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

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

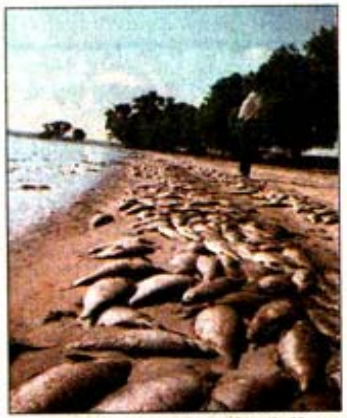
News Release

- **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

Catch of the Day

Pelicans putative of transport agents of *Prymnesium* to Colorado

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

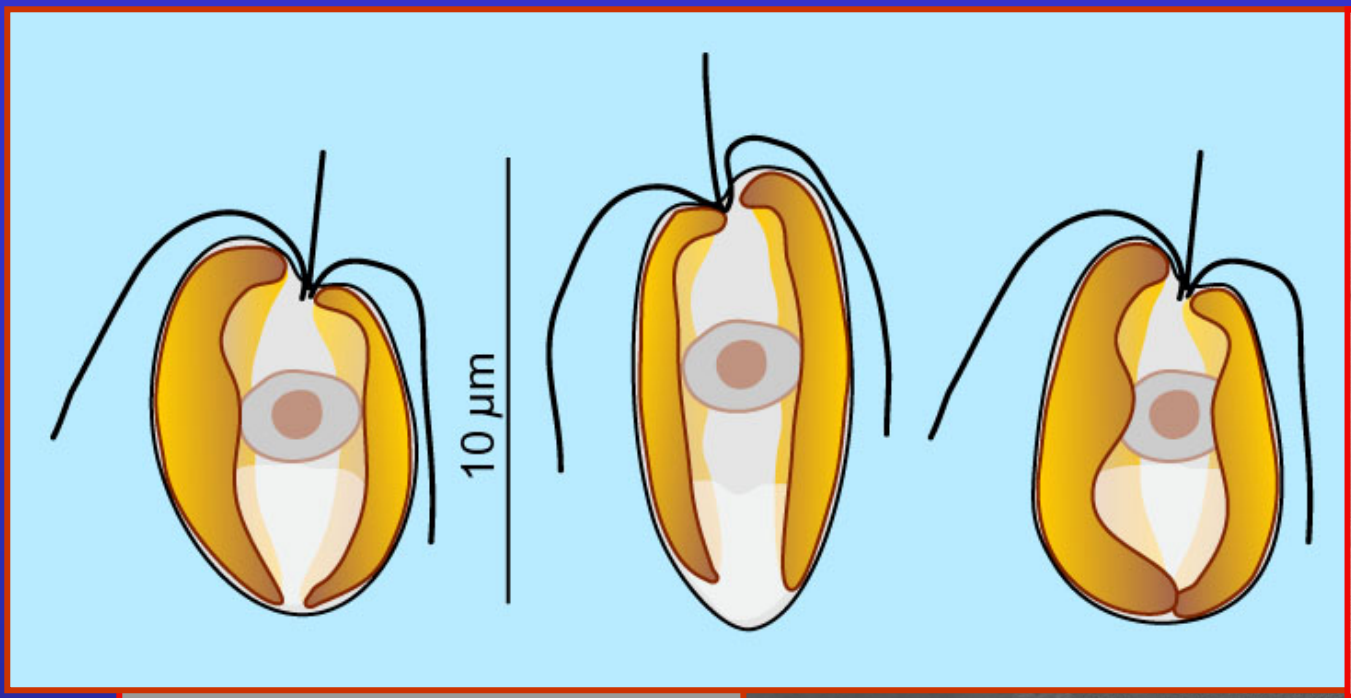
Prymnesium Strains in Culture

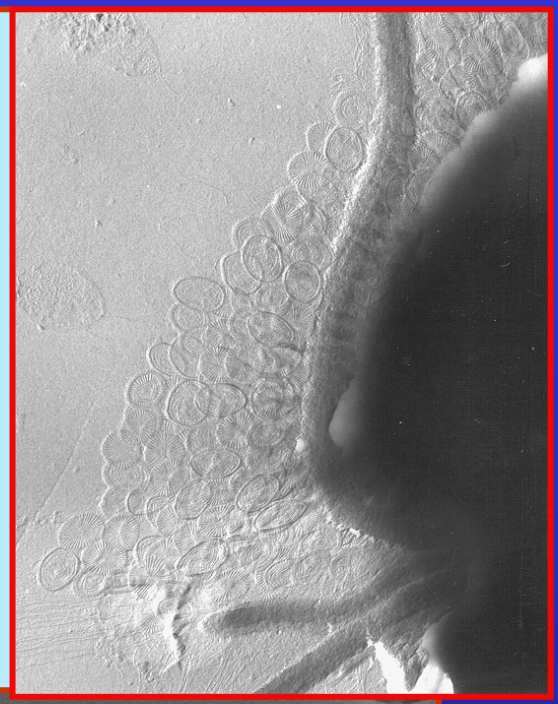
- UTEX - 995 Plymouth, England
- Texas Isolate
- Texas Isolate Lubbock Canyon
- Wyoming Isolate Twin Buttes Lake
- Latvian isolate - Jurmala, Baltic Sea

