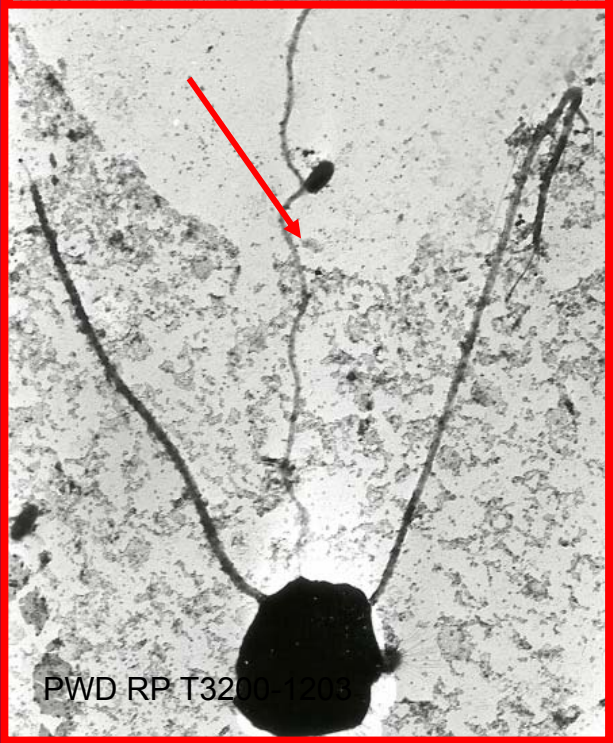
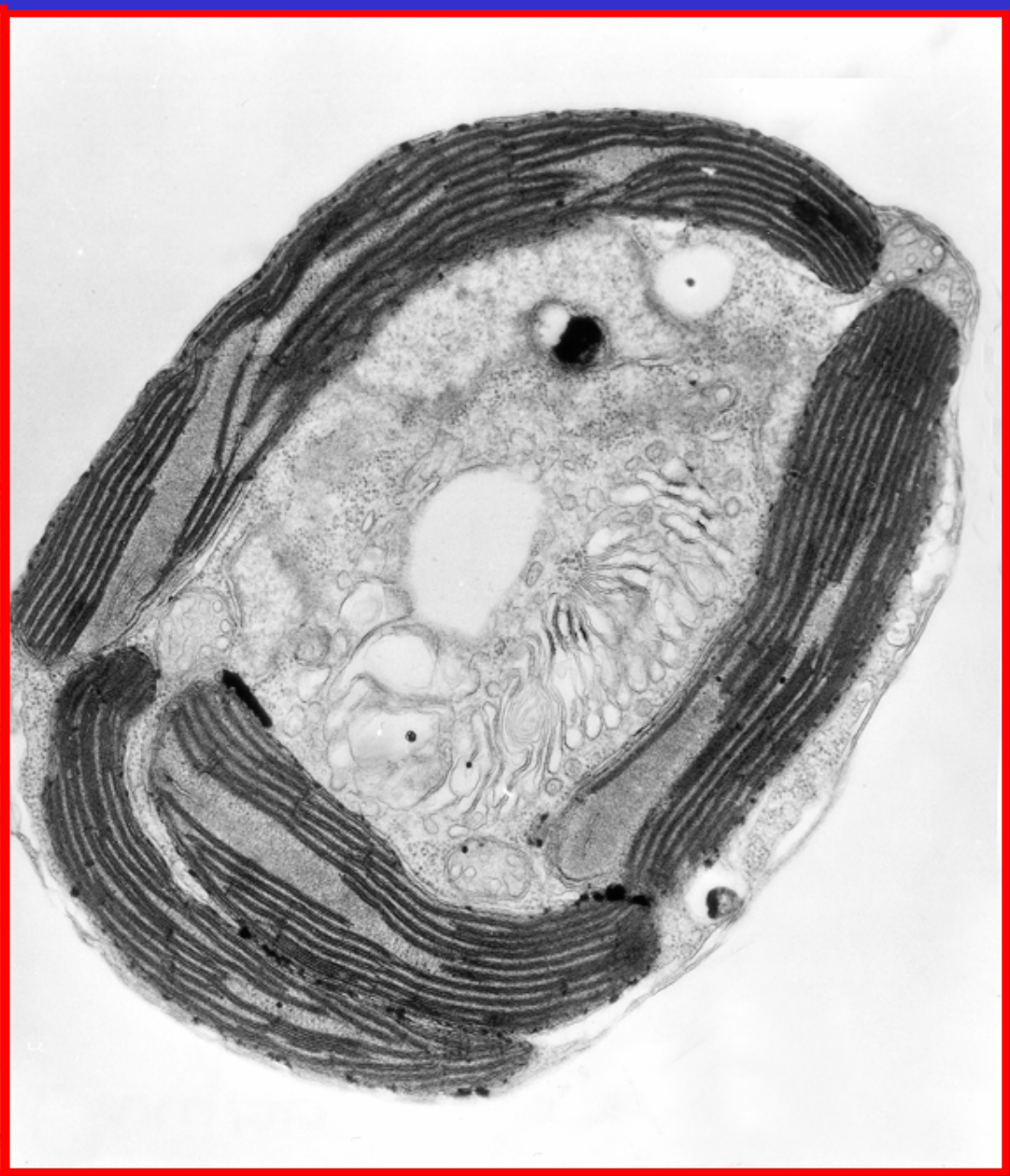
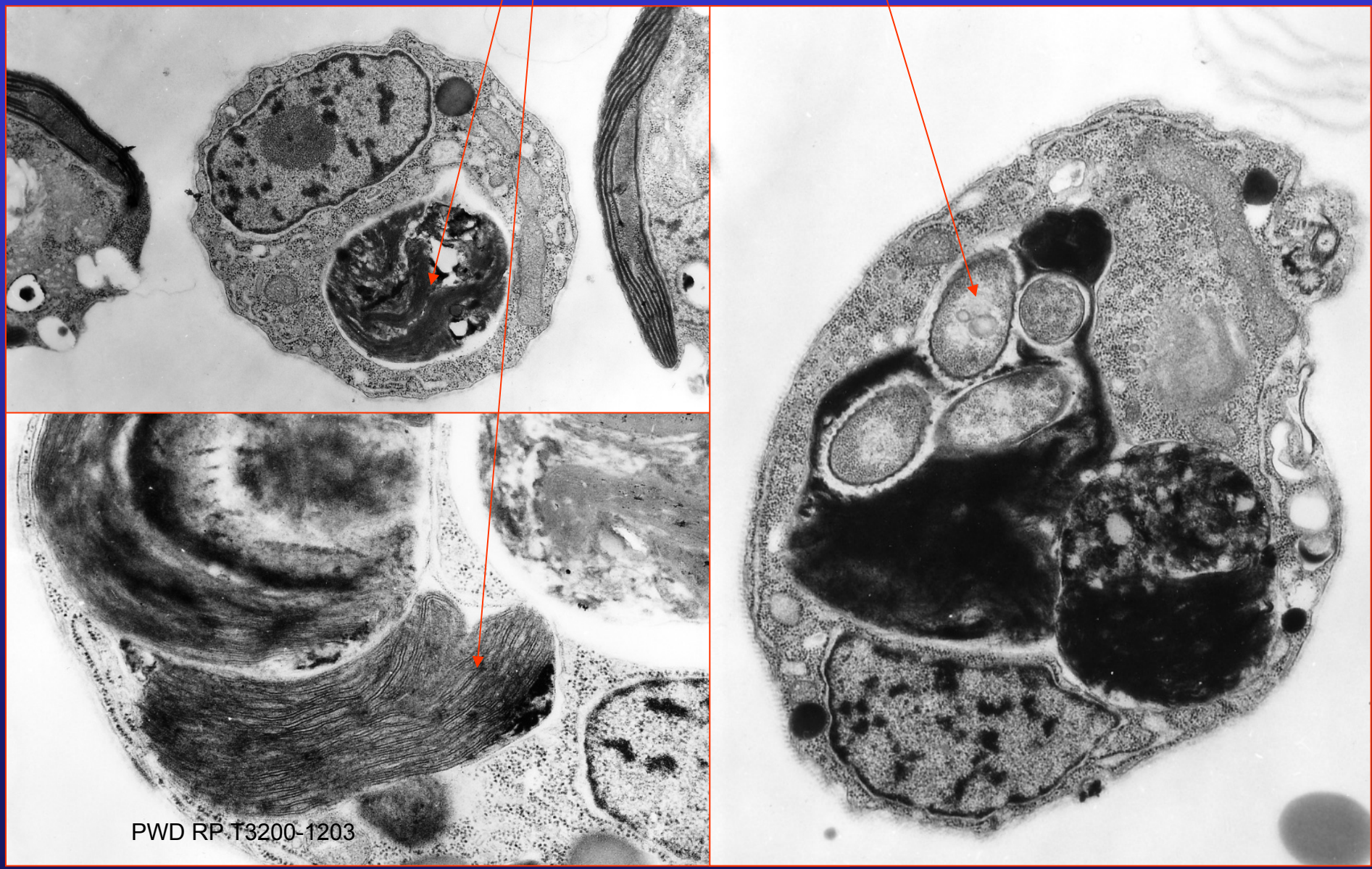
Chrysochromulina parva







Ingested *Chrysochromulina* & some bacteria in *Kathablepharis*



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Biocontrol of *Prymnesium*?

- ***Kathablepharis ovalis*** **could be isolated & exposed to *Prymnesium* to see if the selective feeding response can be adapted**
- **Grows at salinities that *Prymnesium* tolerates**
- **Has been implicated in *Chrysochromulina* bloom disappearance**
- **Decimates dense *Chrysochromulina* cultures within two weeks**

- **Not found in any of Texas samples examined**

Return to Prewitt

- *Prymnesium parvum* was marine in origin
- Able to grow inland only in high salinity waters
  - Grew equally well in seawater & Twin Buttes media
  - Calcium sulfate most common salt in plains lakes of Wyoming – salinity 11 ppt
- In Texas due to increase in the salinity of freshwater
  - By evaporation & agricultural runoffs
  - Buffalo Springs 11 ppt
  - Lubbock Canyon Reservoir – 6 ppt

Prewitt Reservoir Explanation

- **Presence of *Prymnesium* never confirmed.**
- **Low salinity in Prewitt precludes growth of *Prymnesium* – 1 ppt**

- **Therefore, what could have caused the fish kill in Prewitt Reservoir?**

Prewitt Reservoir Fact

- **Five days following fish kill**
- **Phytoplankton bloom consisted of over 4.5 billion cells/Liter**

Mystery

- **Samples taken 5 days after the fish kill did not reveal any *Prymnesium***
- **One week old samples also were examined**
  - **Collected by Colorado Division of Wildlife Fish Pathologist & kept refrigerated**
  - **Did not reveal any *Prymnesium***
  - **A few cells of *Chrysochromulina* sp. were noted**
- **Lack of *Prymnesium* attributed to a sudden die-off of this alga after the fish kill (5 days?)**

Prewitt Reservoir Explanation

- **Phytoplankton bloom five days after the fish kill consisted of over 4.5 billion cells/Liter**
  - **Dominated by cryptomonads**
  - ***Campylomonas reflexa***
  - ***Plagioselmis nanoplanctica***

Dominant Cryptomonads

Campylomonas reflexa

Plagioselmis nanoplanctica

Prewitt Mystery Solved?

- ***Campylononas*** **&/or** ***Plagioselmis*** **could be a toxin producing algae**
- **Due to high algal cell numbers the fish kill could be due to oxygen depletion**
  - **Primarily due to abundance of cryptomonads, not** ***Prymnesium*****!!!!!**
  - **DO was not determined at the time of the fish kill**

Solution to Fish Kill

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Future *Prymnesium* Problems in Wyoming?

- Isolated from highly productive trout lakes
- Decline in fishery observed in Twin Buttes Lake?
- Could spread north & east through interconnected waterways

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THE END

Rapid Tests for the Detection of *Prymnesium parvum* and Its Toxins

Linda K. Medlin

Alfred Wegener Institute, Am Handelshafen 12 D.-27570 Bremerhaven, Germany

Abstract.--Oligonucleotide probes or signature sequences are phylogenetic determinative tools in environmental microbiology. rRNA genes are so variable that they offer regions that are species or even strain specific. These regions can be targeted for the design of probes to recognize species or even strains. We present three platforms for the detection of species, in this case *Prymnesium parvum* using rRNA probes. DNA microarray technology provides a tool that allows the parallel analysis of large numbers of molecular probes. The application of such DNA-chips for the cultivation-independent analysis concerning the biodiversity of phytoplankton-samples would save a lot of time in comparison to other well-established technologies e.g. dot-blots. Therefore we are currently developing a DNA-microarray-based method for the high throughput analysis of phytoplankton-communities. DNA-chips have been developed by spotting probes targeting the 18S-rDNA of six different phytoplankton classes. In preliminary experiments it was possible to detect specific and some unspecific hybridization-signals with PCR-products from nine different species hybridized to the DNA-Chips. Species level probes were used for the development of an early warning system for use with a hand-held DNA microchip reader with electrochemical detection with sandwich hybridization. Results from field trials show excellent correlation with cell numbers and with total RNA content from laboratory cultures. Shelf life and stability of the microchips has been tested through 5 months and offer our prototype excellent possibilities for marketing. The hand-held device is ca. 25 Euros and the disposable microchip is 2.50 Euros. Theoretically, up to 400 probes can be spotted on the chip with the specificity of the chip determined by the signal probe. It would be conceivable to custom design the chip to cater to local HAB needs. Using standard FISH hybridization on filters we employed the ChemScan solid phase cytometry to scan filters within 30 seconds and record all positive signals. The microscope driven computer software enables each positive signal to be retrieved and validated for authenticity. Haemolytic substances produced by ichthyotoxic algae are unknown in molecular structure or specific mechanism of toxicity. Detection and quantification of such substances is dependent on bioassays, using markers that are sensitive for haemolytic impairment and generation of a recordable response. The erythrocyte lysis assay (ELA) represents an advantageous bioassay in this respect, as the lytic response can be measured photometrically by the amount of released haemoglobin. This bioassay has been adapted to a microtiter plate platform for rapid analysis of haemolysis.

View the presentation

Rapid tests for the detection of Prymnesium parvum and its toxins

Linda Medlin, Gundula Ellers, Kerstin Toebe, & Katja Kerkmann
Bremerhaven, Germany

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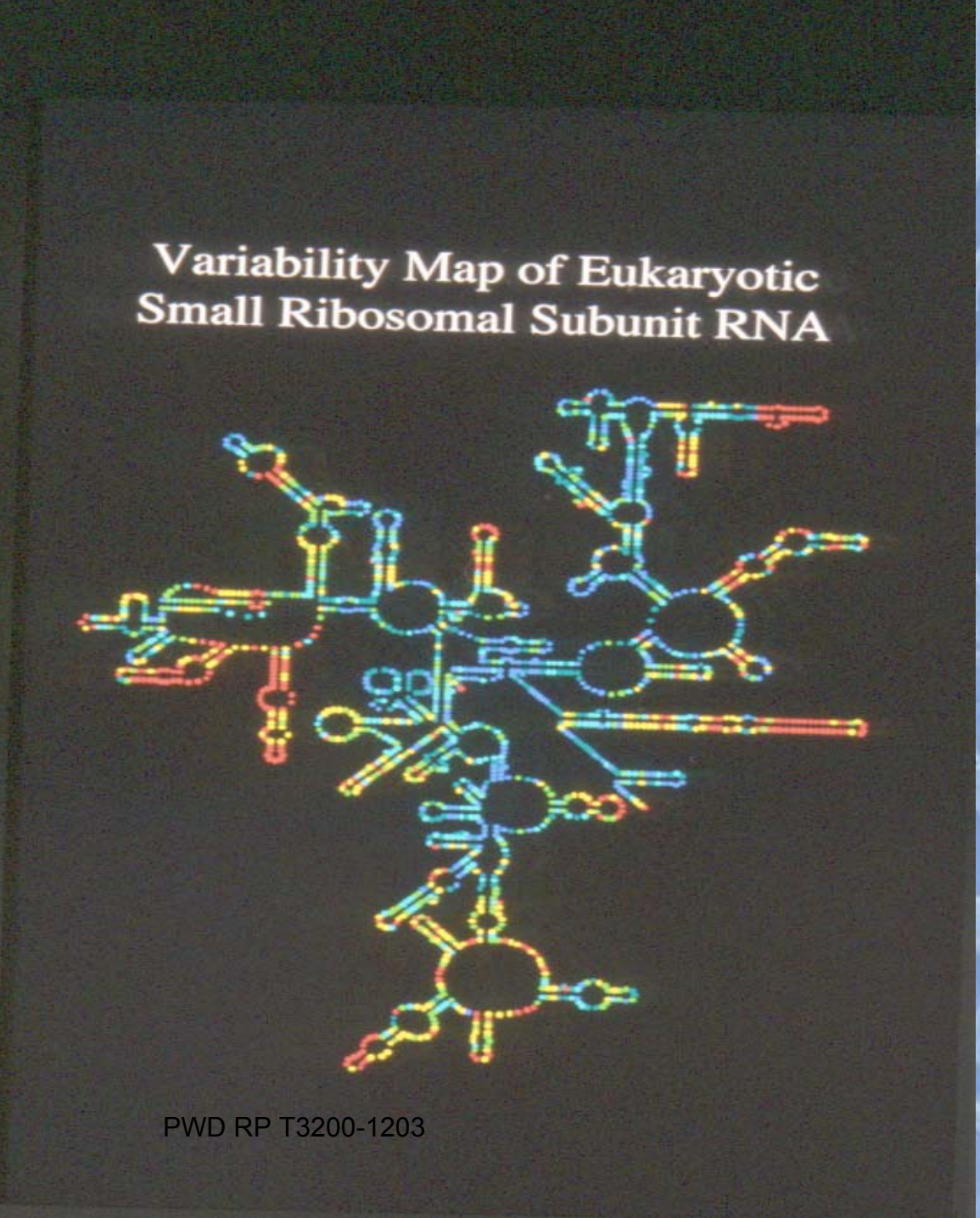
Why rRNA probes?

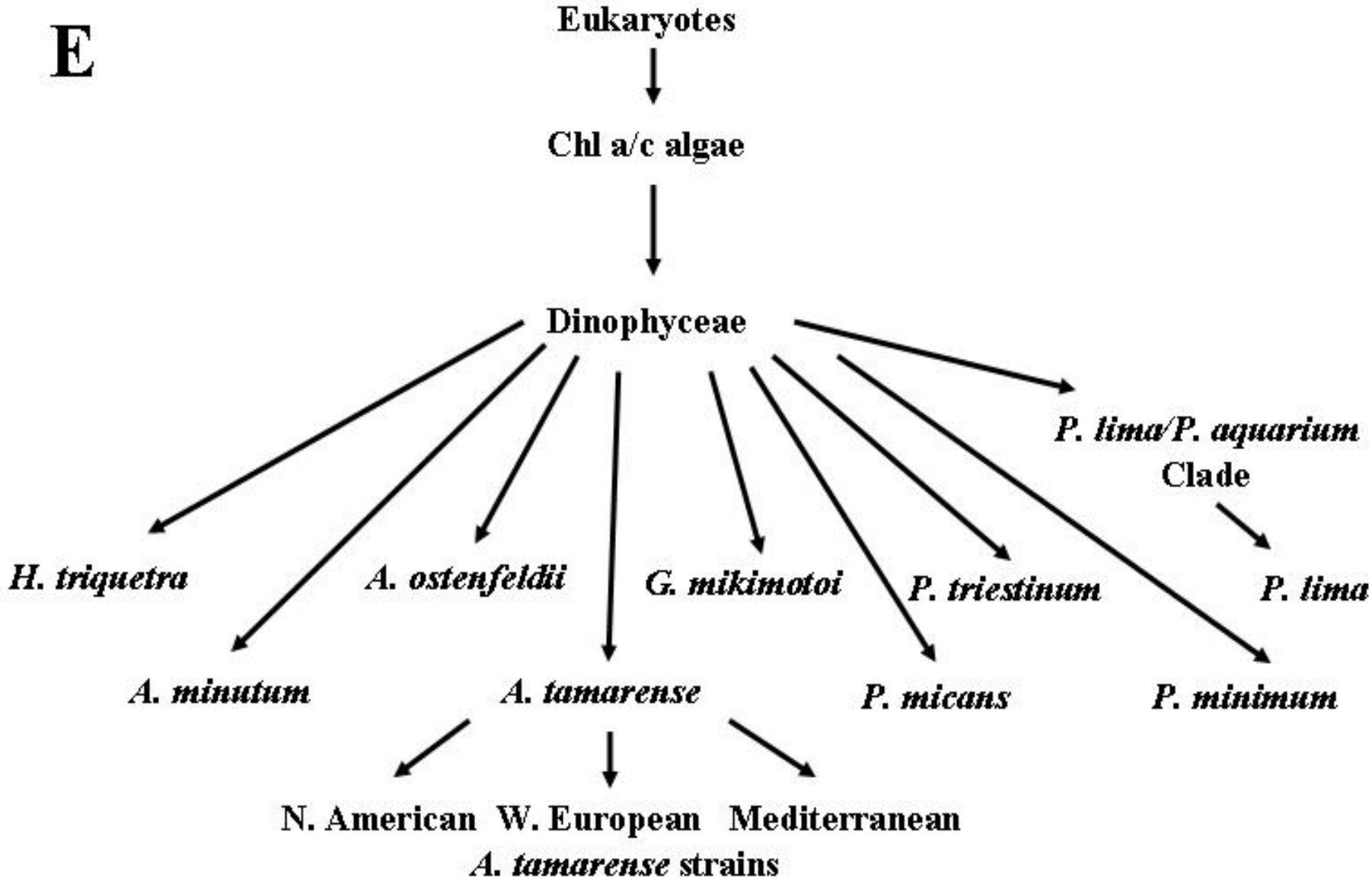
* universally found

* high target numbers per cell

* variable and conserved regions (can make nested probes for quantification)



Make hierarchical rRNA probes


E
Eukaryotes
Chl a/c algae
Dinophyceae
P. lima/P. aquarium
Clade
H. triquetra
A. ostenfeldii
G. mikimotoi
P. triestinum
P. lima
A. minutum
A. tamarense
P. micans
P. minimum
N. American
W. European
Mediterranean
A. tamarense strains

How to design and test a probe

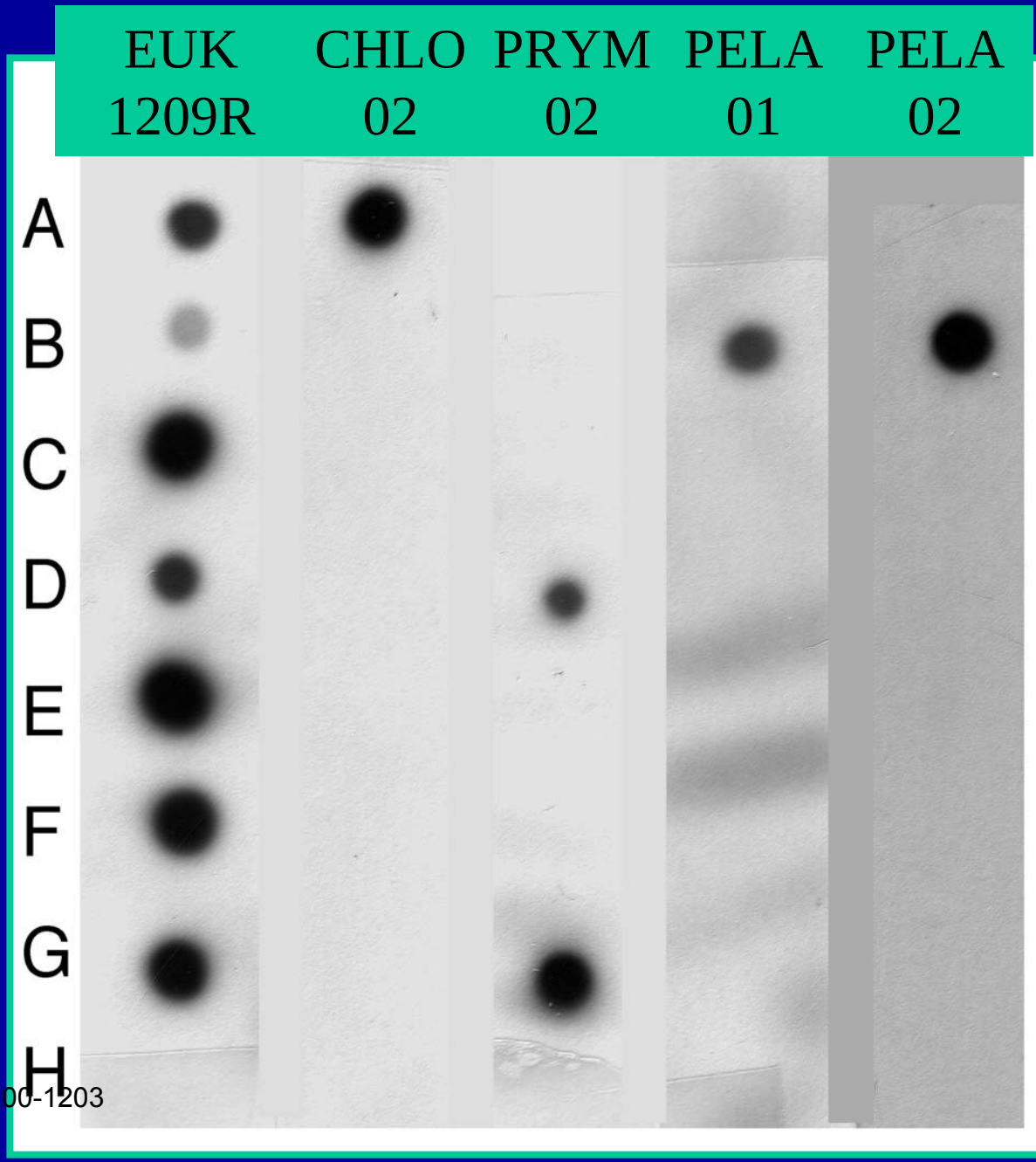
* Amass data bases from rRNA sequences

* use ARB program to design probe

* check probe for possible matches in RDP and Genbank

* test specificity in dot blot (DIG-labelled probe) and in situ (FITC or CY3 labelled probe) tests

* final check with flow cytometer


EUK 1209R
CHLO 02
PRYM 02
PELA 01
PELA 02
A
B
C
D
E
F
G
H

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Change from PFA to ETOH Saline

Change from SDS to Nonidet P-40

Without Dimethyl Formamide

With Dimethyl Formamide

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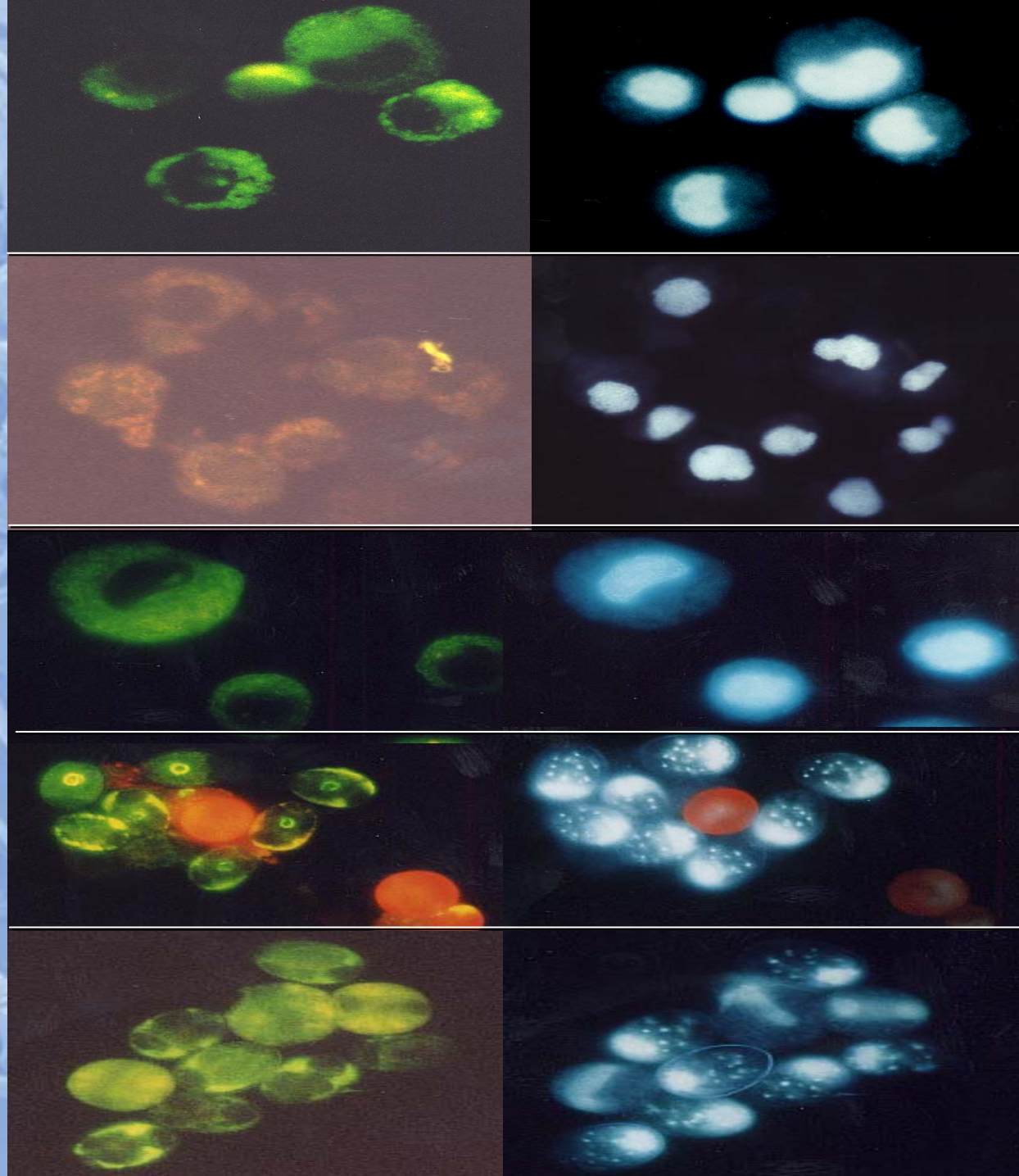
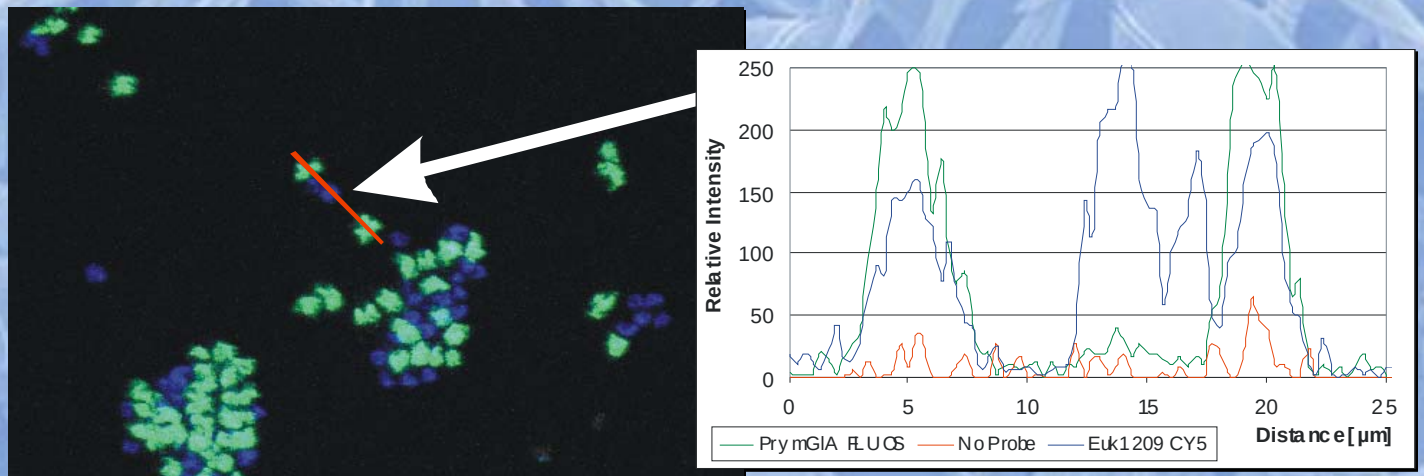


Double Staining of Cells to differentiate cells hierarchically



The cells were hybridised with the universal eukaryotic probe (labelled blue) and the genus specific probe for *Prymnesium* PrymGl01A (labelled green). Mixture of E.huxleyi and Prymnesium parvum

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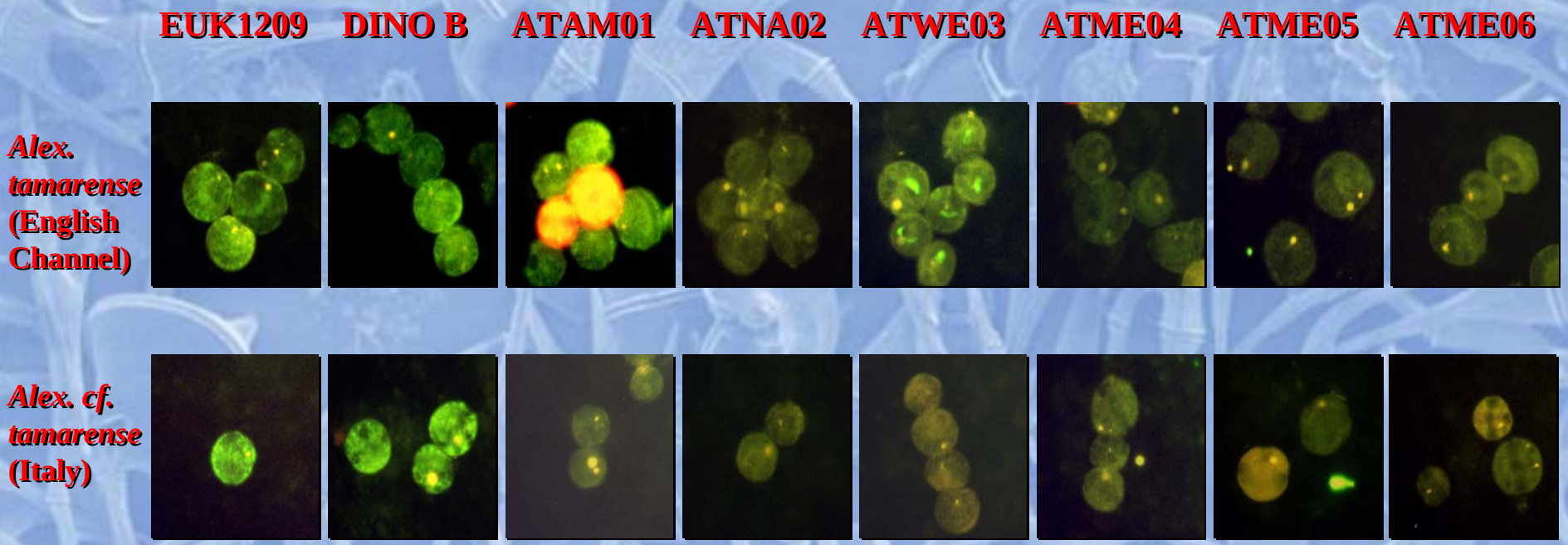
Analysis of *Alexandrium* strains from European waters using hierarchical probes



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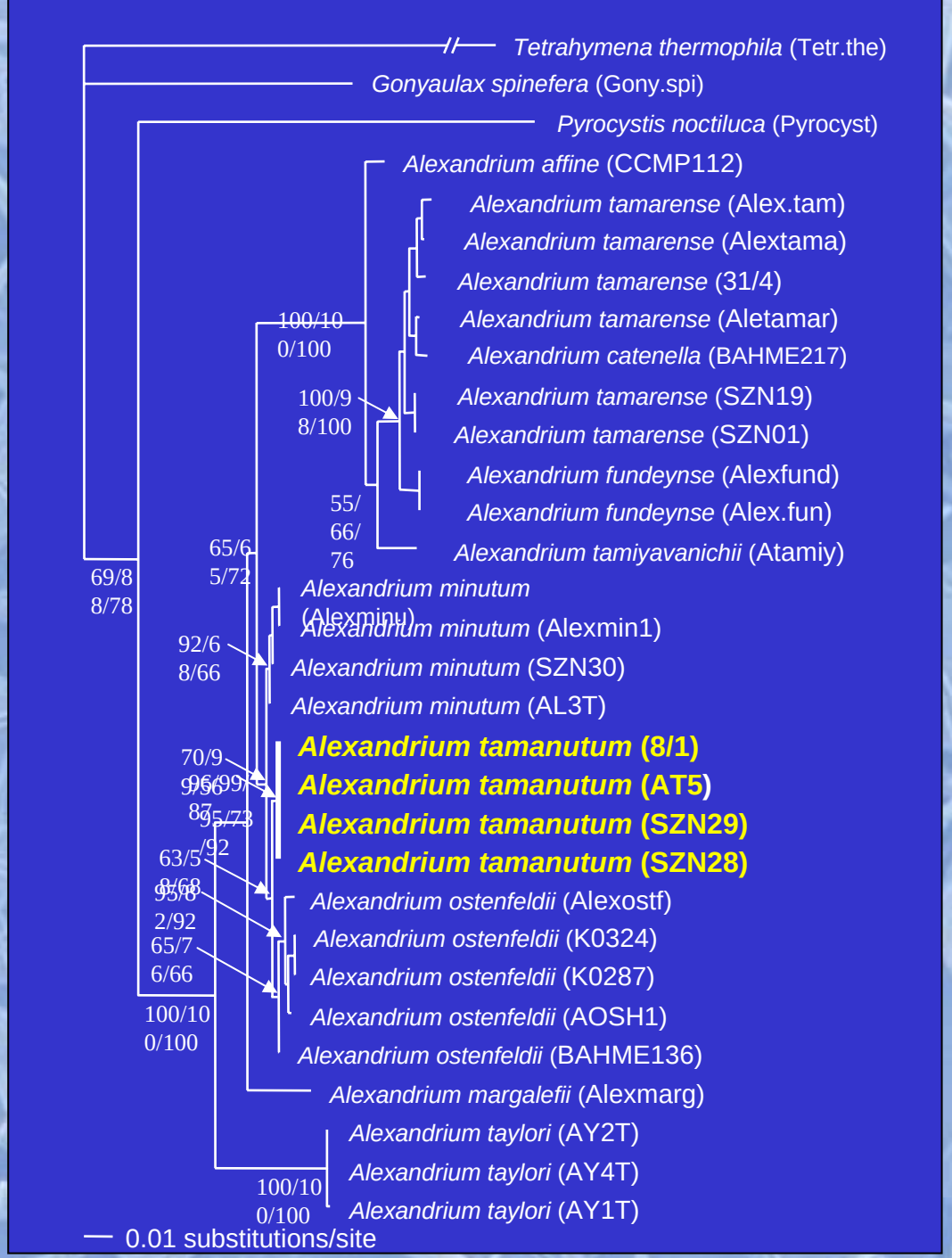
Phylogenetic tree of *Alexandrium* (18S rRNA)



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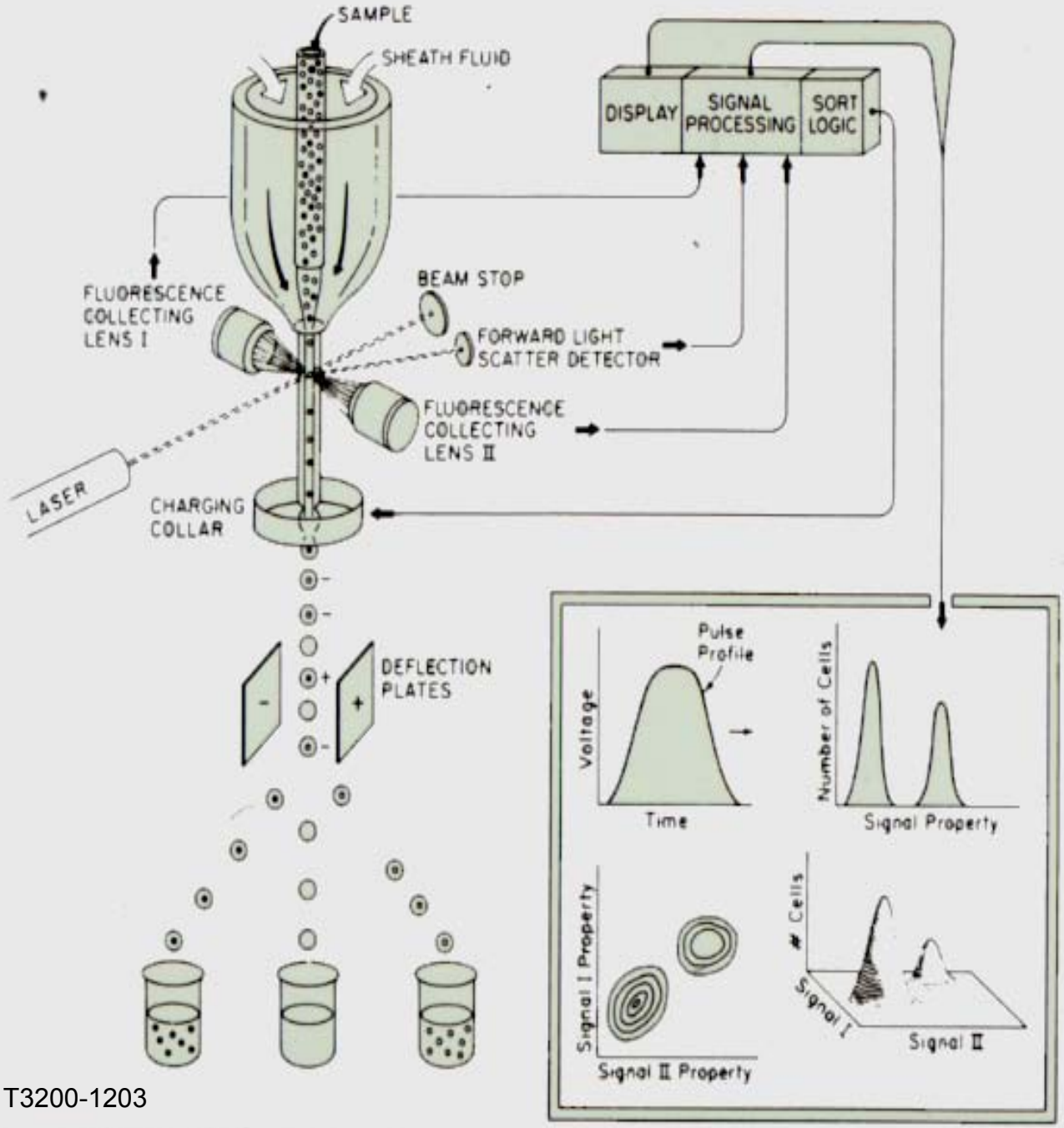

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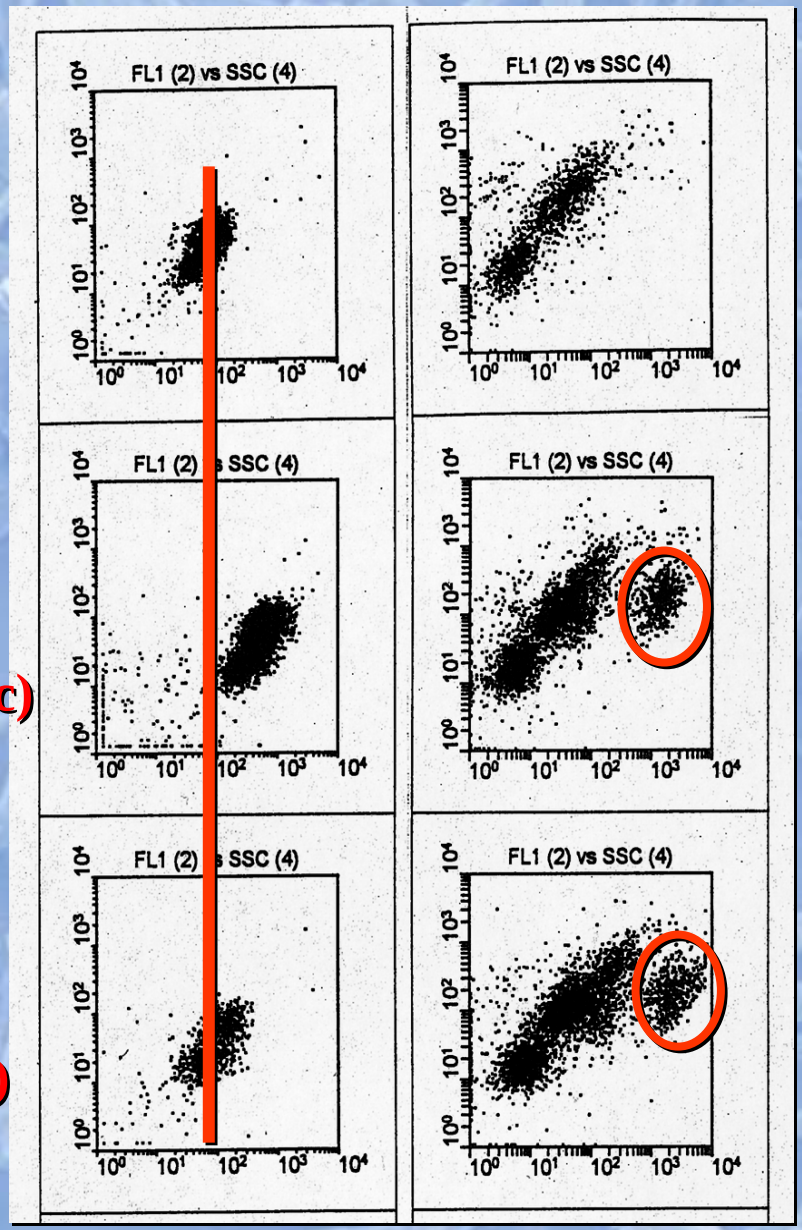
Identification of *A. tamarense* in Lab Cultures and Field Samples by Species- & Strain-specific Probes

no probe

ATAM01
(species specific)

ATNA02
(strain specific)