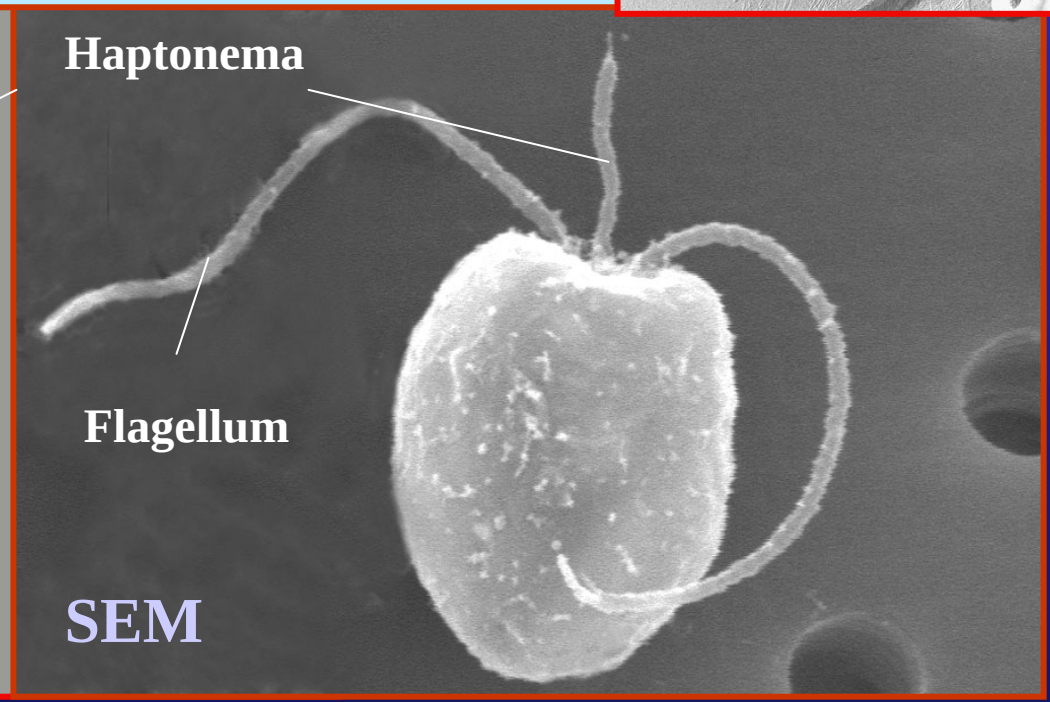
Strains of *Prymnsium parvum* Used in this Study

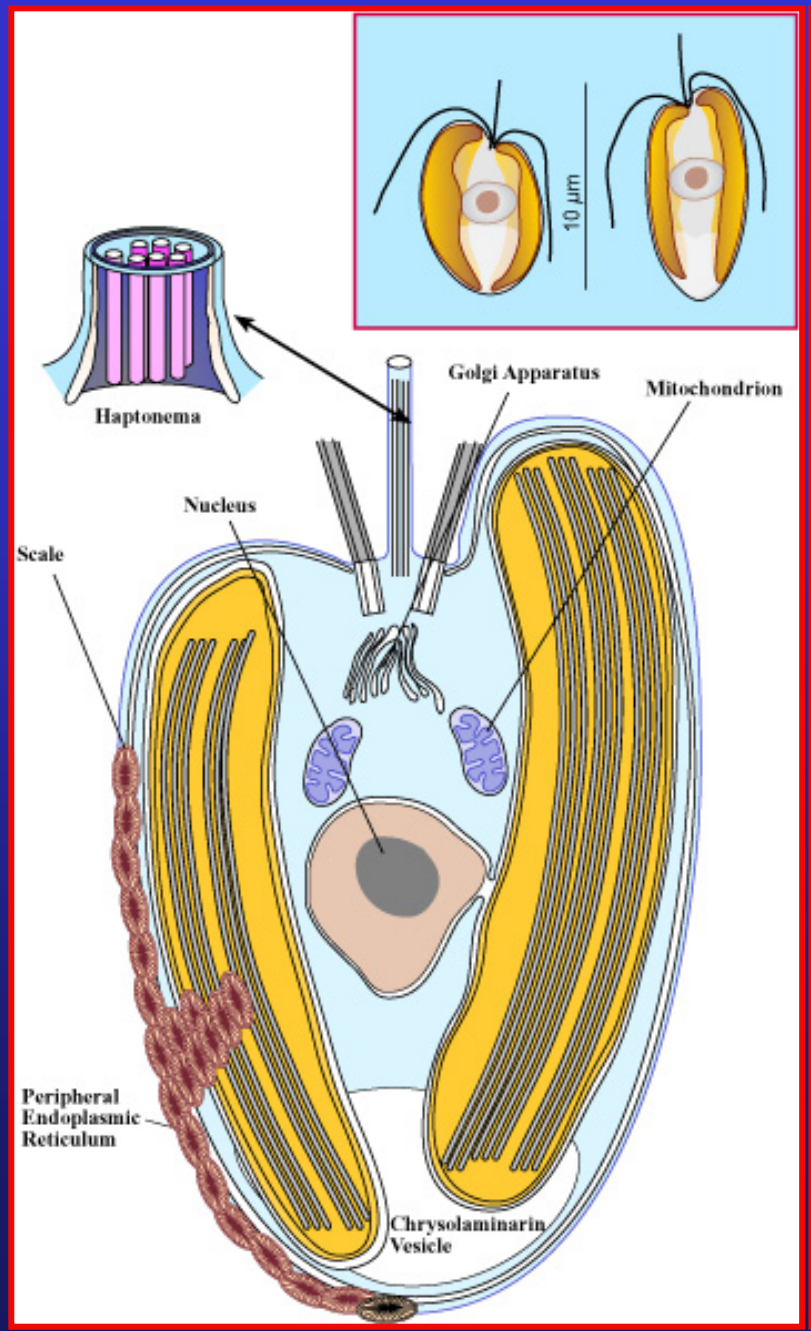
- **UTEX - 995**
- **Texas Isolate**
- **Wyoming Isolate - Twin Buttes Lake**