A. tamarense (WE strain) Field sample (Orkney Islands)

J. Brenner, unpubl. results

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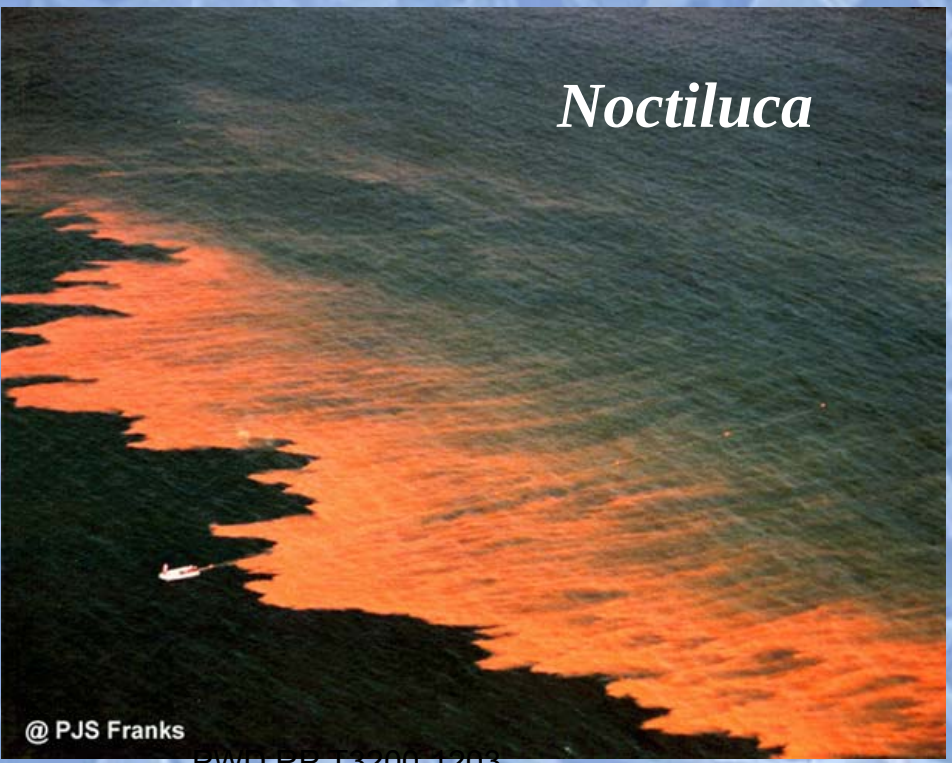
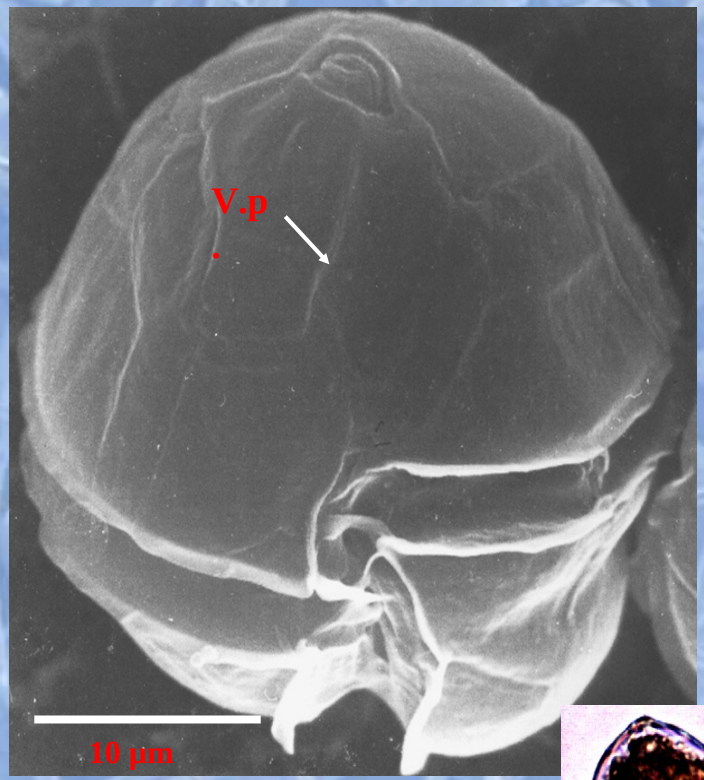
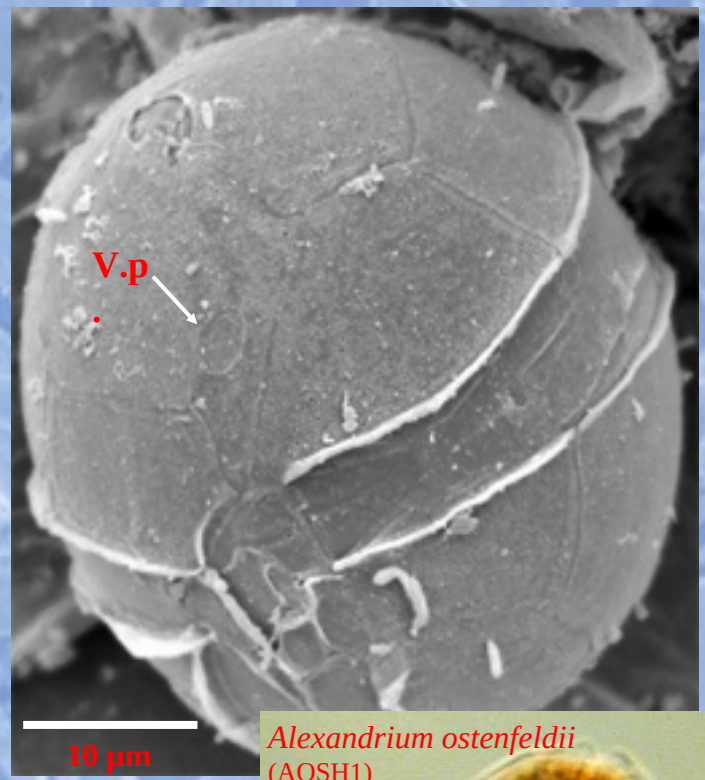
Harmful Algal Blooms

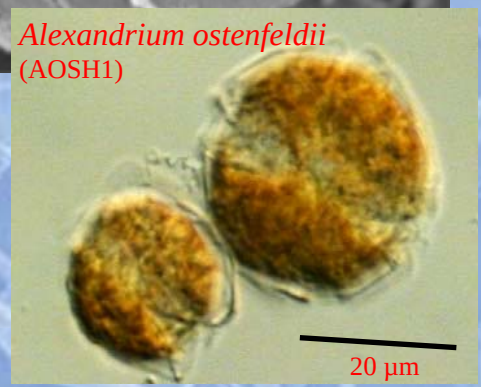


Alexandrium tamarense



A.ostenfeldii





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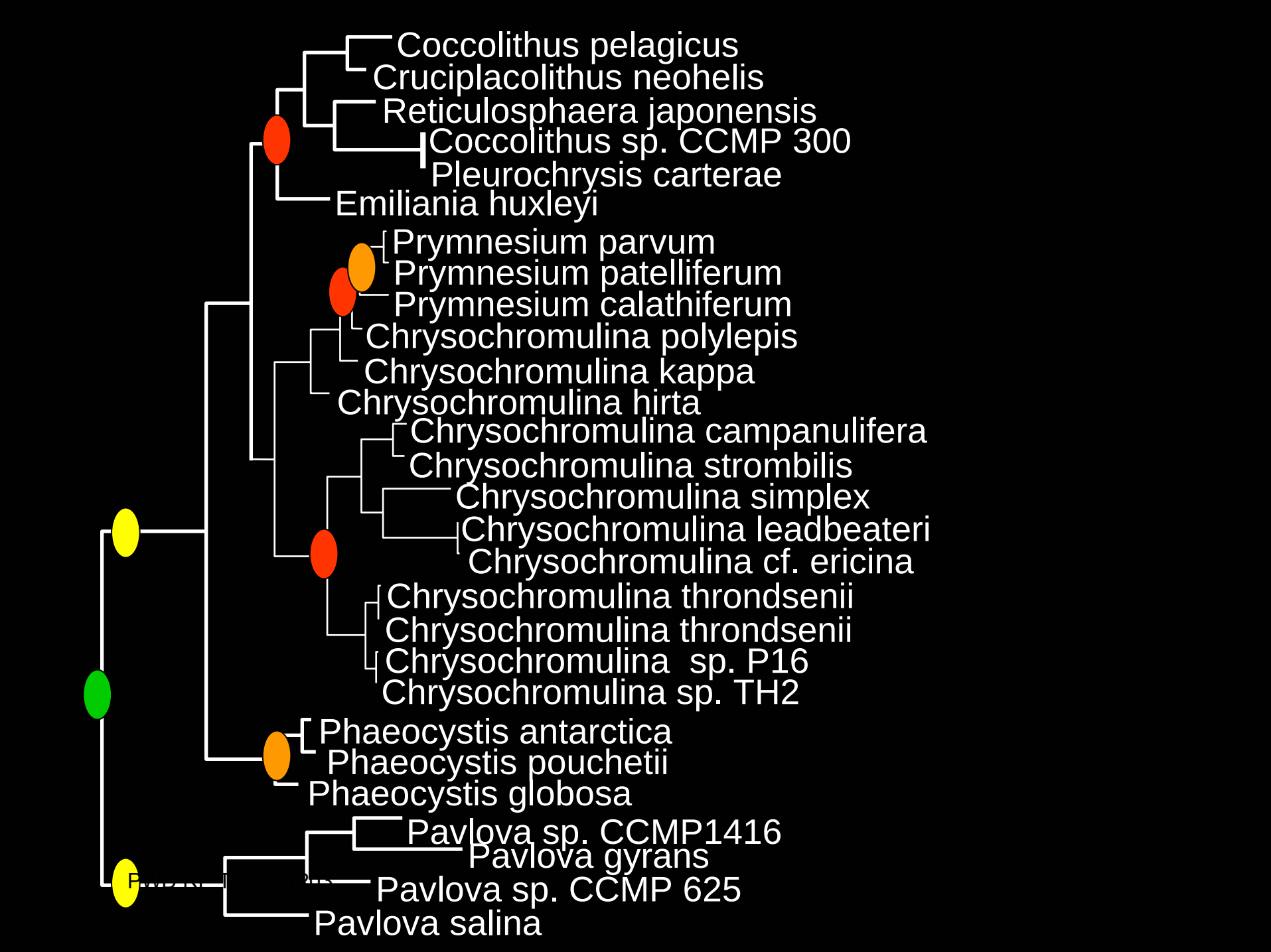
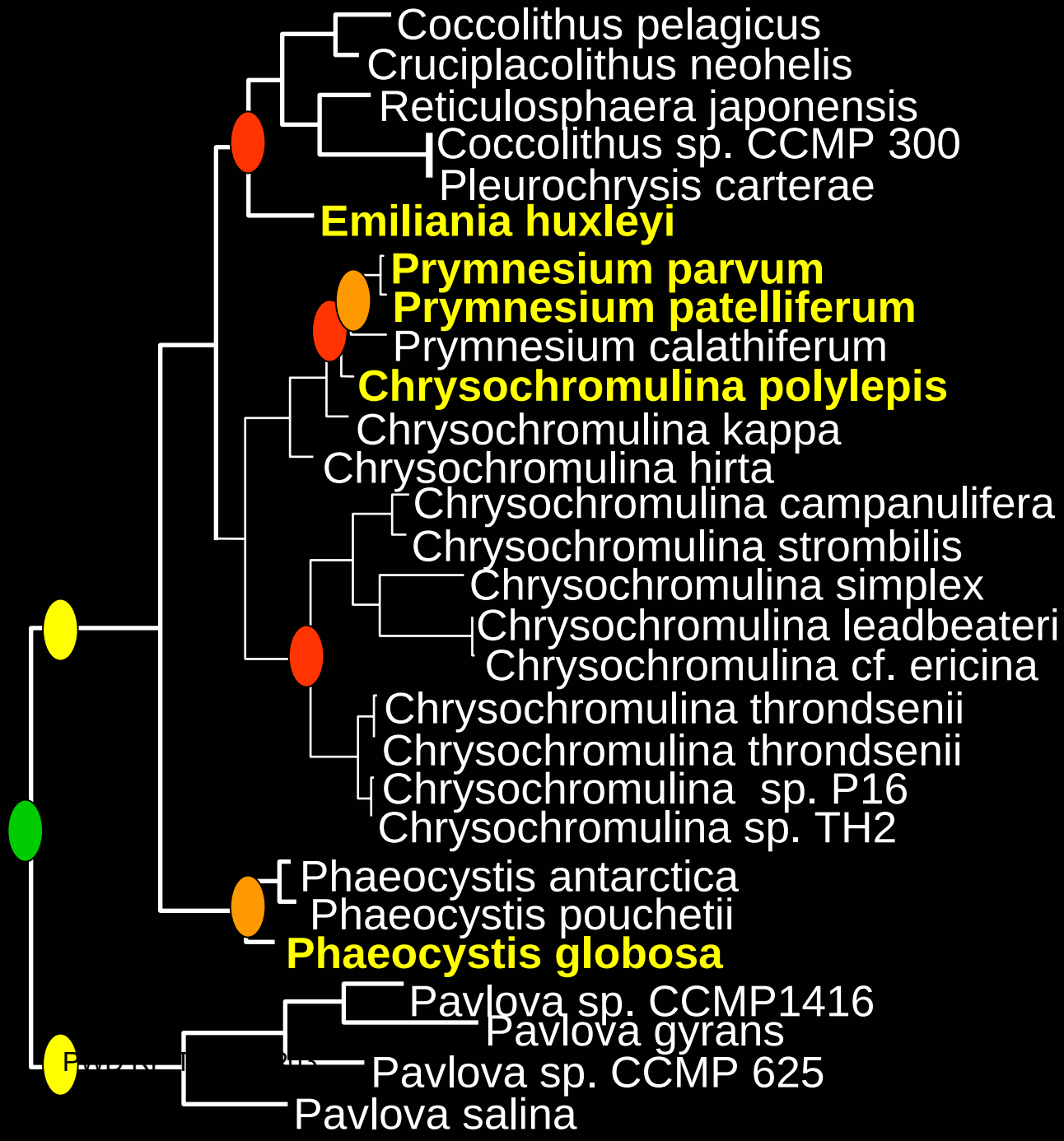

Coccolithus pelagicus
Cruciplacolithus neohelis
Reticulosphaera japonensis
Coccolithus sp. CCMP 300
Pleurochrysis carterae
Emiliania huxleyi
Prymnesium parvum
Prymnesium patelliferum
Prymnesium calathiferum
Chrysochromulina polylepis
Chrysochromulina kappa
Chrysochromulina hirta
Chrysochromulina campanulifera
Chrysochromulina strombilis
Chrysochromulina simplex
Chrysochromulina leadbeateri
Chrysochromulina cf. ericina
Chrysochromulina throndsenii
Chrysochromulina throndsenii
Chrysochromulina sp. P16
Chrysochromulina sp. TH2
Phaeocystis antarctica
Phaeocystis pouchetii
Phaeocystis globosa
Pavlova sp. CCMP1416
Pavlova gyrans
Pavlova sp. CCMP 625
Pavlova salina


Coccolithus pelagicus
Cruciplacolithus neohelis
Reticulosphaera japonensis
Coccolithus sp. CCMP 300
Pleurochrysis carterae
Emiliania huxleyi
Prymnesium parvum
Prymnesium patelliferum
Prymnesium calathiferum
Chrysochromulina polylepis
Chrysochromulina kappa
Chrysochromulina hirta
Chrysochromulina campanulifera
Chrysochromulina strombilis
Chrysochromulina simplex
Chrysochromulina leadbeateri
Chrysochromulina cf. ericina
Chrysochromulina throndsenii
Chrysochromulina throndsenii
Chrysochromulina sp. P16
Chrysochromulina sp. TH2
Phaeocystis antarctica
Phaeocystis pouchetii
Phaeocystis globosa
Pavlova sp. CCMP1416
Pavlova gyrans
Pavlova sp. CCMP 625
Pavlova salina

Application & detection methods for rRNA probes

- **DNA dot blots**
- ***In situ* hybridization / Fluorescence Microscopy**
- ***In situ* hybridization / Flow Cytometry liquid & solid**
- DNA microchips

Euk 1209

PrymGenus Probe
18S rRNA

Prym Species Probe
28S rRNA

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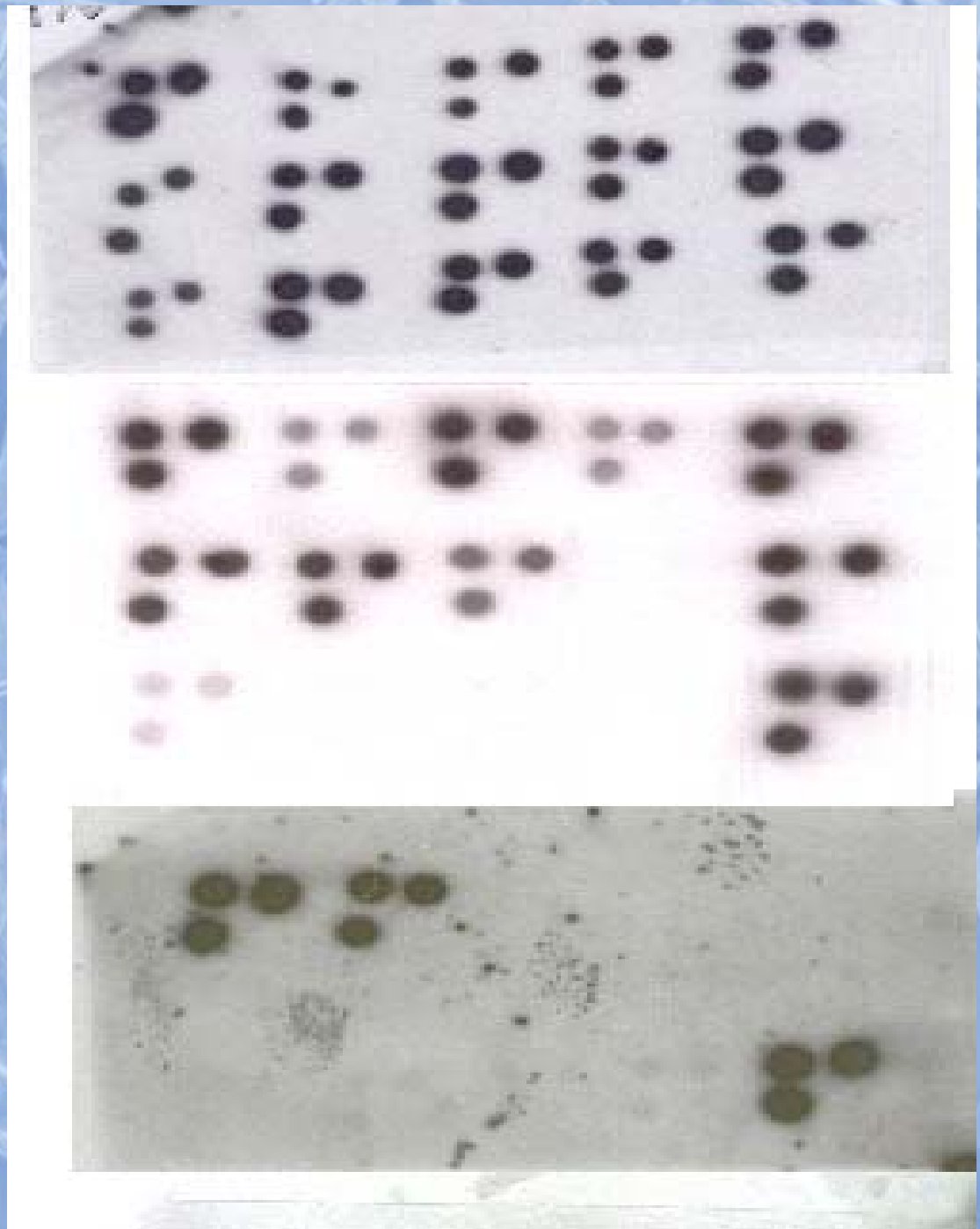

Conclusions

- Specific probes could be made for different groups of phytoplankton (from higher group down to species and strain level)
- More than a dozen probes for toxic algae are available or under development
- It is possible to use the probes with lab cultures and with field samples
- The probes could be used with different kinds of techniques (dot blots, fluorescence microscopy, flow cytometry, DNA chips, etc.)

Platforms for the detection of toxic algae

Linda Medlin, G. Eller, K. Toebe,
R.Groben, M. Lange, & K. Kerkmann
Bremerhaven, Gernmany

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1. Chem*ScanRDI*, a laser based system to quickly analyse FISH-experiments

2. Electrochemical detection of toxic algae via a handhold device

3. Development of DNA-microarrays for monitoring phytoplankton composition

FISH detection of toxic algae via the Chem*Scan, solid phase cytometer*

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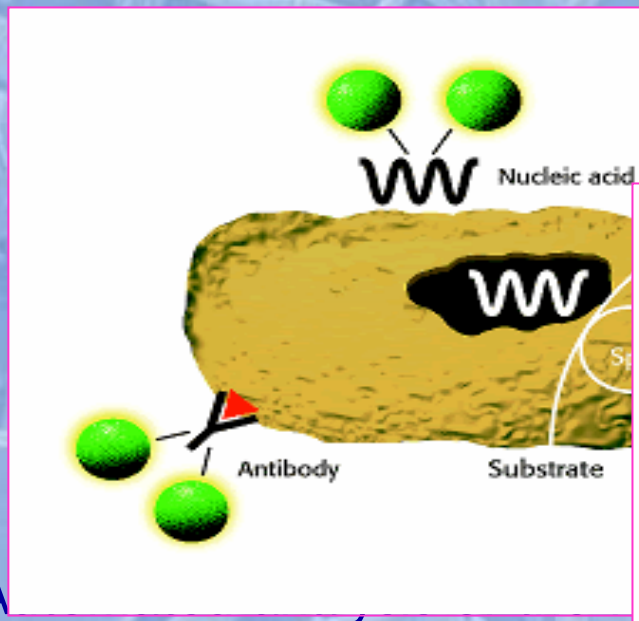
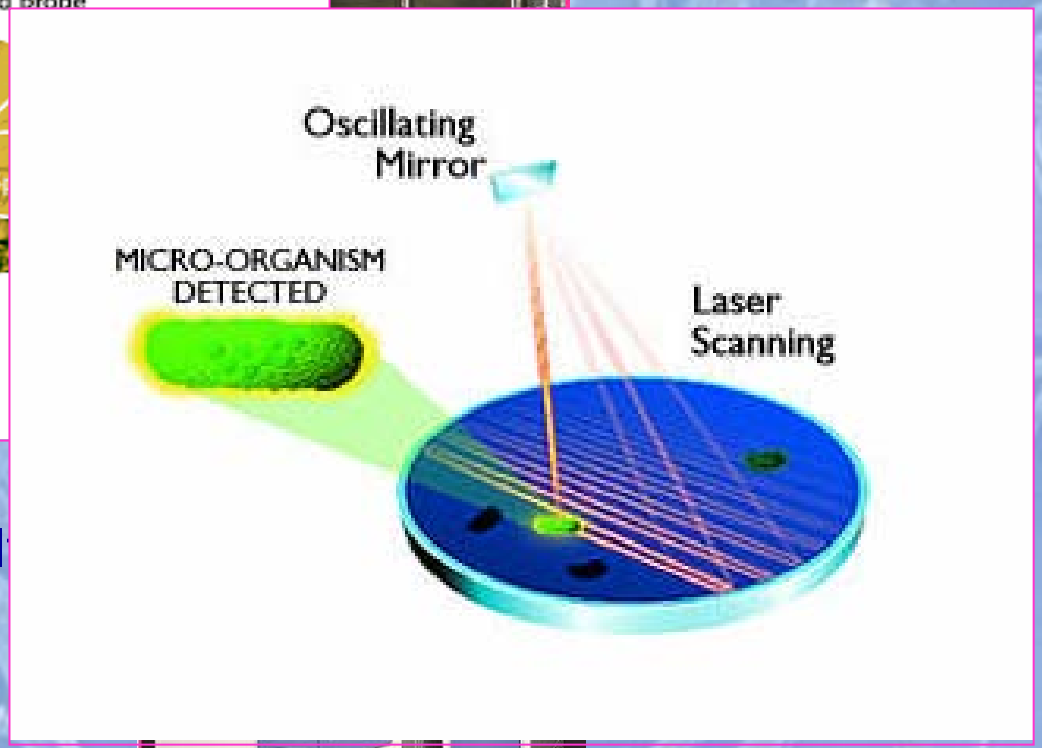
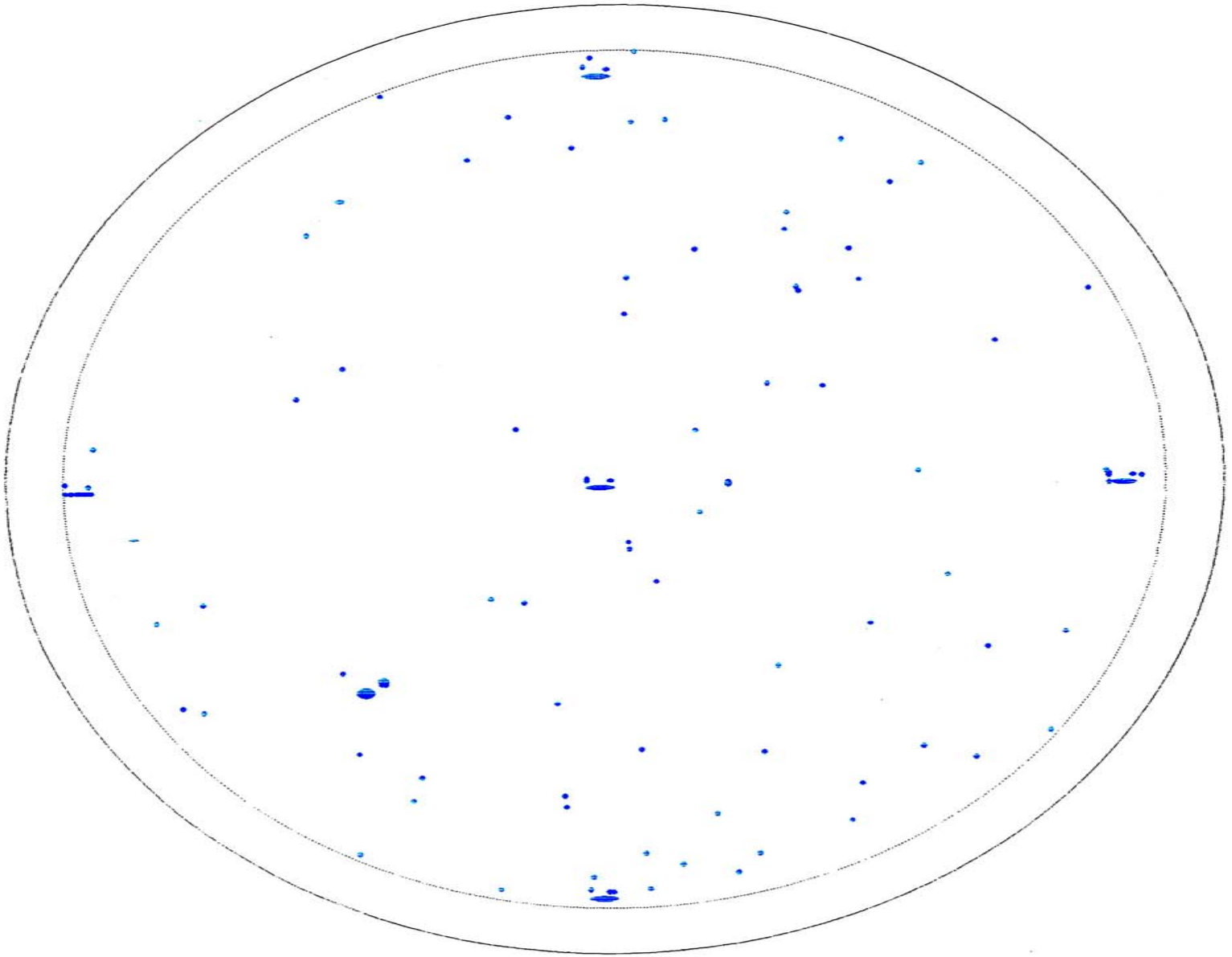
ChemScanRDI combines fluorescent cell labelling with laser scanning





3. A
cells by Chem*Scan RDI*
(Chemunex Inc.; Maisons-Al



All Data: Total Objects Seen: 107

Electrochemical detection of toxic Algae via a handheld device

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A Handheld Device for the Detection of Harmful Algae

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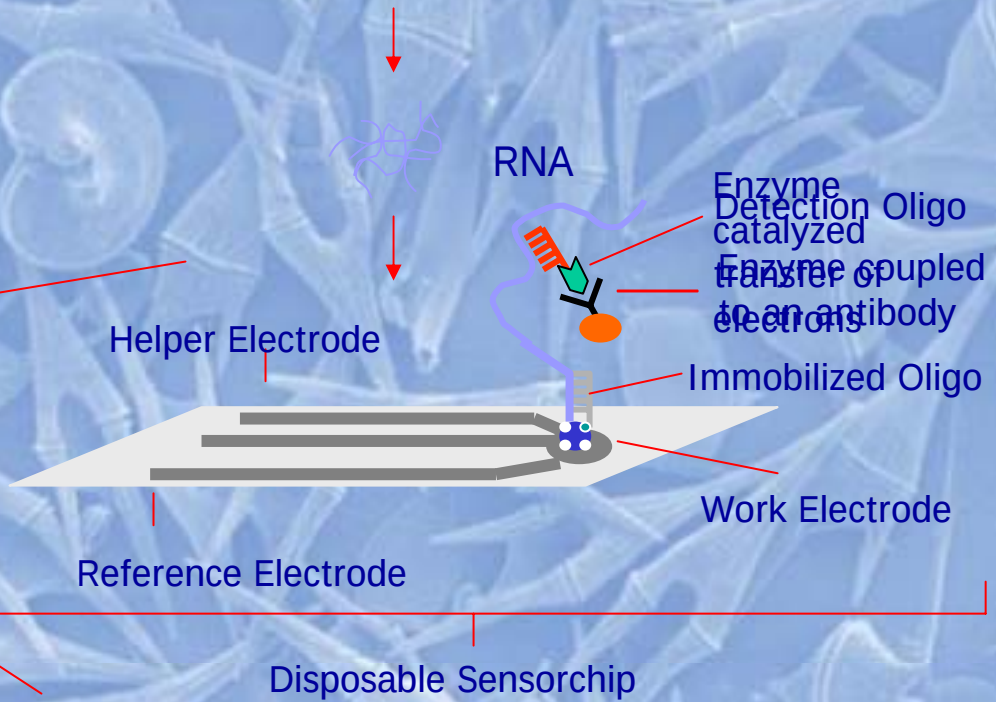
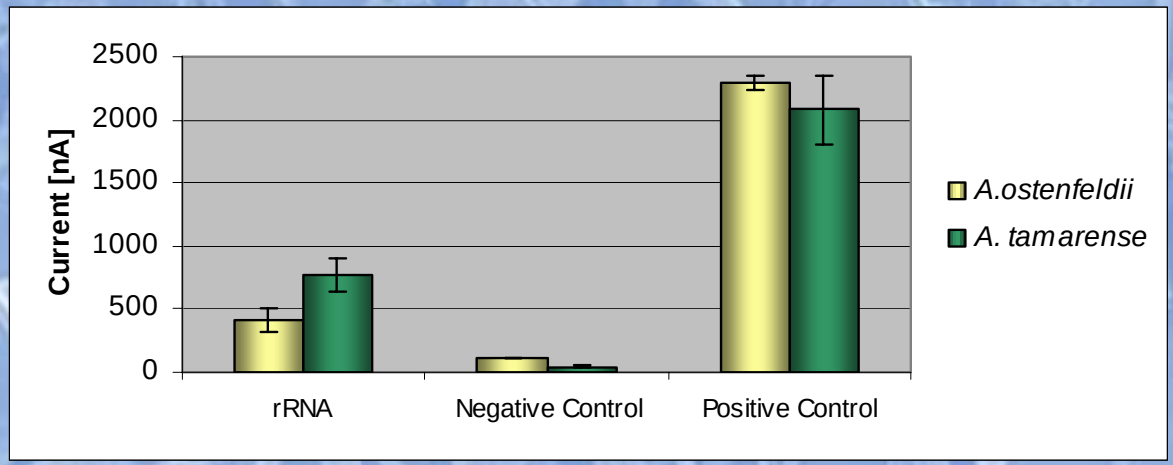
- *Alexandrium tamarense*
- *Alexandrium ostenfeldii*

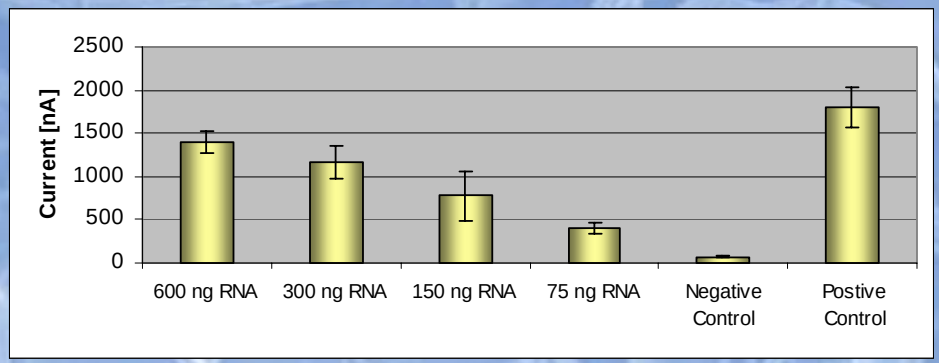


Electrochemical Detection of rRNA from *Alexandrium* Species

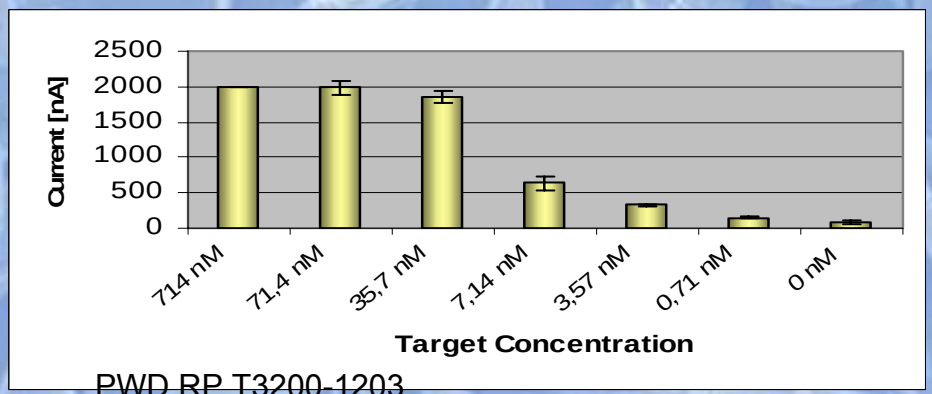


- ~ 500 ng rRNA have been hybridized to probes
- The probes were directed against ribosomal RNA of *A. tamarense,* respectively *A. ostenfeldii*

Concentration Series of Decreasing Amounts of Target



A. Decreasing amounts of A. tamarense rRNA hybridized to a *A. tamarense* probe



B. Decreasing amounts of a 70 bp Oligonucleotide hybridized to a *A.ostenfeldii* probe

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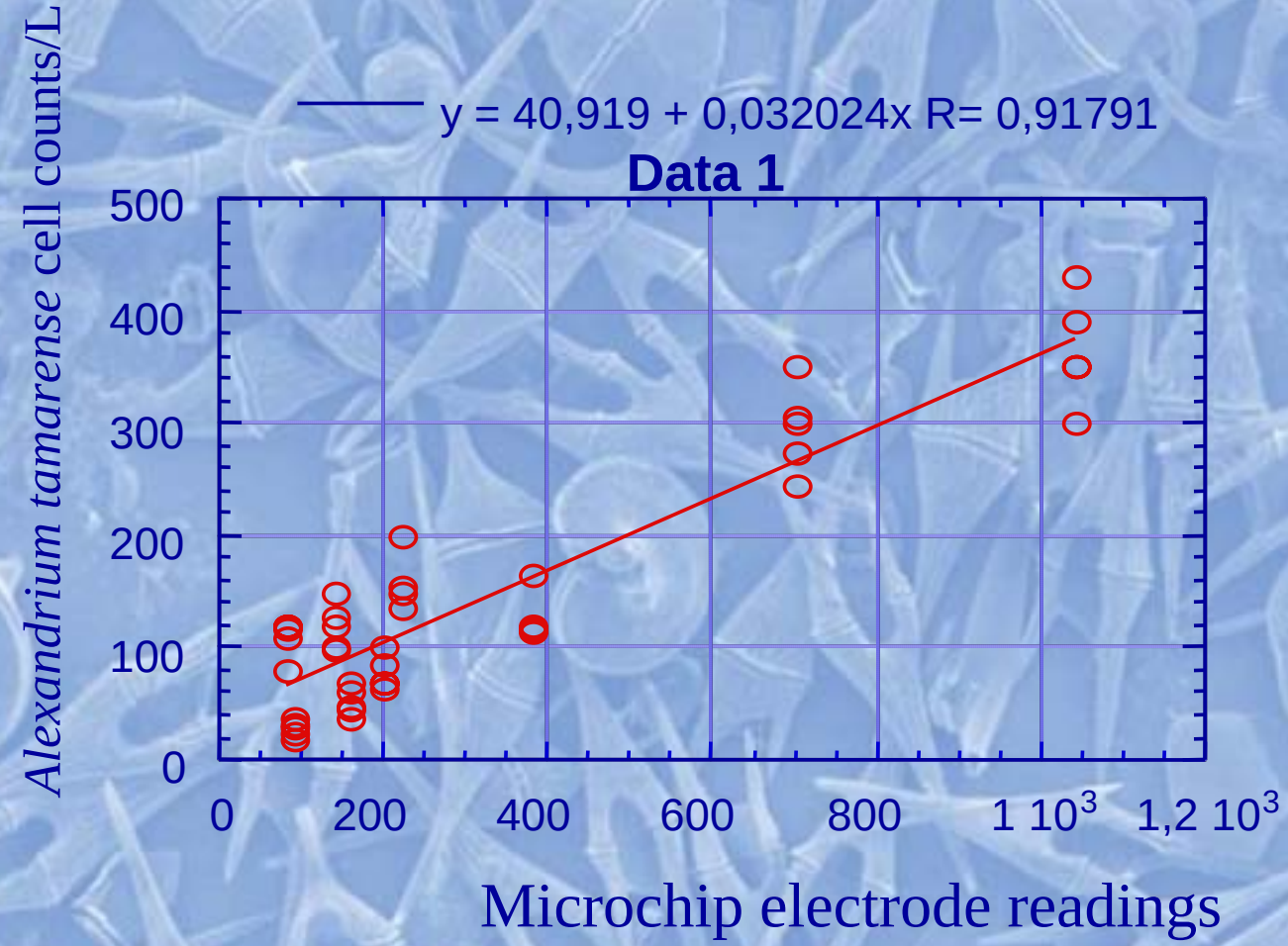
Comparison of cell counts with electrode readings



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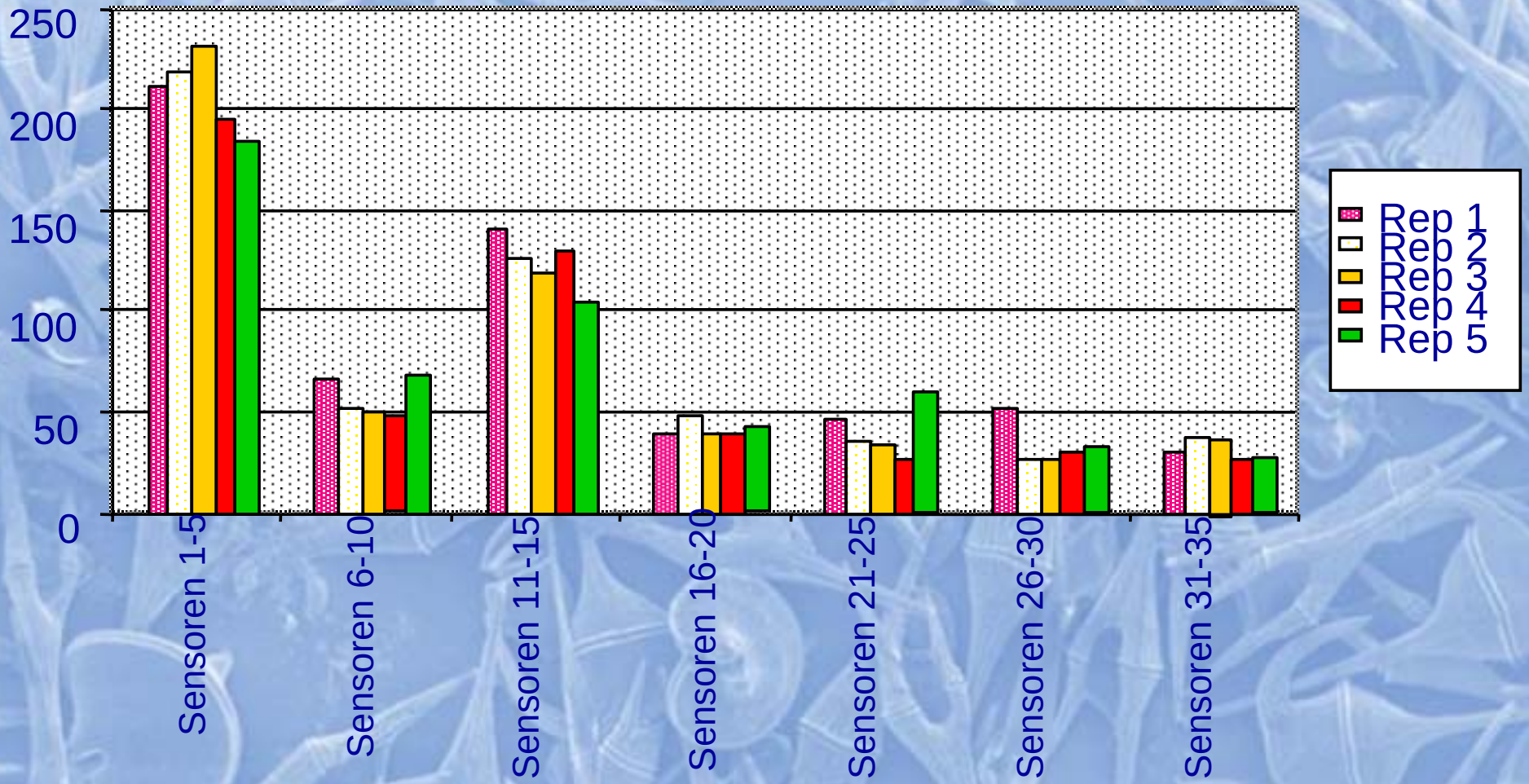
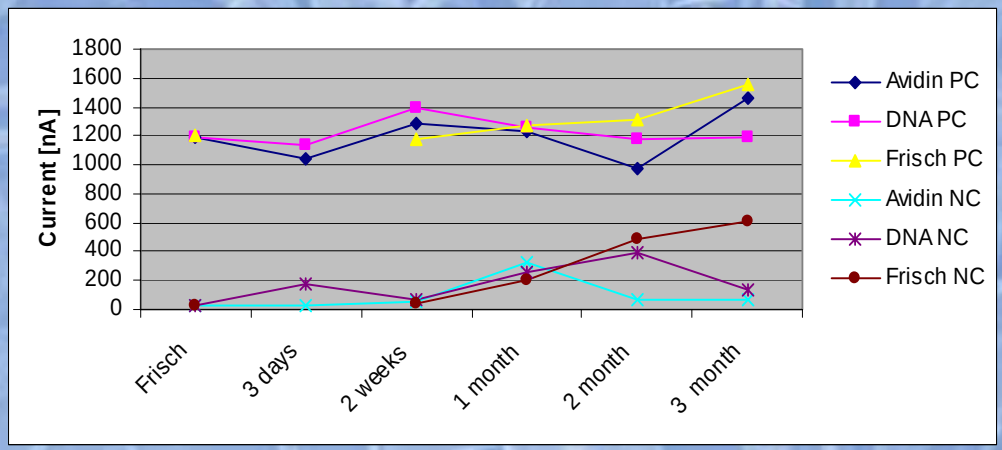


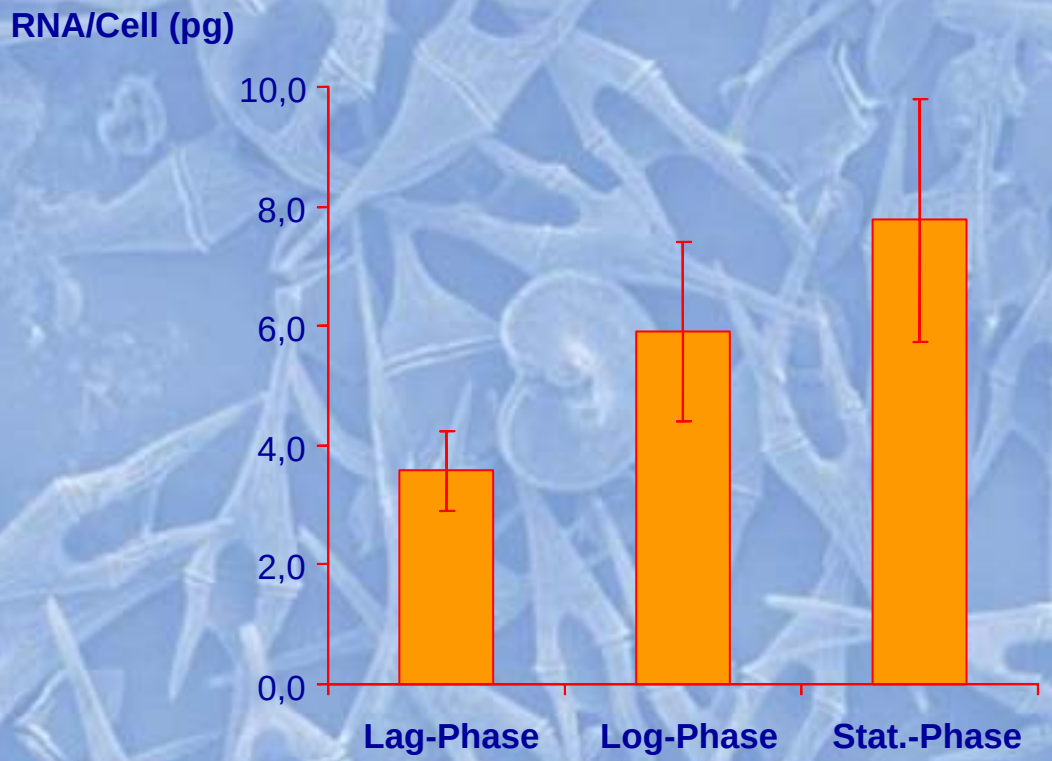
C. Detection of rRNA in natural samples from the Orkneys islands (column sets 1-4) as compared to rRNA from Prorocentrum mexicanum (column sets 5&6) and hybidized with Alexandrium tamarense probe.

Long term stability of treated sensors



- Sensors have been treated with Avidin (Avidin) and Avidin/Probe (DNA)
- The coated Sensors were then stored at 4°C over the indicated times
- Freshly prepared sensors have been prepared before each hybridization as positve controls for the experimental conditions (Frisch)
- To control the stability of the coated sensors, a hybridization was carried out with a 70 bp oligonucleotide (PC)
- For the negative control (NC) a hybridization was carried out without target-DNA

Alexandrium ostenfeldii K0324
RNA per Cell in Log-, Lag- and Stationary Phase



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Development of DNA-microarrays for monitoring phytoplankton composition

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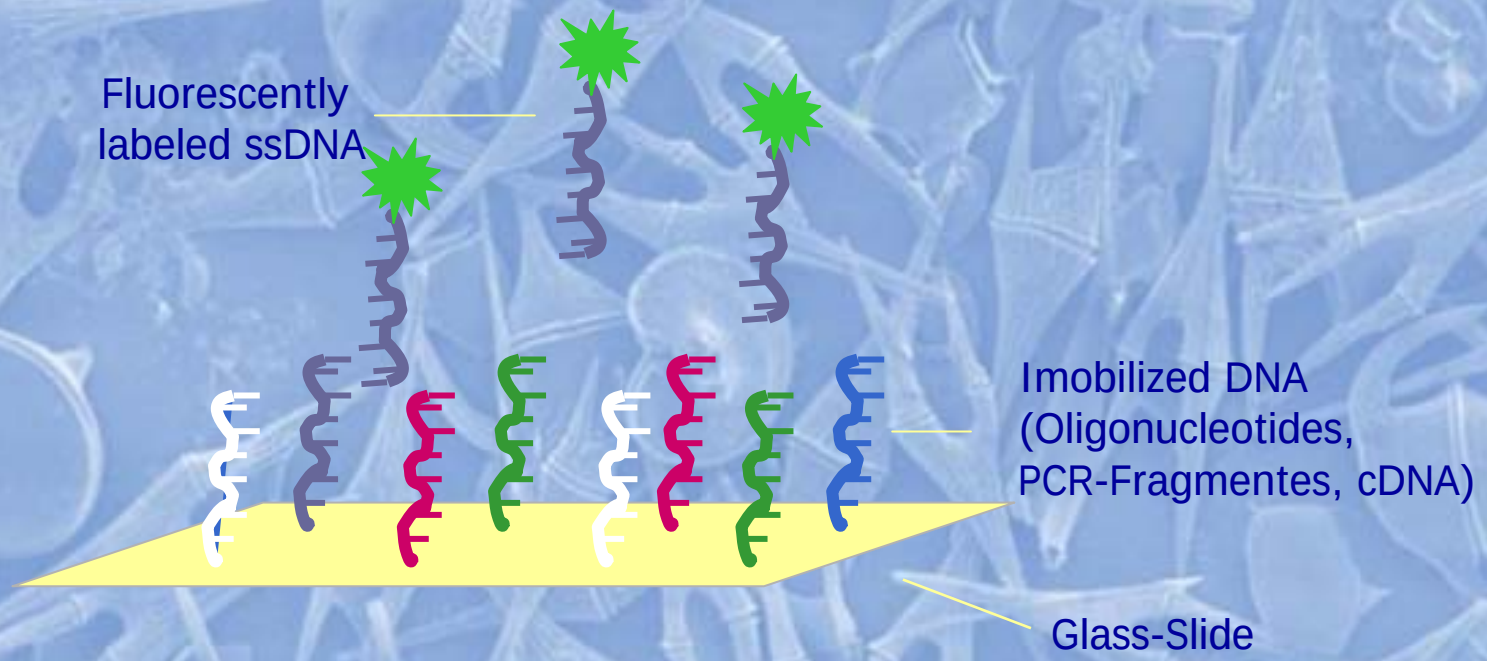
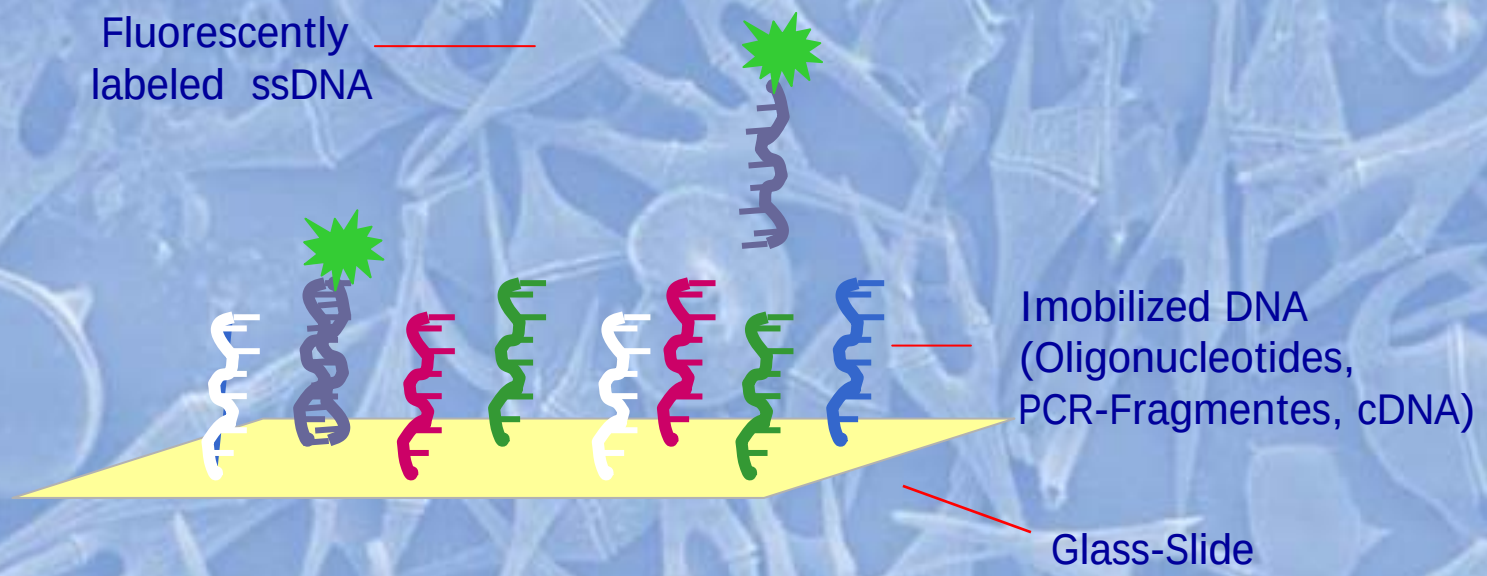
Scheme of a DNA-Chip Experiment

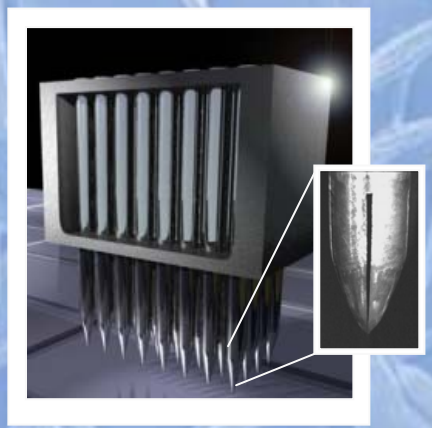


Scheme of a DNA-Chip Experiment



Low Density Chips

- ~ 625 Spots per cm^2
- Spotdiameter: ~ 200 µm
- Spotting with needles (A) or piezotechnology (B)



A.

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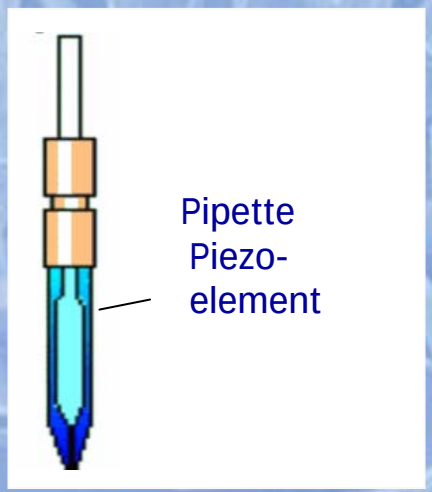
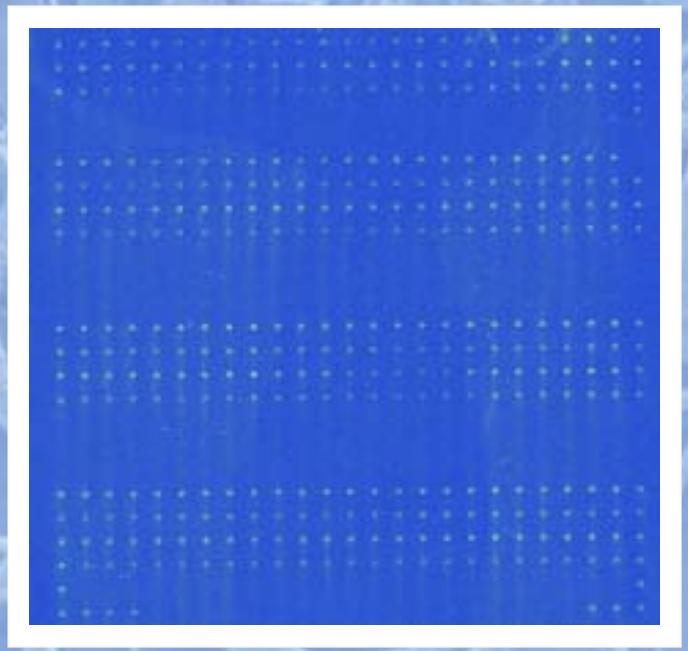
B.





500 µm

Monitoring Phytoplankton composition with DNA-Chip Technology

Phytoplankton samples

↓

Isolation of genomic DNA from the sample

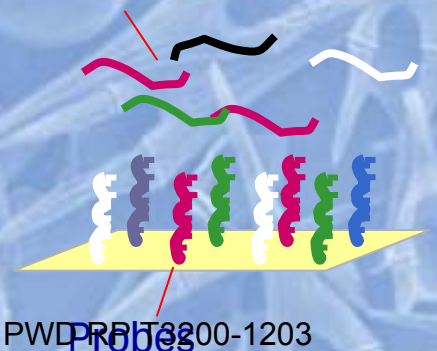
↓

Amplification of the 18S rDNA

↓

Hybridization of the 18S PCR-products with a DNA-Chip that contains probes initially designed for FISH

18S- PCR products

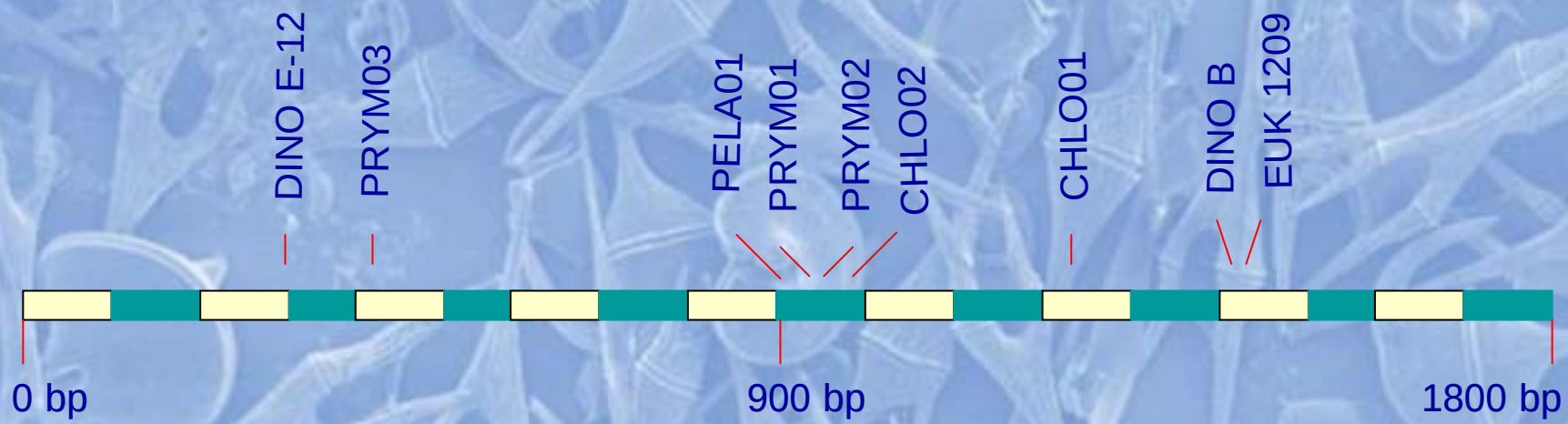


Probes

Probes and Targets used for preliminary Chip-Experiments

Class	Probe	Species
Dinophyceae	DINO B	▪ *Alexandrium tamarense* ▪ *Prorocentrum minimum*
	DINO E12	
Prymnesiophyceae	PRYM01	▪ *Prymnesium patelliferum*
	PRYM02	
	PRYM03	
Chlorophyceae	CHLO01	▪ *Dunaniella salina* ▪ *Pyramimonas obovata*
	CHLO02	
Pelagophyceae	PELA01	▪ *Coccoid pelagophyte* ▪ *Pulvinaria spec.*
Bolidophyceae	BOLI01	▪ *Clone. No. 151 PICODIV*
	BOLI02	

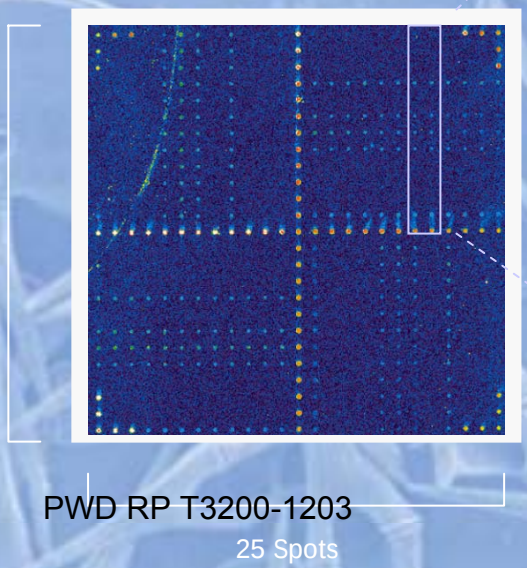
Localization of the Class-level probes in the 18S-Sequence

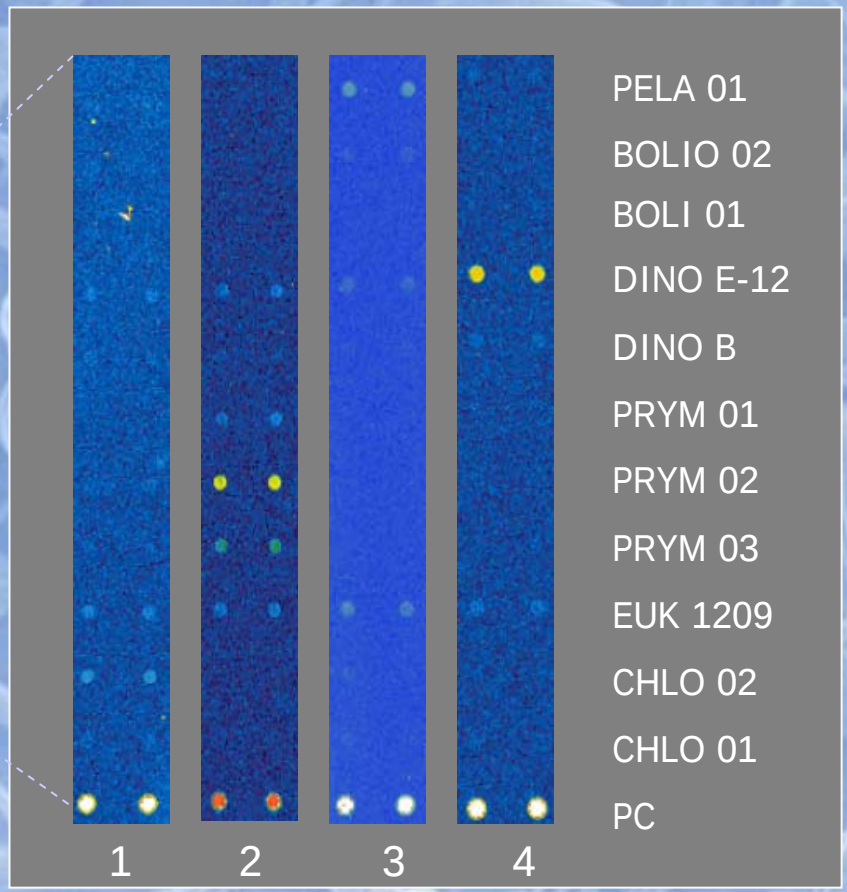


18S-DNA ~ 1800 bp

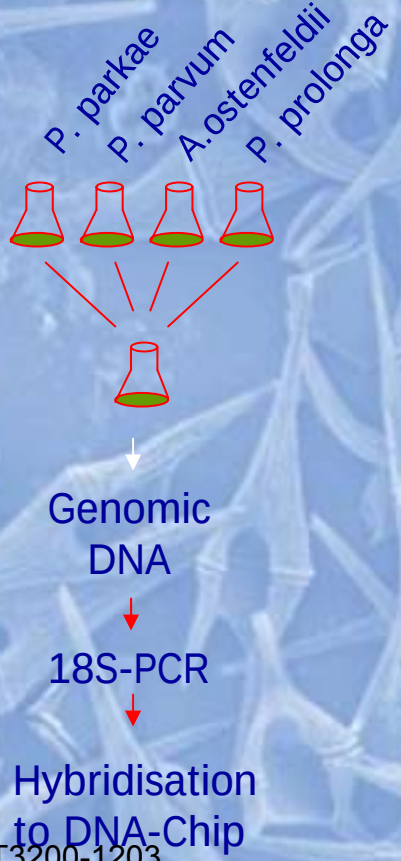
Preliminary results of a DNA-Chip with Class-level Probes

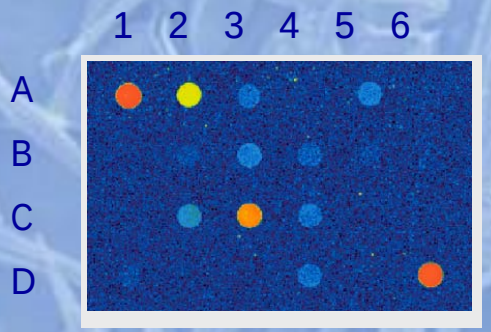
1. *Dunaniella salina*
2. *Prymnesium patelliferum*
3. *Coccoid pelagophyte*
4. *Alexandrium tamarense*



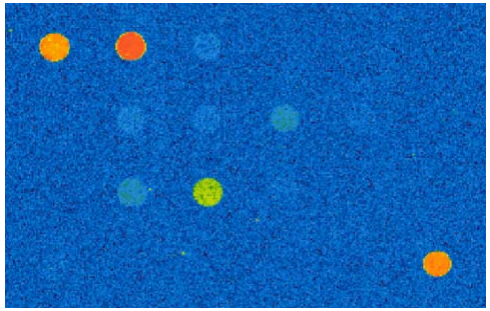


Identification of Phytoplankton on Class-level in a Mix of Laboratory Strains





A1- PC	C1- Pela 02
A2- Euk 328	C2- Dino B
A3- Euk 1209	C3- Dino E-12
A4- Chlo 01	C4- NS 03
A5- Chlo 02	C5- NS 04
A6- Hetero 01	C6- Pras 04
B1- Boli 01	D1- Bathy 01
B2- Boli 02	D2- Micro 01
B3- Prym 01	D3- Ostreo 01
B4- Prym 02	D4- Crypto B
B5- Prym 03	D5- NC
B6- Pela 01	D6- PC



A1- PC C1- Pela 02
A2- Euk 328 C2- Dino B
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A4- Chlo 01 C4- NS 03
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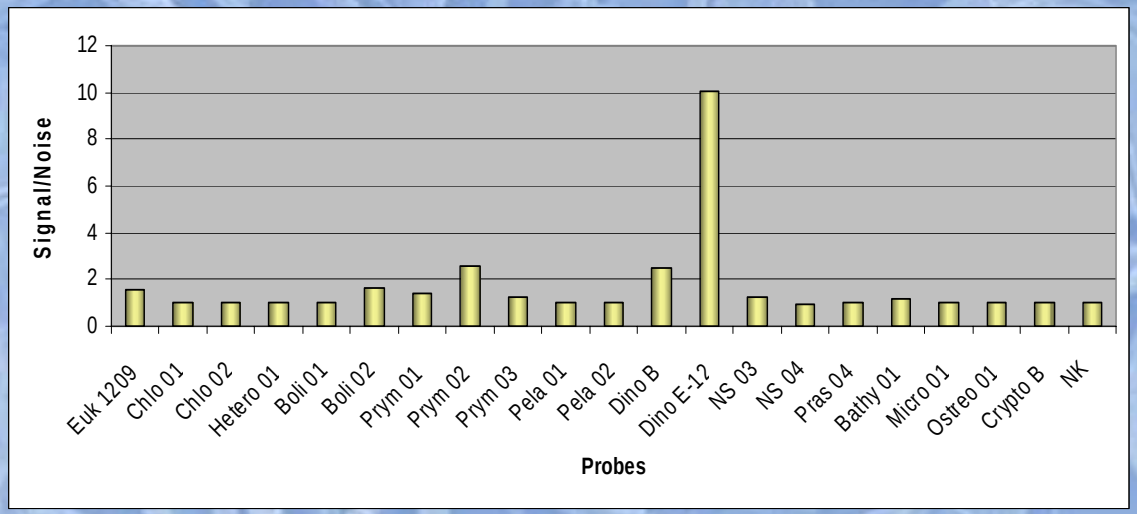
B1- Boli 01 D1- Bathy 01
B2- Boli 02 D2- Micro 01
B3- Prym 01 D3- Ostreo 01
B4- Prym 02 D4- Crypto B
B5- Prym 03 D5- NC
B6- Pela 01 D6- PC

- *Prymnesium parvum*
- *Alexandrium ostenfeldii*
- Threshold for a positive signal: signal/noise ratio ≥ 2



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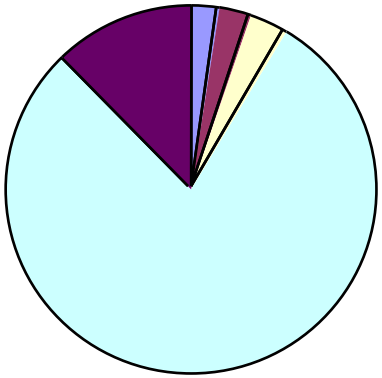
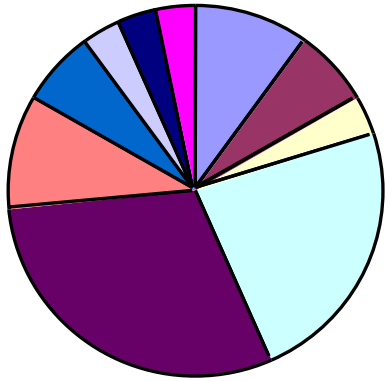
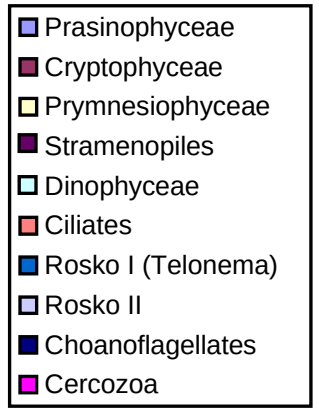
DNA CHIP

Clone librairy

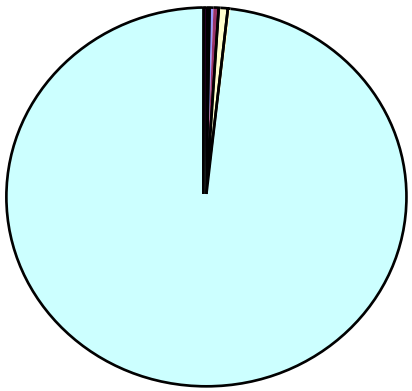
Dec 2000

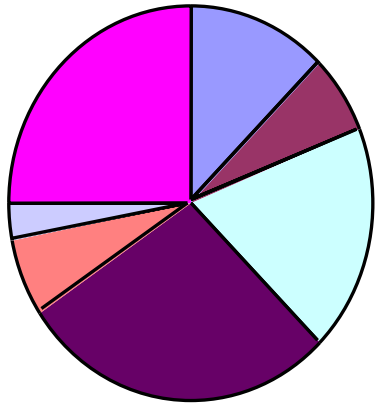


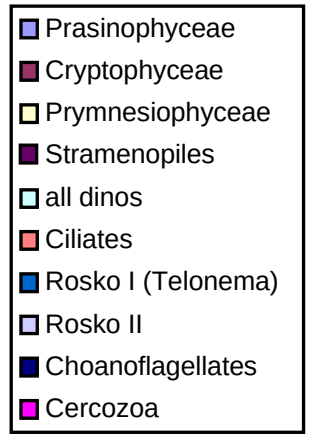




April 2001







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Summary

- The ChemScanRDI is a laserbased system that reduces the time required for FISH due to an automatic analysis, visual recovery of cells with positive signals
- It is possible to detect toxic *Alexandrium* species via a handheld device
- DNA-Chip technology provides the possibility to analyse numerous hybridzations in parallel

Research Needs

- Monitoring of toxic phytoplankton populations is an important scientific issue
- Efficient monitoring requieres quick and reliable techniques
- Currently the identification of species is done mainly by light or electron microscopy
- New tools are needed to be developed which cut down the time necessary for toxic phytoplankton classification
- Methods that involve oligonucleotide probes have the potential to fulfill these needs

People involved in the projects

Dr. Gundula Eller
Dr. Kerstin Toebe FISH/ ChemS*canRDI*

Susanne Huljic Handheld device/ *A.ostenfeldii*

Dr. Katja Kerkmann DNA-Chip Technology

Dr. Martin Lange Handheld device/ *A. tamarense*

Dr, Rene Groben Probe Hybridisation optimisation

Dr. Linda Medlin Principal investigator

Rapid Tests for the Detection of Haemolytic Compounds

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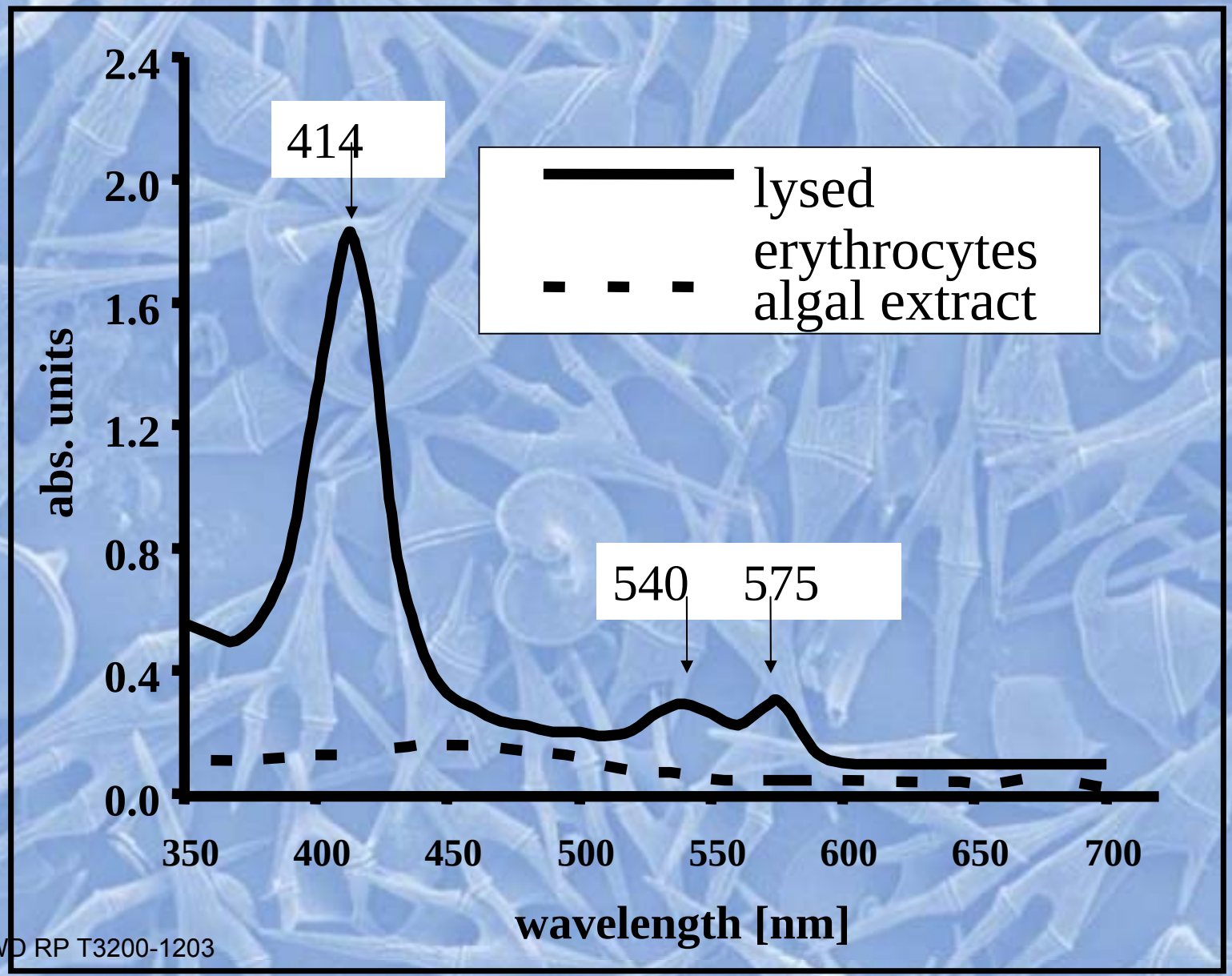

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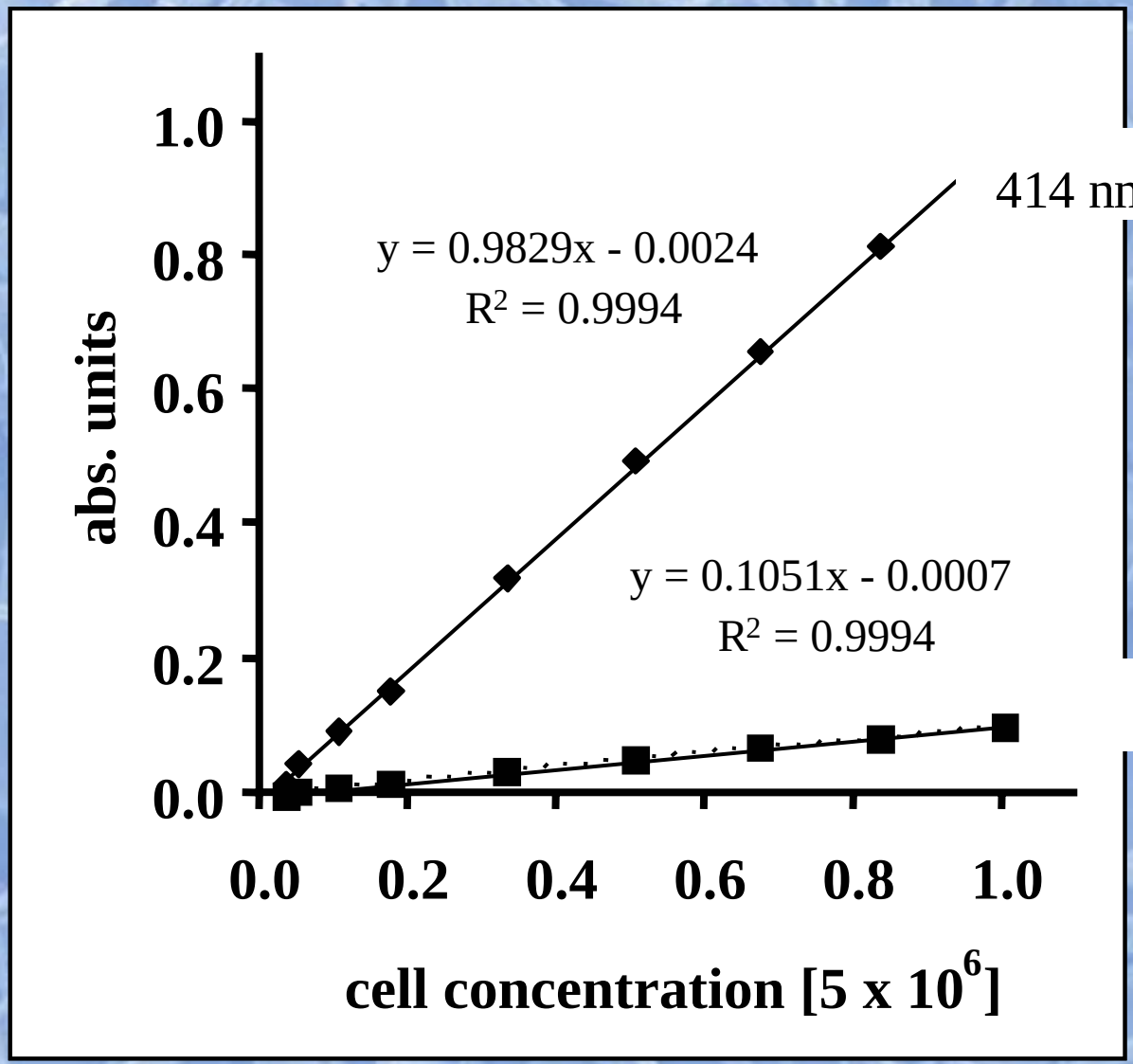

414 nm:

540 nm:

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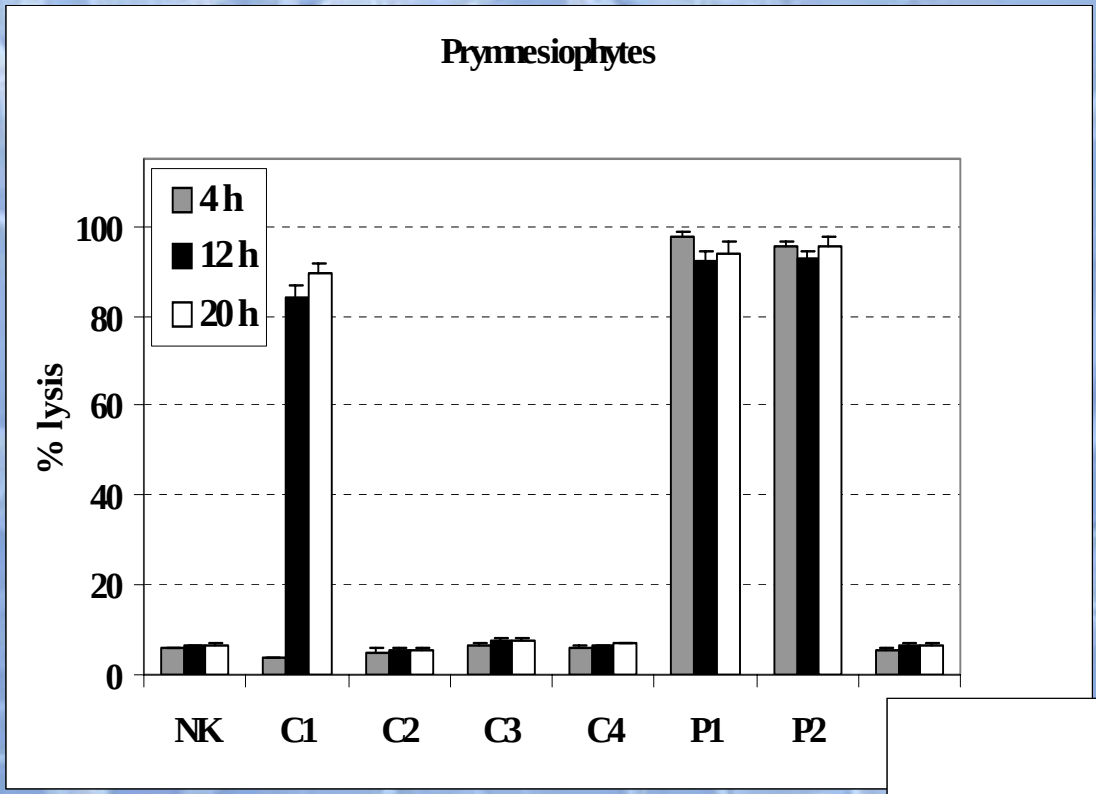

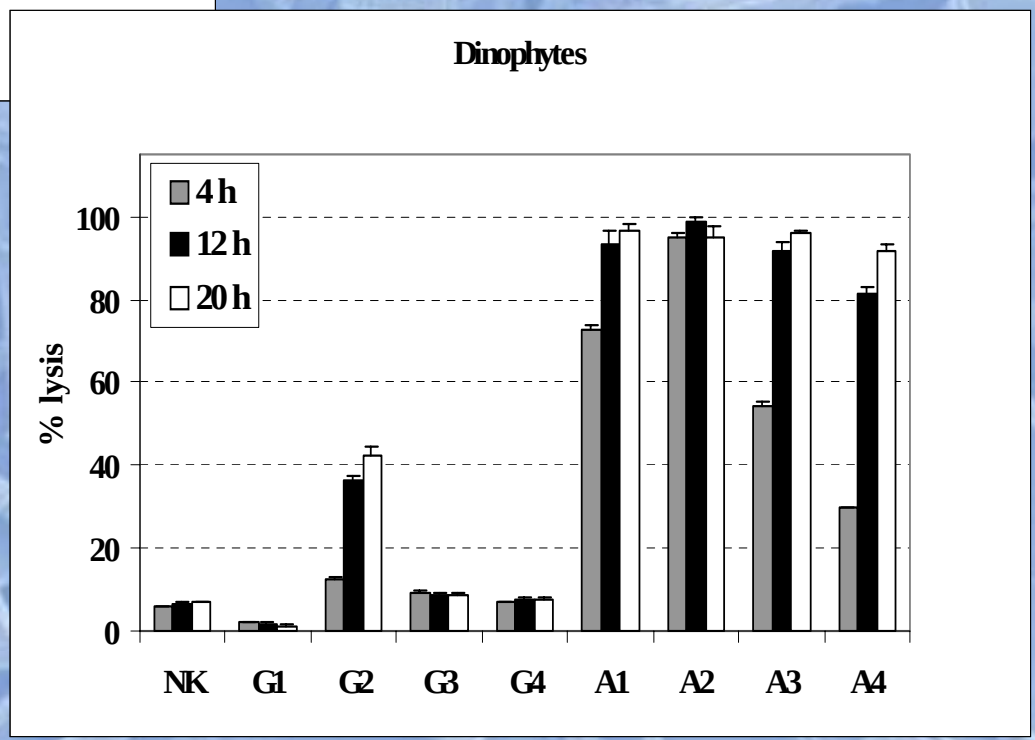

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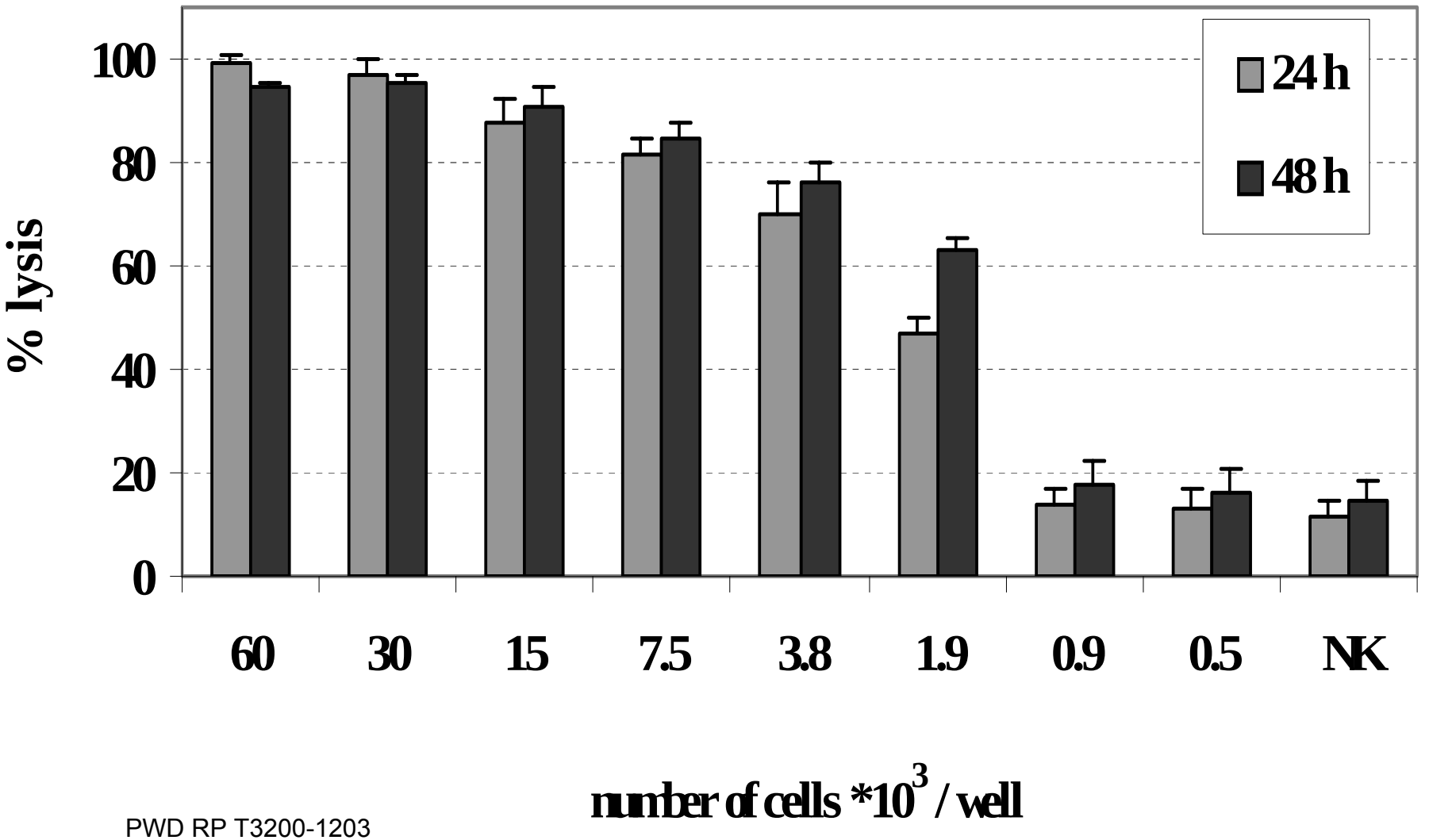
Prymnesium parvum RL10



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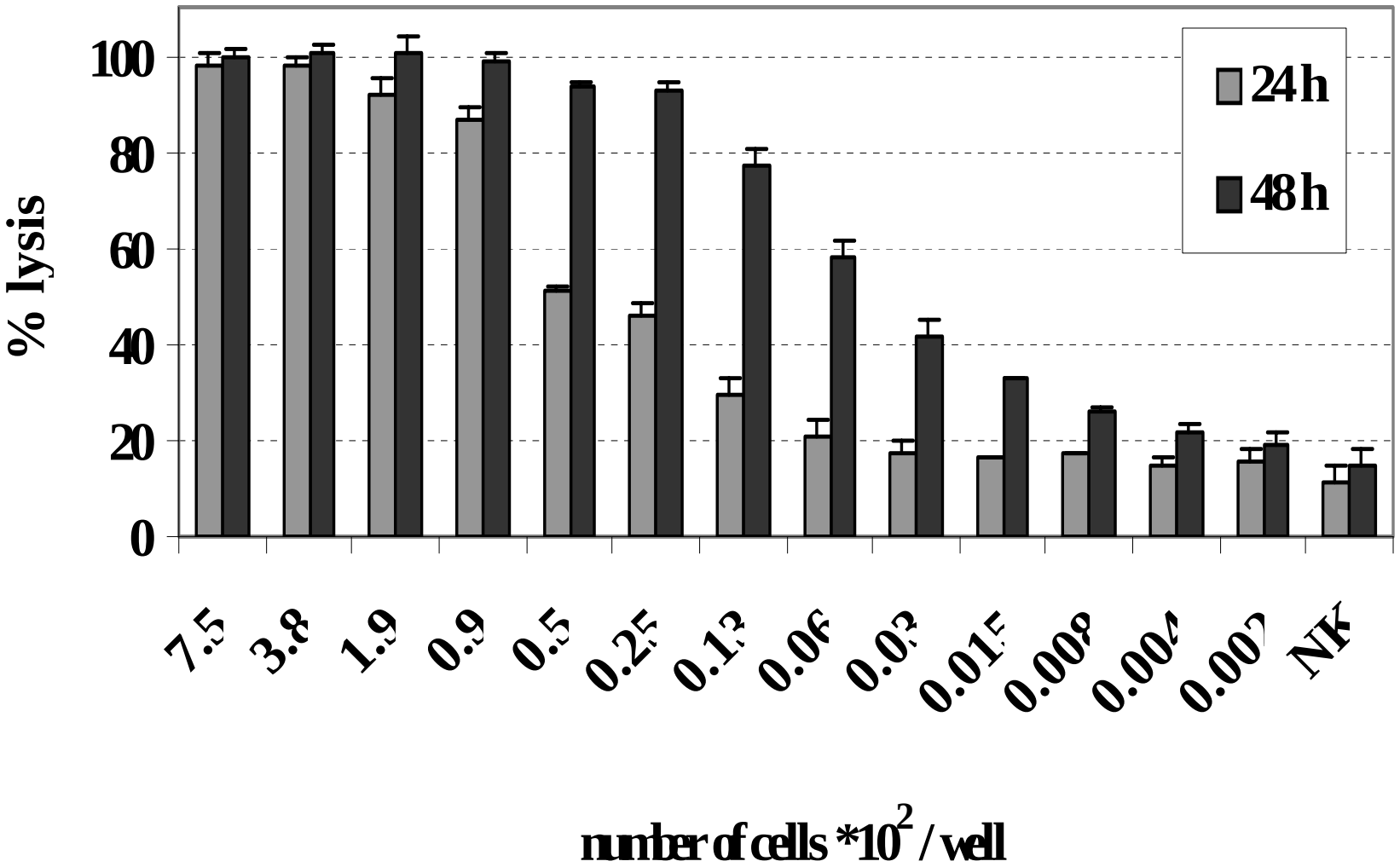
Alexandrium tamarense CCMP115



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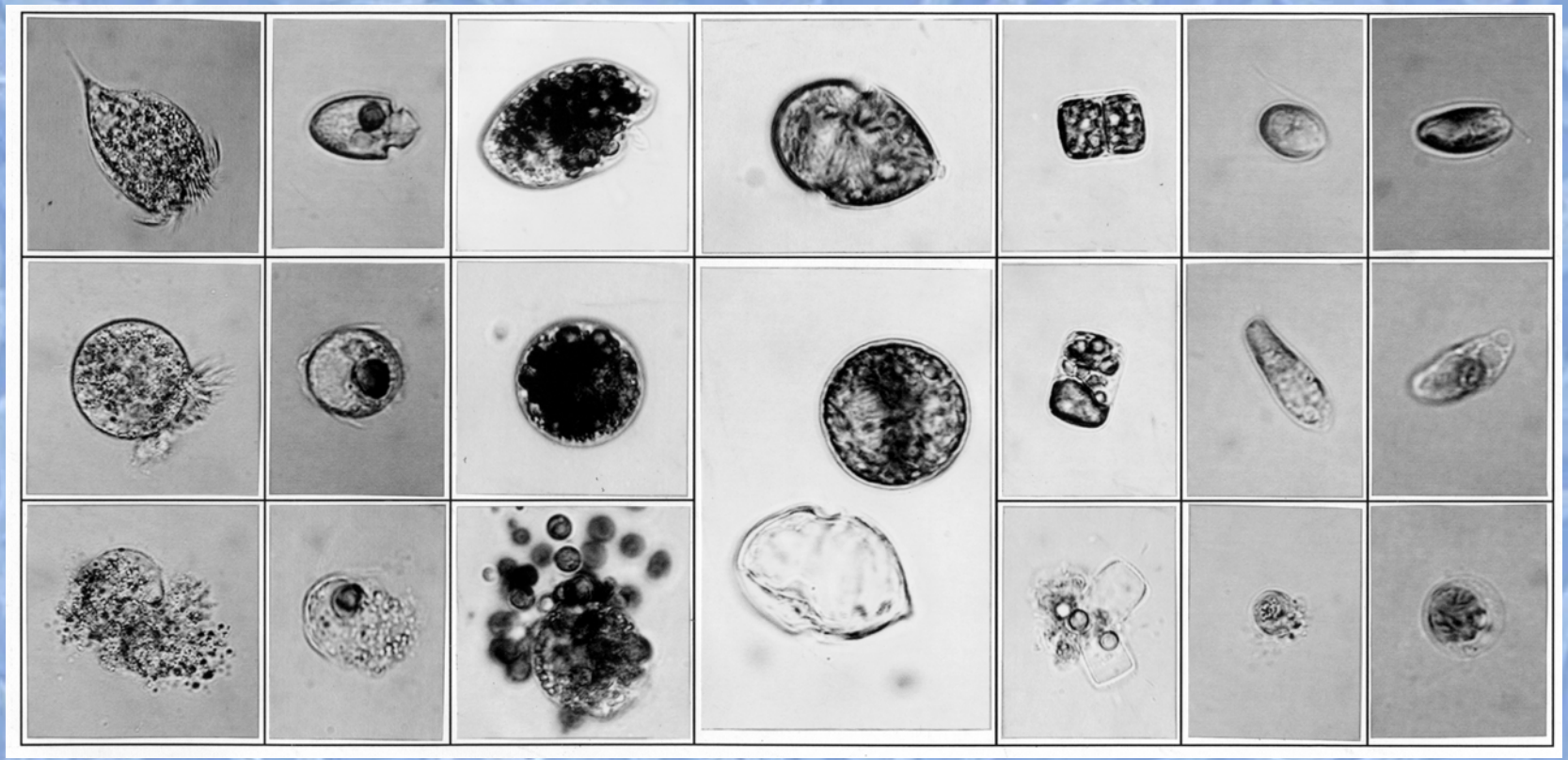
Allelochemical effects



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ELA Tests provide rapid means for detecting haemolytic compounds but these must be coupled with species tests for 100% Reliability

Controlling Harmful Algal Blooms

Donald M. Anderson and Mario R. Sengco

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Abstract.--Efforts to manage harmful algal blooms (HABs) throughout the world have focused mainly on their prevention, and the mitigation of their impacts on public health, fisheries, and important aquaculture industries. While critical and effective, such management programs only address the apparent "symptoms" of HABs without dealing with the causative organisms directly. Currently, there are few strategies to control an existing or persistent outbreak that threatens coastal resources. Bloom control strategies have included the use of ozone and various algicidal chemicals (e.g. copper sulfate, barley straw). Ultrasonic devices induce species-specific algal lysis in small volumes (e.g. ponds and aquaria), but have not been fully explored for use in large-scale brackish or marine systems. The biological control of HABs – through the introduction of algal pathogens (i.e. viruses, bacteria), parasites, competitors and grazers – has been proposed, but not yet attempted at meaningful scales. Chemicals such as alum and a wide assortment of organic flocculants have been tested to determine whether blooms can be removed from the water column by promoting algal flocculation and rapid settling. In marine systems, attempts to use chemical flocculants have been reported in Asia, although results were limited due to rapid dilution and high cost. A variant to this chemical-flocculation approach is the addition of clay minerals to HABs to flocculate and settle the organisms directly. Essentially, these minute (< 2 μm), but dense minerals bind and act to ballast the organisms to promote cell sinking, despite the organisms' motility and buoyancy. Underlying cells are further removed by entrainment into the settling aggregates. The high removal efficiency, rapidity, cost effectiveness and low environmental impacts of clay dispersal have made it one of the most promising control methods under investigation. The potential use of clays to control harmful algal blooms (HABs) has been explored in Japan, South Korea, China, Australia, the United States, and Sweden. In Japan and South Korea, minerals such as montmorillonite, kaolinite, and yellow loess, have already been used in the field effectively, to protect fish mariculture from *Cochlodinium* spp. and other blooms. In the U.S., several clays and clay-rich sediments have shown high removal abilities (e.g. > 80% cell removal efficiency) against *Karenia brevis*, *Heterosigma akashiwo*, *Pfiesteria piscicida* and *Aureococcus anophagefferens*. Benthic impact studies in the laboratory, and early studies in the field, have revealed some repercussion on survival and growth of certain species, and on the composition and abundance of benthic communities. However, these impacts have to be interpreted in the proper context to evaluate the acceptability of the clay control method. In Sweden, phosphatic clay (at 4 g/L) can remove up to 100% of *Prymnesium parvum*, but cell removal was influenced by cell concentration and physiology (N- or P- deficiency). Initial experiments with European clays showed promise through the use of raw, unincinerated clay and the addition of polyaluminum chloride (PAC) to enhance clay adhesiveness. A related mitigation method uses a proprietary clay formulation to scavenge phosphorus from freshwater systems, and to "lock" phosphorus in bottom sediments through the formation of a relatively impervious clay layer at the sediment surface. This technology will be described and discussed in the context of its possible utility during *P. parvum* blooms. The potential control of *P. parvum* with clay will be discussed, emphasizing the challenges of applying control methodologies developed from marine HABs to a low salinity environment.