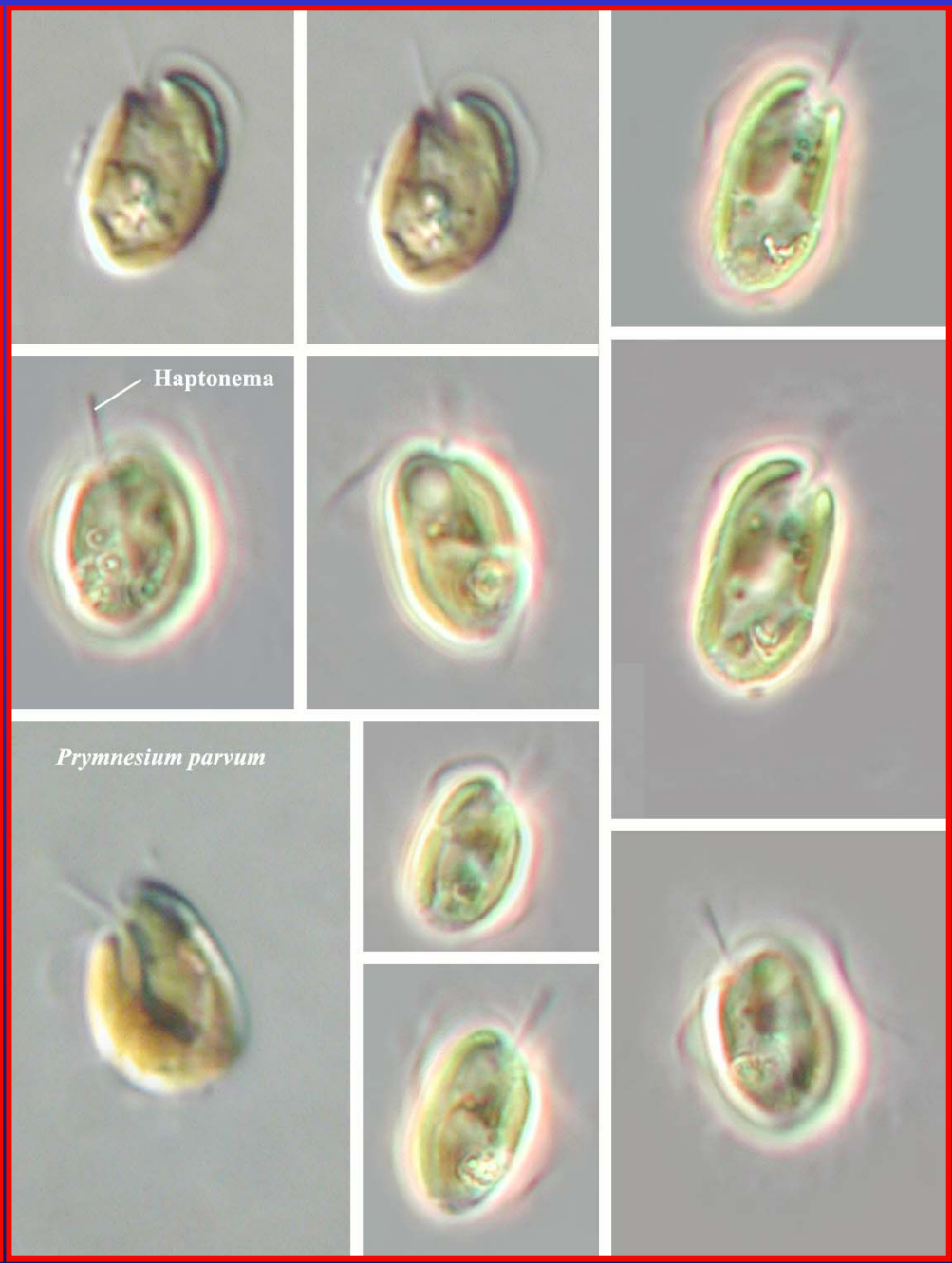
Structure of *Prymnesium*


10 µm



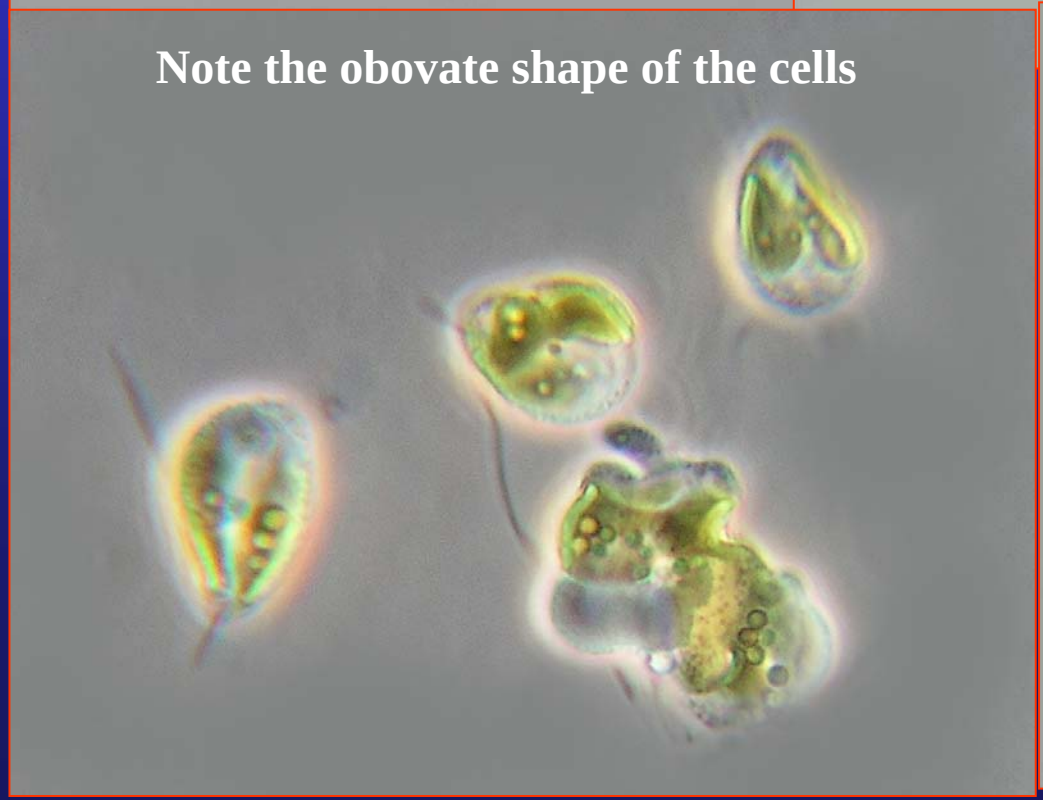

Haptonema
Flagellum
SEM