View the presentation

Bloom Control Strategies for Harmful Algal Blooms

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Management of harmful algae

- ***Prevention***

  options for reducing the incidence and extent of HABs before they begin
  - **alteration of nutrient inputs**
  - **ballast water management**

- ***Mitigation***

  when a bloom is present, reduce the loss of resources and minimize health risks
  - **monitoring for cells and toxins**
  - **forecasting and public communication programs**
  - **transfer of fish pens to clean sites**

- ***Control***

  during an outbreak, methods that target and attack the causative organisms
  - **biological**
  - **chemical**
  - **ultrasonics**
  - **ozonation**
  - **chemical flocculation**
  - **clay flocculation**

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Chemical Control

Inorganic chemicals

- **$CuSO_4$, $KMnO_4$, $FeCl_3$, chlorine,**
- **ozone**
- **NaOCl (from electrified seawater)**
- **H_2O_2 (against cysts)**
- **…….others**

Organic chemicals

- **APONIN (from alga *Nannochloris* sp.)**
- **Sophorolipids (from fungus *Candida bombicola*)**
- **phlorotanins (from brown alga *Ecklonia kurome*)**
- **Barley straw bales and extract**
- **…….others**

With one or two exceptions, chemical control of HABs has not been attempted on any significant scale in natural marine waters.

Chemical control of freshwater algal blooms
- copper sulfate, algicides, barley straw

Barley straw:

Application rates: Based on pond surface area rather than volume - about 225 lbs/acre.

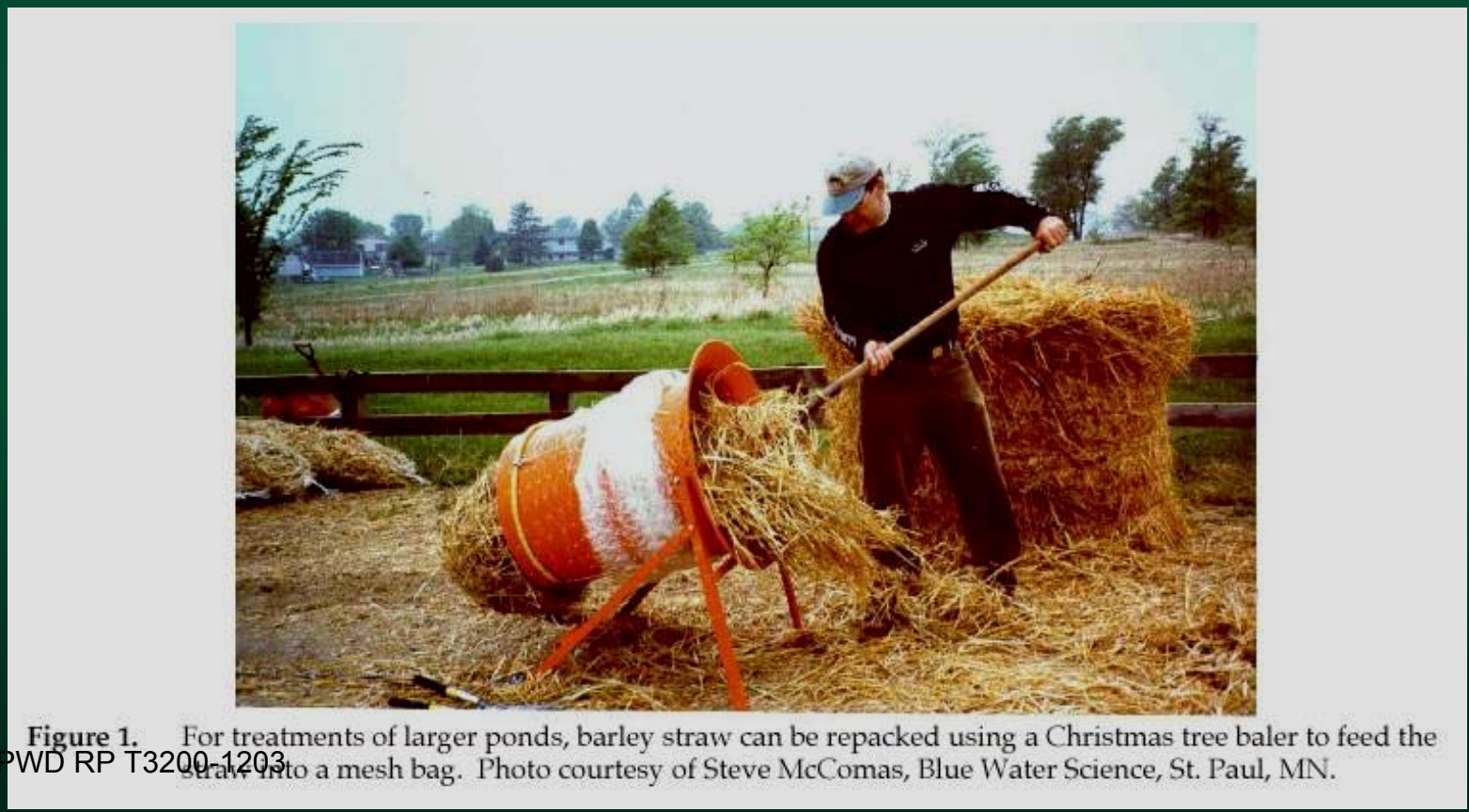


Figure 1. For treatments of larger ponds, barley straw can be repacked using a Christmas tree baler to feed the straw into a mesh bag. Photo courtesy of Steve McComas, Blue Water Science, St. Paul, MN.

PWD RP T3200-1203

***Source*: Lembi, C.A. Aquatic Plant Management, Purdue Univ. Cooperative Extension Office, APM-1-W, 8/02**

- **Decomposing barley straw releases inhibitory compounds, possibly oxidized polyphenolics derived from lignins and tannins. It is considered more environmentally benign than other chemical treatments.**
- **These do not kill the algae, but limit or prevent cell proliferation.**
- **Effects seen days to months after use, and can last several months.**
- **This method is used in freshwater systems. Very little work has been done on brackish, estuarine or marine environments.**
- **Some controversy remains regarding mode of action and effectiveness.**
- **Will this work on *P. parvum*, and especially, in the winter?**

Figure 2. A barley bale being anchored into a lake. Photo courtesy of Steve McComas, Blue Water Science, St. Paul, MN.

IWD-RP-T3200-1203

***Source*: Lembi, C.A. Aquatic Plant Management, Purdue Univ. Cooperative Extension Office, APM-1-W, 8/02**

Hydrobiologia **340:** 307–311, 1996.
J. M. Caffrey, P. R. F. Barrett, K. J. Murphy & P. M. Wade (eds), Management and Ecology of Freshwater Plants.

The control of diatom and cyanobacterial blooms in reservoirs using barley straw

[1]P. R. F. Barrett, [2]J. C. Curnow, [3]J. W. Littlejohn
[1] *8 Sunderland Avenue, Oxford OX2 8DX, England*
[2] *Environmental Medicine, Grampian Health Board, Aberdeen, Scotland*
[3]*Grampian Regional Council, Depatment of Water Services, Aberdeen, Scotland*

- This (small) reservoir had a long history of cyanobacterial blooms, with well-recorded observations of algal types and cell counts.
- During 17-mo. trial, level of tested chemicals remained within acceptable limits and there were no customer complaints
- A marked reduction in algal populations occurred over the 2 summers with straw application. However, no definite conclusions can be drawn due to lack of a legitimate control.

Table 1. Mean Monthly Algal Counts in Reservoir 1 Cells per ml

Year	1991	1992	1993	1994
January	N/C	10,000	10,000	400
February	13,000	18,000	17,500	6,200
March	21,500	28,000	22,800*	7,800
April	57,400	38,000	29,000	8,700
May	67,500	25,200	14,500	3,400
June	45,000	16,500	6,800	106*
July	N/C	17,700	3,000	57
August	2,000	10,500	4,500	440
September	N/C	OS	1,500	
October	4,000	OS	1,000	
November	10,000	4,000	1,000	
December	2,000	7,000	1,000*	

* Straw introduced after the sampling dates in March and December 1993 and June 1994.

PWD RP T3200-1203

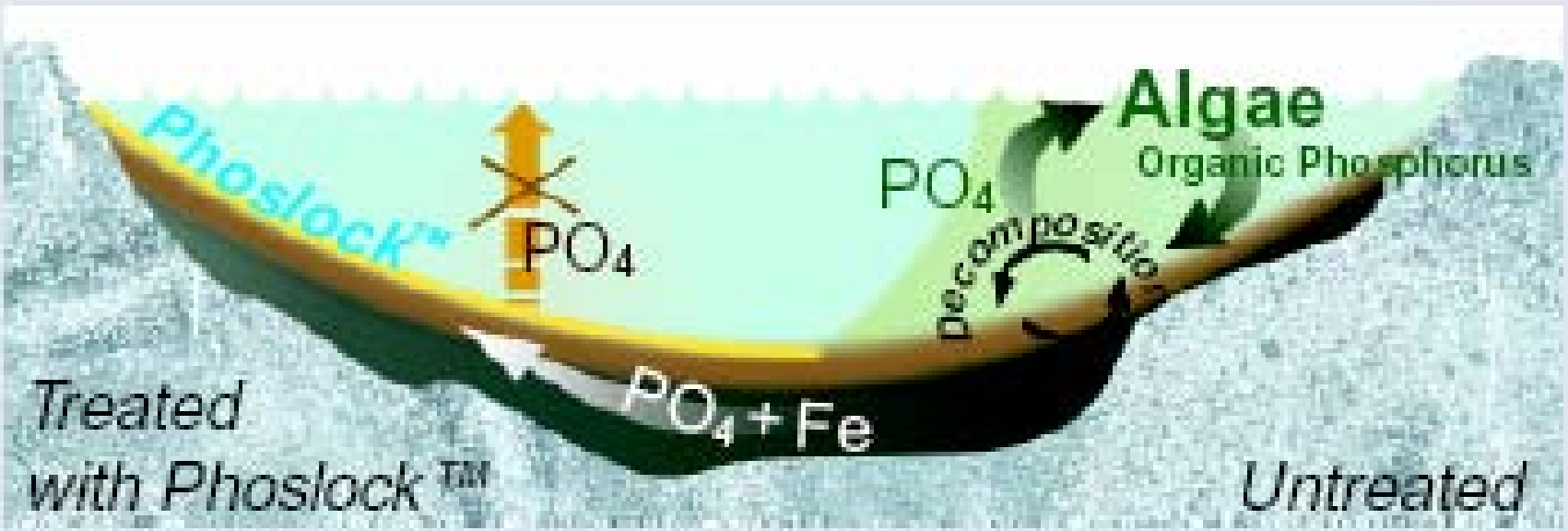
Source: Barrett et al., 1996

Chemical flocculants - Phosphorus Control

alum

polyaluminum chloride

Phoslock (clay-based)

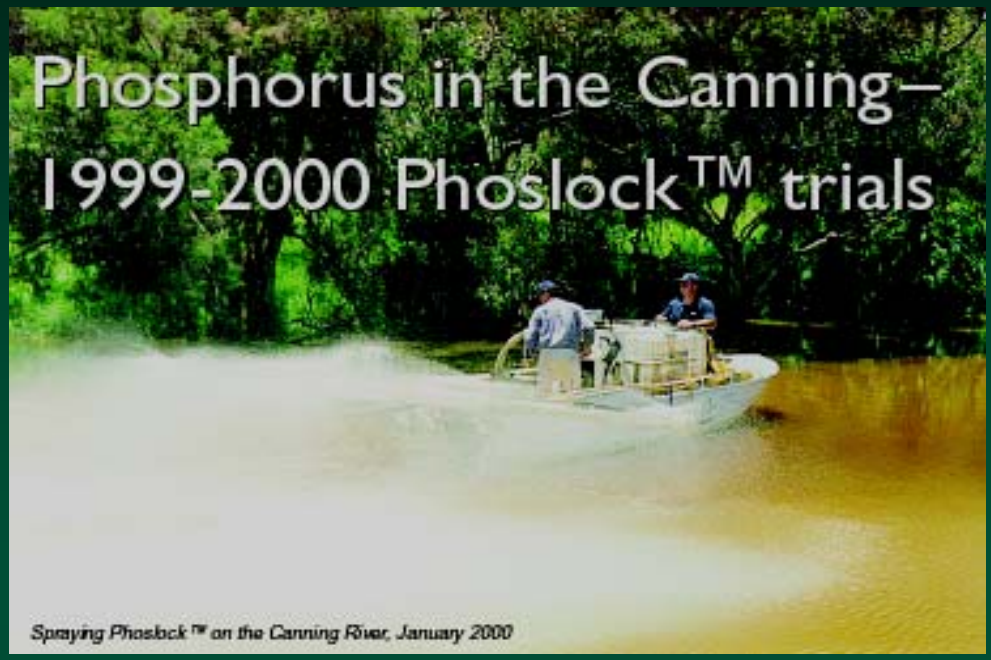


Phoslock™ forms a reactive layer on the sediment that binds phosphorus as it is released from the decomposition of organic matter, or transported into the water body from the catchment. This reduces the amount of phosphorus available to algae, and in theory should reduce algal growth.

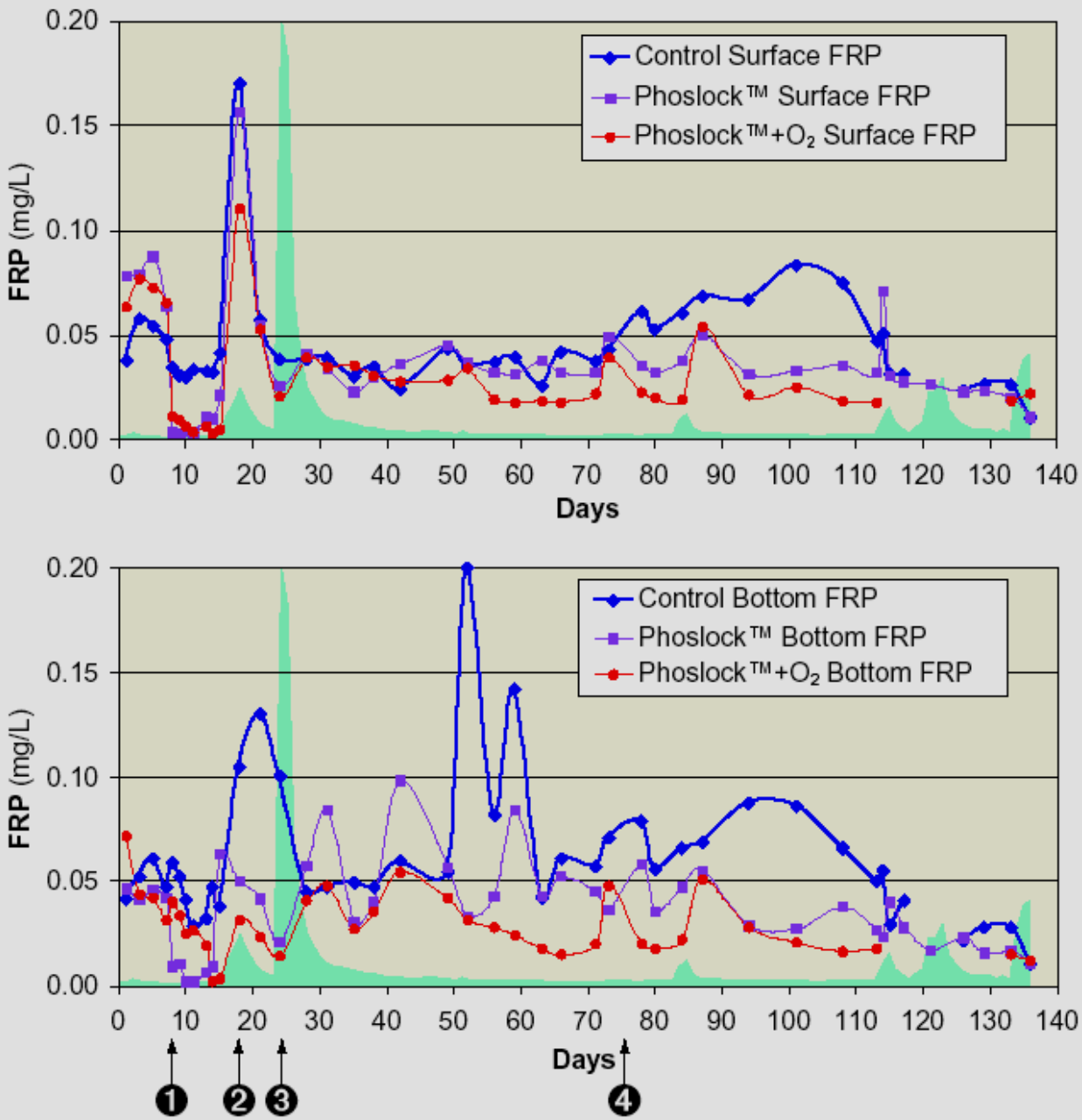
PWD RP T3200-1203

from: *River Science*, Issue 17, 2001

Phosphorus control in Australian using “Phoslock”



from: ***River Science,*** **Issue 17, 2001**



PWD RP T3200-1203

Figure showing the variation in FRP concentrations with time. The green shaded area shows relative flow rates in the Canning River. Point 1 is the application of Phoslock on 5 January 2000. Point 2 is the first rainfall and first flush of nutrients. At point 3 high flow rates flush the trial area. At point 4 FRP concentrations in the bottom water of Phoslock treated areas are consistently less than those of the control area.

from: *River Science*, Issue 17, 2001

Biological control

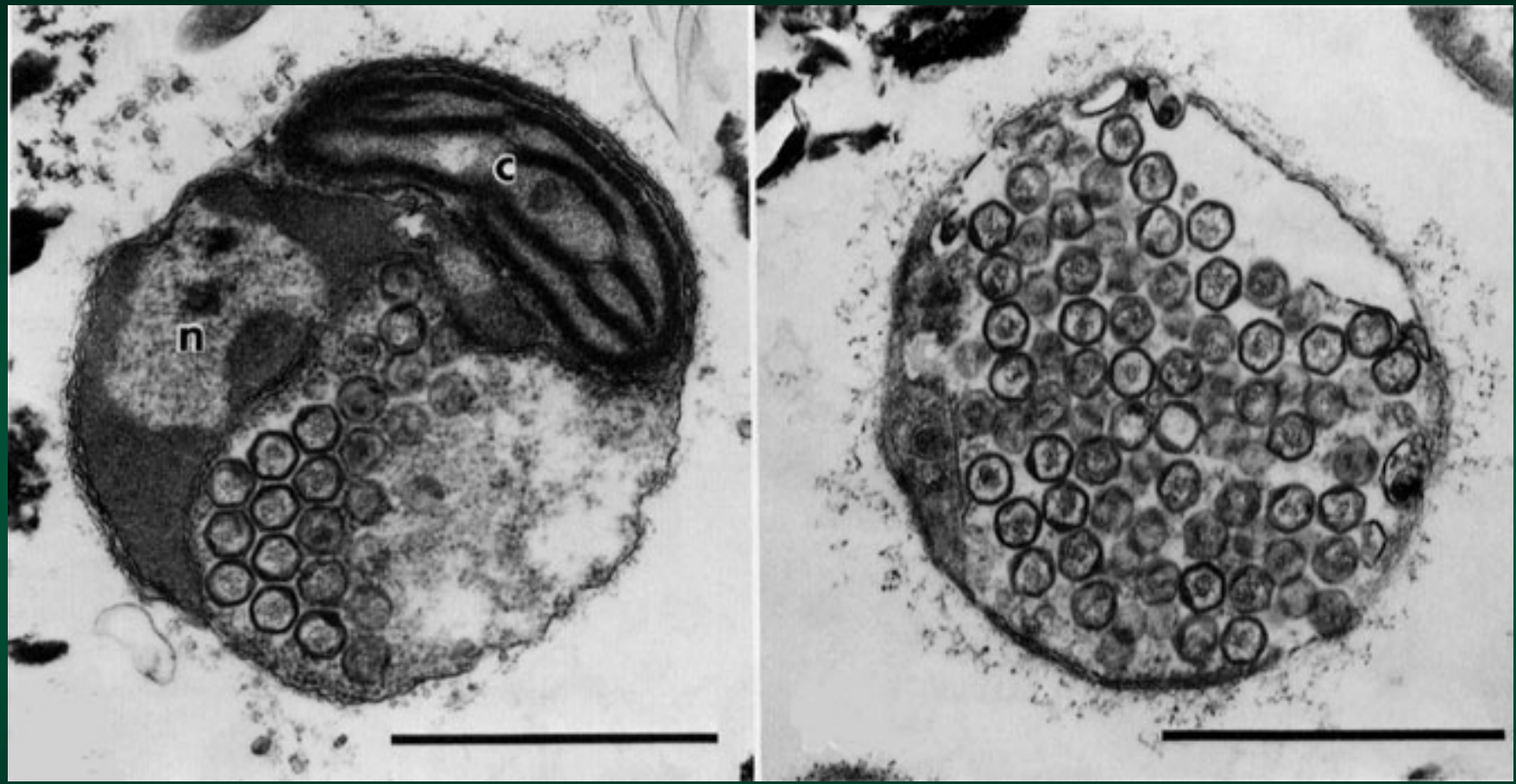
Introduction of non-native predatory or pathogenic species or enhancement of native species.

- Researchers have not yet attempted to use biocontrol in the ocean
- Concerns center on the potential for the introduced species to impact organisms other than the original target species.
- After a long and mixed history on land, biocontrol is receiving increased scrutiny for marine applications, motivated in large part by the proliferation of introduced species.

Biocontrol of HABs? Is it possible?

Yes – we have host-specific predators, parasites and pathogens for many HAB species

Biological Control - Viruses



***Aureococcus anophagefferens* virus**

Source: Gastrich et al., 1998, *Phycologia*

Virus-like particles

E. huxleyi

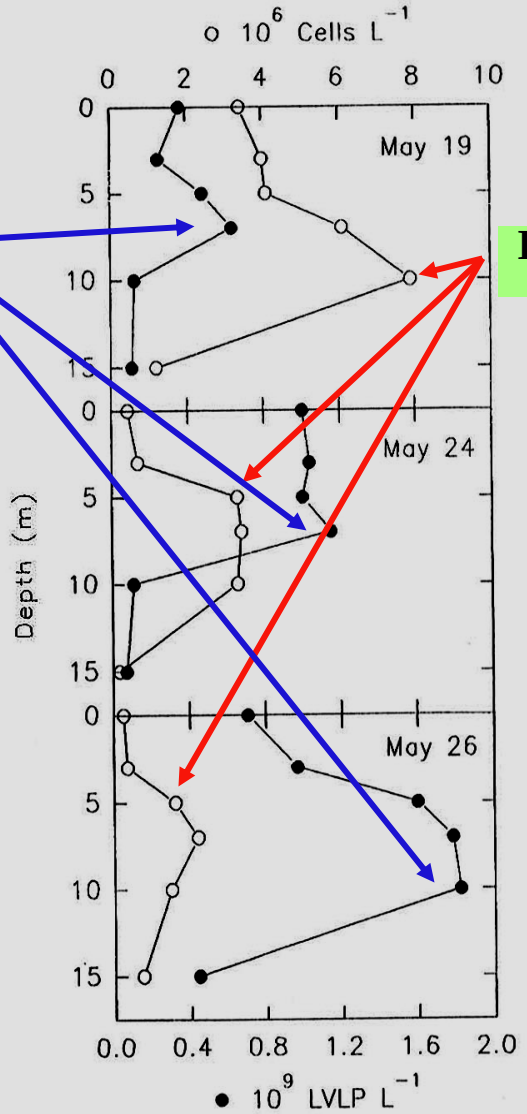


Fig. 2. Dynamics of LVLPs and *E. huxleyi* during one week in May 1993 in Fauskangerpollen, western Norway. The collapse of the *E. huxleyi* bloom corresponded in time and space with an increase in LVLP abundance. Simultaneous changes in other phytoplankton populations were small (for further details see Bratbak et al., 1995).

Source: Bratbak et al., 1996

Viruses for HAB species

Target species	Agent	Reference
Heterosigma akashiwo	virus HAV01	Nagasaki et al., 1999
	virus HaNIV	Lawrence et al., 2001
Heterocapsa circularisquama	virus HcV	Tarutani et al., 2001
Aureococcus anophagefferens	VLP	Gastrich et al., 2002
Alexandrium catenella	VLP	Onji et al., 2000
Gymnodinium mikimotoi	VLP	Onji et al., 2000
Tetraselmis sp.	VLP	Onji et al., 2000
Lyngbya majuscula	virus	Hewson et al., 2001

VLP = virus-like particles

Pros: **extreme host specificity, rapid proliferation**

Cons: **extreme host specificity, general distrust of biocontrol in ocean**

==>Potentially effective, but not yet tested in field applications

Biological control - algicidal bacteria

Mode of action:

- direct physical contact, leading to cell lysis
- release of algicidal compounds

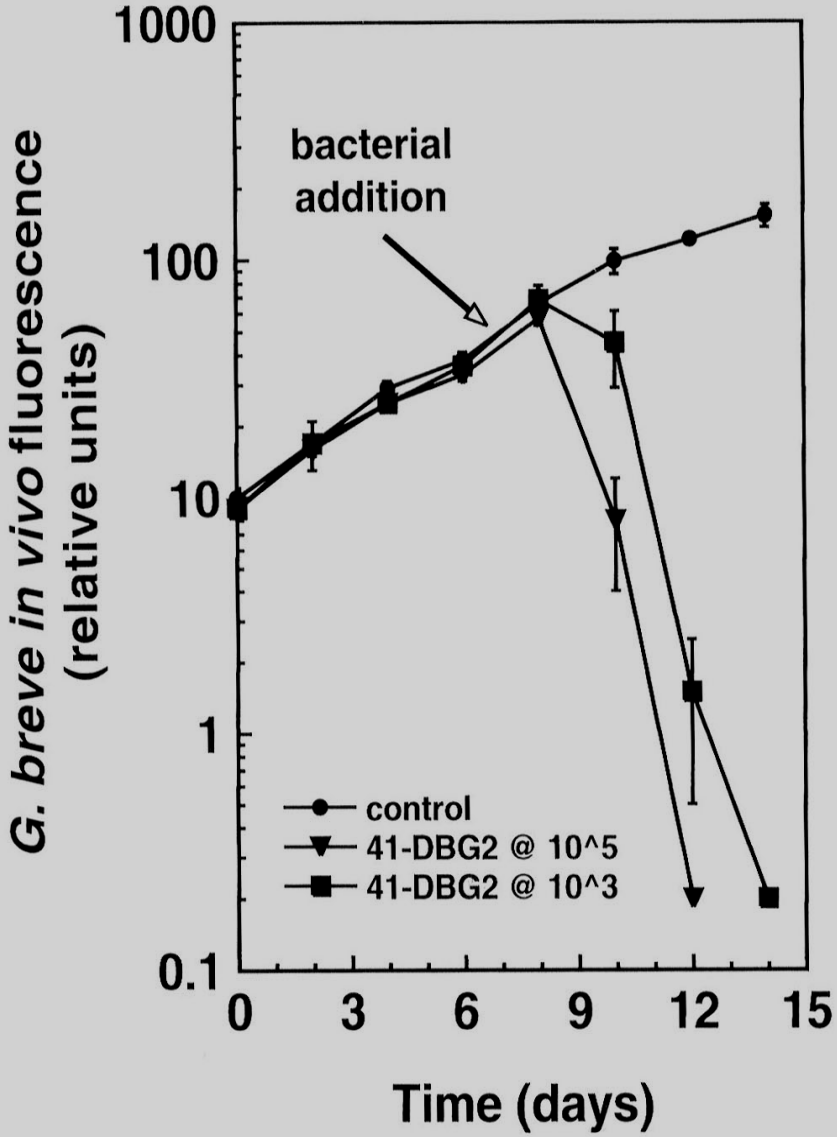


FIG. 1. *Gymnodinium breve* Davis (strain C2). Growth curves of algal cultures as measured by *in vivo* fluorescence, showing response following additions of bacterial strain 41-DBG2 (arrow) at two concentrations (▼ = 10^5 cells·mL^{-1}, ■ = 10^3 cells·mL^{-1}) relative to a no-addition control (●). Values are mean ± SD (n = 3).

Source: Doucette et al., 1999

Biological Control - Bacterial pathogens for HAB species

Target species	Agent	Reference
Heterocapsa circularisquama	Cytophaga sp AA8-2	Nagasaki et al., 2000.
Heterosigma akashiwo	H. akashiwo-killing bacteria (HAKB) H. akashiwo-killing bacteria (HAKB)	Kim et al., 1998 Yoshinaga et al., 1998
Cochlodinium polykrikoides	Micrococcus sp. LG-1	Park et al., 1998
Chattonella ovata	Altermonas sp. strain S, strain R, Cytophaga sp J18/M01	Imai 1997
Chattonella verruculosa	Altermonas sp. strain S, strain R, Cytophaga sp J18/M01	Imai 1997
Karenia mikimotoi	28 strains	Yoshinaga et al., 1997
Karenia brevis	bacterium 41-DBG2	Doucette et al., 1999

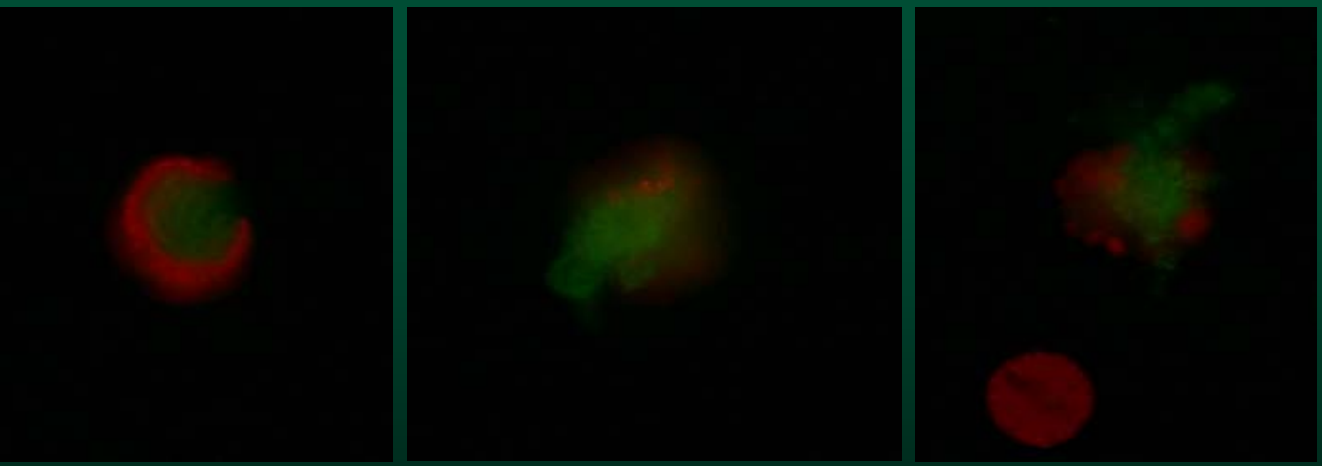
Pros: high host specificity, rapid proliferation of pathogen

Cons: general distrust of biocontrol in ocean, logistical concerns

==> Potentially effective, but not yet tested in field applications

Biological Control - Parasites

Target species	Agent	Reference
Peridinium balticum	*Coccidinium duboscqui*	Chatton and Biecheler, 1934
Dinophysis sp.	*Parvilucifera infectans*	Noren et al., 1999
Alexandrium spp.	*Parvilucifera infectans*	Noren et al., 1999
Alexandrium catenella	*Amoebophrya ceratii*	Taylor, 1968
	Amoebophrya ceratii	Nishitani et al., 1984
Alexandrium tamarensis	*Amoebophrya ceratii*	Jacobson, 1987
Dinophysis norvegica	*Amoebophrya ceratii*	Fitz and Nass, 1992
	Amoebophrya ceratii	Janson et al., 2000
Akashiwo sanguinea	*Amoebophrya ceratii*	Coats and Bockstahler, 1994
Gyrodinium uncatenum	*Amoebophrya ceratii*	Coats et al., 1996
Prorocentrum minimum	*Amoebophrya sp.*	Maranda, 2001



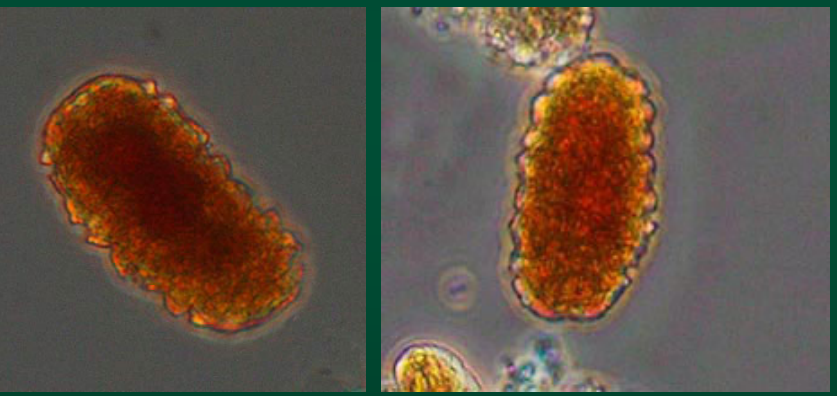
Pros: high host specificity, rapid proliferation of pathogen

Cons: general distrust of biocontrol in ocean, logistical concerns

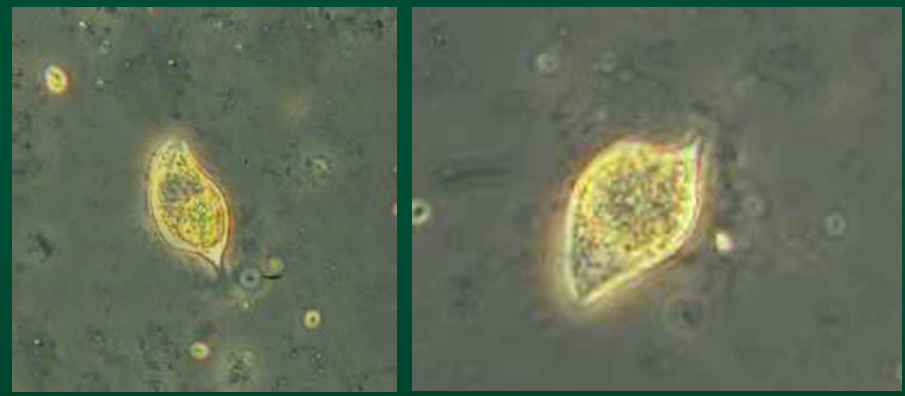
==> Potentially effective, but not yet tested in field applications

Biological Control - Grazers

Target species	Agent	Reference
Karenia brevis	ciliates	Martin et al., 1973
algal blooms	intact benthic community (San Franscico Bay)	Cloern, 1982
algal blooms	*Acartia clausi* (copepod) and bivalves	Shirota, 1989
Aureumbra lagunensis	planktonic grazers	Buskey et al., 1996
Gymnodinium catenatum	*Polykrikos kofoidii* (heterotrophic dinoflagellate)	Jeong et al., 2003
Heterosigma akashiwo	*Oxyrrhis marina* (heterotrophic dinoflagellate)	Jeong et al., 2003



Polykrikos kofoidii



Oxyrrhis marina

<u>Pros:</u> moderate specificity, natural predator

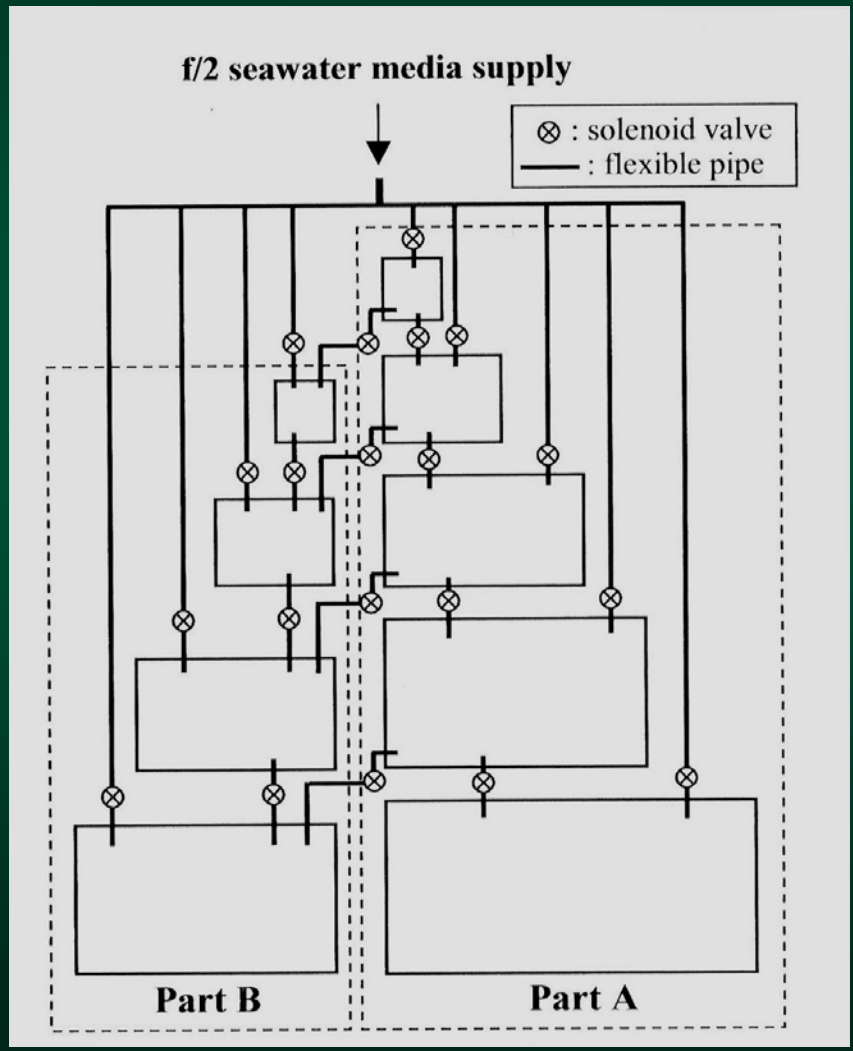
<u>Cons:</u> slow proliferation, logistical concerns for growth and delivery

==> unlikely to be used in practical bloom control efforts

J. Eukaryot. Microbiol., 50(4), 2003 pp. 274–282

Feeding by the Heterotrophic Dinoflagellate *Oxyrrhis marina* on the Red-Tide Raphidophyte *Heterosigma akashiwo*: a Potential Biological Method to Control Red Tides Using Mass-Cultured Grazers

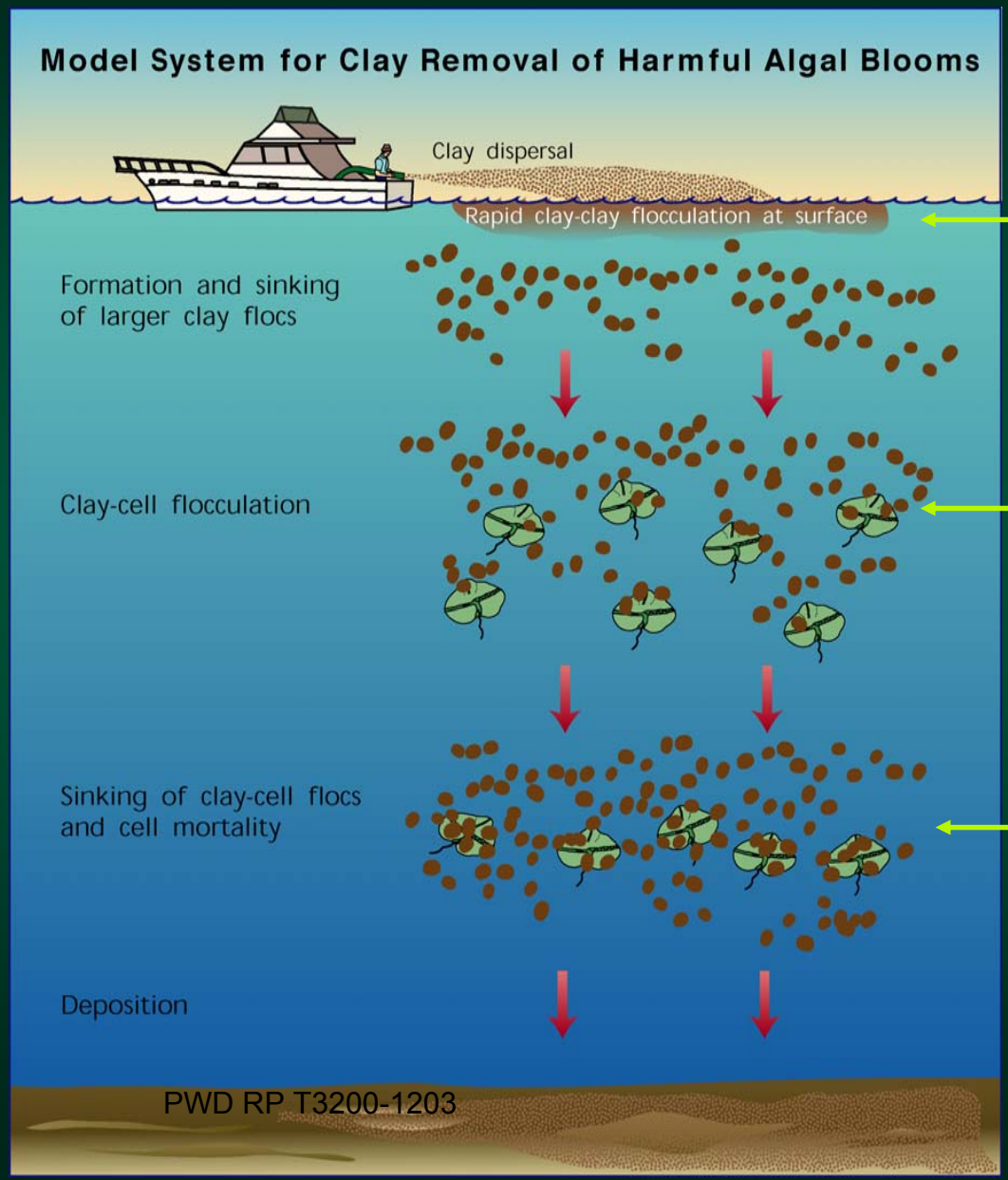
HAE JIN JEONG,[a] JAE SEONG KIM,[a] YEONG DU YOO,[b] SEONG TAEK KIM,[a] TAE HOON KIM,[a] MYUNG GIL PARK,[c] CHANG HOON LEE,[c] KYEONG AH SEONG,[d] NAM SEON KANG[d] and JAE HYUNG SHIM[c]



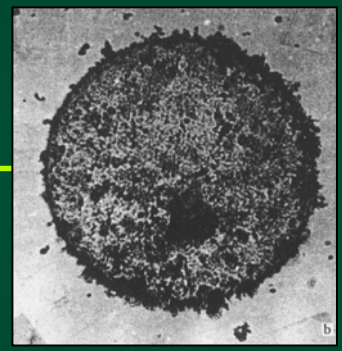
PWD RP T3200-1203

Diagram of an automatic system for growing daily 300 L of *Oxyrrhis marina*

Clay control of HAB species

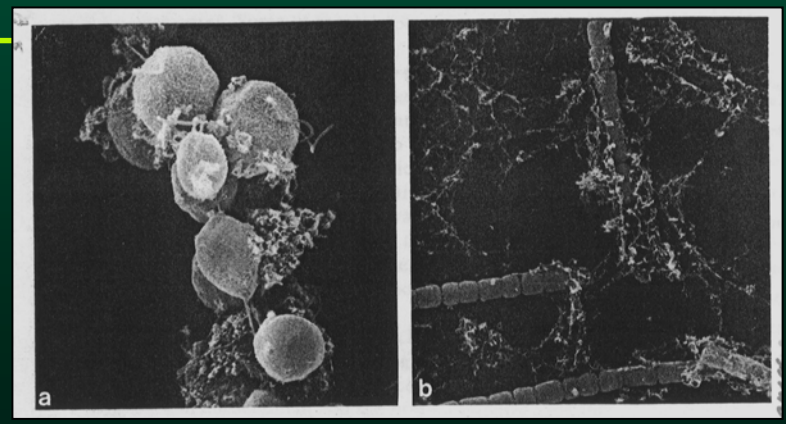


clay minerals



clay/cell flocculation

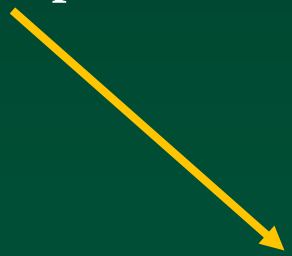
"sweep floc"



Clay control research in the United States

How effective are domestic clays at removing U.S. HAB species?

What are the impacts of clay dispersal on water quality and benthos?