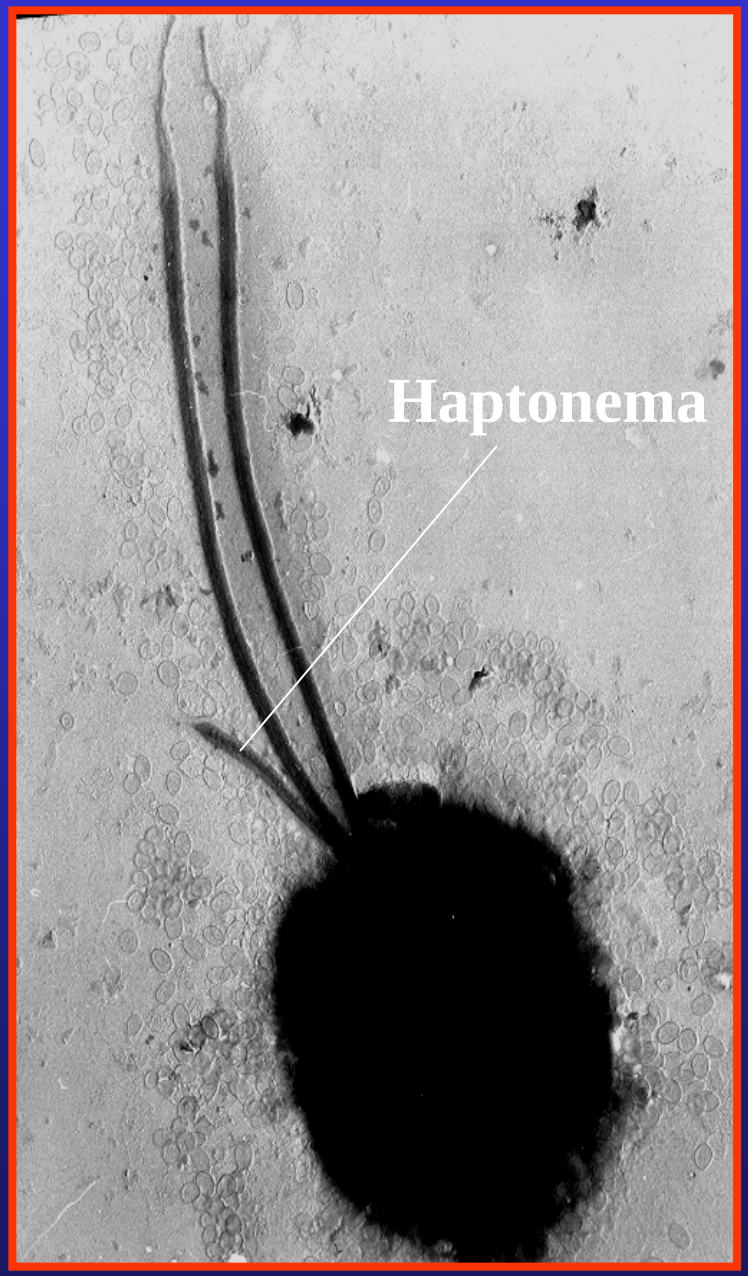

10 µm
Haptonema
Golgi Apparatus
Mitochondrion
Nucleus
Scale
Peripheral
Endoplasmic
Reticulum
Chrysolaminarin
Vesicle

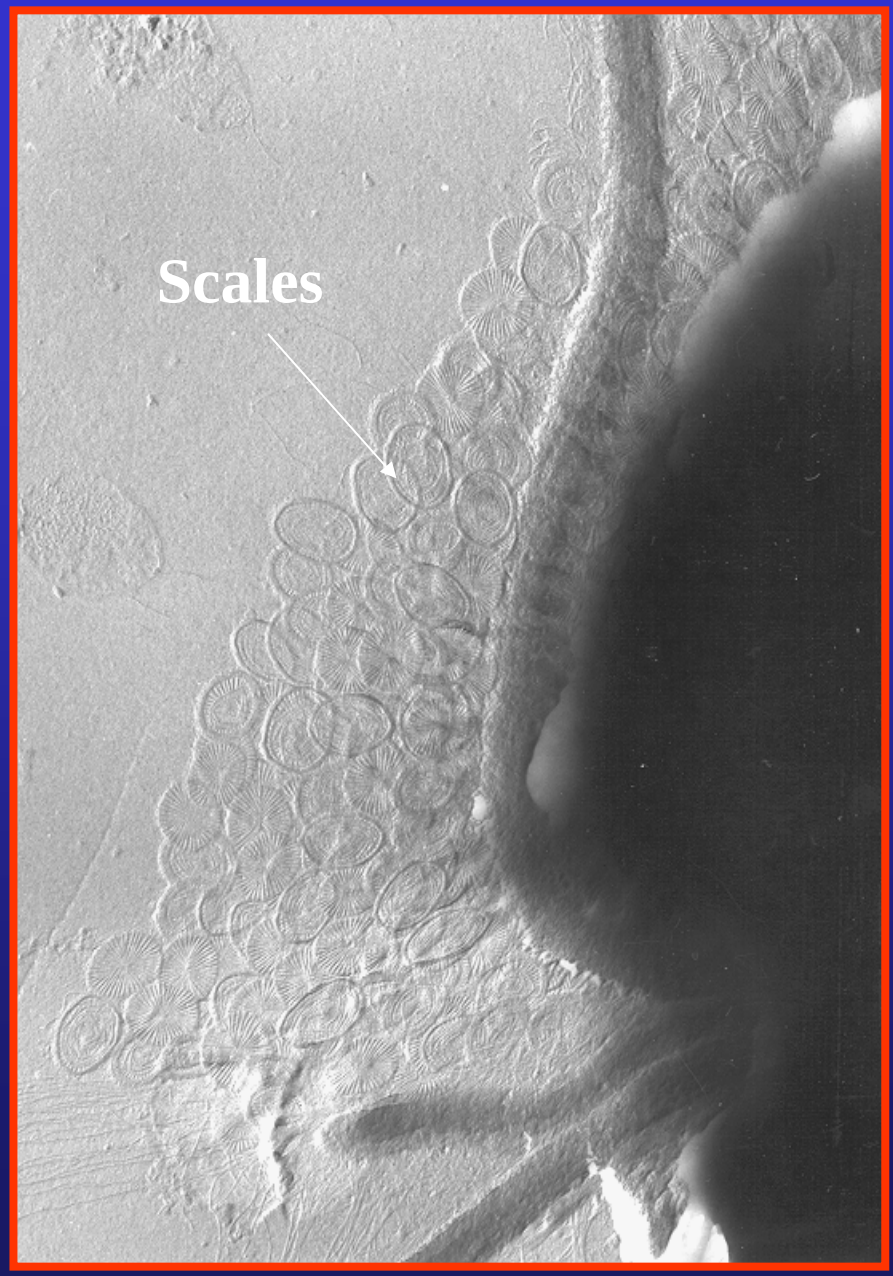

Haptonema
Prymnesium parvum

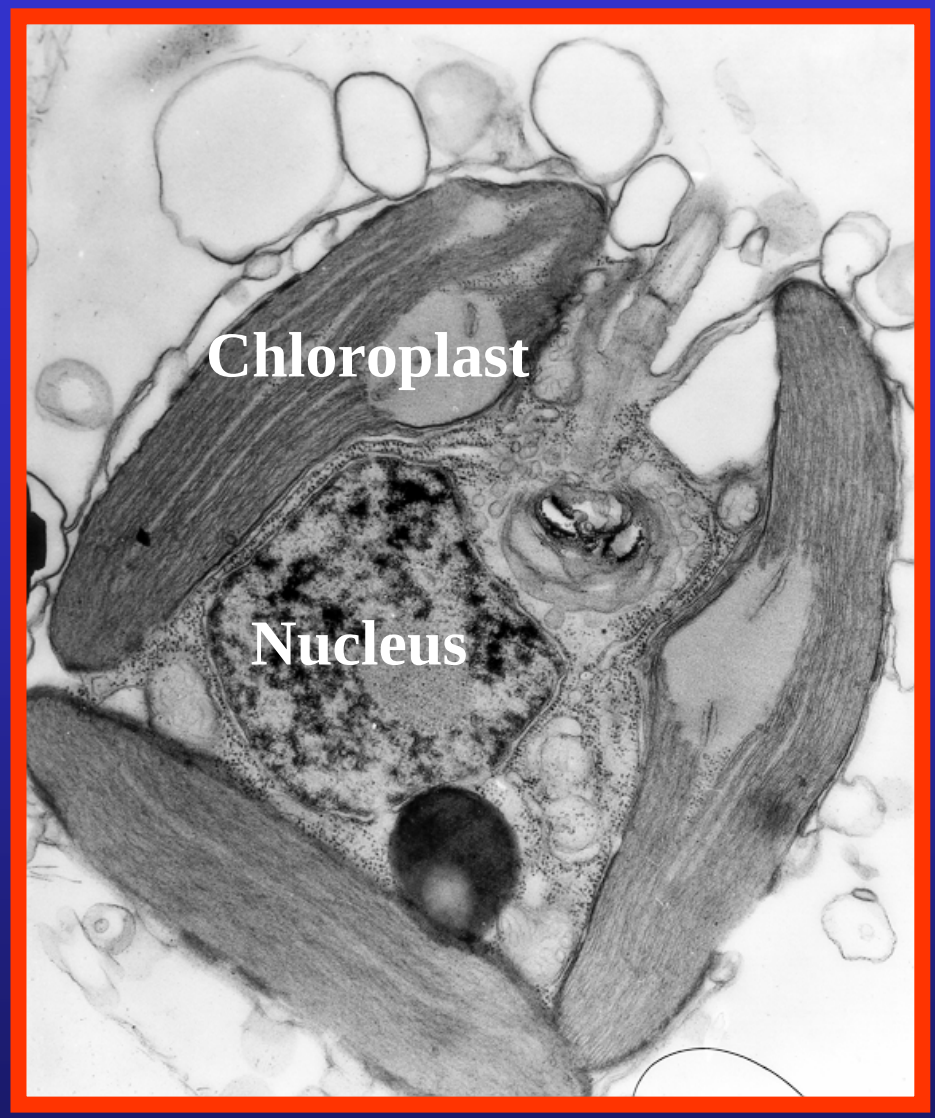
Texas *Prymnesium parvum*

Note the obovate shape of the cells




Haptonema


Scales


Chloroplast
Nucleus

Cyst Formation

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

Media Used

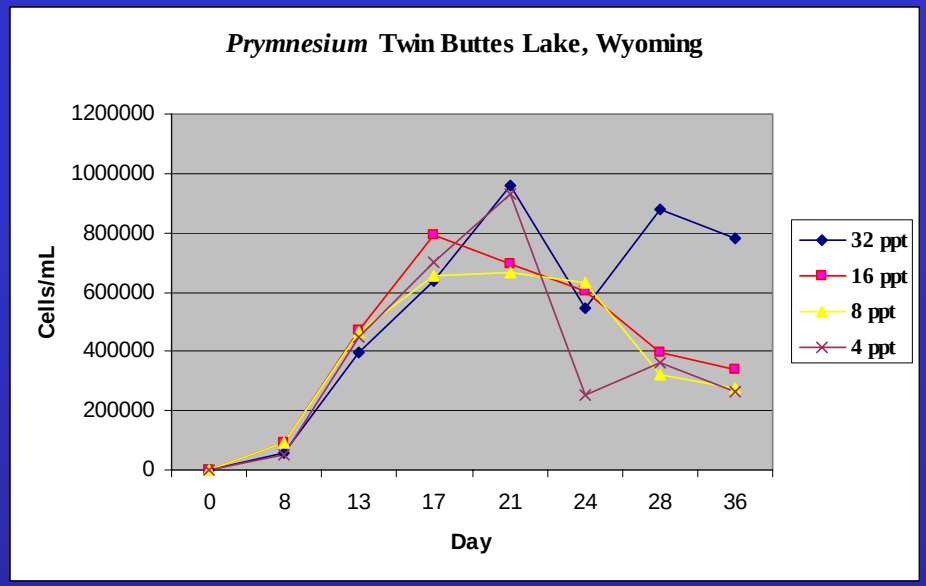