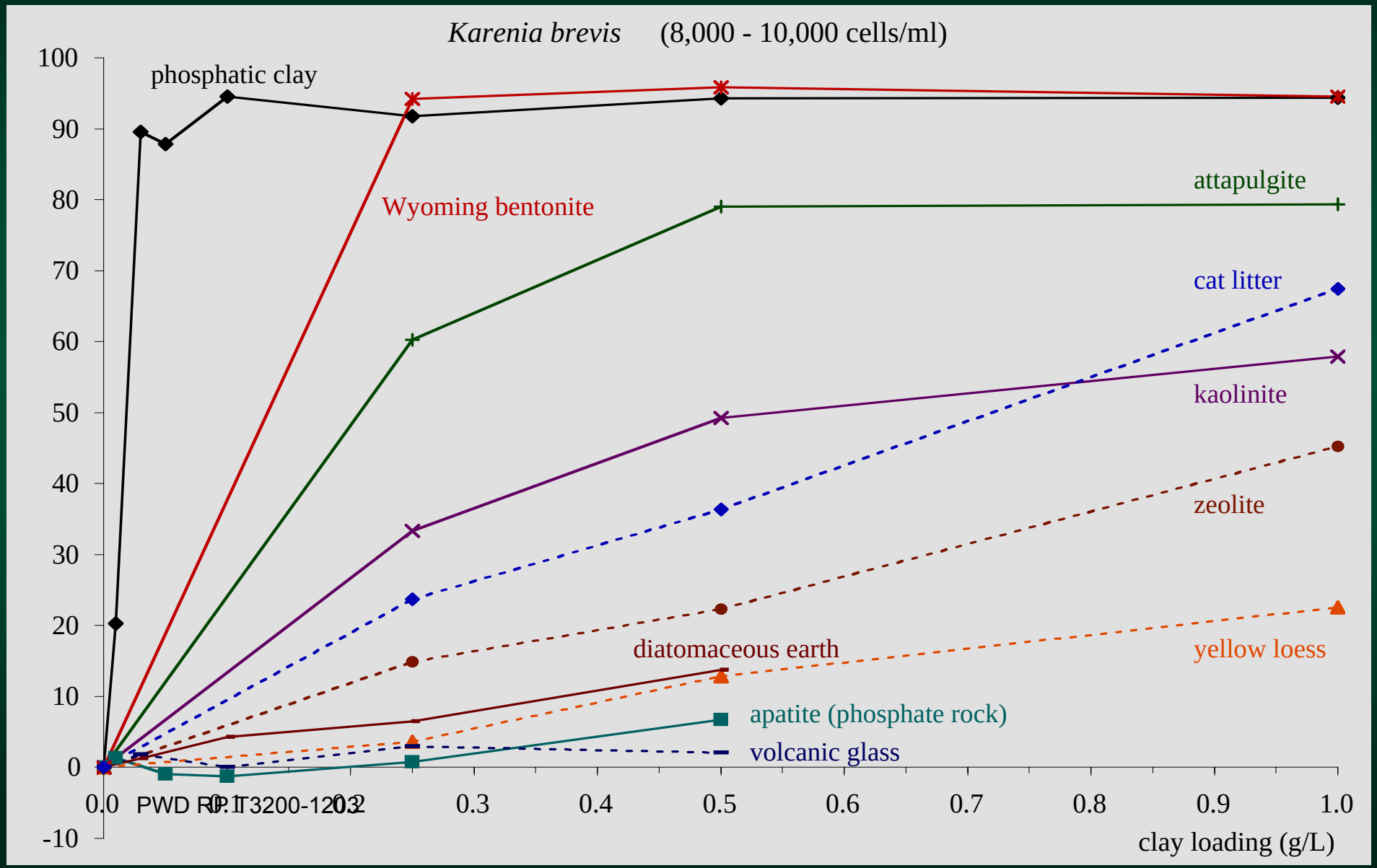


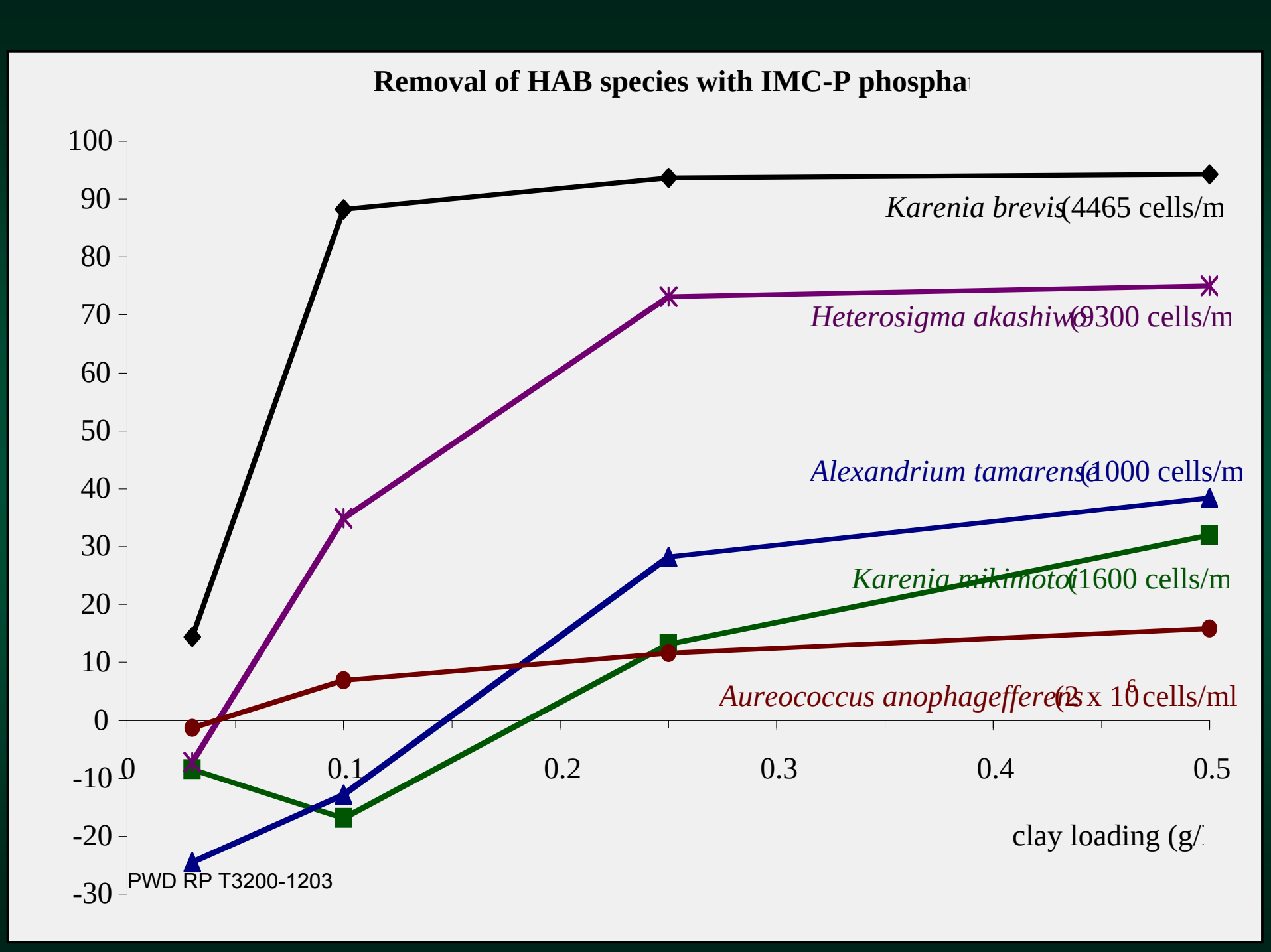
Can we recommend clay control as a means of HAB management?

<u>Approach:</u> laboratory cultures ==> "mesocosms" ==> field trials
enclosures
limnocorrals
flumes

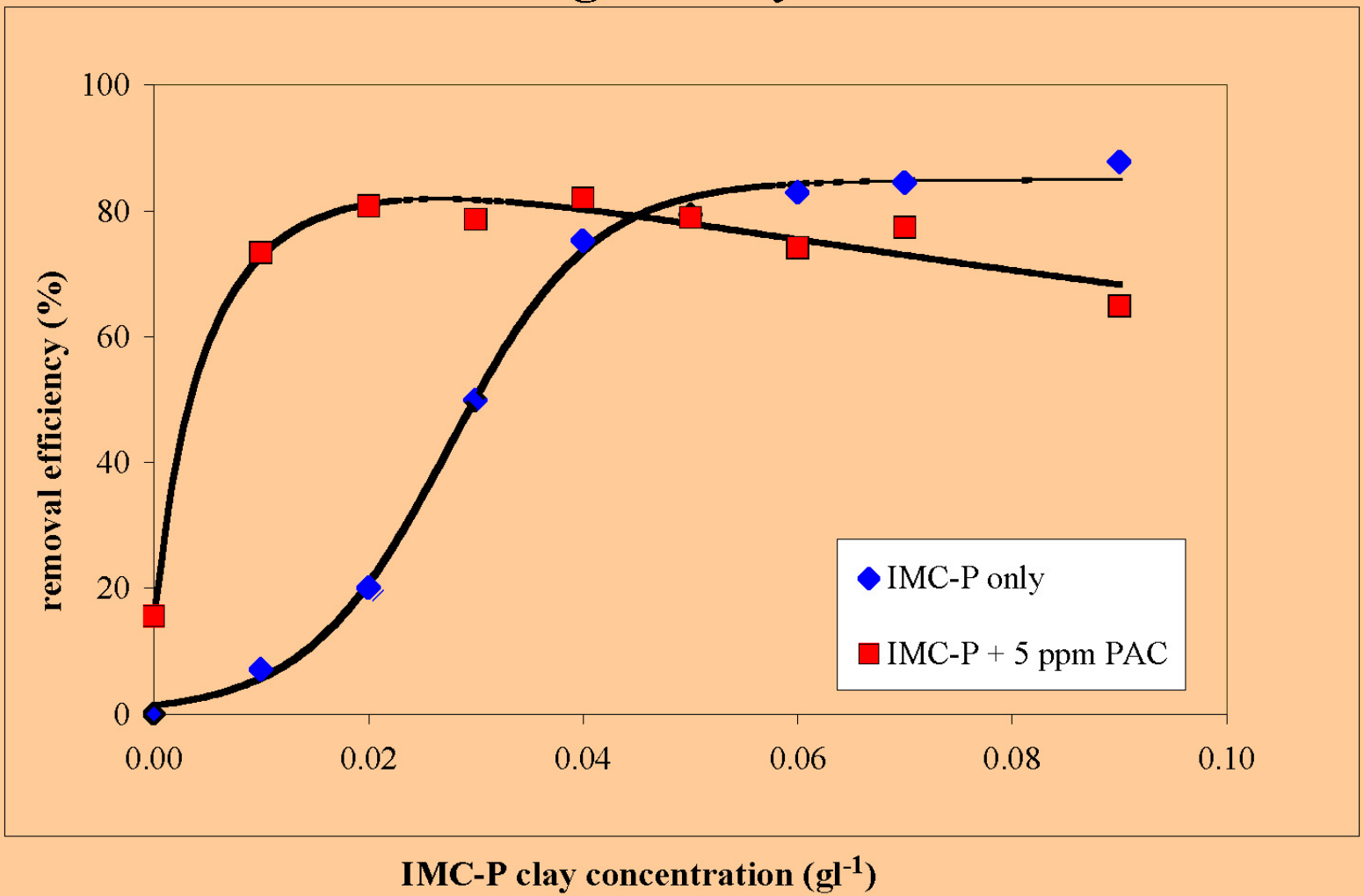
PWD RP T3200-1203

Variable removal ability of domestic clay and non-clay minerals




Removal of HAB species with IMC-P phospha
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
0
0.1
0.2
0.3
0.4
0.5
Karenia brevis (4465 cells/m
Heterosigma akashiwo (9300 cells/m
Alexandrium tamarense (1000 cells/m
Karenia mikimotoi (1600 cells/m
Aureococcus anophagefferens (2 x 10^6 cells/ml
clay loading (g/
PWD RP T3200-1203

Removal efficiency of IMC-P alone and IMC-P2 treated with PAC against *Gymnodinium breve*

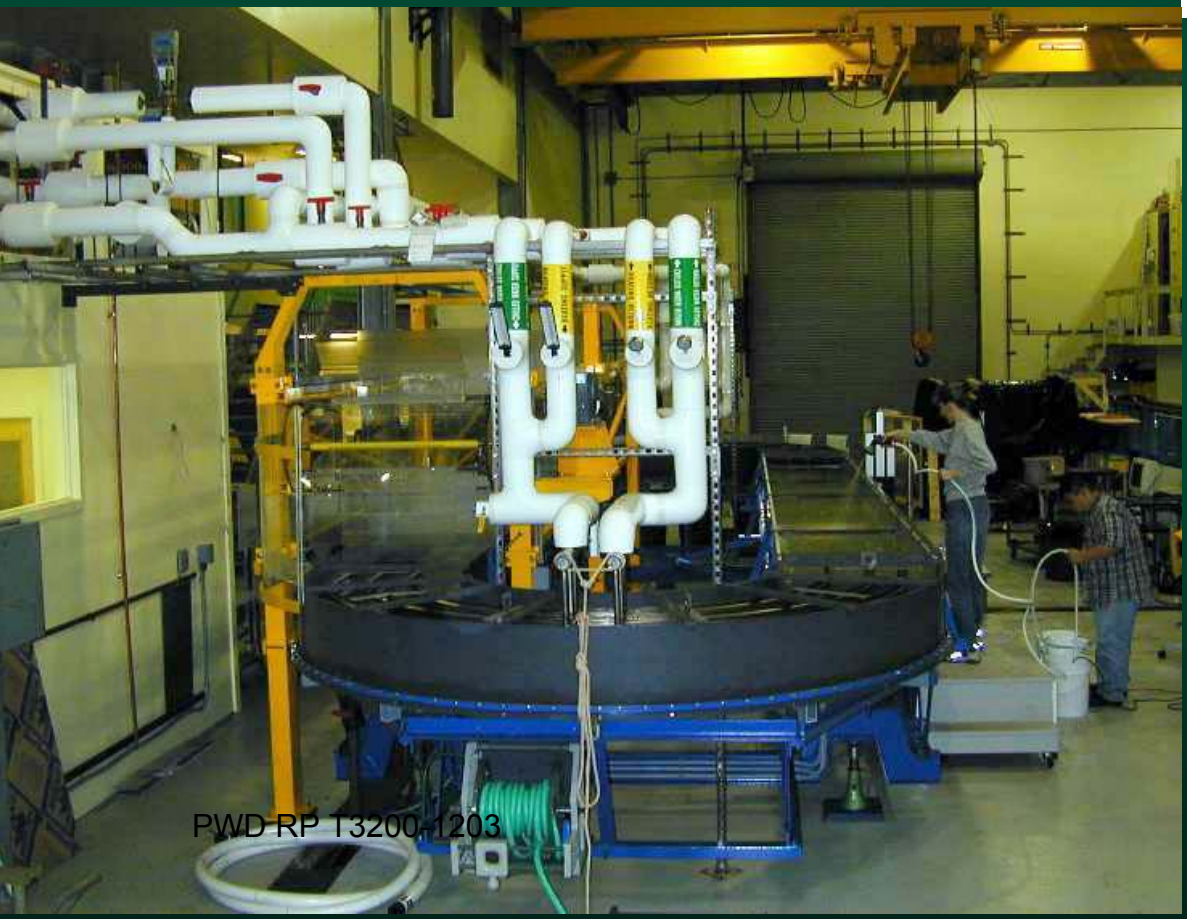


PWD RP T3200-1203

Source: Sengco et al., 2001, Mar. Ecol. Prog. Ser.

Removal efficiency
at intermediate scales

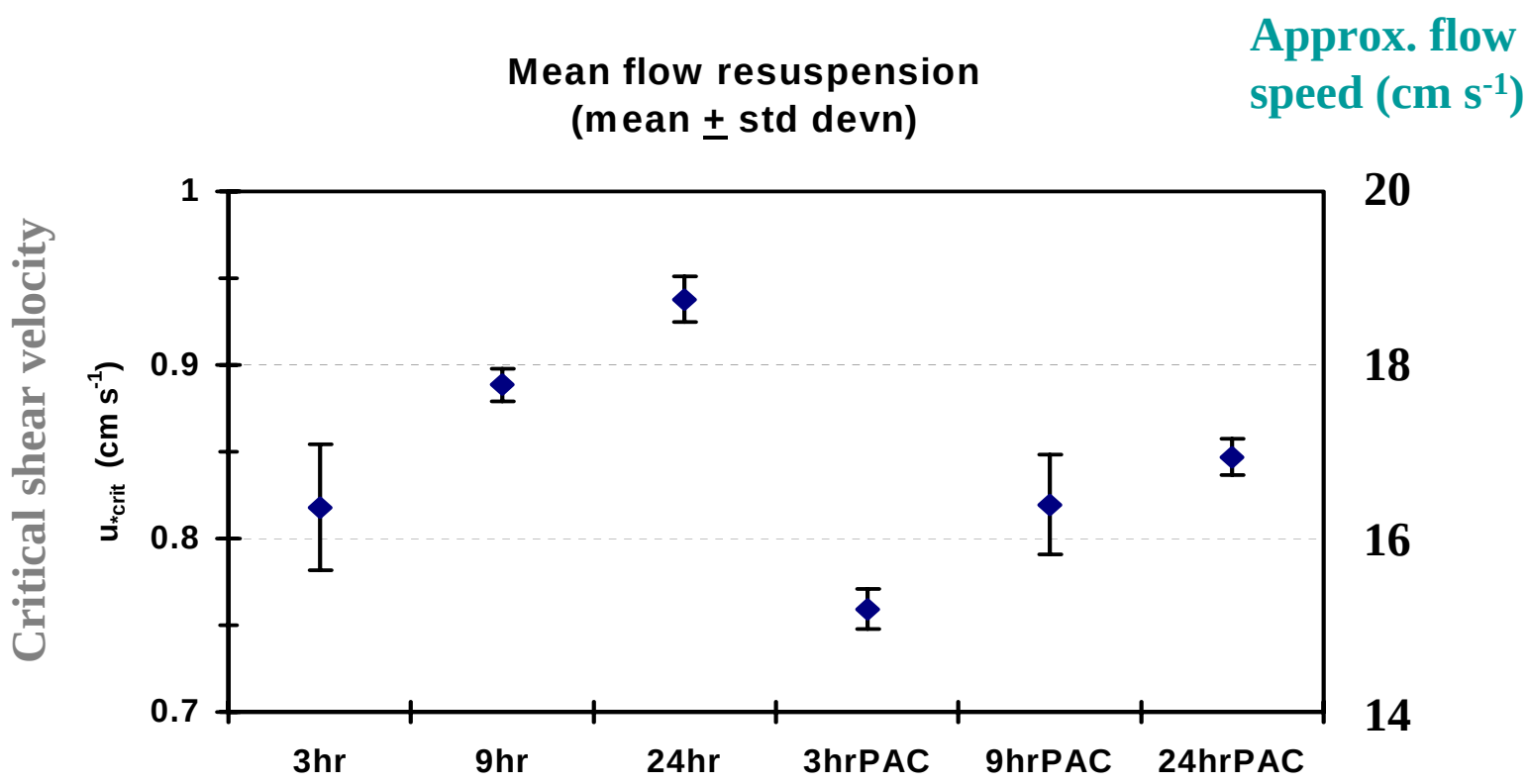
Flume studies, WHOI



PWD RP 13200.1203

(Beaulieu et al., submitted to *Harmful Algae*)

Erosion and Resuspension



1) Sedimented flocs are more difficult to resuspend the longer they sit in a layer on the bottom
2) PAC flocculant makes it easier to resuspend the clay/algal flocs
3) Flocs do not settle as rapidly with PAC flocculant

(Beaulieu et al., submitted to *Harmful Algae*)

Brevetoxin analysis

	cell concentration (cells/L)	loading (g/L)	PAC (ppm)	toxin removal rel. to con
intact	10^6			
intact	10^6			
intact	10^6			
lysed	10^6			
lysed	10^6			
intact	10^6			
	10^6			

==> phosphatic clays can remove 68% - 80% dissolved brevetoxins

PWD RP T3200-1203

(Pierce et al., submitted to *Harmful Algae*)

Impacts - Benthic fauna

Lewis et al., 2003. *Harmful Algae*

test organisms	***Ampelisca abdita* (infaunal amphipod)** ***Leptocheirus plumulosus* (infaunal amphipod)** ***Palaemonetes pugio* (grass shrimp)**
clay	**phosphatic clay (0.25 g/L)**
coagulant	**polyaluminum chloride (0.50, 5, 50 ppm)**
HAB organism	***Karenia brevis* (3.9 to 5.4 x 10^6 cells/L)**

main conclusions

(1) The use of phosphatic clay and coagulant are not likely to have a detectable toxic effect on the benthos. Field validation needed.

(2) Survival of the test species to clay, PAC and *K. brevis* was species-specific. Survival, with one exception, was similar to *K. brevis* alone.

Impacts - Benthic fauna

Archambault et al., *in press*. *Marine Biology*

test organism	***Mercenaria mercenaria***
clay	**phosphatic clay (0.25 g/L)**
organism	***Heterocapsa triquetra***

M. mercenaria, notata strain

RESULTS: Sedimented clay/cell floc (non-toxic)

-No mortality occurred in any of the trials

-Oxygen levels remained >85% saturation.

-Significant growth in shell length and soft tissue occurred in all trials

-Clams quickly recovered siphon contact with the overlying water column

RESULTS: Suspended clay/cell floc (14 days)

-A highly significant growth effect (~90% reduction in shell and tissue growth) *with suspended clay* compared to no-clay controls.

-Repeated clay applications in the field are likely more detrimental to clams under flow conditions leading to prolonged *in situ* resuspension of clay than under conditions that promote rapid sedimentation.

What is the status of clay control for marine HABs?

- Most results suggest that clay flocculation is a viable strategy for certain types of HABs in certain locations. Cells, and some dissolved toxins, can be removed effectively from the water column

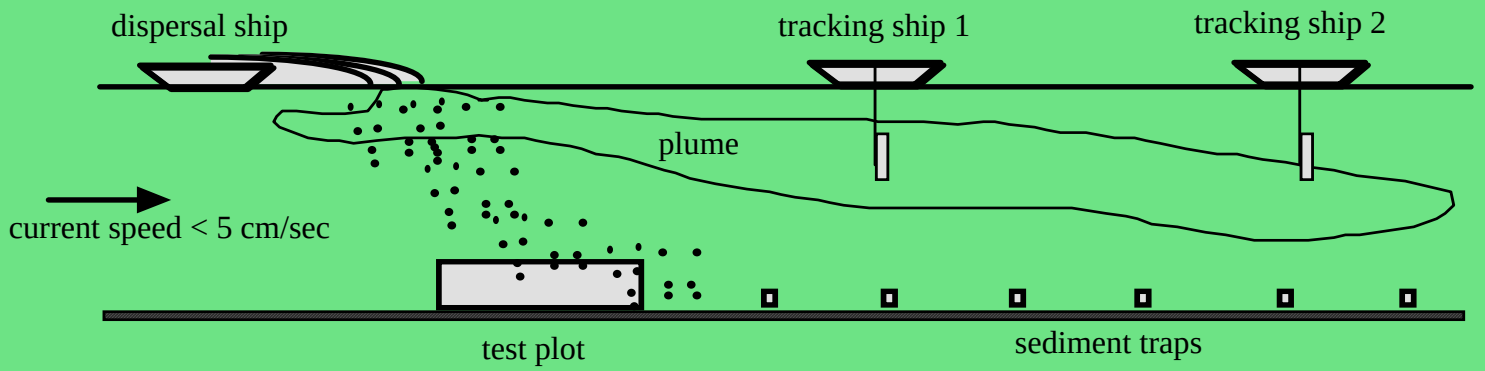
- More impact studies are still needed, especially on the fate of algal toxins and organic matter enrichment of the sediments

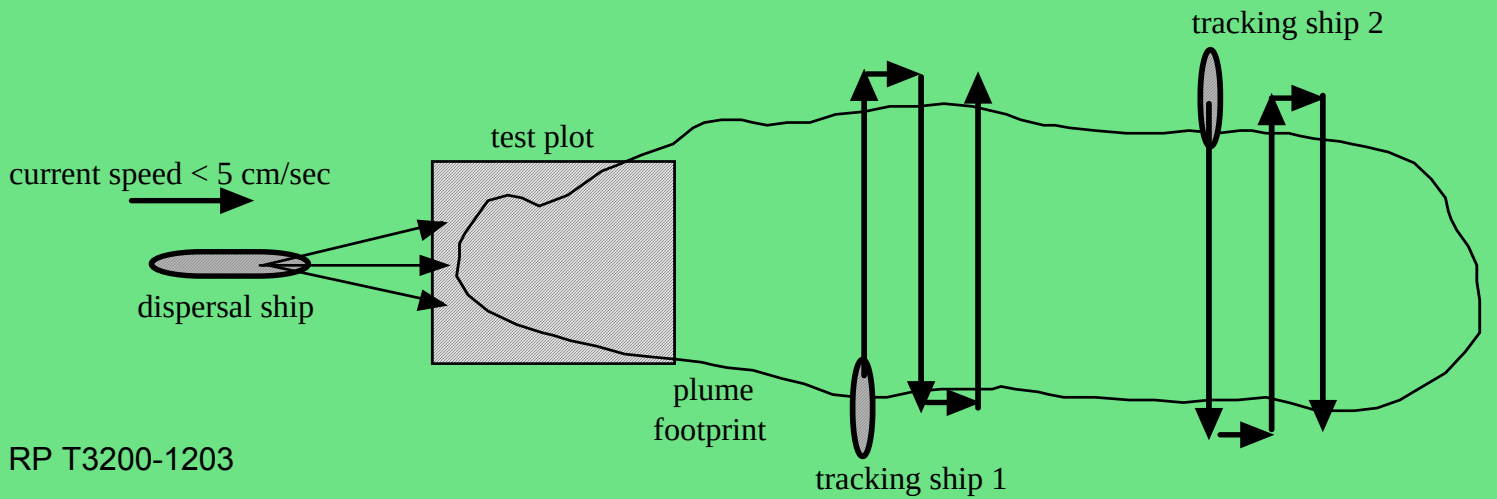
- Need to resolve whether PAC or other flocculants should be used in the field
  PRO: enhance cell removal, minimize toxin/nutrient release
  CON: increase erosion, decrease settling, unknown impacts

- Logistical challenges and economic costs generally unknown

Future directions:

Cell removal, settling, and viability in flow - more flume studies
Removal, degradation and bioavailability of brevetoxins on clay
Impact of flocs on other bivalves and benthic fauna
Pilot-scale treatment of a *Karenia* bloom in unbounded waters





PWD RP T3200-1203

Anderson et al., unpublished data

Experiments on removal of *Prymnesium parvum* with clay

Kalmar, Sweden

Hagstrom and Graneli, submitted to *Harmful Algae*

When the cells reached exponential phase (NP sufficient), and in stationary phase (N or P deficient), the cultures were placed in 30 ml flasks (in triplicate).

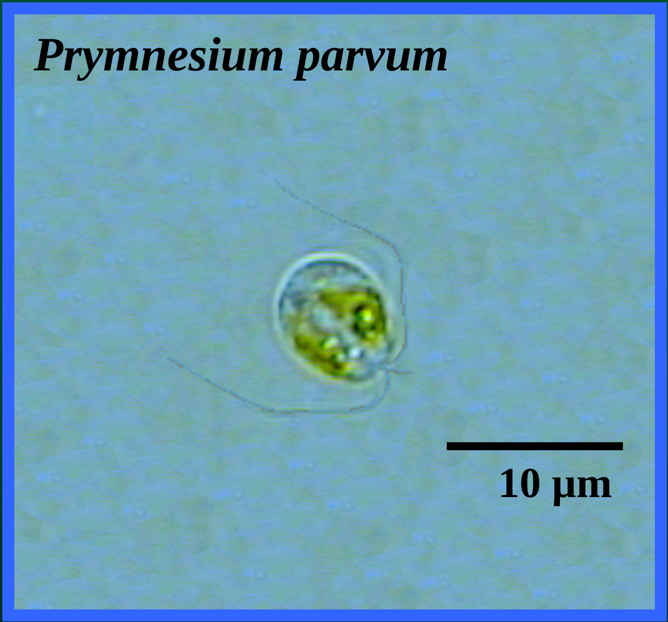
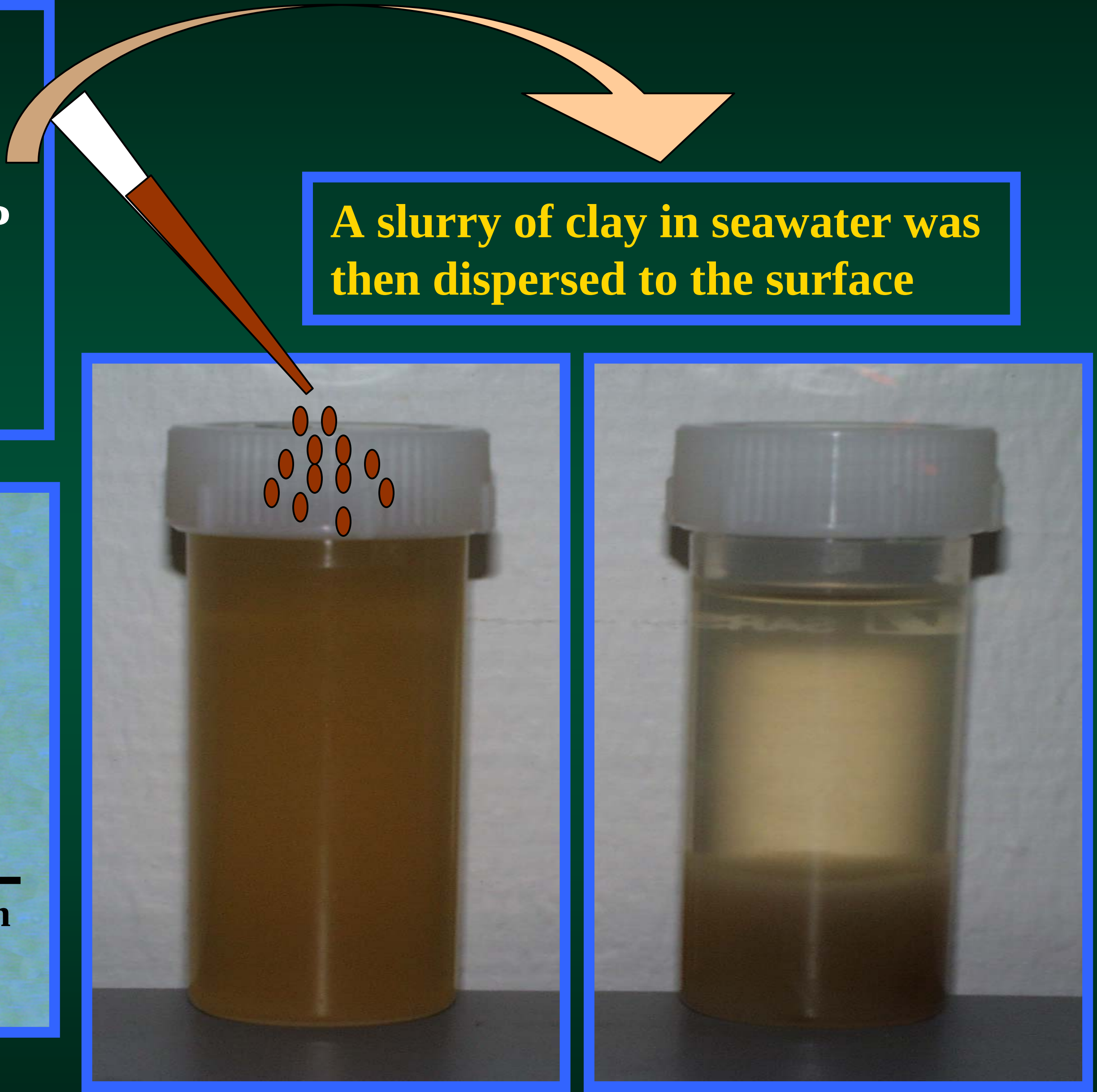


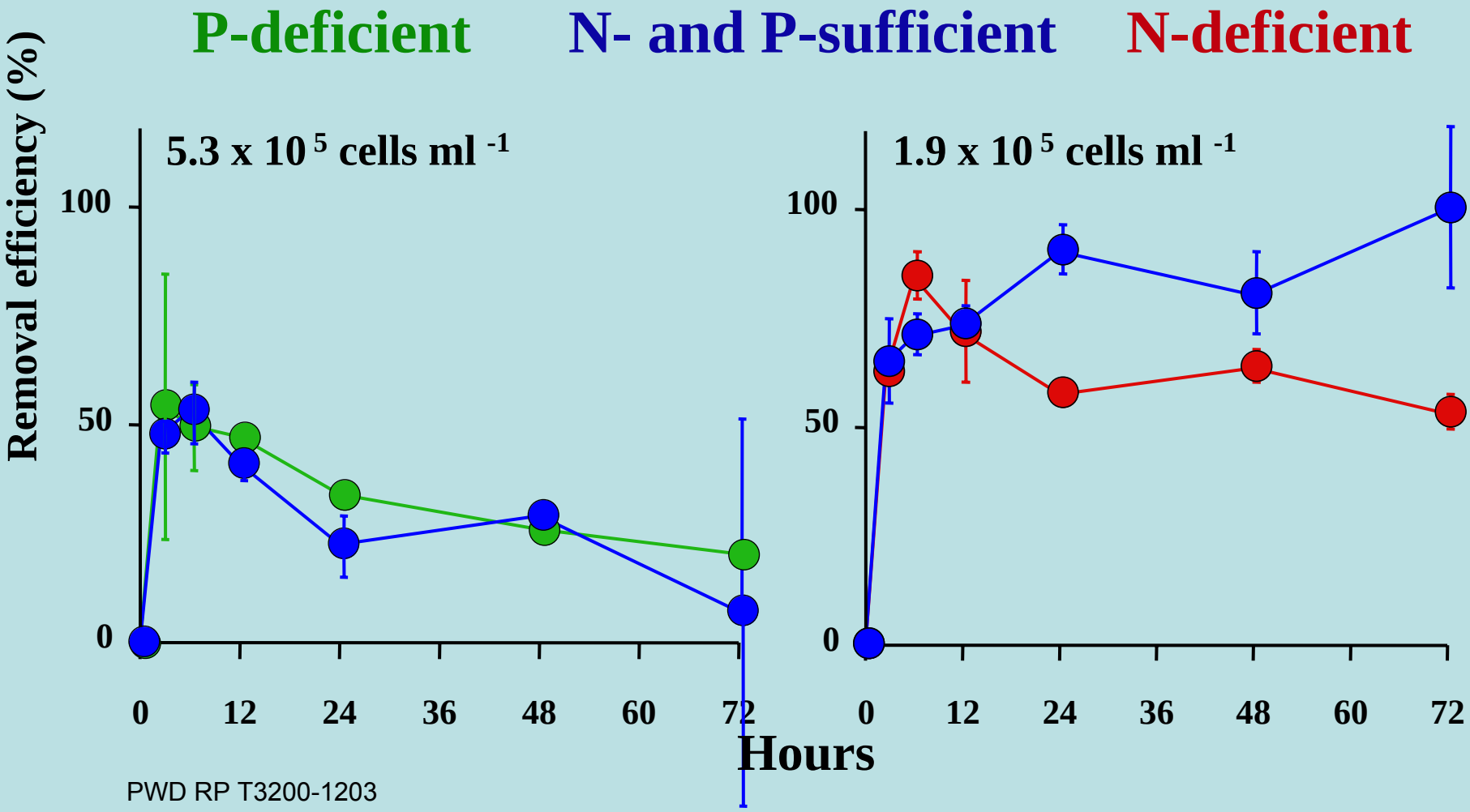
Photo C. Esplund

A slurry of clay in seawater was then dispersed to the surface



Florida Phosphatic clay

4 g/L phosphatic clay + 5 ppm PAC



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Hagstrom and Graneli, submitted to *Harmful Algae*

Conclusions
Kalmar Experiments

- **Phosphatic clay can, in a few hours, remove 100% of the *Prymnesium parvum* (grown with sufficient nutrients) using 4 g/L of clay and 5 ppm polyaluminum chloride**

- **Lower RE's for nutrient-deficient cells**

- **The method may be promising for bloom mitigation, but the clay loadings required are very high. (But, there is an explanation for this).**

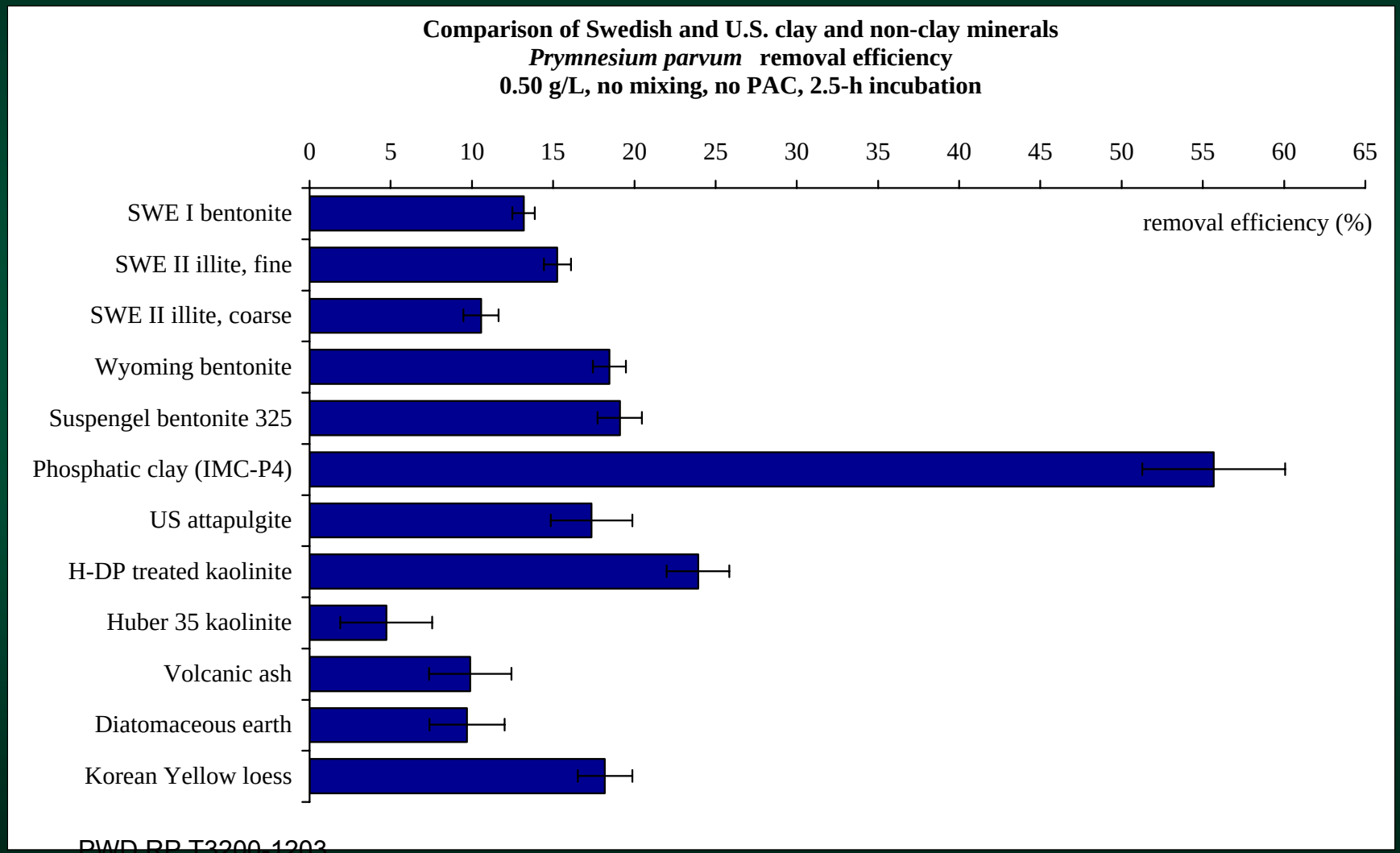
- **In the Baltic Sea, expect low RE as algae are N deficient**

Clay Control Experiments
Tvarminne Zoological Station, Finland

Prymnesium parvum and Swedish clays

Experiments at Woods Hole (in collaboration with J. Hagström)

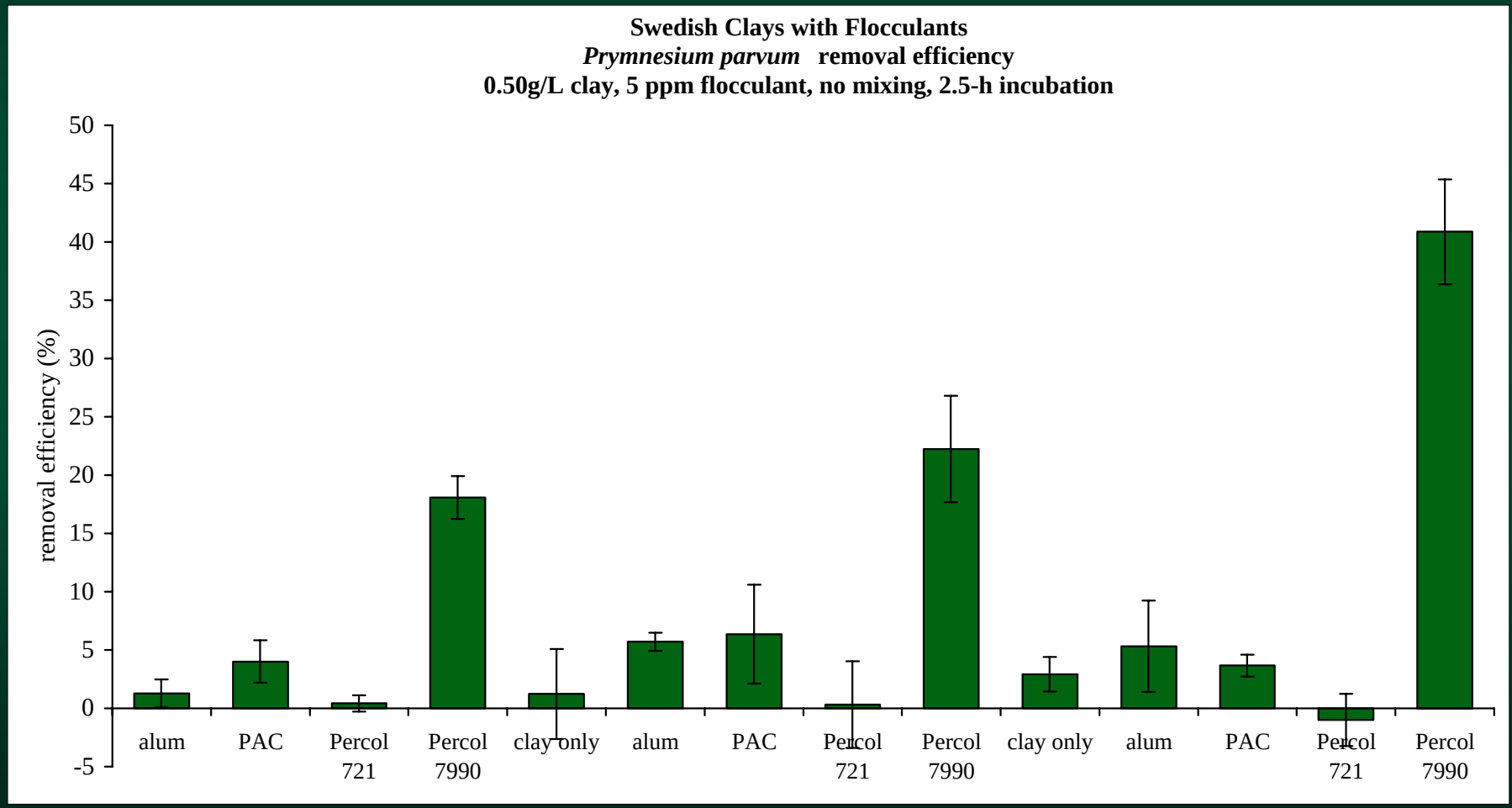
Extended clay screening (clay only - no flocculants)



Source: Hagström, unpublished data

Experiments at Woods Hole (data from J. Hagström)

Alternative flocculants (no clay)



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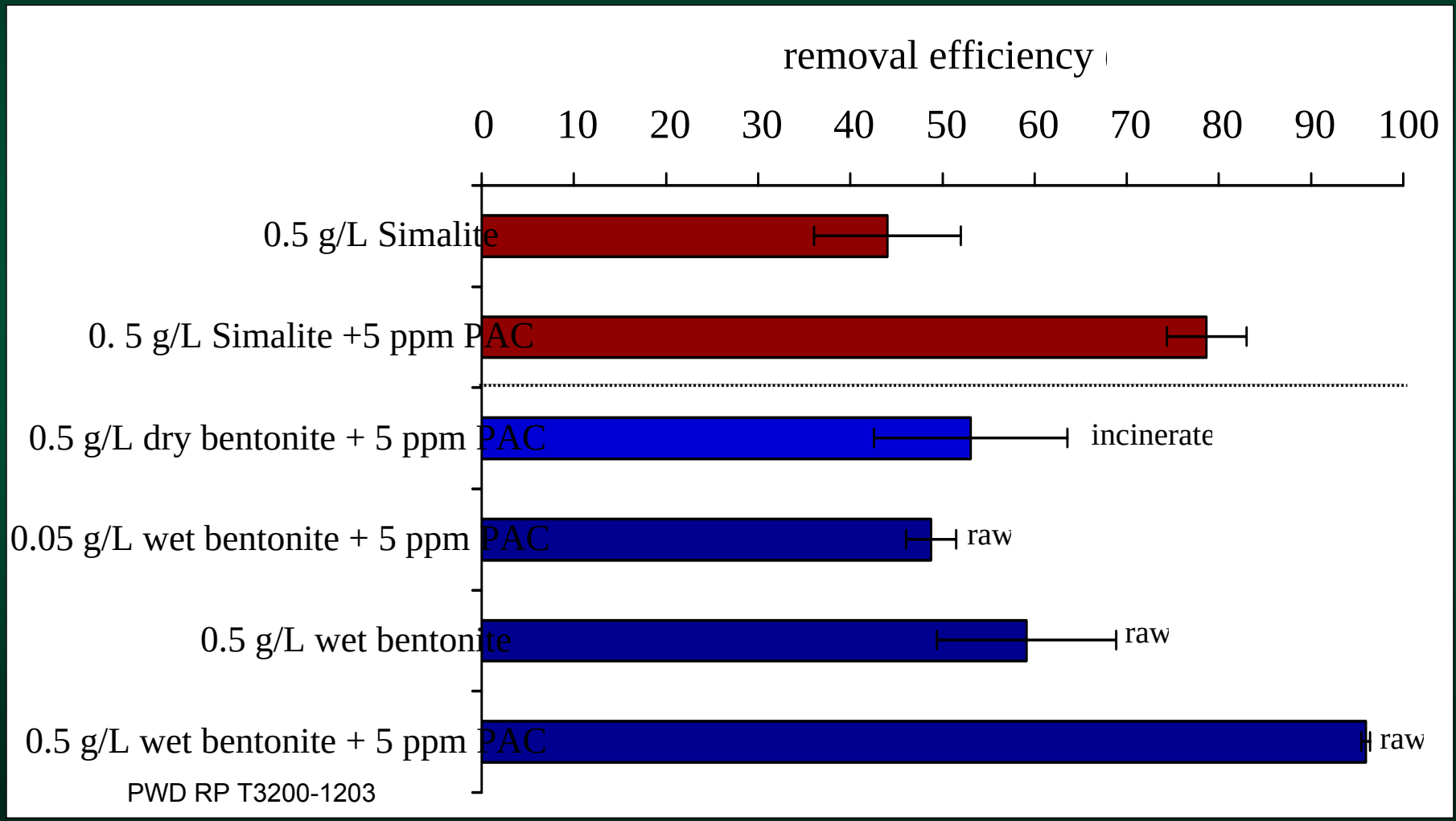
Source: Hagström, unpublished data

Recent work in Kalmar (data from J. Hagström)

Un-incinerated (raw) vs. incinerated Swedish clays (with and without flocculants



Source: Hagström, unpublished data

Conclusions - general

1) Preventive strategies should be pursued to keep blooms from happening, but these will take decades to implement
2) Bloom control research is not well advanced for marine HABs
3) Biological control options are possible in theory, but are far from the application stage
4) Chemical control is also possible, but is not likely due to broad lethality and other environmental concerns
5) Clay flocculation is promising for certain HABs (or certain HAB toxins) in certain locations or situations
6) More research is clearly needed

Conclusions - control of Prymnesium

1) **Consider barley straw and other simple bloom suppression methods in small reservoirs and hatchery ponds**
2) **Consider Phoslock treatments, if phosphorous is shown to be a controlling parameter (but will this increase toxicity?)**
3) **Consider testing local clays against *Prymnesium parvum* - begin freshwater removal efficiency studies Low salinity (ionic strength) directly influences flocculation rates, reducing cell removal. Flocculants will likely be needed.**
4) **Although particle aggregates form with flocculants, floc density may be too low for good settling and cell retention (cell escape, lack of floc settling).**
5) **Explore methods to increase interparticle collisions for clay to work better with *Prymnesium***
6) **Can clays remove *Prymnesium* toxins?**

A Review of Fish-killing Microalgae: Causes, Impacts, and Management with Emphasis on *Prymnesium*

Jan H. Landsberg

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Abstract.--Harmful algal blooms (HABs) cause mass animal mortalities, shellfish and tropical fish poisonings, respiratory irritation, and neurocognitive disease in humans. Globally, HABs have been directly or indirectly associated with fish kills in a variety of aquatic systems. At least 60 species are ichthyotoxic, and more than 30 species are harmful to fish. The sudden appearance of toxic planktonic blooms, such as red tides that lead to acute, mass fish mortalities, has been historically documented since the mid 1800s. Increased reports of HAB-associated fish kills signify their expanding effects in aquatic systems. Microalgae can affect fish in a number of ways: the production of lethal or sublethal toxins can cause neurological impairment, behavioral change, or neurointoxication; the production of bioactive compounds (e.g., hemolysins) can cause cellular damage, or impair respiration and other physiological functions; toxins can be transferred up the food chain via predation, bioaccumulation, and lethal bioconcentration; microalgal anatomical structures can cause mechanical damage and pathology; microalgae can act as vectors for disease pathogens; they can cause immunosuppression and increase the susceptibility of fish to disease; and they can negatively influence water quality (e.g., low dissolved oxygen, increased ammonia). Historically, many fish kills have been associated with the low dissolved oxygen levels produced by nontoxic algal blooms. At all trophic levels, fish chronically exposed to microalgal toxins can experience lethal or sublethal effects such as impaired feeding, avoidance behavior, physiological dysfunction, impaired immune function, reduced growth and reproduction, pathological effects, and mortality. The wide variety of life strategies adopted by many HAB species suggests that fish in numerous trophic niches can be affected. Traditionally, only planktonic HABs have been recognized as having acute effects, but benthic and predatory HAB species can also kill or harm fish. The majority of fish kills are caused by dinoflagellates such as *Karenia*, *Karlodinium*, *Gymnodinium*, *Gyrodinium*, and *Pfiesteria* and others are caused by the raphidophytes *Chattonella*, *Fibrocapsa*, and *Heterocapsa* and the prymnesiophytes *Chrysochromulina* and *Prymnesium*. At least four *Prymnesium* species have been reported to be ichthyotoxic, with the majority of fish kill reports involving *P. parvum*. *Prymnesium* produce bioactive glycosides known as prymnesins. Prymnesins are released into the water naturally during *Prymnesium* blooms with even higher concentrations released during stressful conditions, or after the cells die. Fish are affected directly because prymnesins are absorbed across the gills. Other gill-breathing organisms such as amphibian larvae and shellfish can also be susceptible to prymnesins. Unlike many other toxic microalgae, the toxins from *Prymnesium* do not affect mammals, so less attention has been drawn to their effects because they are not a public health risk. However, *Prymnesium* blooms have caused significant ecological and economical impacts. Primarily responsible for high-density blooms and fish kills in aquaculture systems and fish farms, *Prymnesium* species have also been responsible for significant natural kills in brackish waters. Strategies for management include chemical and biological controls, and predictive monitoring that uses a combination of sensitive bioassays, traditional monitoring methods, and innovative technologies.