- **Medium Variations**
- **Twin Buttes water (high salinity – primarily calcium sulfate)**
- **Prewitt Reservoir Water (low salinity)**
- **Dowdy Lake water (low salinity)**
- **Seawater**
- **Artificial Seawater Medium**

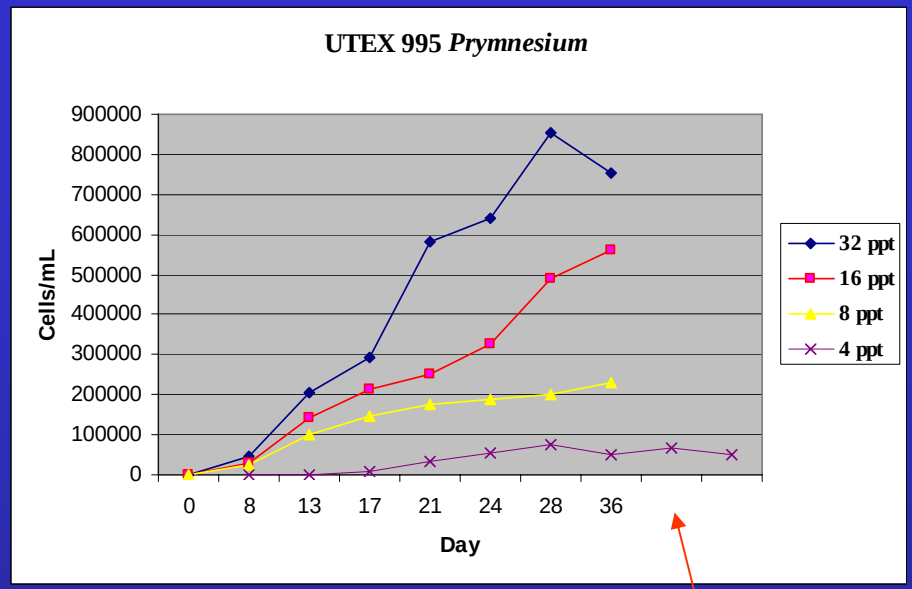
Growth Observations 0n *Prymnesium* strains from Texas & Wyoming