View the presentation

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A review of fish-killing microalgae: causes, impacts and management with emphasis on *Prymnesium*

Jan Landsberg

Florida Marine Research Institute (FMRI)

Fish and Wildlife Conservation Commission (FWC)

St. Petersburg, Florida

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Etiology of aquatic animal mortalities

- toxic microalgae
- contaminants
- water quality
- pathogens
- fishery by-catch
- mechanical damage
- natural

Toxic/harmful microalgae

- dinoflagellates**
- diatoms
- cyanobacteria
- raphidophytes**
- prymnesiophytes**
- dictyophytes
- chrysophytes

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** ichthyotoxic species

HABs and fish kills

- global
- all habitats
- > 60 ichthyotoxic species known
- > 30 species harmful to fish


Fish kills
PWD RP T3200-1203

Harmful mechanisms

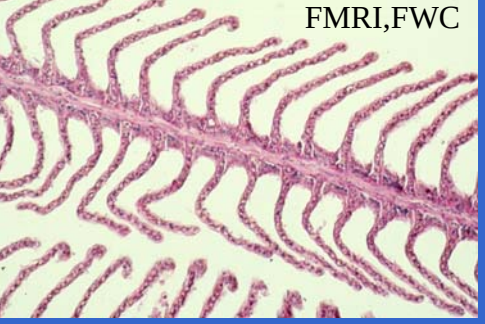
- toxins
- enzymes
- reactive oxygen species
- mechanical
- physical
- anoxia/hypoxia
- NH_4 toxicity
- allelopathy
- starvation
- predation

Ichthyotoxic species

- *Karenia brevis*
- *K. mikimotoi*
- *Karlodinium micrum*
- *Gymnodinium pulchellum*
- *G. aureolum*
- *Amphidinium* spp.
- *Cochlodinium* spp.
- *Pfiesteria piscicida*
- *P. shumwayae*
- *Alexandrium monilatum*
- *A. tamarense*
- *Chrysochromulina* spp.
- *Heterosigma* sp.
- *Fibrocapsa* spp.
- *Prymnesium* spp.
- *Chattonella* spp.

Impacts of ichthyotoxic species

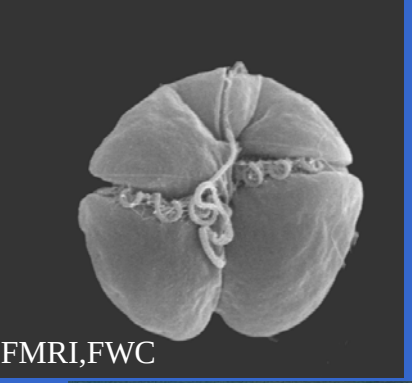
- public health
- direct mortalities
- indirect losses – disease, growth, fecundity, loss of recruitment
- economic
- ecological



Exposure routes

- gills – absorption of soluble toxins from water
- skin – absorption of soluble toxins from water
- ingestion – direction consumption of cells/ bioaccumulation of toxins

Karenia brevis


FMRI,FWC


FMRI,FWC

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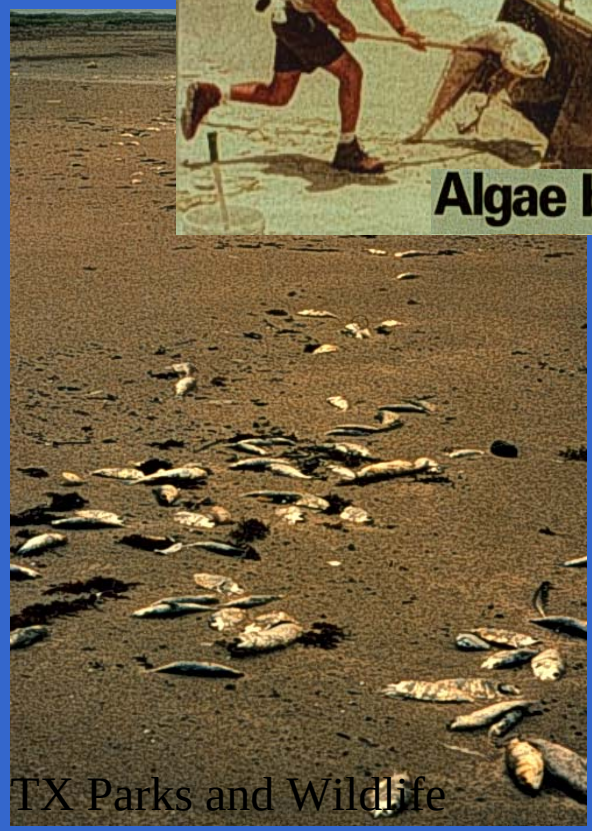
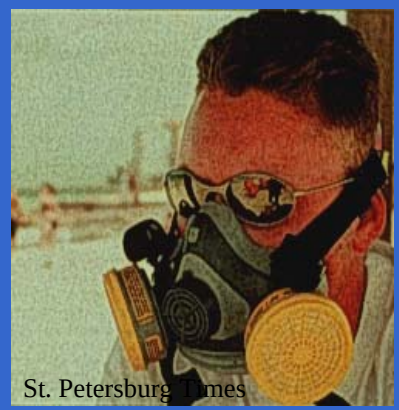
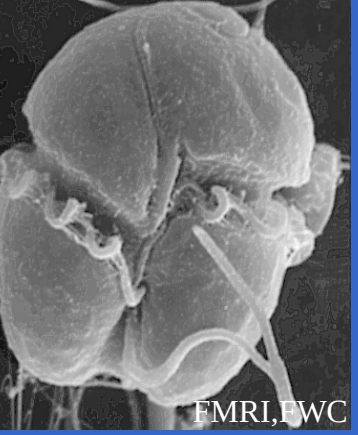
St. Petersburg Times


TX Parks and Wildlife

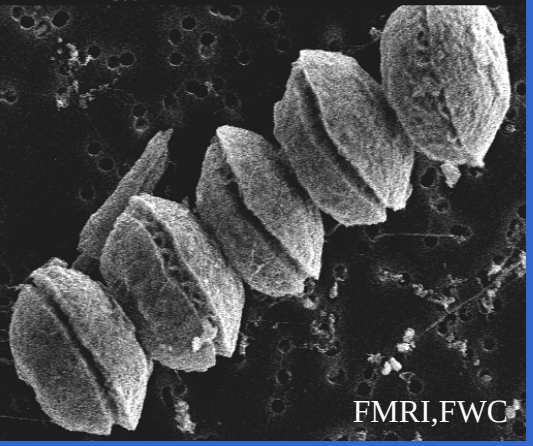

St. Petersburg Times

Brevetoxins - neurotoxins and hemolysins



Gymnodinium pulchellum (brevetoxins)

- natural mortalities of fish and mortalities in aquaculture
- respiratory irritation in humans



Alexandrium monilatum (hemolysins)

- reduced filtration in oysters and clams
- decreased byssus production in molluscs
- moribund shellfish
- mortality in oysters
- fish mortalities

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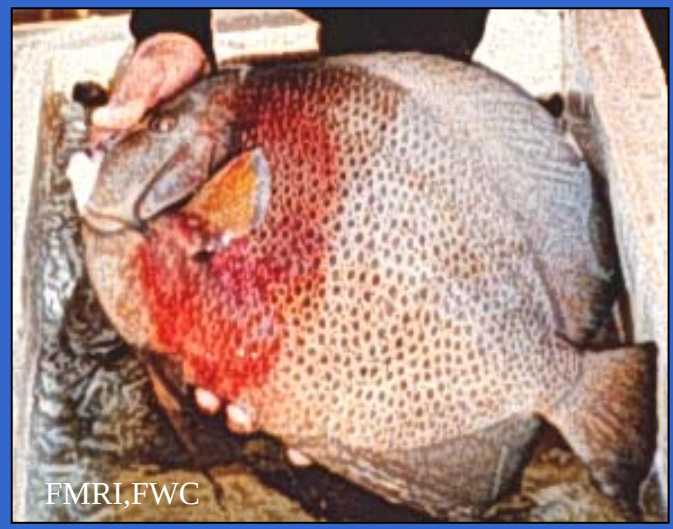
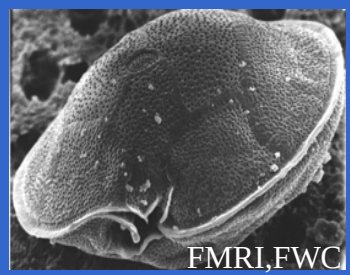
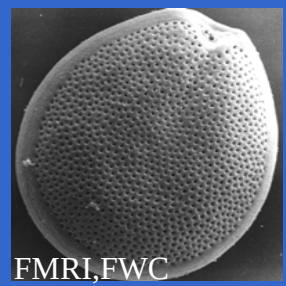
Reef fish disease - Caribbean, Florida







Microalgae

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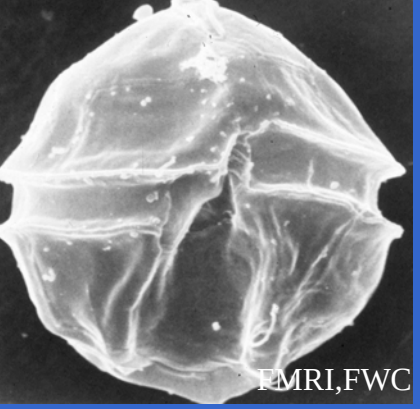
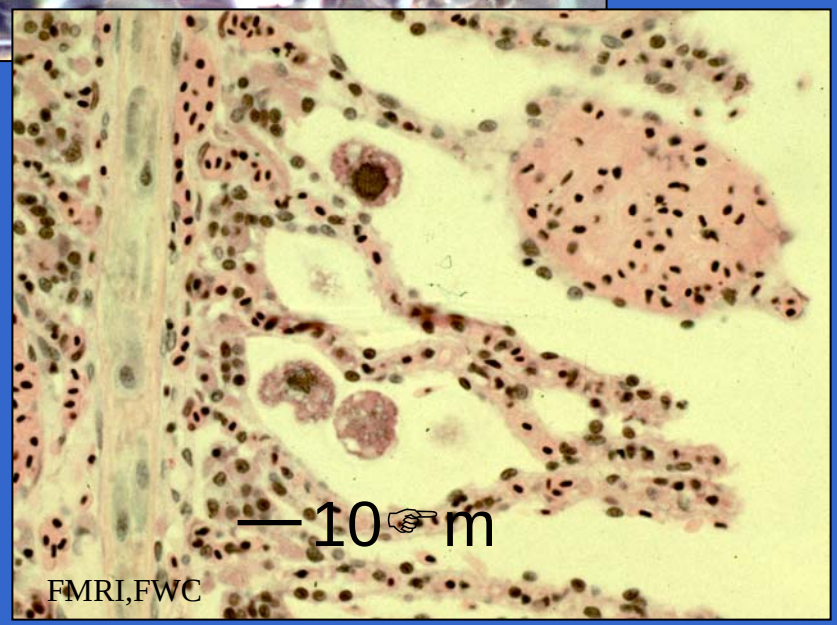
From Landsberg 1995

Scrippsiella sp.



- fish mortalities
- ?toxic
- low dissolved oxygen
- acute gill pathology



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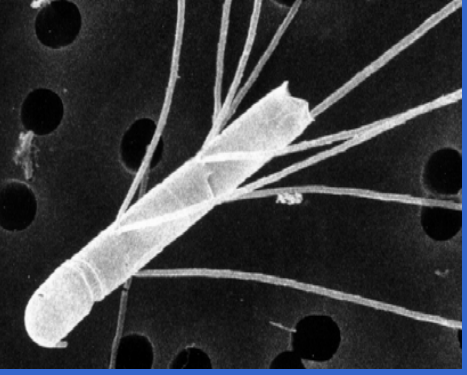
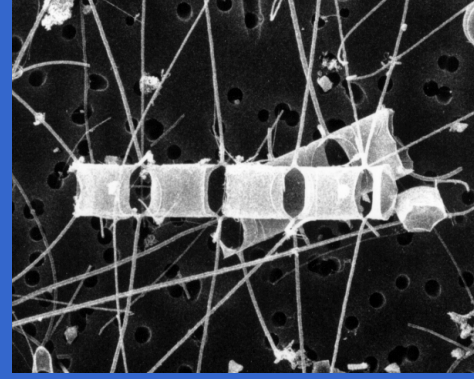
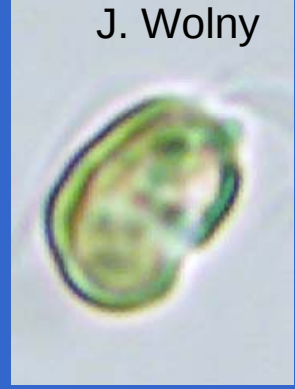


Diatoms



- physical damage to gills by spines, barbs
- gill lesions, excessive mucus, asphyxiation
- marked edema
- change in blood parameters
- immunosuppression - susceptibility to vibriosis

Prymnesium



- at least 3 ichthyotoxic species - globally
- primarily *P. parvum* associated with kills
- brackish water – aquaculture systems
- fish exposed to prymnesins in the water
- no transfer of toxins up the food chain or in drinking water

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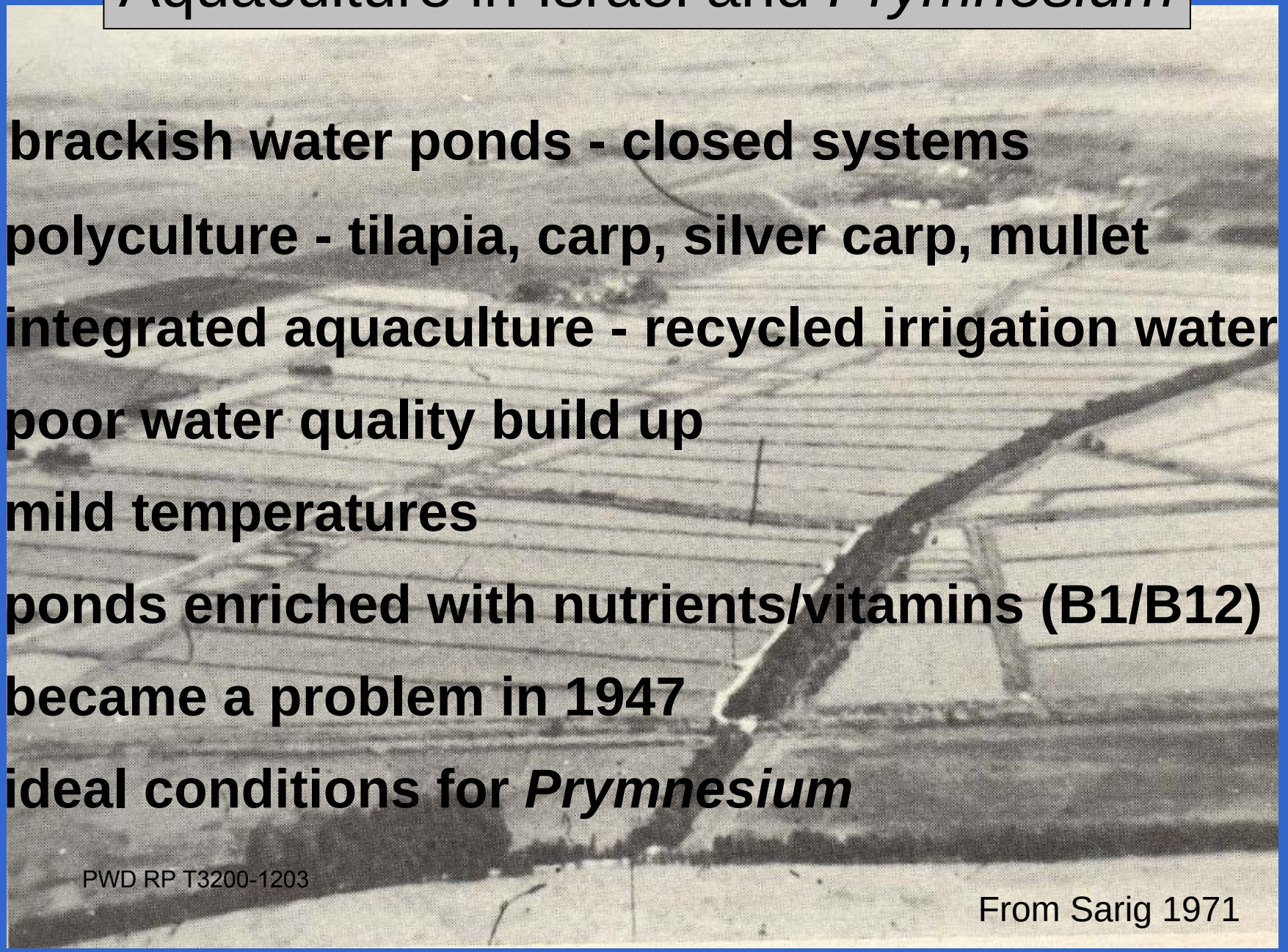
Aquaculture in Israel and *Prymnesium*



- **brackish water ponds - closed systems**
- **polyculture - tilapia, carp, silver carp, mullet**
- **integrated aquaculture - recycled irrigation water**
- **poor water quality build up**
- **mild temperatures**
- **ponds enriched with nutrients/vitamins (B1/B12)**
- **became a problem in 1947**
- **ideal conditions for *Prymnesium***

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From Sarig 1971

From Sarig 1971

Heavy carp mortality in 5 hectare pond due to *Prymnesium parvum* in Israel

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Prymnesium parvum from Israel

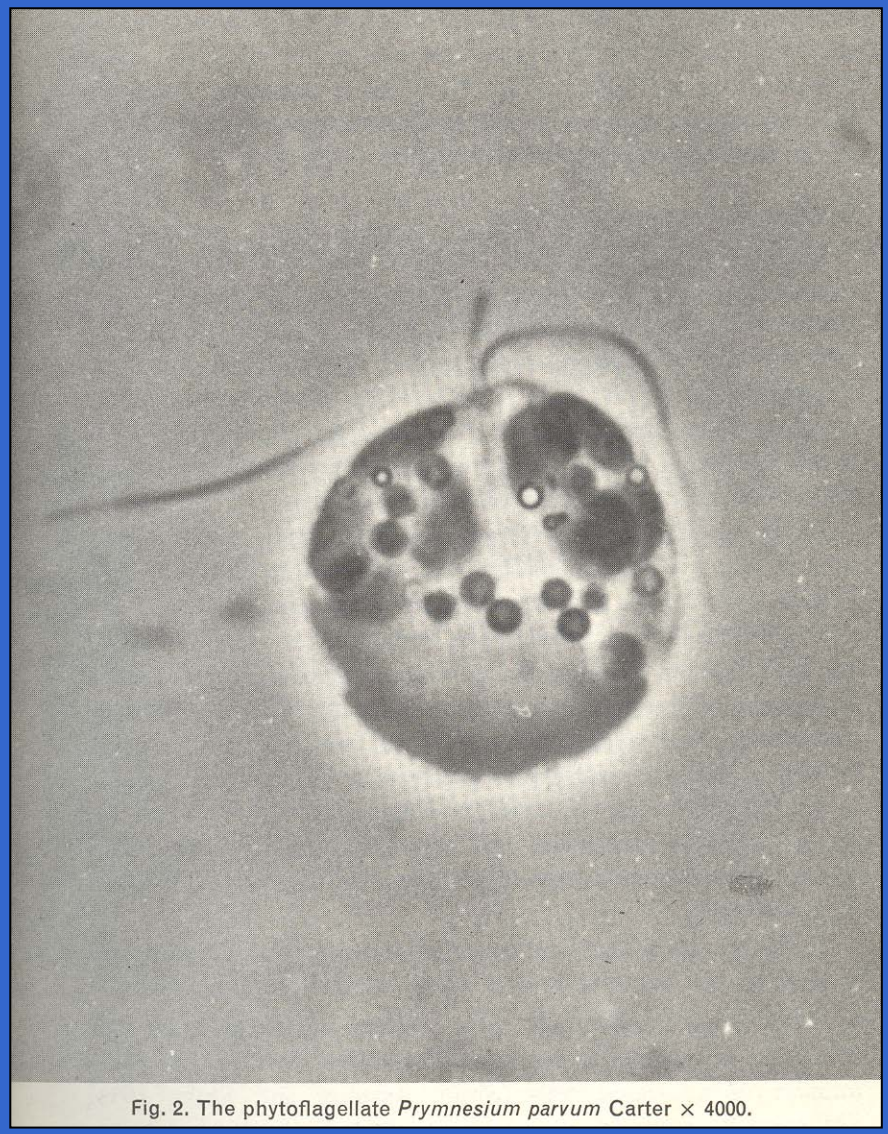

Fig. 2. The phytoflagellate *Prymnesium parvum* Carter × 4000.

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From Sarig 1971

Prymnesium impacts

- gill breathing organisms sensitive – larval amphibians, finfish, bivalves
- non selective
- restricted by habitat type
- seasonality
- acute effects only – direct through the gills

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Prymnesium parvum blooms in Israel

- lack of correlation between blooms, toxin, fish kills
- sporadic fish kills
- requires vitamins B_{12} and thiamine
- can tolerate freshwater with chloride 250-625 ppm
- no growth below 0.1% salinity
- typically rare in natural habitats

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Prymnesium parvum toxin

- hemolytic and ichthyotoxic components
- toxin synthesized during late stage of logarithmic growth and in early stationary phase
- intracellular > extracellular
- biosynthesis and extracellular stability affected by environmental conditions
- light essential for toxin formation
- phosphate limitation > toxin production
- toxin inactivated by change in pH, absorption on various colloids, exposure to UV and short wave light

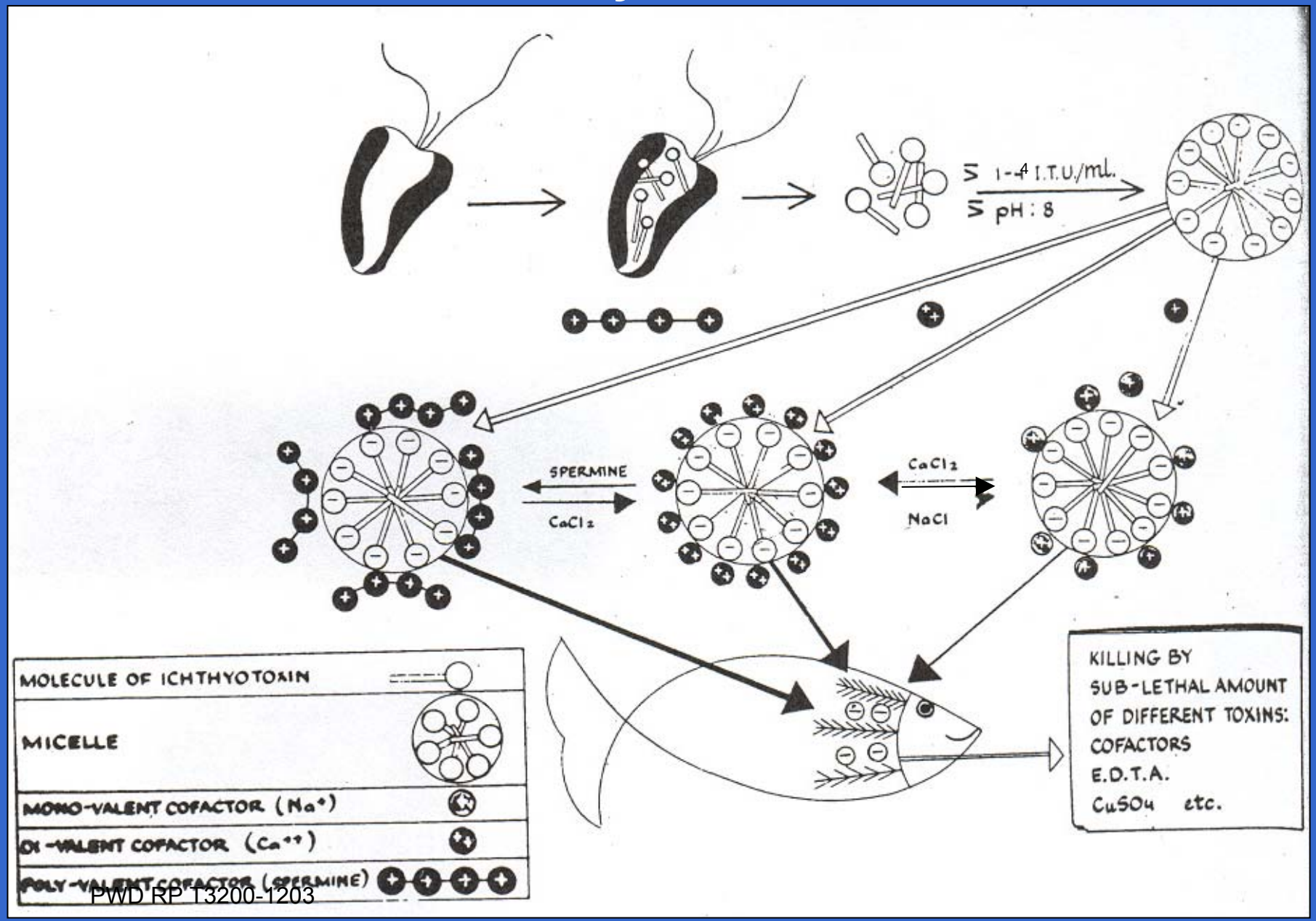
From Sarig 1971

Prymnesium parvum toxicity

- activity of prymnesin requires cationic cofactors
- Na, Mg, Ca and salinity determine toxicity
- non toxic by dialysis or cationic exchange column
- ichthyotoxicity restored on addition of the dialyzate
- dialyzed cation salts e.g. Ca/Mg restore fish toxicity
- streptomyin, spermine, detergents (DADPA)
  enhance toxicity of *Prymnesium* preparations
- relative activities of various cations different
- inverse relationship between toxicity and salinity

From Sarig 1971

Mode of action of *Prymnesium* toxin on fish



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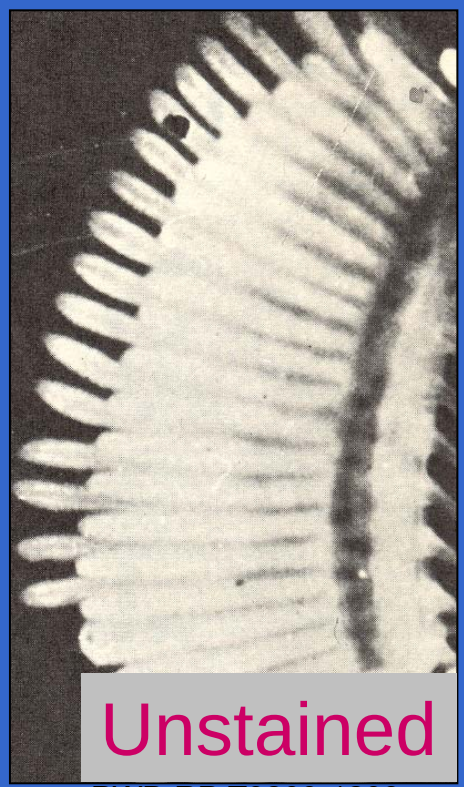
From Sarig 1971

Mode of toxin action

- fish affected within minutes of exposure

unexposed fish

fish exposed to toxin-cation mixture



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trypan blue

increase in gill permeability

From Sarig 1971

Mode of toxin action

- increased gill permeability only in conditions in which ichthyotoxin activity is cation activated
- pH dependent -- requires higher pH
- toxic activity inhibited by NaCl
- damage to gill permeability and consequent sensitization to toxic agents is reversible
- intoxication in two stages

1) reversible specific damage resulting in the loss of selective gill permeability

2) response of sensitized fish to an array of toxins

Fish bioassay

- dependence of toxin activity on various cations lead to sensitive bioassay
- assay based on minimal toxin concentration killing *Gambusia*
- in the presence of 3’3 diaminodipropylamine (DADPA) as a cationic activator

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From Sarig 1971

Add 1 ml DADPA (0.003M) + tris buffer (0.02M), pH = 9

50 ml pond H_2O

28°C

40 ml distilled H_2O
+ 10 ml pond H_2O

2 hours exposure

50 ml distilled H_2O (control)

Death = 1 ITU

1/25 lethal dose in ponds

Death = 5 ITU

1/5 lethal dose in ponds

Recommend treatment

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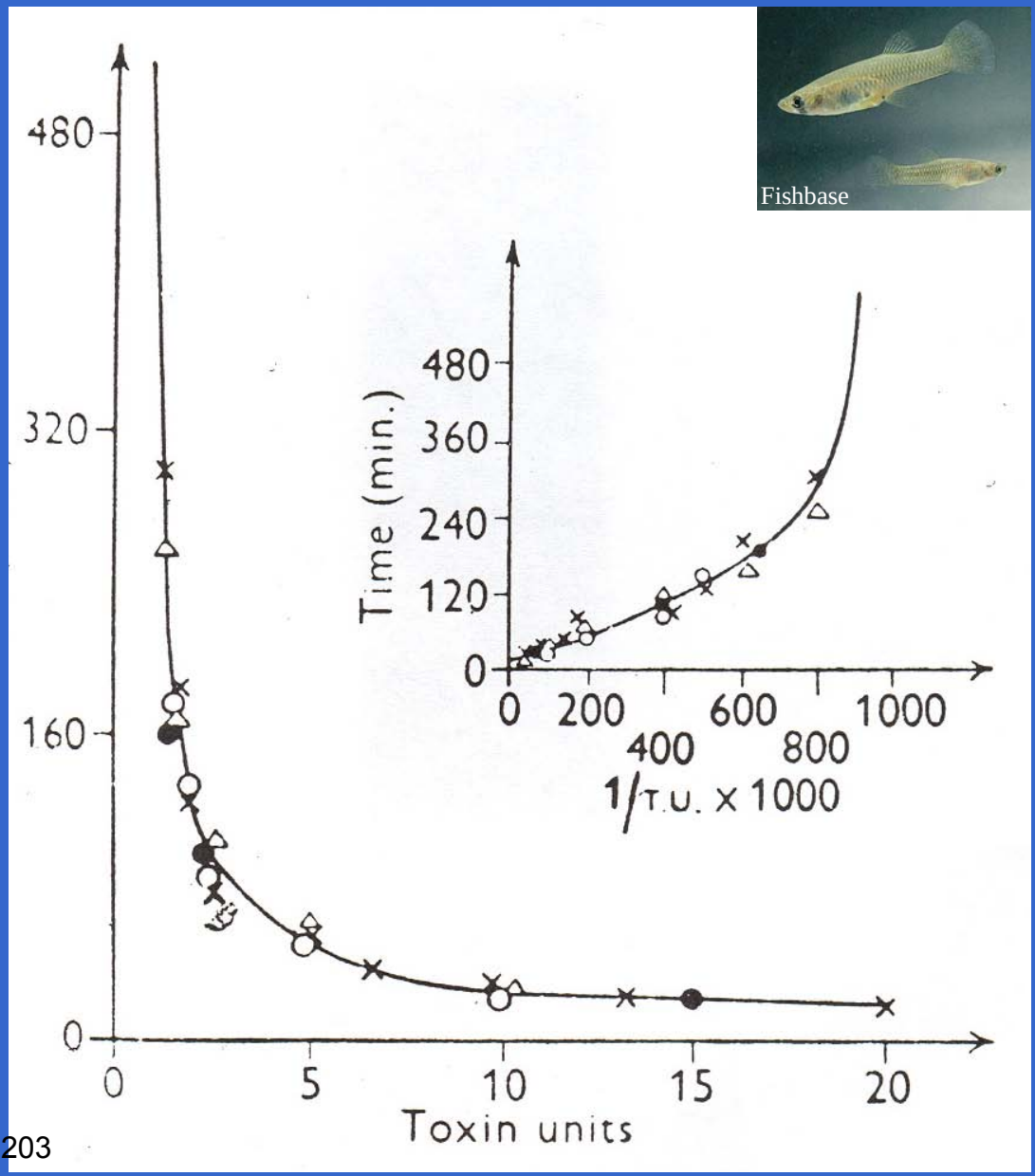
1 ITU = minimal amount of ichthyotoxin/ml that kills fish

Relationship # toxin concentration and time for loss of equilibrium



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From Sarig 1971

Testing chemical applications on *Prymnesium*

- 10 ppm $(NH_4)_2SO_4$ lytic effect
- low cost, high solubility, ease of dispersion
- *Prymnesium* lysis > with temperature and pH
- decreased activity of $(NH_4)_2SO_4$ in winter
- ammonia responsible for cell lysis
- diurnal changes in pH max. at noon
- control added few hours before pH peak
- Cu_2SO_4 not dependent on pH or temp
- $(NH_4)_2SO_4$ (acid fertilizer) lowers pH
- treatment strategy varies with conditions

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From Sarig 1971

Management of *Prymnesium* blooms in Israel

- proactive monitoring
- fish bioassays
- test for sublethal *Prymnesium* concentrations
- treat ponds with liquid ammonium
- ammonia concentrates in *Prymnesium*
- shift in pH > water entry > swelling > cell lysis
- best results at temps < 20^{o}C and pH < 8.5
- aqua ammonia is alkaline and raises the pH

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From Sarig 1971

From Sarig 1971

Algicides and *Prymnesium*

pH	Temp (°C)	Liquid ammonia	Ammonium sulfate	Copper sulfate
>9.0	> 20	-	10-12	-
	17-20	10-12	15	2
	10-17	14	25	2-3
8.6-9.0	> 20	10-12	13-15	-
	17-20	12-14	20	2
	10-17	14	-	2-3
<8.6	> 20	12-13	15-17	-
	17-20	13-14	25	2-3
	10-17	-	-	2-3

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per Kg/1000m^3 pond water = 1million liters or 265,000 US gallons

Management strategies

- prevent blooms
- inactivate or remove toxin
- separate fish from blooms

Needs

- dynamics of toxin production in different systems
- determine triggers for bloom formation
- spatial and temporal variations in toxicity
- are *Prymnesium* effects only acute?
- impacts on recruitment?
- economic assessment for management strategies

How to Use the Past to Plan for the Future

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Abstract.--Harmful algal blooms occur worldwide and in some cases have been documented for hundreds of years. Others have only been documented recently in a specified location although the causative organism may have been resident at low levels for years. Funding has historically been directed more toward the species that directly impact human health. Others may be fish killers of major proportions and impact aquaculture industries. *Prymnesium parvum* is an ichthyotoxic brackish water microflagellate that has caused fisheries, aquaculture and economic losses around the world. Additionally, it has been found in west Texas inland rivers, lakes, and reservoirs as well as fish farms. How can we use what we know about HAB species/events, including *Prymnesium*, to frame an approach to needed *Prymnesium* studies in west Texas saline freshwaters? This workshop will present much of the known information on *P. parvum* and then identify and prioritize research and monitoring needs. A resulting action plan could also include a preliminary economic assessment, proposed education and outreach component, and suggested data management for monitoring. The plan can be the product of an agency or a governmental multirepresentative task force and can be used as documentation for local and state financial support. The task force should cross boundaries of fish and wildlife, water resources, and other agencies as well as universities, fishing groups, environmental groups, aquaculturists, and others. It should include managers, scientists, and activists. Florida has had a HAB task force to address several major HAB species and was funded $3,330,000 over a four-year period by the state legislature. The Task Force plan with recommended action items was a major instrument for creating a HAB Task Force funding program through a state agency. In addition, it provided recurring additional funds for monitoring of HABs by the state agency and a contractor. Having diverse support from stakeholders strengthens the multiyear funding request. When a state is confronted with a new or increasingly occurring HAB species, there are certain questions that need to be addressed whether it is marine or freshwater. They constitute the basic building blocks of a plan. The plan, perhaps five years, needs to be specific to a geographic area, e.g., Texas. What species is it? Could similar species of the same genus be involved? Is its toxin ichthyotoxic, neurotoxic, hemolytic, or cytolytic? Are there any public health concerns? Are there differences in toxicity and potency? Is toxicity influenced by environmental cues such as nutrients, light or other factors? Where did the species originate – locale or introduced? What is the best realistic monitoring method - microscopy, species probes, or toxin probes? Can platforms with automated sensors be used to monitor? Can a volunteer monitoring program be set up? Is economic data available for losses? Can the species be successfully controlled by clay, ammonium compounds, ozone, viruses or other means without adverse impacts? What is the organism's complete life cycle and how are the stages influenced by environmental variables? Can the stages be easily identified by microscopy or molecular probes? Are there hot spots in a lake, reservoir or other freshwater environ that are linked to resting stages? Is there any seasonality for presence in the water column, blooms and toxic populations? What environmental variables affect the resting stage and its emergence to the water column? Most of these questions can be applied to any HAB species.

View the presentation

PWD RP T3200-1203

HOW TO USE THE PAST TO PLAN FOR THE FUTURE

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Florida Marine Research Institute

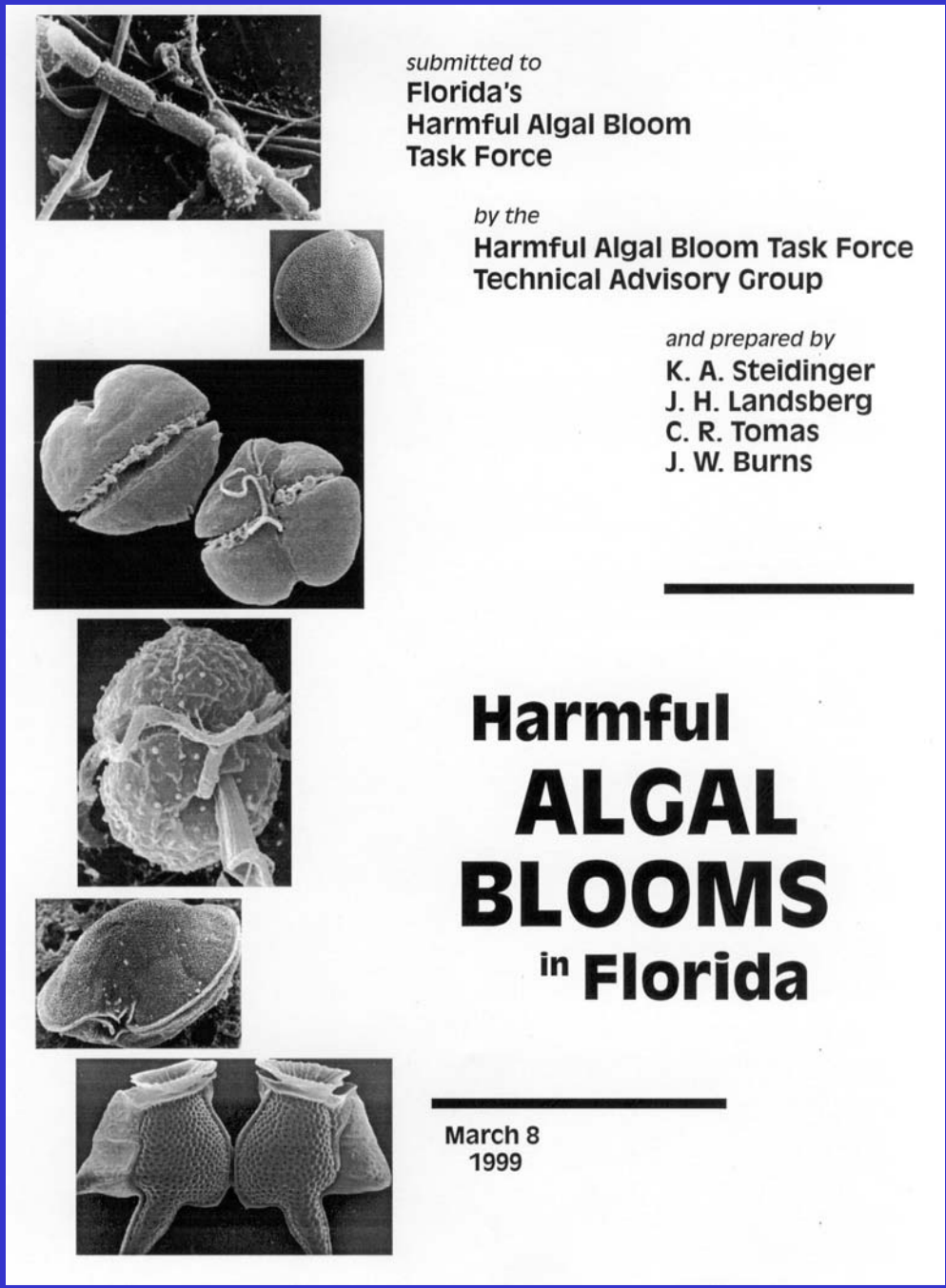
PWD RP T3200-1203

How can studies on other harmful algal species and events help structure an action plan directed toward monitoring, mitigation, and management

- Like Texas, Florida's experience with red tides and other HABs has helped to determine direction when new HABs emerge
- Like Texas, Florida brought in experts to discuss different red tides and the biology and ecology of the causative organisms
- Texas has a Harmful Algal Bloom Committee consisting of state agency personnel, academia, and interested parties that prepared a report outlining specific research needs

❖Task Force report identified 7 HAB groups as requiring further research and identified research topics

❖The Task Force itself prioritized research thru the funding process by identifying which topics would be funded



PWD RP T3200-1203

- **10 million was provided by the Florida legislature to address HAB issues over a 5 year period with 3.3 million contracted to outside investigators at the recommendation of the Task Force and the remainder going to a joint FMRI/Mote Marine Laboratory HAB program**

- **The responsible state agency with its research scientists and collaborators (>20) were successful in being awarded ECOHAB and MERHAB federal grants to supplement state funds for major red tide programs.**

ECOHAB:Florida

A 5-year federal, state, academic, and private laboratory partnership to understand the development of Florida red tides and be able to predict their occurrence, movement, and landfall through coupled biophysical models

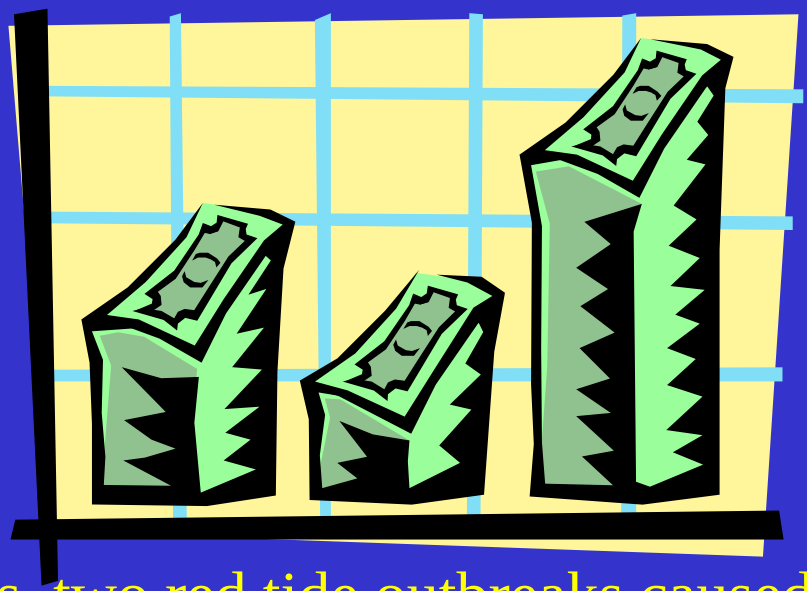
13 institutions including the University of South Florida, Mote Marine Laboratory, and the FWC Florida Marine Research Institute and 23 Principal Investigators

PWD RP T3200-1203

WHAT DRIVES FUNDING FOR HAB MONITORING, MITIGATION, AND MANAGEMENT

- Public health
- Living resources and Fisheries
- Economic losses
- Area covered and frequency
- Constituency concerns and complaints

ECONOMIC IMPACT



In the 1970s, two red tide outbreaks caused by the toxic dinoflagellate *Karenia brevis* affected several west coast counties for 3 to 5 months and caused an estimated 15- to 20-million-dollar impact to those counties. Recently, a WHOI report estimated that from 1987-1992 an average annual cost for total USA HABs would have been 49 million in Year 2000 dollars.

TEXAS	FLORIDA
Website w/current info	Website w/ current info
Fish kill hotline	Fish kill hotline
Kill and Spill Team	Event response /volunteers
HAB committee	HAB Task Force
workshops	workshops
Action plan	Action plan
Lobbying	Lobbying
	Red Tide Alliance

What is Needed Based on What is Known

- Species and strains in Texas
- Toxins and toxicity of Texas strains
- Influence of nutrients, light and other factors
- Persistence of toxins in the environment

What is Needed Based on What is Known

- Life cycle stages, particularly bottom resting stages
- Environmental influence on “excystment”
- Does life cycle influence spread of *Prymnesium*
- Documenting basins with cysts

What is Needed Based on What is Known

- ***Prymnesium parvum*** **and its life stages – microscopic detection or molecular probes and arrays, sentinel monitoring stations or autonomous platforms**

- **Will mitigation or control measures have to be applied annually because of resting stages**

What is Needed Based on What is Known

- **Does Texas *Prymnesium* have any affinities with populations in Europe or elsewhere – is it native or introduced**

- **Can mitigation and control treatments used elsewhere be applied to Texas waters, are there new methods being tested**

What is Needed Based on What is Known

- **What are the forcing variables – biotic and abiotic – for bloom initiation, growth, and maintenance**
- **What are the species-species interactions including predator-prey relationships**
- **What are the forcing variables for *Prymnesium* bloom termination in different environs**

Prymnesium monitoring, mitigation, and management will need

- An action plan
- Collaborative research efforts
- Protocols
- Agency or Committee direction
- Targeted funds, recurring
- Communication and coordination
- Public outreach

Golden Alga Workshop Notes:
Presentation Question & Answer Sessions
Facilitated Discussions and Recommendations

Presentation Question & Answer Sessions:

1. Joan Glass: Historical Review of Golden Alga (*Prymnesium parvum*) Problems in Texas

When you look at historical patterns, are they [fish kills due to toxic golden alga] moving east/west or randomly occurring?

The Pecos came first and then the Brazos. (Both have seen fish kills related to golden alga for years.) We're not sure if it's [golden alga] being moved or was already there. The dead fish look like a low dissolved oxygen kill, so it is often misidentified. We have a limited understanding on if *P. parvum* is endemic or introduced and a poor baseline to make conclusions.

What about silicon in the region? It appears to be decreasing due to damming in the rivers according to some studies.

There is currently not a good answer to this question. Patterns appear similar in Texas and Sweden, but this has not been studied for *P. parvum* due to funding limits.

Is there any data indicating why a pH in excess of 7 is significant?

The toxin does not finish forming in low pH; it requires cations, and they aren't available when pH is below 7. This explains part of the reason the toxin is not there.

Have you developed any intuition or suspicion about where or when the next toxic golden alga [bloom] will happen?

- There appears to be some susceptibility in having many lakeside houses with septic tanks and thus high nutrient loading. This would be one factor, although no reliable indicators for nutrient loading have been established.
- The months of October through February are times of the greatest potential risk with blooms going into April.
- Stress (drought and other weather) may be a contributor.
- We are adding in studies to see if there are existing cysts and are doing a historical review of past blooms.

2. Greg Southard: Overview of Texas Hatchery Management of Golden Alga, *Prymnesium parvum*

What about treatment following an ammonia shock?

With existing high levels (>0.2 mg/L un-ionized ammonia), adding additional ammonia will not help.

What size are the hatchery ponds?

About an acre.

Does continuous piping connect the ponds at a hatchery?

There is a common water supply to the hatchery ponds.

Do cutoffs exist to segregate the flow pond water, or is there continuous flow through?
We have a mechanism for cutting off flows (i.e. water does not flow from one pond to another).

Once a pond is clear how often does it become reinfested?
The pattern is unknown and random. We have seen recurrence within 2 months if a cyst stage is present. It appears to be dependent on water quality.

3. Bente Edvardsen and Aud Larsen: Phylogeny, Life History, Autecology and Toxicity of *Prymnesium parvum*

Do we have any idea of how long the cysts are viable?
Very little is known; they have only been found in a few locations in Norway. Blooms have occurred there every year from 1985 to 1995. It is believed the cysts can live through the winter.

Why avoid aeration?
Toxin production can increase dramatically during aeration. This should be avoided!

Do you ever collect enough cysts to run a ploidy analysis?
No.

Under low toxicity, one organism eats the other; under high toxicity, the situation is reversed. Are there any indications why this happens?
Toxin production could be a function of grazer protection, or allelopathy. For example, at low toxin levels, grazers eat *P. parvum*; at high toxin levels, *P. parvum* eats others, including the grazers.

How does *P. parvum* protect itself from its toxins?
We don't know at this stage. Cholesterol may play a role.

4. Carmelo R. Tomas: *Prymnesium parvum*: An Overview and Questions

Do hatcheries in Texas have the capability to reduce bloom potential by drilling freshwater wells? (Would the bloom potential be reduced in Texas by drilling freshwater wells?)
The Elizabeth City, NC resolution was only successful when it [the facility] was re-flooded with fresh water, dropping salinity to virtually zero.
(TPWD Hatchery Personnel, post-conference: The groundwater around the affected hatcheries is brackish, making this a nonviable solution for us.)

What happens during the dark cycle? What is being regenerated at night?
Some toxin genes are turned on just before daylight. The dark cycle may be a recharge or a trigger that activates an aspect of the physiology of the organism.

What agents have been used to combat *P. parvum*?
In Artesian Aquafarms (4 ppt salinity water), Diuron and Loicidyn were both effective but illegal in the concentrations necessary. Loricidyn (a ham-curing product) was effective and approved.

Do you know anything about brood stocks for the ponds?
Private aquaculture isn't always comprehensive about record-keeping and sometimes forgets to mention certain variables. Insuring that no other water was coming into the

facilities was a problem in North Carolina. By being close to the Atlantic in Elizabeth City, part of the introduction could be the result of birds. Introducing fry might be another. We really don't know, but there are 2, possibly 3, ways this might have been introduced.

5. Richard L. Kiesling: Analysis of *Prymnesium parvum* Blooms in Lake Whitney, Texas

Were nutrient bioassays included?

Single assays were performed.

Did you have a total nutrient control in the experimentation?

No full nutrient controls were performed.

Have you considered nutrient bioassays by exclusion?

We have never done this. It should be discussed and pursued. It looks promising. A limited number of replicates that could be run on an exclusionary basis should be considered.

How much chlorophyll was generated in blooms?

15 -10 micrograms/liter

Typically, one approach that works in a study like this is adding three to five-fold amounts of ambient nutrients to avoid stressing the populations. Managing phosphorus production should reduce bloom production. This is an important takeaway message.

Were the incubations stirred or mixed in any fashion?

Achieving replication or maximum growth is the main objective here. Algae don't respond well to any handling or storage conditions, although they are pretty good at adapting to nutrient pulses. Bioassays are done in large pools that are gently mixed. All bioassays are somewhat artificial. It is believed that there are nutrient effects on the bloom. This must be studied further. "Dinoflagellate whiners" can pose a challenge!

6. Edna Graneli: Kill your Enemies and Eat Them: The Role of *Prymnesium* Toxins

What are the observations from reservoirs and lakes on the phytoplankton community during a bloom? Are the blooms initially monospecific?

Glass: When the blooms start, we have mixed communities, and the toxicity is low. As they progress, the toxicity increases, and the community becomes monospecific.

7. Paul Kugrens: *Prymnesium parvum* Laboratory Studies: Structure, Reproduction, Salinity Tolerance and Bioassay

Have you found the small, immobile cells in the Colorado research?

Cyst-like structures ¾ the size of a cell have been identified in some of the Texas samples. They are not orange but rather greenish with very small chloroplasts.

The variability in the organism, depending on the geographic location it originates from, is remarkable. The Wyoming strain has distinctly different shapes than Texas and Colorado samples.

How was the Colorado fish kill attributed to *Prymnesium*?

The head fish pathologist contacted Texas. The Colorado organism was a similar shape (i.e. a Chrysophyte) but not *Prymnesium*.

Has any DNA sequencing been completed on the Wyoming sample?
Not yet. Blame Wyoming.

8. Linda Medlin, Gundula Ellers, Kerstin Toebe, and Katja Kerkmann: Rapid Tests for the Detection of *Prymnesium parvum* and Its Toxins

What is the limit of detection on the hand-held unit?
60 cells/liter is the lowest field sample limit of detection.

Could the filter be rotated for solid phase assays?
No, the shape of the probe limits rotation and interferes with measuring oblong cell shapes.

9. Donald M. Andersen and Mario R. Sengco: Bloom Control Strategies for Harmful Algal Blooms

- Barley straw has been used in the United Kingdom. It provided habitat for microplankton that cleaned up the algae. There are counter-arguments pointing to an active ingredient responsible for this effect.
- Leftover Christmas trees have been placed to increase habitat. The fish population thrived (possibly due to an increase in tannins), but algae didn't grow. The cause and effect of this should be investigated.

Why does the requirement for only using incinerated clay in Europe exist? There is nothing similar in the United States.
European clay incineration is a requirement to kill nematodes for tulips.

How much clay per acre is recommended?
350-400 grams/square meter is the loading amount for per unit area.
This clay will only be effective in the top 4 meters. Tiny particles have to flocculate to be effective. The Koreans are using much higher concentrations and are achieving success.

How does the clay treatment affect cyst formation?
There are two issues. Cyst-forming dinoflagellates take days to form and reproduce. This won't happen in the two hours of a clay treatment. Temporary cysts that occur in the life cycle may remain dormant for longer periods. This has not been noted in the Woods Hole research, but some freshwater dinoflagellates may exhibit different behavior. Closer study is needed.

Marine hard clam studies are radically different organisms than rare and endangered freshwater mussels. Silt is a problem for them [rare and endangered freshwater mussels]; clay may be devastating.
Situational triage may have to be put in play. Responders must decide which is worse: a *Prymnesium* bloom or the effects of the clay. Remember, we will not be talking about a pristine environment when making these calls. More valid comparisons should be made with the effects of red tide and algae blooms.

At what water depths have the Koreans achieved success?
15-20 meters deep with good flushing. The clay does not persist with tidal patterns. They have not seen a major re-bloom of algae following major treatments. Consistently reducing the bloom cysts may reduce the propensity for future blooms (1995-1996).

Korean fisheries are a $1 billion industry that has sustained over $1,000,000 in red tide losses.

Is there evidence suggesting there is a rebound of species after applications?
Yes.

What permits are required for application?
Environmental Protection Agency/FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) permitting has been required. Additional permits have been obtained from the Florida Department of Environmental Protection. The issue in Florida is closely linked to phosphatic clay (a mining byproduct and a toxic waste). There is concern that by offering permits for limited small-scale application in Florida, additional requests may be triggered to dump 100,000 times that amount into the Gulf of Mexico by mining interests who want to dispose of a byproduct. This would be a problem.

How important is multiyear funding?
Critical. [$1.2 million] is not a lot of money. It is crucial to establish recurring funds for ongoing monitoring. Recurring general revenue funding is the best of all possible worlds.

10. Jan Landsberg: A Review of Fish-Killing Microalgae: Causes, Impacts, and Management with Emphasis on *Prymnesium*

Since the Israelis have a limited supplies of freshwater, is dilution less of an alternative there?
Most systems are brackish, and there is less access to freshwater. In Texas the question is less one of access than of timeliness. Freshwater for dilution near hatcheries does exist, but it has to be delivered rapidly in order to dilute blooms if it is to be effective.

Do you know how often the Israelis monitor (daily or some other schedule)?
It generally depends on the individual farmer and his area of responsibility. They have become more proactive in identifying emerging problems. In the past, most of this was triggered by fish behavior. There may be a brief window of opportunity for treatment (2-3 hours in the morning to afternoon) if response is rapid. Treatment and recovery can be possible if the symptoms are recognized and acted upon quickly. Fishermen do most of the monitoring themselves, and there is a much greater recognition of the problem. The smaller systems there lend themselves more readily to monitoring and treatment.

One of our strategies should include increasing the size of the volunteer monitoring.

Shrimp farms exist along areas of the Pecos that have experienced *P. parvum* blooms. Is there any reason NOT to expect shrimp mortality if there is a *P. parvum* bloom?
We would expect them to be affected. If they are NOT, it would be a basis for further study. The sodium/calcium channels of vertebrates vs. invertebrates should be examined. We do not recall this being an issue in Israel.

Could you elaborate on the cation exchange process for deactivating the toxin?
If any cation that could be complexed with the toxin could be removed, it should make a difference. Is it the *P. parvum* that is complexing with various cations? What are the implications of pH on the relationship? Many combinations of this matrix should lead to greater contact with Israeli partners.

Bioassay variability must take the variability of chemical composition into account. More needs to be known about the chemistry of the water.

Edvardsen: *P. parvum* toxins appear to be easily broken down with light. Reducing salinity to prevent the growth of *P. parvum* cells could be one key.

Glass: In Texas, one of the keys to a kill is that the aquatic insects remain alive and well. They appear to be unaffected by a *P. parvum* bloom.

Audience Comment: With respect to reducing salinity, reducing it even a few parts per thousand requires a significant volume of freshwater. It sounds terrific as a lab approach but can be potentially very expensive in the U.S.

Shrimp/crabs were salvaged by putting them in cages. Why were the crustaceans unaffected?

What animals were used as controls for bioassays?

Gambusia. There were many fish in the channels between the ponds in Israel, and they are very easy to collect.

Although magnesium and calcium are involved in increased toxicity, and sodium is involved in decreased toxicity, might NaCl be an alternative for reducing toxicity?

Clays are often high in magnesium and calcium. Could there be binding with the clays because of these tendencies?

Edvardsen: In Norway, high salinities reduced toxicity, but growth of the organism was stopped at very low salinities.

Are fish kills caused by a toxin that is free in the water and accumulating through time, or are they caused by bursting cells? How much time would it take for the toxin to recur to a level that was lethal?

The dynamics of the toxin need to be studied and identified. At the beginning of the bloom, the fish can be exposed by ingesting the toxin. With time (1-2 days) and lysing of cells, more extracellular toxin appears (depending on a range of variables). When all of the toxin is intracellular, we are not clear on the risk factors for fish consuming the algae.

Exposure by ingestion needs additional study.

Gambusia were used as test fish. They are generally a surface fish, which explains why they survive longest. Fathead minnows tend to swim deeper and are a more rapid indicator of *P. parvum* toxicity. If you have deep reservoirs, be aware if the benthic fish are being affected. Identify how the toxin is circulating within the system. It is crucial to verify how fish are being exposed and what they are being exposed to. Multiple effects must be sorted through.

11. Karen A. Steidinger: How to Use the Past to Plan for the Future

How important is recurring funding?

Critical. Having to go to the Legislature year after year is difficult, time-consuming, and tends to erode credibility over a long period of time.

Friday October 24, 2003 Panel Discussion Notes:
Each of the panel members was asked to offer specific recommendations for managers.

Don Anderson:
- We've got to learn much more about bloom dynamics and what's going on in reservoirs. It's crucial to understand why blooms are declining, what happens that triggers their end, and the role of nitrogen and phosphate limitation.
- Cell and toxin detection techniques must be advanced. We need the ability to identify and measure a single toxin, or 4-5 multiple toxins simultaneously. This is a complex, difficult challenge.
- Mitigation using basic approaches (e.g. barley straw bales) should be studied in appropriate systems. This can be started almost immediately in hatcheries.
- More research needs to be directed toward the modeling of bloom development and migration, and more meetings like this one will speed the understanding and dissemination of findings.

Edna Graneli:
- Hand-held probes are needed for field sampling. Using them for regular sampling will be useful in data collection and situational analysis.
- Experimenting to determine the effects of strong light for 24 hours (especially in hatcheries) would establish the effectiveness of this approach as a mitigation strategy.

Are you suggesting we should count the occurrence of empty cells as indication of allelopathy?
Yes.

Carmelo Tomas:
- This kind of problem will require great coordination among multidisciplinary task force members. Greater organization and cooperation is needed. Identifying flexible funding alternatives will be one of our first tasks. State, federal, non-government agencies, and public health departments all will have roles to play in addressing this issue.
- Overall management efforts must be closely coordinated. Inter-jurisdictional issues may complicate our response, but they must be addressed.
- The field component will provide the background and frontline data gathering. The lab component (using cell cycles) will help us understand the biological side.
- The chemical dimension of toxins must be identified. A greater understanding of toxin half-life, persistence, and lethal levels is required.
- We must be able to concisely and persuasively explain the importance of study and action to Legislatures. Making the case for why research is crucial may require creative "packaging" of our messages. Researchers and practitioners must be able to speak with one voice on this issue. Confusion and inconsistency of messages can be fatal to lobbying efforts.

Should field work concentrate on the originating locations instead of where the organisms may have moved downstream? Wyoming headwaters may be a case; residents there are not aware they have Prymnesium yet.
They should be contacted. This is a good and potentially an ideal location for comparisons and study with the Texas organism.

- Establishing a history of origin is vital. We should begin to develop solid data that correlations can be drawn from.
- Predictable trends will emerge if better records can be kept and shared.

Paul Kugrens:
- We need greater increased knowledge about the alga, including algal ID, biology, phycology, lifecycles, and classes.
- Texas experts on phycology should be contacted more frequently. List, communicate, and correspond with them.

Steidinger: We must emphatically state that this isn't a 2-year problem when approaching the Legislature. Expectations should be set that it is at least a 10-year problem. Funding requests should be calibrated to long-term, step-by-step study and analysis. Don't expect a silver bullet.

Karen Steidinger/Jan Landsberg:
- The distribution of the organism throughout the system (horizontal, vertical and benthic distribution) must be better understood.
- Dispersal and meteorological influences should be studied.
- In monitoring programs, map and identify the resting stages of *P. parvum*. Can we identify populations that are on the bottom?
- Hand-held species identification kits are really needed. We need to establish priorities for the capabilities and performance standards for them.

Richard Kiesling:
- SWAT teams need to be organized to follow the entire life cycle from bloom to resolution. They can implement several objectives real time.
- Having a pre-defined action plan requires a major commitment of time and resources. This is difficult but can be potentially very beneficial.

Bente Edvardsen (Post Conference Suggestions):
Recommendations for future work and measures:
- Collect all monitoring data that have obtained through the years. Make a database for the data and a comprehensive report.
- Publish paper(s) in international journals on blooms and environmental conditions during the bloom (including algal concentrations, chemical and physical conditions, fish kills).
- Plan a thorough monitoring program for affected lakes, reservoirs, and rivers as well as non-affected adjacent lakes for comparison. The parameters that should be considered to be measured are:

  Physical parameters (measurement in each meter): water temperature, conductivity, salinity/chlorine concentrations, oxygen, and irradiance in water and at surface

  Chemical parameters: pH, dissolved reactive nitrogen/phosphorus/silicate, particulate nitrogen and phosphorus, total nitrogen and phosphorus, total organic carbon, chlorophyll a, calcium ions, and magnesium ions

  Biological parameters: concentration of *P. parvum*, total phytoplankton counts, and bio-volume.

- Design a sampling program with different stations and depths. Sampling should be done all year (around every 14-30 days or more often during a bloom event).
- Get funding for monitoring. Possible sources include the following:
  National funding for research (ex: National Science Foundation)

  State funding for research and training

  State and county funding for monitoring

  Private companies such as hydroelectric power plant companies in affected areas

  Hatcheries and fisheries companies for monitoring

  Water works (drinking water) for monitoring of water quality (Regulation of rivers in reservoirs and increased tapping of ground water are probable causes for increased salinity in the water, and if so, are partly responsible for the blooms of *P. parvum*.)
- Start training staff in limnological and phycological methods (sampling, field measurements, microscopy, chemical analyses, etc.). Alternatively, hire people with expertise in limnology or hydrology.
- Start monitoring.
- Use the results from monitoring to establish an early warning system to inform the public through a web page, reports, and a telephone service (automatic information for different areas).
- Use results in research to improve the understanding of the environmental conditions for bloom formation, toxin production, and fish kills (e.g. the nutrient concentrations causing blooms), as well as the *P. parvum* concentrations causing fish kills.
- Consider appropriate measures to reduce problems in the future. If salinity is important, can it (e.g. in some fish ponds) be reduced? If nutrient concentrations are exceptionally high, consider replacing private leaking septic tanks with waste water treatment plants. If flow in reservoirs is important, consider changing the water flow regime.
- Consider more direct measures such as additions of barley, Christmas-trees, clay, or fertilizers to fish ponds. These measures should not be considered for reservoirs or lakes though, since the cure could be worse than the cause.
- Scientists and Texas Park and Wildlife Department staff should aim to establish collaborative research.

Gaps in knowledge (Examples of future research topics):

- Genetics and phylogeny: Is *P. parvum* in Texas native or has it been introduced? Does *P. parvum* in Texas represent one homogenous population or several populations?
- Life cycle: Are benthic cysts important for bloom formation? Under which conditions are cysts formed, and under which conditions do they hatch? Does sexual reproduction occur in these systems, and what is the significance of this in the life cycle of *P. parvum*?
- Autecology: Which conditions are optimal for growth? What are the conditions where *P. parvum* cannot grow?
- Nutrition: What are the nutrient requirements for toxic *P. parvum* blooms? What is the limiting nutrient for growth of *P. parvum*? Can the biomass of *P. parvum* be reduced by lowering the nutrient levels in reservoirs?
- Toxic activity: Which environmental conditions promote toxin production or activate the toxin(s) and which reduce toxin production or activation?

- Toxins: Which of the toxins is responsible for the each of the different effects observed? How can the toxins be detected and quantified in an effective way?
- Effects on natural systems and other organisms in the system: What are the effects of *P. parvum* blooms on the ecosystem? What effect does high fish biomass have on the formation of toxic *P. parvum* blooms?

Additional Panel Questions and Discussion (Grouped by topic):

Additional Data Needs and Suggestions:

What future data collections (presently planned or new) would help fill in information gaps?

Both a remote sensing approach and a hand-held diagnostic unit could help follow populations of *P. parvum*.

- Can we identify the forms of data that are routinely collected by managers? Make this a 2-way dialog with researchers.
- Edvardsen: Continue a thorough monitoring program for bloom formation and toxic production. More insight on the chemistry, physics, and phytoplankton production will shed light on the causes and effects of blooms. This understanding will enable us to better warn the public when blooms may be imminent and confirm when it is safe to fish and eat fish.
- Monitoring will help to understand what is happening with the dynamics. In-depth and closer sampling of bloom characteristics is needed to determine potential cause and effect relationships. Ultimately, this should lead us to predictive strategies. A comprehensive database will be needed for effective partnerships.
- Understanding the spatial and temporal context for *P. parvum* blooms is crucial. We must have a better understanding if this a new organism or an existing one. Long-term longitudinal sampling is recommended.
- A more systematic approach to the study of blooms would be useful.
- Investigate the effect of residence time in situations where water flow rates can be altered.
- The Chinese are examining shrimp culture ponds. Growers there use very shallow areas that are drained semi-annually. This may represent a situation for expanding our knowledge and understanding.
- Graneli: Adding the missing nutrient may reduce toxicity.
- Some phytoplankton experts have been slow to embrace the idea and explore evidence that chemical signaling may trigger some response in density.

In terms of modeling, do you mean statistical or numerical modeling?

Anderson: Numerical modeling coupled with physical and biological models will offer the greatest insight. Modeling can help identify the minimum set of parameters necessary.

Audience Comment: Modeling an autotrope is tough. A mixotrope is much more difficult, several magnitudes of difficulty higher.

What about impacts to other species? Will we potentially observe impacts on a broader range of endangered and other species?

We have some figures on recreational fishing, but *P. parvum* is nonspecific in impact. There are probably impacts we are not detecting or monitoring.

What are your recommendations for immediate control of the status quo?

Massive fish kills excite the general public. We need recommendations and guidance for addressing current problems.

Research and monitoring are expensive; strategies will need to be developed. We will explore the Florida example on Saturday.

Clay Flocculation

Are there clay flocculation studies on small water bodies with low flow?

Anderson: In Korea, this material was carried off. In contained systems, it may accumulate. The keys are using the correct proportions of flocculent and applying it properly. The amounts required should be relatively small, and application should not have to be done often.

Flocculation may cause an accumulation of algae if it doesn't kill it. Is this a problem?

Anderson: If the clay amount is correct, the algae will be killed. Selecting the correct clay and/or other material helps.

Cysts

Why not begin with sediment cores from the oldest known bloom sites and examine cysts from these cores?

We do not know what the cysts look like; they are difficult to identify at sites. This appears to be a good idea on the surface, and it might work if the sample is anoxic.

How can we adapt methodology such as ultrasound for examining living cysts in natural sediments?

This is really tough because they are small, and we do not know what other problems, if any, ultrasound will cause. Cysts are tough to form in the lab for a variety of reasons.

Efforts in Norway

Is Norway analogous to Korea in terms of effects on long-term aquaculture? Is Norway examining control measures for *P. parvum*?

Edvardsen: Blooms occurred between 1989 and 1995. During this time, fish cages were moved to areas of greater salinity to preserve them. These efforts were largely successful. What was learned in the fiord systems was that hydropower plants only released water briefly in the summer time. *P. parvum* grew during these periods, and greater water releases flushed the organisms out of the system. Water residence time in the system was found to be an important variable.

Saturday October 25, 2003 Subgroup Recommendations:
Subgroups met to consider 5 separate questions and to recommend actions.

1. What research protocols do you believe would provide the greatest "bang for the buck?" (Include ideas on modeling here.)

Actions:

- Recognize that much general research and knowledge is still needed.
- Collect monitoring data, including full cycle of blooms (beginning to end).
- Establish more standardized protocols for effective data analysis and comparison.
- Develop additional information on numbers, affected species, and range.
- Establish if there is an association with introduced species in Texas.
- Investigate allelopathy events during and after the event.
- Increase sampling frequency to the maximum feasible levels (weekly/biweekly).
- Establish how many strains are loose in Texas and if they are the same or related.
- Dovetail lab input with monitoring efforts more closely.
- Determine which methods work and which environments are needed by conducting trials on small scales.
- Facilitate research that enables us to attack the source(s) of the problem, especially nutrients.
- Consider variables in sedimentation as contributory factors.
- Increase work on experimental exposures and toxin study.
- Build a model for the mixotrophy/phagotrophy of the event; do more than cell counts.
- Establish a database the HAB community can contribute to and utilize.
- Encourage greater coordination of agency and academic efforts.
- Establish and publish the current "knowns" NOW.

Specific Research or Data:

- Sampling of community structures: zooplankton, phytoplankton, and bacterial
- Irradiance profiles
- Sediment profiles for persistence
- Nitrogen, Phosphorus, available CO_2 and O_2 concentrations, pH, Biochemical Oxygen Demand, Chemical Oxygen Demand
- Silicates, Cobalt, B12
- Specific ions and Conductivity
- Data management with Geographic Information System (GIS) databases are needed for georeference.

Discussion

- Groups in the Pacific Northwest have been successful changing flows. Explore similarities here.
- Integration of state databases will have to be considered. (Look at Florida's experience.) This is a major undertaking, but ultimately it can yield huge results. Get started now with planning for data management wants and needs. (TPWD is developing the Resource Information System to assist in this.)
- Coordinate efforts between groups such as Texas Commission on Environmental Quality and the Texas Clean Rivers Program. Coordinate monitoring meetings. Texas Parks and Wildlife Department should be participating in these.

- Rapid progress is made where there is a chemical/analytical facility that can deliver quick turnarounds.
- Establish foundation techniques for the chemical identification and study of toxins. Closer is better.

2. From a research perspective, what work must be done to provide practical solutions to *P. parvum* challenges?

- Acquire hand-held detection devices for cells and toxins ASAP.
- Perform toxin identification and classification of toxin dynamics in the field.
- Focus comprehensive monitoring on a specific bloom from start to finish with daily sampling.
- Plan mitigation with strategies, risk assessments, and flow charts of roles and responsibilities.
- Discover and implement short-term mitigation controls in aquaculture and natural systems.
- Identify control mechanisms.
- Determine long-term bloom causes such as nitrogen/phosphorus ratios and loading conditions; work towards prevention.
- Analyze historical blooms; include data-mining of bloom and non-bloom areas.
- Compare meteorological data and long-term relationships.

3. What are the top 5 tools or research findings that managers need to address the *P. parvum* problem? (Not funding)

Tools:

- An easy-to-use hand-held detection device for early warning
- Predictive models for blooms defining why blooms start and what changes in a system
- Tools and techniques that predict conditions for possible future blooms
- Statewide coordination of water quality data
- Full understanding of the range of HAB parameters that must be monitored

Actions:

- Identify statewide data distributions.
- Determine if there are meaningful controls to stop or contain the spread of HABs.
- Develop cost-effective, viable counter-measures and tools to combat and prevent blooms.
- Determine if reasonable controls on septic tanks are likely to help.
- Establish fish refugia sites during stress and HABs.
- Probe for *P. parvum*'s weakest link.
- Better understand and communicate the total economic impact of blooms and economically viable treatment alternatives.
- Focus on developing an understanding of HAB origins and triggers.
- Understand water system operations and the implications of changes such as artificial flush reductions; all changes have political dimensions that have to be considered.
- Maintain a long-term goal to prevent future blooms; avoid "Band-Aid" solutions.

4. Identify funding strategies, sources, and potential partners for *P. parvum* research

- Understand that partnerships come in several levels, including affected stakeholders (anglers, lakeshore residents, guides, businesses, etc. These will comprise the main support group for solutions.), state HAB partners (It will be essential to speak with the same voice and share resources effectively.), federal partners, university partners, and others.
- Include international partners; the problem is bigger than Texas. Efforts between the National Science Foundation and the European Community on HABs should be continued. Remain engaged.
- Clearly identify the problem in terms all stakeholders can comprehend. Economic impacts will need to be at the top of everyone's list.
- TCEQ (Texas Commission on Environmental Quality) is working toward long-term monitoring on the Pecos River and other locations. More cooperation is needed and planned.
- USDA South Texas Agricultural Research Laboratory in Weslaco has an aircraft which may be available for bloom monitoring.
- Articulate a plan for engaging partners to work together.
- Barley bales, hay bales, and Christmas trees all offer opportunities for experiments with partners and constituents. The worst thing that can happen in these experiments may be that we add some new fish habitat! Results here will generate positive momentum and give us tangible results to show for our efforts.
- Identify and leverage River Watch partners and other volunteer groups. Provide them with tools and techniques to assist in monitoring and data collection.
- Use Texas Parks and Wildlife Department resources wisely. Use the funding review process to select the best of the best from proposal alternatives.
- Raise the level of funding by encouraging other partners to contribute resources.

5. Identify messages that must be communicated to the public, the government, and the press on the "knowns" of *P. parvum*

- Develop a focused, consistent message that many can deliver and reinforce.
- Communicate that this is NOT a public health threat.
- Be willing to state what is Not known.
- Deliver messages in terms of animal and pet health.
- Deliver messages rapidly; provide as much information as possible immediately.
- Always be proactive: get messages out earlier rather than waiting for complete or perfect information.
- Include small community papers in the communication strategy.
- Anticipate distrust and skepticism from the general public.
- Emphasize that *P. parvum* has been around for a long period of time.
- Discuss where it exists without causing kills or other problems.
- Communicate that the impacts of *P. parvum* may be severe, but they are temporary.
- Include a "Top 10" list of Frequently Asked Questions.
- Deliver messages in terms of economic impact, especially the potential number of lost jobs in Texas or the affected areas.
- Be up-front about uncertainty; we have a lot to learn.

- Maintain a Golden Alga web site. (This offers interactivity for researchers and the public.)
- Offer "after-hour" points of contact for the media and citizens.
- Emphasize that the problem and solutions are complex; learning is continuous.
- Communicate how we will address causes of blooms and sources of environmental problems.
- Enlist the support of all Texans in solving the problem.
- Frame the issue in terms of health of the reservoir (ex: usually all the fish in the reservoir were not lost).
- Highlight what is still there.
- Leverage California's invasive species example: what it is, what it does, what we know. Use this at boat ramps.
- Communicate the results of today's session to the press. State results and communicate regularly.

List of Research Needs for the Study of *Prymnesium parvum* in Texas

The list below was developed from the Golden Alga Workshop recommendations and was reordered by the TPWD Golden Alga Task Force based on priority criteria and funding feasibility. This list will be used in determining proposals to be funded by TPWD and for seeking additional funding and cooperative studies. Procedures for applying for research funding from TPWD will be listed in the golden alga website portion of the TPWD website (www.tpwd.state.tx.us).

Research Needing Funding:

- **Develop cost-effective, viable mitigation for bloom and toxin treatment in large natural ecosystems.** A current list of treatment alternatives includes the following:
    a. Use of decomposing barley straw to limit or prevent cell proliferation
    b. Use of Christmas trees to limit or prevent cell proliferation
    c. Use of clay flocculation in certain locations
    d. Comparative advantages of PAC or other flocculant application
    e. Biological control options (including *Kathablepharis*)
    f. Ultrasonics, ozonation, and chemical flocculation
    g. "Last resort" chemical control alternatives (may be appropriate in specific situations where severe lethality and other environmental concerns take a back seat)
    h. Water system operation and potential alternatives for river flow alteration (Consider political dimensions.)

- **Monitor affected systems for specific blooms** (start to finish); include sampling during non-bloom times for baseline data. (Some parameters include physical data such as temperature, salinity, alkalinity, light, turbidity, flow levels; chemical data such as dissolved oxygen, dissolved and particulate nitrogen, phosphorus, and carbon; and biological data such as phytoplankton composition and concentrations.) A Rapid Response plan should be developed.

- **Research golden alga bloom causes**, particularly related to nitrogen and phosphorus ratios and nutrient loading conditions. Explore if reasonable controls on septic tanks are likely to help or not; include cost/benefit analysis. Establish if there is an association of golden alga with introduced species in Texas.

- **Explore bloom triggers and ending points for *P. parvum***; understand the importance of grazing and nutrient limitation (including carbon dioxide availability) for *P. parvum* growth.

- **Investigate the conditions and triggers necessary for toxin production and fish kills**; include the roles/dependencies of obligate needs for a dark cycle, nutrients and their interactions, conflicting temperature/salinity evidence, fish-stimulated production, and *P. parvum* densities.

- **Develop predictive models, tools and techniques** to recognize conditions supporting future blooms and prevent them. (Models will also spotlight gaps in knowledge to direct future research.)

- **Develop hand-held field tools to identify alga and toxins.** (Possibilities include electrochemical detection of toxic algae, rapid detection rRNA probes for toxic algae,

Chem*ScanRDI* (a laser based system to quickly analyze FISH-experiments), DNA-microarrays for monitoring phytoplankton composition, and oligonucleotide probes.)

- **Establish and communicate reliable economic statistics for golden alga impact and remediation.**
- **Communicate and engage stakeholders about golden alga issues in Texas**; include a public outreach strategy.
- **Study and understand the "lethal cocktail" of toxins present from *P. parvum*** and the dynamics of toxin production in different systems. These would include hemolysins, neurotoxins, fast-acting ichthyotoxins (cycloamines), reactive oxygen species H_2O_2, O_2^- and OH^-, DMSP, and toxic fatty acids; study spatial and temporal variations in toxin levels.
- **Study and communicate the strengths and weaknesses of current mitigation strategies in hatcheries and contained systems**; explore other mitigation strategies in these systems.
- **Identify and study the resting stage of *P. parvum*, including distribution in Texas.**
- **Investigate methods and procedures to prevent the spread of golden alga** to an uninfected water body; include potential transfers via boats.
- **Coordinate water quality and other data statewide**.

Research Presently Funded:

The following initiatives were identified at the workshop and are included in an already-funded State Wildlife Grant to TPWD for golden alga issues. While additional funding may be needed in the future, these actions are already being undertaken.

- **Conduct a historical analysis and data mining of bloom and non-bloom areas**; include meteorological data and spatial and temporal variations in toxicity. Data management with Geographic Information System (GIS) databases are needed for georeference.
- **Develop information on distribution of golden alga in Texas rivers and reservoirs.**
- **Host a scientific workshop to bring together professionals interested in golden alga in Texas**. Create a public and professional website to enhance communication.
- **Explore the genetic strain of Texas golden alga and compare to worldwide golden alga.** Establish how many strains are in Texas and if they are the same or related.

Golden Alga Workshop Summary

The Golden Alga Workshop was a groundbreaking effort to bring researchers and practitioners together to chart a course of action for understanding and combating golden alga (*Prymnesium parvum*) blooms. This document includes workshop highlights, observations, and potential recommendations, summarizes the output of the workshop planning process, and outlines a framework for moving forward. The Texas Parks and Wildlife Department (TPWD) Golden Alga Task Force will take the results and recommendations from the workshop to help develop priorities and action plans for addressing golden alga issues and funding efforts.

The workshop facilitators (Group Solutions) drafted a summary of the results from the Golden Alga Workshop for the TPWD Golden Alga Task Force to review and revise for a basis for moving efforts along to address golden alga issues. Several facilitated discussion groups were held for identified stakeholder groups. The following lists were compiled from the results of those group discussions. This first step has been taken to create a living document that incorporates discussion group output and recommendations from the workshop's sessions.

Develop A Statement On Research Direction.

The research community and front-line managers clearly stated what they believe needs to be done. Future research and field work should be targeted for developing golden alga management strategies. Proposed research should be examined using the following criteria. If it does not directly contribute to one of the criteria, it may not represent an activity that is addressing identified needs.

- Predicting future golden alga blooms
- Preventing golden alga blooms and their dispersal
- Treating and mitigating blooms in natural systems with approved techniques
- Containing the release of toxins when blooms occur
- Protecting hatchery stocks

Create An Action Plan With Defined Roles And Responsibilities.

The most effective role for TPWD will be that of a facilitator for gathering and focusing a broad range of state, national, and international stakeholders to address this issue…not that of the unilateral problem solver. Multiple stakeholders will have roles to play in addressing and advancing the knowledge on this issue. TPWD should focus on increasing the size and scope of the dialog and the problem-solvers working toward a solution.

Identify Collaborative Research Priorities.

Initial research priorities were jointly developed by teams of scientists and front line managers. These priorities are not listed in any order of importance but include the following:

Priorities identified by researchers:

- **New field tools are needed to accelerate identification and classification of toxic alga, and toxin dynamics.** Hand-held detection device for cells and/or toxins need to be evaluated for field suitability, cost-effectiveness, and accuracy compared to conventional lab techniques. The ones showing greatest promise are:
  - Electrochemical detection of toxic algae via a hand-held device
  - Rapid Dectection rRNA probes for toxic algae
  - Chem*ScanRDI*, a laser based system to quickly analyze FISH-experiments

- DNA-microarrays for monitoring phytoplankton composition
- Oligonucleotide probes

- **More systematic monitoring is needed that focuses on the complete history of specific blooms start to finish.** Affected water systems should be monitored at least monthly to establish better baseline data. Daily sampling would be preferable for many researchers. While no single partner may be able to handle the entire task, a coalition of volunteers working together can show progress. A preliminary parameter request list includes:
  - Physics (temperature, salinity, alkalinity, light, Secchi depth),
  - Chemistry (dissolved oxygen, dissolved and particulate nitrogen, phosphorus, and carbon)
  - Phytoplankton composition and concentrations

  These will need to be "reality-checked" against the measurements that can reliably be delivered if field volunteers with limited technical experience are used. Some compromises may need to be made.

  Research and data protocols must be established to enable the exchange and correlation of data from independent studies.

- **Historical analysis and data mining of bloom and non-bloom areas will allow for better data and may lead to defensible recommendations.**

- **The strengths and weaknesses of current mitigation strategies must be studied and communicated.** Specific recommendations are needed for hatcheries, contained systems, and large natural ecosystems.

- **Additional research is needed to identify control mechanisms for golden alga blooms. Some prioritization may be required.**

- **Long-term bloom causes and effects related to nitrogen and phosphorus ratios and nutrient loading conditions must be established.** Ultimately, these will lead to prevention strategies. These may lead to difficult discussions in the future on changes in land, water, and agricultural practices. Anticipating and reaching out to willing representatives of these communities may be beneficial. If involved early in the process, the likelihood of achieving future consensus-based solutions may go up.

- **Meteorological data and long-term relationships should be compared.** Data management with Geographic Information System (GIS) databases are needed for georeferencing information gathered.

- **The "lethal cocktail" of toxins released from *P. parvum* and the dynamics of toxin production in different systems needs to be understood.** These include hemolysins, neurotoxins, fast-acting ichthyotoxins (cycloamines), reactive oxygen species (H_2O_2, O_2^-, OH^-, and DMSP), and toxic fatty acids.

- **The conditions necessary for toxin production must be investigated.** These include the roles, dependencies, and need for a dark cycle; nutrients and their interactions; conflicting temperature and salinity evidence; and possible fish-stimulated production.

- **The spatial and temporal variations in toxicity of the golden alga need to be understood.**
- **The importance of grazing and nutrient limitation for controlling *P. parvum* populations must be understood.**
- **Toxin levels as a function of *P. parvum* density, conductivity, and nutrient limitations should be assessed.**
- **Biocontrol of *Prymnesium* should be investigated.**
- **Carbon dioxide availability as a limiting factor in *Prymnesium* growth needs to be investigated.**

Priorities identified by front-line managers:

- **Predictive models, tools, and techniques that enable reliable recognition of the conditions that can support future blooms must be developed in order to prevent them.** Focus on golden alga origins and triggers, specifically the alteration of nutrient inputs and potential means of dispersal. Being able to predict and act on emerging problems is the single most important issue to those who interact with the public.

  These models will be used to spotlight information gaps that will help direct future research on preventing or reducing the severity of blooms and to fully understand the full range of golden alga parameters that require monitoring.
- **Controls to prevent and contain the spread of golden alga blooms must be evaluated.**
- **The number and relationship of strains in Texas must be established.** Additional information on numbers, affected species, and range must be developed.
- **Associations, if any, with introduced species in Texas need to be established.**
- **Cost-effective, viable mitigation for bloom treatments must be developed.** The logistical and economic implications of each treatment alternative and parameters for appropriate use must be defined. A current list of treatment alternatives includes the following:
  - Use decomposing barley straw to limit or prevent cell proliferation.
  - Explore the use of clay flocculation for in certain locations.
  - Investigate comparative advantages of PAC (or other flocculants) application with clays.
  - Evaluate and explore biological control options.
  - Determine if ultrasonics, ozonation, and chemical flocculation offer promise.
  - Determine what "last resort" chemical control alternatives may be appropriate in specific situations where severe lethality and other environmental concerns take a back seat.
- **Managers want an easy-to-use hand-held detection device for cost-effective early warning in the field.**
- **Statewide coordination of water quality data must be provided.**

- **Reliable economic statistics for golden alga bloom impacts and remediation must be established and communicated.** Cost/benefit analyses should be established to definitively state if reasonable controls (e.g., septic tanks, etc.) are likely to help or not.

- **Water system operation and potential alternatives for river flow alteration should be explored.** All have political dimensions that have to be considered.

Secure Needed Funding.

The present level of funding was acknowledged as probably not being sufficient to address all the issues and needs raised at the workshop. The need for continued funding was expressed. Potential components of a funding strategy were identified by a discussion group and include the following:

- Clearly identify the problem in terms all stakeholders can comprehend.
- For interagency harmful algal bloom partners, speak with the same voice, deliver consistent messages on impact of the harmful algal bloom (HAB) challenge and share resources effectively. This will be essential.
- Define baseline assumptions surrounding economic impact and a series of common yardsticks. Validate immediately with potential partners and stakeholders.
- Align potential strategies with appropriate partnerships of affected stakeholders (anglers/lakeshore residents/guides/businesses). These will comprise the main support group for solutions.
- Develop a list of potential resource agencies including state/federal HAB partners. Specific agencies that were mentioned included the Texas Commission on Environmental Quality (TCEQ) that is working on long-term monitoring of the Pecos River and other locations. More cooperation is needed and planned with this agency. The USDA South Texas Agricultural Research Laboratory in Weslaco may have aircraft available for bloom monitoring that can be contributed and leveraged.
- (TPWD) Help coordinate monitoring meetings between TCEQ and Texas Clean Rivers Program partners.
- Include international partners; the problem is bigger than Texas. Efforts between the National Science Foundation and the European Community on HABs should be continued. Remain engaged on this front.
- Immediately assemble a "coalition of the willing" for rapid response. Barley straw bales, hay bales, and Christmas trees all offer opportunities for experiments with partners/constituents. The worst thing that can happen in these experiments may be adding some new fish habitat. Results may generate momentum, establish grass-roots support, and establish tangible results.
- Identify River Watch partners and other volunteer groups that can assist with data collection. Provide them with tools and techniques to assist in monitoring and data collection.
- Use TPWD resources wisely. Use a review process to select the best of the best from proposal alternatives.
- Raise the level of funding as other partners contribute resources.

Funds are limited and it will be critical to award research funds to those that can generate tangible results. Invest in proposals that can produce the greatest short-term

"bang for the buck." Use these results to build momentum and establish credibility that a successful plan is being built.

One potential strategy for screening research requests would be to use a simple matrix scoring potential research and action using 1 to 10 criteria. A starting point for this evaluation list might include the following:

- How well does it address a specific priority identified by the task force?
- What is the likelihood of tangible results within 12 months?
- What is the Cost Effectiveness?
- To what extent is new ground being broken?
- To what extent can TPWD funds be used as "seed money" to attract additional resources?

Define Roles And Responsibilities For Communication And Coordination.

Central to the success of this effort is establishing consistent messages that can be agreed to by key stakeholders and reinforced by repetition. Specific distribution lists have been requested for statewide data distribution. Additional distributions may be required for regional, national, and international partners.

Create A Public Outreach Plan and Communication Strategy.

Important first steps have been taken to establish a comprehensive website that will become the central source of information for citizens and stakeholders. The website may prove useful as a central "data portal", providing scientists and other interested people an entry point to all research and data. There appears to be a site already available through the TCEQ that can be linked to for historical water quality data in many water bodies.

The discussion group identified a preliminary list of communication messages, strategies, and guidance:

- Golden alga blooms are NOT a public health threat.
- HABs and the golden alga have been around for a long time. Their impacts may be severe, but they are temporary.
- Typically golden alga blooms seldom kill all the fish any single reservoir.
- This is a big problem affecting millions of anglers and businesses.
- Blooms are bad news if you have gills, but don't physically affect animals or people.
- There is no silver bullet; solutions will require time, resources, and patience.
- The knowns are…
- Research is needed to better understand….
- The golden alga can exist without causing kills or other problems.
- The problems and solutions are complex; learning is continuous, and we have a lot to learn.
- Communicate the root CAUSES of blooms that can be addressed through their sources.
- Develop a "Top 10" list of Frequently Asked Questions for the website.
- Maintain a golden alga website to offer interactivity for researchers and the public.
- Provide as much information as possible immediately, but make it "scannable". Minimize the number of clicks to get to desired information.
- Offer "after-hours" points of contact for the media and citizens.
- Develop a focused, consistent message that everyone can deliver and reinforce.

- Be proactive; get messages out earlier rather than waiting for complete or perfect information.
- Frame needed messages in terms of possible animal (and pet) health issues.
- Frame messages in terms of economic impact, especially the potential number of lost jobs in the affected counties or regions.
- Anticipate distrust and skepticism from the general public.
- Enlist the support of all Texans in solving the problem.
- Include small community papers in the communication strategy.
- Use California's invasive species pamphlet as an example: what it is, what it does, what we know. Use at Boat ramps. Be concise. Be simple - just the facts.
- Communicate the results of today's session to the press. State results and communicate regularly.

Guiding Principles

To supplement the action plan, several important guiding principles may be useful to keep in mind when choosing between the many action alternatives with which the Golden Alga Task Force will be faced.

- Do not duplicate the successful work of others. Global partners in Israel, Korea, Scandinavia and Florida have a wealth of experience that can be leveraged. Related lessons learned from red tide and brown tide can be applied in the development of "knowns" and best practices.

- *"How can we help <u>you</u> solve this problem?"* should become a mantra for the Golden Alga Task Force. TPWD needs to define its role as facilitator, organizer, and flight controller of larger issues. Making this a "big tent" that accommodates local, state, federal, and international partners will help.

- Well-crafted plans of attack that are well-understood and widely supported get funded.

- Stretch conventional definitions. Some creativity may be useful in making funding efforts successful. Consider creative definitions of the problem when applying for partner funds. There may be linkages with invasive species, homeland security, or public health work that could prove useful. A little might be able to go a long way.

Golden Alga Task Force Members

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Golden Alga Workshop Attendees, continued

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Jerry	Guajardo	Lower Colorado River Authority	Austin, TX
Gayle	Haecker	Brazos River Authority	Waco, TX
Dee	Halliburton	Texas Parks & Wildlife Dept	Austin, TX
Pamela	Hamlett	Texas Parks & Wildlife Dept	San Marcos, TX
Scott	Haney	Texas Parks & Wildlife Dept	Ft Worth, TX
Paula	Hawkins	Texas Parks & Wildlife Dept	Austin, TX
Dean	Heffner	Possum Kingdom Guide	Graford, TX
Vern	Herr	Group Solutions / Facilitators	Alpharetta, GA
David	Herrin	University of Texas	Austin, TX
Larry	Hodge	Texas Parks & Wildlife Dept	Athens, TX
David	Holub	Red River Authority	Wichita Falls, TX
Mark	Howell	Texas Parks & Wildlife Dept	Wichita Falls, TX
Bob	Howells	Texas Parks & Wildlife Dept	Ingram, TX
Tom	Hungerford	Texas Parks & Wildlife Dept	Ft Worth, TX
Bruce	Hysmith	Texas Parks & Wildlife Dept	Pottsboro, TX
Jake	Isaac	Texas Parks & Wildlife Dept	Graford, TX
Marc	Jackson	Inks Lake Federal Fish Hatchery	Burnet, TX
Mike	Johnson	Nikon Instruments	Smithville, TX
Jim	Keffer	Texas House of Representatives	Austin, TX
Richard	Kiesling	U.S. Geological Survey	Austin, TX
Harry	Knight	Applied Biochemists	Cullman, AL
Christine	Kolbe	Texas Comm. on Environmental Quality	Austin, TX
Keith	Koupal	Nebraska Game and Parks Commission	Kearney, NE
Paul	Kugrens	Dept of Biology Colorado State University	Ft Collins, CO
Gerald	Kurten	Texas Parks & Wildlife Dept	Athens, TX
John	LaClaire II	University of Texas	Austin, TX
Jan	Landsberg	Florida Marine Research Institute	St Petersburg, FL
Dick	Luebke	Texas Parks & Wildlife Dept	Ingram, TX
Wayne	Lynch	H2O Sales & Service	Boca Raton, FL
Tom	Mabry	University of Texas	Austin, TX
Robert	Mauk	Texas Parks & Wildlife Dept	Wichita Falls, TX
Roger	McCabe	Texas Parks & Wildlife Dept	Waco, TX
Larry	McKinney	Texas Parks & Wildlife Dept	Austin, TX
Linda	Medlin	Alfred Wegener Institute	Bremerhaven, Germany
Lythia	Metzmeier	Texas Comm. on Environmental Quality	Houston, TX
David	Mohr	Nikon Instruments	Smithville, TX
Tiffany	Morgan	Brazos River Authority	Waco, TX
Dan	Mosier	Kansas Dept of Wildlife & Parks	Farlington, KS
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Golden Alga Workshop Attendees, continued

First Name	Last Name	Affiliation	Location
Melissa	Mullins	Texas Parks & Wildlife Dept	Waco, TX
Charlie	Munger	Texas Parks & Wildlife Dept	Canyon, TX
Eduardo	Nunez	Texas Parks & Wildlife Dept	Graford, TX
Anjna	O'Connor	US Army Corps of Engineers	Clifton, TX
Toni	Oldfather	Texas Parks & Wildlife Dept	Austin, TX
Chetta	Owens	Lewisville Aquatic Ecosystem Research Fac	Lewisville, TX
John	Paret	Texas Parks & Wildlife Dept	Graford, TX
Chad	Pernell	Canadian River Municipal Water Authority	Sanford, TX
Kevin	Pope	Texas Tech University	Lubbock, TX
Kip	Portis	Texas Parks & Wildlife Dept	San Marcos, TX
John	Prentice	Texas Parks & Wildlife Dept	Ingram, TX
Bill	Provine	Texas Parks & Wildlife Dept	Austin, TX
Robert	Ramirez	City of Fort Worth	Ft Worth, TX
Daniel	Roelke	Texas A&M University	College Station, TX
David	Rutledge	TXU Energy	Dallas, TX
David	Sager	Texas Parks & Wildlife Dept	Austin, TX
Gary	Saul	Texas Parks & Wildlife Dept	Austin, TX
Mike	Schaub	Environmental Protection Agency - Reg. 6	Dallas, TX
Warren	Schlechte	Texas Parks & Wildlife Dept	Ingram, TX
Liz	Singhurst	Texas Parks & Wildlife Dept	Austin, TX
Mike	Smart	Lewisville Aquatic Ecosystem Research Fac	Lewisville, TX
Dennis	Smith	Texas Parks & Wildlife Dept	Electra, TX
Greg	Southard	Texas Parks & Wildlife Dept	San Marcos, TX
Bob	Spain	Texas Parks & Wildlife Dept	Austin, TX
Gary	Spicer	TXU Energy	Dallas, TX
Karen	Steidinger	FWC / Florida Marine Research Institute	St Petersburg, FL
Vicki	Stokes	City of Fort Worth	Ft Worth, TX
Jeff	Terhune	Auburn University	Auburn, AL
David	Terre	Texas Parks & Wildlife Dept	Tyler, TX
John	Tibbs	Texas Parks & Wildlife Dept	Waco, TX
Sidne	Tiemann	Texas Comm. on Environmental Quality	Austin, TX
Elroy	Timmer	H2O Sales & Service	Boca Raton, FL
Carmelo	Tomas	UNC @Wilmington/Center for Marine Sci.	Wilmington, NC
Jason	Vajnar	Texas Parks & Wildlife Dept	Electra, TX
Brian	Van Zee	Texas Parks & Wildlife Dept	San Angelo, TX
Vernon	Vandiver	Vandiver Consultants Corporation	Gainesville, FL
Tracy	Villareal	University of Texas / Marine Science Inst.	Port Aransas, TX
Deborah	Wade	Texas Parks & Wildlife Dept	Athens, TX
Dan	Warren	Texas Comm. on Environmental Quality	Abilene, TX
Adam	Whisenant	Texas Parks & Wildlife Dept	Tyler, TX
Tom	Wyatt	Texas Parks & Wildlife Dept	San Marcos, TX
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Vendors at the Golden Alga Workshop

Company	Location	Representatives
Applied Biochemists	Cullman, AL	Harry Knight
H2O Sales & Service	Boca Raton, FL	Wayne Lynch
		Elroy Timmer
Natural Golf Solutions		Mancil Davis
Nikon Instruments	Smithville, TX	Mike Johnson
		David Mohr
Nordevco Associates, Ltd.	Winnipeg, Manitoba	Brian Bostic
	Canada	Johan DePrez
		Paul DePrez
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Literature Review of the Microalga *Prymnesium parvum* and its Associated Toxicity

Sean Watson, Texas Parks and Wildlife Department, August 2001

Introduction

Recent large-scale fish kills associated with the golden-alga, *Prymnesium parvum*, have imposed monetary and ecological losses on the state of Texas. This phytoflagellate has been implicated in fish kills around the world since the 1930's (Reichenbach-Klinke 1973). Kills due to *P. parvum* blooms are normally accompanied by water with a golden-yellow coloration that foams in riffles (Rhodes and Hubbs 1992). The factors responsible for the appearance of toxic *P. parvum* blooms have yet to be determined.

The purpose of this paper is to present a review of the work by those around the globe whom have worked with *Prymnesium parvum* in an attempt to better understand the biology and ecology of this organism as well as its associated toxicity. I will concentrate on the relevant biology important in the ecology and identification of this organism, its occurrence, nutritional requirements, factors governing its toxicity, and methods used to control toxic blooms with which it is associated.

Background Biology and Diagnostic Features

Prymnesium parvum is a microalga in the class Prymnesiophyceae, order Prymnesiales and family Prymnesiaceae, and is a common member of the marine phytoplankton (Bold and Wynne 1985, Larsen 1999, Lee 1980). It is a uninucleate, unicellular flagellate with an ellipsoid or narrowly oval cell shape (Lee 1980, Prescott 1968). Green, Hibberd and Pienaar (1982) reported that the cells range from 8-11 micrometers long and 4-6 micrometers wide. The authors also noted that the cells are sometimes slightly compressed with the posterior end rounded or tapered and the anterior end obliquely truncate. An individual *P. parvum* cell has two equal flagella and a well-developed haptonema (Lee 1980). The flagella are used for motility and the haptonema may be involved in attachment and/or phagotrophy (McLaughlin 1958, Prescott 1968). Green, Hibberd and Pienaar (1982) found that the flagella range from 12-15 micrometers long and the flexible, non-coiling haptonema ranges from 3-5 micrometers long. These authors noticed that each cell has body scales of two types found in two layers with scales of the outer layer having narrow inflexed rims and those of the inner layer having wide, strongly inflexed rims. The scales are an important diagnostic feature invaluable in distinguishing *P. parvum* from closely related algal species, and the flagella-to-cell length ratio and the haptonema-to-cell length ratio are also important diagnostic features that aid in identifying this organism, especially when collected in mixed algal blooms (Chang and Ryan 1985).

In *P. parvum*, the nucleus is located centrally between two chloroplasts, one being lateral and the other parietal, that are usually yellow-green to olive in color (Green et al. 1982). Lee (1980) noted that a two-membrane chloroplast endoplasmic reticulum is present with the outer membrane of the chloroplast ER being continuous with the outer membrane of the nuclear envelope**.** The author also found a large Golgi apparatus located at the anterior end of the cell. This single polarized Golgi apparatus is always located between the bases of the two flagella and the nucleus (Bold and Wynne 1985). A

contractile vacuole is also sometimes found at the anterior end of *P. parvum* cells (Lee 1980). The reserve metabolite chrysolaminarin is found in posterior vesicles (Green et al. 1982, Lee 1980). Peripheral muciferous bodies and lipoidal globules may also be present, and the cysts formed by *P. parvum* have been reported as having an oval shape (Green et al. 1982).

Bold and Wynne (1985) described the microalga *P. parvum* as photosynthetic with possible heterotrophic growth (phagotrophy) when cells sink below the euphotic zone. They also found that it is a euryhaline and eurythermal organism tolerating a broad range of salinities and temperatures.

Global Occurrence of Fish Kills

Prymnesium parvum was first identified as the culprit of mass fish mortalities in the brackish waters of Denmark and Holland (McLaughlin 1958, Shilo and Aschner 1953). According to records of these mortalities, thousands of pike, perch, roach, eels, bream, and tench were killed in 1938 in the Ketting Nor off the coast of Jutland, and again in 1939 in the Selso So located on a peninsula of Sjalland Island (Reichenbach-Klinke 1973). In 1947, Israel reported mass mortalities in carp ponds, and it has been a reoccurring problem (Shilo and Shilo 1953). *P. parvum* has been implicated in fish kills in Palestine, in rock pools of Scotland, Germany, Spain, Bulgaria and in South Africa as well (Comin and Ferrer 1978, Dietrich and Hesse 1990, Johnsen and Lein 1989, Linam et al. 1991, Rahat and Jahn 1965, Reichenbach-Klinke 1973).

Bales, Moss, Phillips, Irvine and Stansfield (1993) noted that well-documented accounts of multiple fish mortalities associated with *P. parvum* were recorded in the River Thurne system (Norfolk Broads, England) starting in 1969 and becoming less severe until 1975. They stated that large kills occurred in mid-August 1969 at Horsey Mere and Hickling Broad, in early September at Heigham Sound, Candle Dyke and the River Thurne, and another large kill occurred in April 1970 with smaller kills in 1973 and 1975. The authors believed that *P. parvum* was stimulated by gull-guano from the large number of black-headed gulls (*Larus ridibundus* L.) nesting in the area. They remarked that guanotrophication may have lead to an abundance of *P. parvum* due to supply of associated organic nutrients and noted that a decline in gull numbers was followed by a decline in *P. parvum* numbers. Anecdotal records described brown-colored water (possibly *P. parvum*) associated with fish kills in 1894, 1911, 1914, 1925 (kill comparable to the 1969 kill), 1934, 1954, 1966, and 1967 in this same area (Holdway et al. 1978).

In July-August 1989, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) died in aquaculture enclosures in the Sandsfjord system (southwest Norway) with fewer of the free-living fish in the brackish water fjord system affected (Kaartvedt et al. 1991). From 1989-1996, mixed blooms of *P. parvum* have occurred every summer in the Sandsfjord system (Larsen and Bryant 1998).

Hallegraeff (1992) noted that since the 1970's, *P. parvum* blooms have been related to recurrent fish kills in Vasse-Wonnerup estuary (W. A.) of Australia with kills most common in January-March. The author remarked that these fish kills, like those of the Sandsfjord system in Norway, show that wild fish stocks are less vulnerable to the *P. parvum* toxins than caged fish since they can swim away from toxic areas. Fish kills in

Oued Mellah Reservoir in Morocco occurred in November-December 1998 and again in September-October 1999 (Sabour et al. 2000). Recurrent kills in carp ponds due to *P. parvum* in the People's Republic of China have also been reported since 1963 (Guo et al. 1996).

Occurrence of Fish Kills in Texas

Mass fish mortalities have occurred in Texas in recent history. In October 1982 in the Brazos River Basin, an estimated 2,300 fish were killed in California Creek with the suspected killer being *Prymnesium parvum* (Glass et al. 1991). The first confirmed fish kill due to *P. parvum* occurred in October and November 1985 on the Pecos River with approximately 110,000 fish dying during this time in the stretch of river between Iraan, Pecos Co., to the mouth of Independence Creek (James and De La Cruz 1989, Rhodes and Hubbs 1992). Additional kills occurred in November and December of 1986 where an estimated 500,000 fish died in the same stretch of the Pecos River, and in November and December of 1988, a number of fish kills resulted in more than a million and a half fish dying in the reach of the Pecos River from Malaga, New Mexico to below the town of Imperial in Pecos County, Texas, as well as in the segment between Iraan and Sheffield (James and De La Cruz 1989, Rhodes and Hubbs 1992). In November of 1988, another 48,000 fish were killed in the Paint Creek tributary of the Brazos River, in Throckmorton and Haskell counties near Abilene, Texas (James and De L Cruz 1989). In April 1989, another fish kill on Paint Creek claimed another 15,000 fish, and an estimated 180,000 fish were also killed in August through October 1989 in a stretch of the Colorado River below Spence Reservoir (Glass et al. 1991).

According to a TPW news release, on January 11, 2001, *Prymnesium parvum* blooms were responsible for the death of approximately 175,000 fish in the Brazos River basin at Possum Kingdom Lake, 261,000 fish at Lake Grandbury since January 26, 2001, and this microalga has been implicated in recent kills at Lake Whitney (Cisneros 2001a and Cisneros 2001b). *P. parvum* has also wiped out the striped and hybrid bass production at Dundee State Fish Hatchery near Wichita Falls recently (Lightfoot 2001).

James and De La Cruz (1989) noted that, during the 1986 Pecos River fish kill, cell densities of 150 million cells per liter were recorded. They also reported that all species of fish in the fish kill areas were affected. The authors noted that some of the species of fish affected include *Cyprinus carpio, Etheostoma grahami, Gambusia affinis, Lepisosteus osseus, Micropterus salmoides*, and *Pylodictus olivaris*. They also remarked that the bivalves of the Unionidae family and the Asiatic clam, *Corbicula fluminea*, were also adversely affected. The authors discerned that *C. fluminea* was once common to the Pecos river with densities in the past as high as 100 per square foot, and that no live *C. fluminea* have been observed since the 1985 kill on the Pecos River. The authors remarked that this suggests a recent introduction of *Prymnesium parvum* to the Pecos River, and noted that results from recent data suggests that *P. parvum* is expanding its range in Texas.

Environmental Requirements

Salinity

A study by McLaughlin (1958) showed that optimal NaCl concentrations for the growth of one Scottish and two Israeli strains *P. parvum* occurred at 0.3%-6% with growth possible at 0.1%-10%. Padilla (1970) observed that low salinities (less than 10%) increased the doubling time of *P. parvum* cells and induced high levels of protein and nucleic acid. Paster (1973) noted 0.3%-5% NaCl as optimal for growth of *P. parvum*. *P. parvum* germinated in the low-salinity environment (4-5%) of the fjord branch Hylsfjorden in the Sandsfjord system of southwest Norway (Kaartvedt et al. 1991). A 1993 study reported an optimal salinity range of 8-25% for a *P. parvum* strain from Denmark (Larsen et al. 1993). Larsen and Bryant (1998) reported that the Norwegian, Danish and English *P. parvum* strains they tested grew over a wide range of salinities each with different optimum growth concentrations, and that all three strains survived salinities from 3 to 30 psu (or .3%-3%). These researchers also speculated that discrepancies from earlier studies could have been due to the unknowing use of different strains of *P. parvum*. The water associated with the fish kill in Morocco was characterized by an elevated salinity of 8.6-12.4% (Sabour et al. 2000).

Dickson and Kirst (1987) speculated that the success of *P. parvum* in variable saline environments may be due to its ability to synthesize compatible solutes. In this 1987 study of osmotic adjustment in marine algae, the researchers found that *P. parvum* showed an increase in DMSP (a tertiary sulphonium compound: *B*-dimethylsulphoniopropionate), as compared to other algae in this study, and an increase in the synthesis of an unknown polyol. The authors suggested that the increasing synthesis of these two molecules may aid in osmoregulation. They concluded that the control of compatible solute synthesis by *P. parvum* may give this microalga an advantage in environments with fluctuating salinities.

Temperature

Shilo and Aschner (1953) observed that temperatures greater than 30 C were inhibitory to the growth of *P. parvum*, and 35 C resulted in lysis. The authors also discovered that *P. parvum* cells survive 2 C for many days. In the 1958 study by McLaughlin, it was found that the three strains of *P. parvum* tested (1 Scottish strain and 2 Israeli strains) showed erratic growth above 32 C with death occurring at 34 C. A separate study by Larsen, Eikrem and Paasche (1993) found that the Denmark strain of *P. parvum* used had a growth temperature optimum of 26 C. The authors noted that this same strain was found to be severely limited at 10 C. The Danish, Norwegian and English strains of *P. parvum* tested by Larsen and Bryant (1998) exhibited a maximum growth rate at 15 C with two of the strains (Norwegian and Danish) tolerating a wide temperature range of 5 C to 30 C. The authors noted that this finding supports the notion that *P. parvum* is a eurythermal organism. The *P. parvum* outbreak in Morocco was characterized by water with moderate temperatures between 15C-23.5C (Sabour et al. 2000).

pH

McLaughlin (1958) found that the success of *P. parvum* growth below pH 7 depended on the adjustment of concentrations of metal ions. The author discovered that

the metal ions Fe, Zn, Mo, Cu or Co, with an increase in the concentration of any of these, resulted in increased growth with the adjustment of Fe concentrations determined to be the most important. The author also noted that, for the three strains tested, growth below pH 5.8 was erratic, and all cells remained viable to pH 5. The *P. parvum* outbreak in Morocco occurred in water with a pH of 7.67-9.04 (Sabour et al. 2000).

Light

Wynne and Rhee (1988) noticed that, in *P. parvum*, the activity of alkaline phosphatase is higher at saturation light intensities. The authors also noted that an increase in light intensities allows *P. parvum* to increase the speed at which it is able to take up phosphate from its environment, and it therefore seems that changes in light intensities have a profound affect on competition. However, it has been found that excessive illumination inhibits the growth of *P. parvum* (Padan et al. 1967).

Growth in the Dark

Rahat and Jahn (1965) discovered that heterotrophic growth of *P. parvum* is possible in the dark with high concentrations of glycerol available. They noted that the optimal concentration of glycerol was found to be lower in the light than in the dark. Chisholm and Brand (1981) found that *P. parvum* divided primarily in the dark period (L:D 14:10), and that this division is phased (synchronized) by the light/dark cycle. Jochem (1999) tested the dark survival strategies of *P. parvum*, and determined that *P. parvum* was a Type II cell when exposed to prolonged darkness (in Type II cells, metabolic activity continues 'as usual' in the dark resulting in a decrease in cell abundance). The author found that the surviving cells needed new energy upon illumination to refill exhausted cellular reserves before the cells could divide, and would therefore not be advantageous in long or short dark periods.

Phosphorous

It is known that phosphate is limiting to phytoplankton growth in the summer (Larsen et al. 1993). McLaughlin (1958) determined that *P. parvum* is able to satisfy its phosphate requirement from a wide range of compounds. The author noted that the three strains of *P. parvum* were indifferent to high or low levels of inorganic phosphate, and speculated that this may be due to the presence of many phosphatases. This obligate phototroph was also found to graze bacteria, especially when phosphate is limiting, and it therefore seems that bacteria may be a source of phosphate for this microalga when phosphate is scarce (Nygaard and Tobiesen 1993).

Nitrogen

McLaughlin (1958) found that ammonia is a good source of nitrogen for *P. parvum* in the acid pH range. The author discovered that in acidic media, ammonium salts, the amino acids aspartic and glutamic acid, alanine, methionine, histidine, proline, glycine, tyrosine, serine, leucine, and isoleucine all can be utilized as a nitrogen source by this organism. In alkaline media, nitrate, creatine, asparagines, arginine, alanine, histidine, methionine and acetyl-urea were found by the author to be good sources of nitrogen. Syrett (1962) reported that *P. parvum* is not able to utilize urea as a nitrogen source. Methionine and ethionine can be utilized as sole nitrogen sources by *P. parvum*, and they are not inhibitory at high concentrations (Rahat and Reich 1963).

Nutrients and Eutrophication

Increases in the concentrations of nitrogen and phosphorous (and other nutrients) in water ultimately leads to eutrophication. The introduction of phosphorous to waterways may be from agricultural runoff (including fish ponds and aquaculture) and domestic sources. Nitrogen also comes from agriculture and is also introduced through airborne nitrogen precipitation from traffic emissions (Finnish Environmental Administration 2001). Holdway , Watson and Moss (1978) noted that there could be a relationship between the degree of eutrophication and population sizes of *P. parvum*. This is likely since eutrophication is known to cause an increase in phytoplankton and algae along with other aquatic plant life (Finnish Environmental Administration 2001).

Holdway, Watson and Moss (1978) noted that, in the Thurne system of Norfolk Broads, England, *P. parvum* competes poorly with *Chlorococcalean*, small cyanophytan and diatoms. They suggested that an increase of phosphorous and nitrogen may ease this competition and allow *P. parvum* to capture available nutrients more quickly. The authors noted that increases in fertility in the Hicking Broad-Horsey Mare-Heigham Sound area of River Thurne system caused eutrophication followed by heavy phytoplankton growth and a decrease in submerged macrophytes in these areas where *P. parvum* blooms occurred (Martham Broad, also in the Thurne system, was noted as having submerged macrophytes and also lower levels of *P. parvum* cells). They believed that the increase in nitrogen in the Thurne system was most probably from agriculture, and phosphorous-loading was likely from a large population of black-headed gulls. The authors remarked that a connection may be seen in the 1938 bloom of *P. parvum* in Ketting Nor, Denmark where it was noted that gulls were polluting the water causing it to turn turbid followed by the disappearance of macrophytes.

Kaartvedt, Johnsen, Aksnes and Lie (1991) noted that currents in the Hylsfjorden branch of the Sandsfjorden system, Norway, were weak which led to a long residence time of the brackish water in this fjord branch. The authors suggested that this resulted in low advective loss of *P. parvum*, relatively high temperatures and depletion in nitrogen and silica derived from freshwater with the low silicate levels favoring the proliferation of flagellates over diatoms. They speculated that low exchange rates and benthic settlement (*P. parvum* was found associated with the benthic green macroalgae *Cladophora* spp., and on nets of fish farms) of *P. parvum* could have facilitated an increased efficiency in the use of nutrients supplied by fish farms. They noted that the discharge of a hydroelectric power plant just after the first observed fish mortalities caused advection of *P. parvum* and its associated toxin throughout the Sandsfjord system. The authors suggested that subsequent large amounts of additional freshwater runoff from other sources aided in the dispersal of the algae, and may have also played a part in phosphorous limitation since the freshwater input contained low concentrations of phosphates. Overall, the authors concluded that fertilization associated with fish farming seems to have created a favorable environment for a *P. parvum* bloom in the Sandsfjord system. The association of *P. parvum* with *Cladophora* sp. and other macroalgae was tested by Johnsen and Lein (1989) with the conclusion that *P. parvum* is attracted to macroalgae (*P. parvum* grown in nutrient-poor solutions swam toward *Cladophora* sp.). The authors offered the possible explanation that *P. parvum* is chemotactic and may attach to macroalgae (via the haptonema) that give off dissolved organic matter when the concentrations of nutrients in the water are low. They also suggested that microalgae

with the ability to utilize organic matter given off by macroalgae would have a definite advantage over other autotrophic algae.

In the Morocco *P. parvum* bloom, the water was high in organic matter, and characterized by elevated levels of total nitrogen, limited concentrations of nitrates and undetectable amounts of orthophosphates. This eutrophic, phosphorous-limiting environment is believed to have lead to the extensive fish mortalities (Sabour et al. 2000).

Wynne and Rhee (1988) found extracellular alkaline phosphatase activity to be highest in *P. parvum* when compared to the other species of algae tested. These authors discovered that phosphate uptake and enzyme activity increased with an increase in the N:P ratio, and concluded that this would give *P. parvum* a competitive advantage in phosphate-limited environments. They also determined that a decrease in phosphate concentrations were found to cause a disruption in the membrane synthesis of *P. parvum* that may lead to leakage of intercellular molecules including toxins.

Vitamins

Past studies indicate that vitamin B12 and thiamine are absolutely required for the growth of *Prymnesium parvum* (McLaughlin 1958, Shilo and Sarig 1989). Biotin was found not to be necessary for growth (McLaughlin 1958). Droop (1962) noted that thiamine is a component of the enzyme thiamine pyrophosphate (cocarboxylase), but no algae are known to be able to utilize the complete enzyme in place of thiamine as some bacteria do (the enzyme is probably less permeable). He found that *P. parvum* requires the pyrimidine component of thiamine, but does not require the thiazole component of thiamine. The author also noted that pyrimidine-requiring organisms require the molecule some 200 times more than vitamin B12. Another study indicates that with a given concentration of vitamin B12, growth in the light equals growth in the dark, and that this outcome may mean that B12 is not required for the immediate metabolism of the photosynthetic product (Rahat and Jahn 1965). Rahat and Reich (1963) discerned that a small portion of the B12 molecule is utilized in methyl metabolism for methionine or methyl group synthesis, and the rest of vitamin B12 (majority) used in other metabolic processes. They suggested that this may be why there is no sparing of B12 in the presence of methionine. The authors also found that some B12 analogs were found to inhibit the growth of *P. parvum*.

Toxin Characteristics

Structure

The toxin of *Prymnesium parvum* has been found to be composed of a collection of substances and not a single component (Shilo and Sarig 1989). It was noted in one study that the *P. parvum* toxin was proteinaceous, acid-labile, thermostable, and non-dialyzable (Prescott 1968). Padilla (1970) noted the finding by Paster in 1968 that hemolysin, the hemolytic component of the *P. parvum* toxin, is a lipopolysaccharide. Padilla observed glycerol enhancement of hemolysin production, and suggested this shows that synthesis of hemolysin is dependent on carbohydrate and lipid metabolism. It was also implied by the author that hemolysin may be a structural component of *P. parvum* membranes; a notion supported by previous research that gave evidence that toxins of *P. parvum* are a heterogeneous mixture of phosphate-containing proteolipids. Dafni, Ulitzer and Shilo (1972) found a correlation between toxin formation (hemolysin)

and presence of membrane vesicles. The authors noted that the observations of this study and past studies suggest that the *P. parvum* toxin appears in conditions where growth factors are limited and growth is disturbed. Because of this, they hypothesized that the toxin may be a product of imbalanced cell membrane metabolism. In one experiment, hemolysin was separated into six components with the major component, hemolysin I, determined to be a mixture of 1'-*O*-octadecatetraenoyl-3'-*O*-(6-*O*-*B*-D-galactopyranosyl-*B*-D-galactopyranosyl)-glycerol and 1'-*O*-octadecapentaenoyl-3'-*O*-(6-*O*-*B*-D-galactopyranosyl-*B*-D-galactopyranosyl)-glycerol (Kozakai et al. 1982).

Yariv and Hestrin (1961) noted that the *P. parvum* toxin, prymnesin, was soluble in methanol and n-propanol water solvent systems thereby distinguishing the toxin from simple protein and polysaccharides. They ascertained that prymnesin was a lipid with both non-polar and polar moieties. The authors recognized that the observed properties of prymnesin (ichthyotoxicity, hemolytic activity, non-dialysability against water, general solubility features, formation of insoluble inactive complexes with certain alcohols, and capacity to precipitate by Mg hydroxide and ammonium sulphate) were similar to the properties of saponins. However, they also noted that the action of prymnesin requires a cofactor whereas saponins do not require a cofactor. Paster (1973) described prymnesin as a high molecular weight glycolipid with a detergent-like structure. It has also been hypothesized that *P. parvum* toxins are plastid components or that toxin synthesis is partially plastid mediated (Guillard and Keller 1984). The hypothesis that the toxin, a proteophospholipid, is a membrane precursor is supported by the fact that there is a 10-to 20-fold increase in the toxins (ichthyotoxin, hemolysin and cytotoxin) when phosphate is limiting (Shilo and Sarig 1989).

Igarashi, Satake and Yasumoto (1999) have recently reported the structural elucidation (Figure 1) of the *P. parvum* toxin. They found that *P. parvum* produces two glycosidic toxins they named prymnesin-1 ($C_{107}H_{154}Cl_3NO_{44}$) and prymnesin-2 ($C_{96}H_{136}Cl_3NO_{35}$). The authors also concluded that prymnesin-1 and prymnesin-2 have biological activities that are almost the same. They noted that both prymnesin-1 and prymnesin-2 express potent hemolytic activity greater than that of a Merck plant saponin, and that both exhibit ichthyotoxicity.

Target and Action of Toxin

P. parvum produces soluble toxic principles: an ichthyotoxin, hemolysin and cytotoxin (Ulitzer and Shilo 1964). The *P. parvum* ichthyotoxin is toxic to gill-breathing species such as fish, mollusks, arthropods, and to the gill-breathing stage of amphibians (Paster 1973). The ichthyotoxin targets the permeability mechanism of the gill (Yariv and Hestrin 1961). Ulitzer and Shilo (1966) noted that toxicity occurs in two stages. The first stage is reversible damage to the gill tissues (i.e. permeability) that occurs only with a cation synergist and suitable pH. They described the second stage as mortality due to a response to toxicants already present in the water including the *P. parvum* toxin itself.

Dissolved potassium and calcium are necessary for developing extracellular micelles important for toxicity (Glass et al. 1991). The ichthyotoxin (now in micelles) requires activation by cofactors such as calcium, magnesium, streptomycin and sodium. (Shilo and Sarig 1989, Yariv and Hestrin 1961). Ulitzer and Shilo (1964) observed that neomycin, spermine and other polyamines can activate ichthyotoxicity with spermine being the most active. They concluded that, in the presence of more than one cofactor,

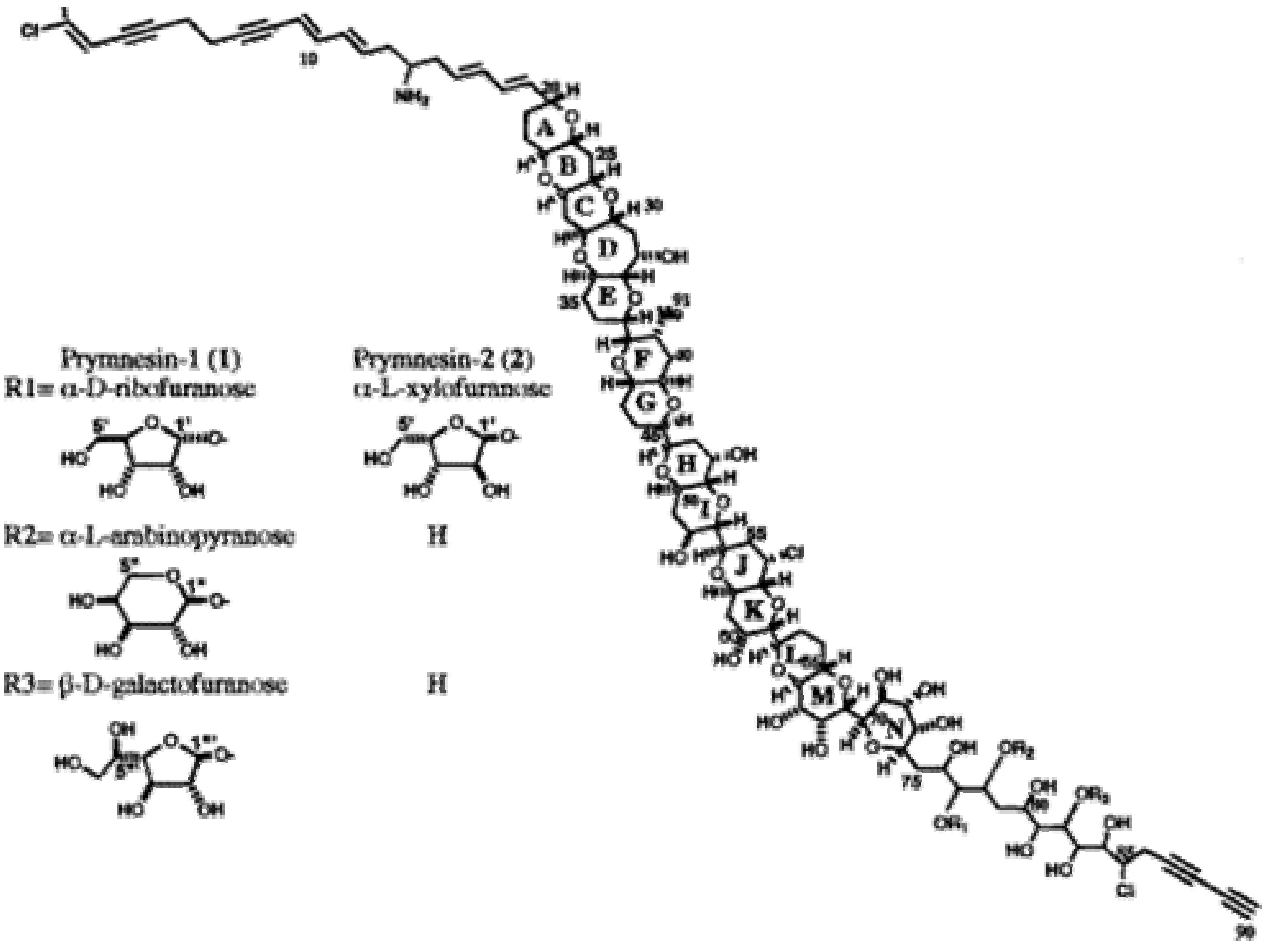

Figure 1. Prymnesin-1 (**1**) and Prymnesin-2 (**2**) structure. Relative stereochemistry is shown for the rings A-N of prymnesin-1 and prymnesin-2 (From Igarashi et al. 1999).

the resulting toxicity was not always additive. Instead, the authors found that the toxicity depends on the specific activity of each cofactor present and their relative concentrations. They also observed that calcium has the ability to mask other cofactors. The authors found that the presence of calcium (low activity) in the presence of a cofactor that normally expresses high toxin activity will cause the activity of the toxin to decrease. Ulitzer and Shilo (1966) discovered that the *P. parvum* ichthyotoxin was also augmented by the cation DADPA (3,3-diaminodipropylamine) in lab with the ichthyotoxin increasing the sensitivity of *Gambusia* to toxicants already present in the media. Padilla and Martin (1973) noted that calcium and streptomycin have proven to be slightly synergistic, neomycin slightly more synergistic, spermine induces a four-fold increase in ichthyotoxicity, and DADPA induces a two-fold increase. They also speculated that the toxin/cation complex could interact with charged groups on toxin molecules reducing the degree of ionization and making them more reactive with the membrane.

Paster (1973) noted that the attachment of prymnesin to gill cell membranes most likely occurs where molecules such as lecithin and cholesterol are found, and attachment

imposes a rearrangement on the membrane making it more permeable. The fact that prymnesin interacts with cholesterol in attack of erythrocyte membranes may support this idea (Padilla and Martin 1973). It has also been speculated that the cofactors may alter the permeability of the gills, thereby increasing the rate of absorption of the toxin into the circulation (Spiegelstein et al. 1969). Increased permeability of the gill membrane imposed by prymnesin causes fish to become more susceptible to compounds in water like CaCl and streptomycin sulphate (Yariv and Hestrin 1961). The increased permeability of the gills may even cause an increased susceptibility to the toxin's cytotoxic and hemolytic activity (Ulitzer and Shilo 1966). Spiegelstein, Reich and Bergmann (1969) used two methods to observe the effects of the ichthyotoxin on *Gambusia*. They found that in the immersion method (fish in an ichthyotoxin solution), the toxicity occurs as follows: the toxin enters the gills (via capillaries), enters the dorsal aortas, and then travels to the brain. The authors noted that in the intraperitoneal injection method, the toxin first enters the circulation where it travels to the liver, then enters the hepatic vein, the heart, the aorta and finally the brain. They recalled that the toxin is acid-labile and suggested that it may be altered (inactivated) in the GI tract and liver. The authors suggested that this could be why the toxin is non-toxic to non-gill breathers, but toxic to gill breathers.

Accumulation

It has been reported that the ichthyotoxin accumulates during the stationary phase of growth, and the hemolytic toxin accumulates during log phase (Padilla 1970). Simonsen and Moestrup (1997) determined that the hemolytic compounds within the *P. parvum* cells are the highest in late exponential growth phase and decreased during stationary phase. The authors then discovered hemolytic activity in the medium during stationary phase.

Population Density

Shilo and Aschner (1953) discovered that fish peptone and egg yolk increases the density of *P. parvum* cultures. The authors ruled out the idea of toxigenic variants due to an observed decrease in toxicity accompanied by an increase in cell proliferation**.** They often observed an inverse relationship between cell count and toxicity. Shilo (1967) also found a lack of correlation between toxicity and cell density. The author hypothesized that the ability to form toxins may be determined by genetic factors. The author based this hypothesis on the knowledge that different strains of *Microcystis aeruginosa* and *Anabena flos-aquae* were shown to differ markedly in their toxin productivity. The author also found non-toxigenic strains of this alga.

Salinity

Reich and Rotberg claimed that the activity of the ichthyotoxin of *P. parvum* is inversely proportional to salt concentrations (Reich and Parnas 1962). Ulitzer and Shilo (1964) also found that a decrease in salinity equals an increase in ichthyotoxicity, and that ichthyotoxicity decreases as salinity increases. In a later study, increased salinity decreased the uptake of trypan blue (i.e. toxicity) in the gills of fish (Ulitzer and Shilo 1966). Paster (1973) observed that a NaCl range of 0.3%-5% was optimal for toxin production in *P. parvum*. However, Larsen and Bryant (1998) noted that variable salinity did not have significant effects on toxicity.

Temperature

Shilo and Ashner (1953) found that at 80 C and 97 C toxicity declined rapidly while 62 C caused a slow decline in toxicity. They also observed that at room temperature and 4 C there was no decrease in toxicity. Yariv and Hestrin (1961) noted that prymnesin solutions in water showed a decrease in titer when kept at 35 C for 60 minutes, but returning the solution to a pH of 4 restored toxin activity. Paster also discerned the thermo-sensitivity of the toxin in 1968 (Stabell et al. 1993). Ulitzer and Shilo (1964) noted that an increase in temperature in the range of 10 C to 30 C caused an increase in the rate of mortality of minnows with the titer of the toxin unaffected. However, Larsen and Bryant (1998) concluded that variable temperatures in the range of 5 C to 30 C do not have significant effects on toxicity thereby contradicting the research by Ulitzer and Shilo in 1964.

Light

Shilo and Aschner (1953) concluded that light augments toxin production. These authors found water containing *P. parvum* to be more toxic in light than in dark. Parnas, Reich and Bergmann (1962) found that the *P. parvum* toxin is sensitive to light. The experiments conducted by these authors revealed that UV causes 100% inactivation of the toxin with the upper limit of inactivation by visible light at 520 nm (50% inactivation). Reich and Parnas (1962) noted that, in their first experiment, ichthyotoxicity decreased gradually with exposure to light. The authors also found that, in the dark, toxicity rises reaching a maximum in 7.5 hours. In the second experiment, the observed similar results: toxicity increased in the dark accompanied by a pH drop in dark to between 7.0 and 7.1. The pH rose in the light to 8.0-8.1 due to, the researchers speculated, photosynthetic activity. They hypothesized that the desistance of ichthyotoxicity was either due to inactivation by light or to the delay of toxin formation due to increased photosynthesis in light.

Rahat and Jahn (1965) observed that dark cultures in their study were more toxic, even with less cells, than light cultures (this agrees with idea that the extracellular toxin is inactivated by light). The authors also concluded that light is not needed to make the *P. parvum* toxin, and that previous assays of the toxin are only the net result of toxin production and inactivation. A study by Padan, Ginzburg and Shilo (1967) showed that the ichthyotoxin and hemolysin are both sensitive to inactivation by light. The authors also concluded that light is needed for the appearance of extracellular hemolysin. They noted that the equilibrium between the appearance of hemolytic activity and inactivation appears to be in favor of toxin accumulation in low light (60 foot candles). Spiegelstein, Reich and Bergmann (1969) determined that the ichthyotoxin is produced in the dark equally well as in the light (light not necessary). Paster (1973) observed inactivation of prymnesin by visible light (400nm-510nm) and UV light (225nm). The toxin of the closely related, flagellated algae, *Phaeocystis pouchetii*, is also believed to be photo-sensitive (Stabell et al. 1999). However, Larsen and Bryant (1998) have concluded that variable salinity, light and temperature do not have significant effects on toxicity. These authors believe that growth phase and nutrient status probably have a greater impact.

pH

Shilo and Aschner (1953) deduced that *P. parvum* toxicity was independent of pH in the range of 7.5-9.0; toxicity decreased rapidly at pH less than 7.5 and was

completely inactive at 6.0. The authors hypothesized that the inactivating effect was due to the hydrogen cation. McLaughlin (1958) concluded that toxicity decreased at pH 6.0-6.5 (or become non-toxic), and toxicity returns when the solution is brought back to a neutral pH. The author also found that acid grown cultures are less toxic than alkaline grown cultures. Ulitzer and Shilo(1964) noted that there is a correlation between elevated pH and toxicity. Ulitzer and Shilo (1966) observed that the gills of *Gambusia* became darkly stained at pH 9, but that no trypan blue staining (i.e. toxicity) was observed at a pH of 7. Padilla (1970) noted an increase in pH caused a decrease in hemolysis (pH range 5.5-8.0). Padilla and Martin (1973) noticed that maximum hemolytic activity occurred at pH 5.5. The authors also found that cytotoxicity is arrested by pH 6.4, and that maximum binding of the toxin was observed in the pH range of 4.6-5.5.

Shilo and Sarig (1989) found that a pH higher than 8 was necessary for cation activation, and a pH of 7 and lower equals little ichthyotoxic activity with the toxicity increasing to a pH of 9. They also determined that, when a cation is complexed with the toxin at high pH, the ichthyotoxicity is expressed even at low pH (6-7).

Phosphate and Nitrogen

Dafni, Ulitzer and Shilo (1972) found that a decrease in phosphate caused an increase in toxicity. The authors speculated that a phosphate-limiting environment could cause a disturbance in the formation of membrane phospholipids that may lead to leakiness (and the toxin escaping). They noted that the cell volume of *P. parvum* increased as the concentration of phosphate decreased, and it was hypothesized that swelling was due to osmotic imbalance (leakiness) or disturbance in regular cell division. Paster (1973) also found *P. parvum* to be more toxic in phosphate-poor media.

Holdway, Watson and Moss (1978) noted that, with substantial concentrations of nitrogen and phosphorous, *P. parvum* will not produce or release toxins. The fish kills in the Sandsfjord system in Norway were determined to be mostly due to phosphorous-limited growth of *P. parvum*; the phosphate-limited environment was considered to be the major factor influencing increased toxicity (Kaartvedt et al. 1991). Larsen, Eikrem and Paasche (1993) found that phosphate-limitation caused an increase in toxicity of a Denmark strain of *P. parvum* in lab. Simonsen and Moestrup (1997) observed an increase in the size of *P. parvum, Chrysochromulina polylepsis, Chrysochromulina hirta,* and *Isochrysis* spp. cells accompanied by increased toxicity when phosphate was limited. They also noted that the dinoflagellate *Alexandrium tamarense* has been noted to show increased toxicity with a decrease in phosphate concentrations. Johansson and Graneli (1999) discerned that nitrogen limitation causes an increase in toxicity, and also found that phosphate limitation causes increased toxicity as well. The authors hypothesized that the N:P ratio could be the governing factor of toxicity in *P. parvum,* and that a change in the N:P ratio by nutrient inputs could lead to toxicity (an unbalanced N:P ratio could result from eutrophication). The authors admitted that the reason for toxin production by *P. parvum* is uncertain, but speculated that the toxin could be produced because of the need to wipeout competition during nutrient limitation. Wynne and Rhee (1986) concluded that changes in the light regime can alter the optimum cellular N:P ratio in *P. parvum* thereby greatly influencing nutrient requirements and species interrelationships.

Glycerol

Glycerol was found to increase the growth rate and toxin synthesis in *P. parvum* (Padilla 1970). Cheng and Antia (1970) found that *P. parvum* is able to metabolize glycerol in high and low concentrations. The authors implied that glycerol pollution may stimulate *P. parvum* thereby causing blooms in light as well as in the absence of light. They found that *P. parvum* responded rapidly to high glycerol concentrations, and appeared to become 'spent out' with rapid cell lysis following an early growth peak.

Toxin Inhibitors

Padilla (1970) noticed that hemolysis is inhibited by high pHs with a maximum toxicity at pH 5.5, 50% at pH 7, and 10% at pH 8. Paster (1973) found that lecithin, cholesterol and cephalin inhibit the hemolytic affect in small quantities, and concluded that these lipid compounds must compete with the toxin for the target site. The author also noted that the bacteria *Proteus vulgaris* and *Bacillus subtilis* decrease the potency of *P. parvum* cultures. Padilla and Martin (1973) inferred that cholesterol, cephalin and the *Gymnodinium breve* toxin exert a protective influence. It has also been observed that NaCl inhibits *P. parvum* toxin activity (Shilo and Sarig 1989).

Successful and Possible Control Methods

Moshe Shilo and Miriam Shilo (1953) noted that ammonium sulphate has a lytic effect on *P. parvum*. They found lytic activity to be a function of temperature in the range of 2 C-30 C with lytic activity increasing as temperature increases. The authors also found that lytic activity decreases dramatically when temperatures below 10 C are reached. The authors also discovered that the lytic activity of ammonium sulphate was a function of pH in the pH range of 6.5-9.5 with activity increasing as pH increases. They suggested that this shows that free ammonia, not the ammonium ion, is responsible for lysis. The addition of ammonia to water for control of *P. parvum* is also effective (Glass et al. 1991). The addition of ammonium sulphate or ammonia to contaminated water controls *P. parvum* in the following manner: trapping and concentration of the protonated ammonia ion in the *P. parvum* cell due to a pH difference between the inside and outside of the cell is followed by the entry of water, swelling and lysis (Shilo and Sarig 1989).

Unslaked lime (CaO) was found to reduce the amount of ammonium sulphate or ammonia needed for complete lysis by a factor of three; unslaked lime markedly enhances the effectiveness of ammonium sulphate and ammonia because it increases the pH of water when added (Shilo and Shilo 1953). However, ammonia and ammonium sulphate are counteracted by an increase in NaCl concentrations (McLaughlin 1958). Removal of fish from contaminated water and then placing the fish in non-contaminated water was found to reverse the gill permeability effect of the toxin (Glass et al. 1991).

Shilo and Aschner (1953) found that oxygen and air decrease toxicity when bubbled through a solution of the toxin. The authors also discerned that potassium permanganate and sodium hypochloride destroy toxicity. They also noted that adsorbents such as kaolin, Norit A (acid washed), activated charcoal, calcium sulphate and pond-bottom soils have also been shown to detoxify cultures of *P. parvum.* In addition, the authors observed that the bacteria *Proteus vulgaris* and *Bacillus subtilis* decreased the

toxicity in cultures by 50% in one hour. Paster (1973) also revealed that *Proteus vulgaris* and *Bacillus subtilis* decreased toxicity of *P. parvum* cultures. Simonsen and Moestrup (1997) speculated that the *C. polylepsis* toxin decomposition in dark may be explained by bacterial activity, and the same may be true for the *P. parvum* toxin.

In Palestine, a 1:100,000 of copper sulphate was used to successfully control *P. parvum* (Reichenbach-Klinke 1973). Introduction of acetic acid and other weak electrolytes is reported to cause *P. parvum* cells to lyse (Glass et al. 1991). McLaughlin (1958) noted that organic algicides or lowering the pH decreases toxicity. Glass, Linam and Ralph (1991) noted that a pH less than 6 and greater than 9 reportedly inactivates the toxin. The authors added that increasing NaCl concentrations decreases toxicity probably by replacing a cofactor (Ca++ and/or Mg++) needed to activate the toxin. They also noted that UV and strong visible light have also been found to destroy prymnesin in lab. Nygaard and Tobiesen (1993) noted that *P. parvum* grazes bacteria when phosphate is limited. These authors believe that *P. parvum* utilizes certain species of bacteria when nutrients are limited. They suggested that the presence of these bacteria could decrease toxicity. Wynne and Rhee (1988) noted that detecting the activity of phosphatase in the water could be used to determine if the environment is limited in phosphate concentrations, and that this could be used to predict toxic blooms of *P. parvum*.

Conclusion and Areas of Further Research

The biological and ecological significance of the synthesis and release of the *Prymnesium parvum* toxin is not clear. However, it is now known that this micoalga produces two glycosidic toxins, prymnesin-1 and prymnesin-2, collectively called prymnesin, and that the two similar toxins have both hemolytic and ichthyotoxic activity (Igarashi et al. 1999). Does prymnesin have negative effects on the competitors of *P. parvum* that would lend an advantage to the growth and success of this flagellate? It has been proposed that a critical concentration of a "growth-initiating factor" is required to start division in this species and yield blooms, but this factor (if it exists) has not been described and is an area of additional research (Glass et al. 1991). If a "growth-initiating factor" is discovered, it could lead to an effective means of controlling *P. parvum*. The targeting of alkaline phosphates for the control of this microalga is another area that will require additional research if it is indeed possible. The synthesis of DMSP and the unknown polyol believed to aid in the osmoregulation of *P. parvum* needs to be studied further. This, too, may lead to an effective control of this microalga.

Additional research is needed to determine which types of bacteria cause a decrease in *P. parvum* toxicity (Nygaard and Tobiesen 1993). These bacteria could be potential biological control agents, and prove to be more practical in the control of *P. parvum* blooms that cover large areas in sensitive aquatic environments.

It seems that the most important factor governing the toxicity of *P. parvum* blooms is the relative amounts of nitrogen and phosphorous found in the water, and that limitation of both of these nutrients seems to cause an increase in toxicity (Johansson and Graneli 1999). Additional research must be conducted in this area to determine if an unbalanced N:P ratio indeed leads to increased toxicity. If the N:P ratio proves to be the most important factor, research must be conducted to determine how the optimum N:P ratio for *P. parvum* can be restored. The sources for the imbalance must also be studied

since it seems that nitrogen and phosphorous inputs from agriculture, aquaculture and other sources may be involved. *P. parvum* has been found to graze bacteria as a source of phosphate (Nygaard and Tobiesen 1993). More research in this area may lead to ways of treating *P. parvum* blooms in phosphate-limited environments by supplying this microalga with bacteria it is known to utilize as a source of phosphate.

Glycerol has been found to enhance the growth of *P. parvum* (Cheng and Antia1970). Are there any sources of glycerol pollution in the areas where fish kills occurred in Texas? The investigation of future fish kills should include the detection of glycerol in the aquatic environment since this may cause *P. parvum* blooms.

The importance of the microscopic algae *Prymnesium parvum* can be seen in the millions of fish killed across the globe, and the ensuing economic losses it creates. The recent appearance of *P. parvum* in Texas, and the recurring fish kills caused by the toxins released, is a cause for concern. This problem is one that must be addressed soon for history has shown us that *P. parvum* is ever present once the organism first appears. Steps must be taken to further understand the ecology of this organism, its toxin and causes of toxic blooms in an attempt to decrease the number of occurrences we see in the future.

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