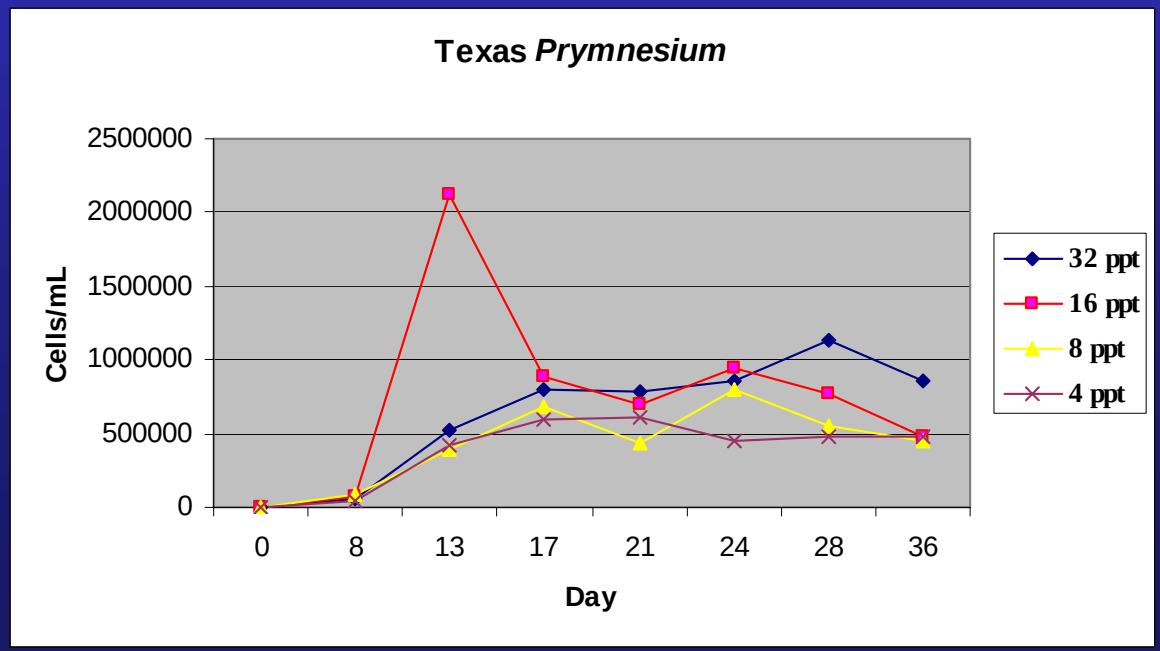
- **Grew well in following media & salt concentrations**
  - **Twin Buttes – 11 ppt**
  - **SW – 32 ppt**
  - **Buffalo Spring Lake – 11 pt**
  - **Lubbock Canyon Lake - 6 ppt**
- **No Growth**
  - **Dowdy Lake Water – >1 ppt**
  - **Prewitt Reservoir – 1 ppt**
  - **Cells hypertrophied & burst within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

Salinity Tolerance

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







Definite Salinity Effect

No growth in 1 ppt for any of the strains

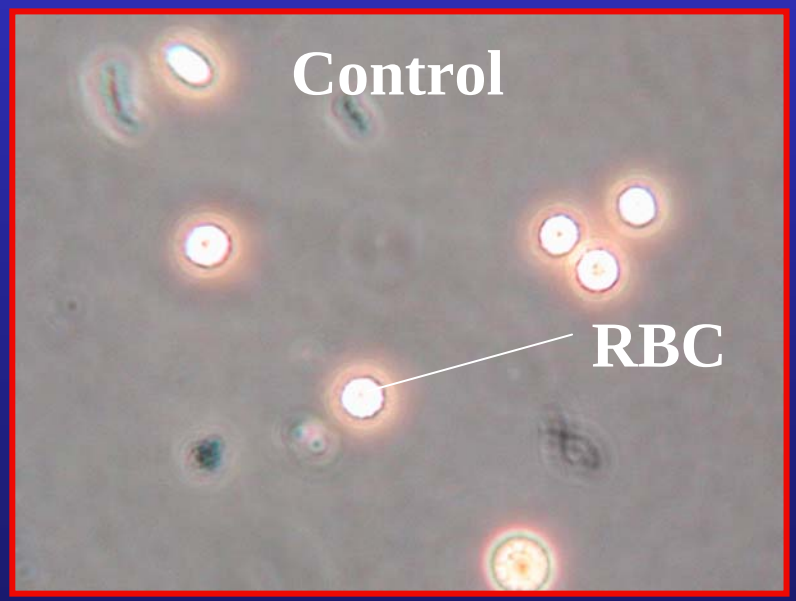
Summary of Salinity Experiments

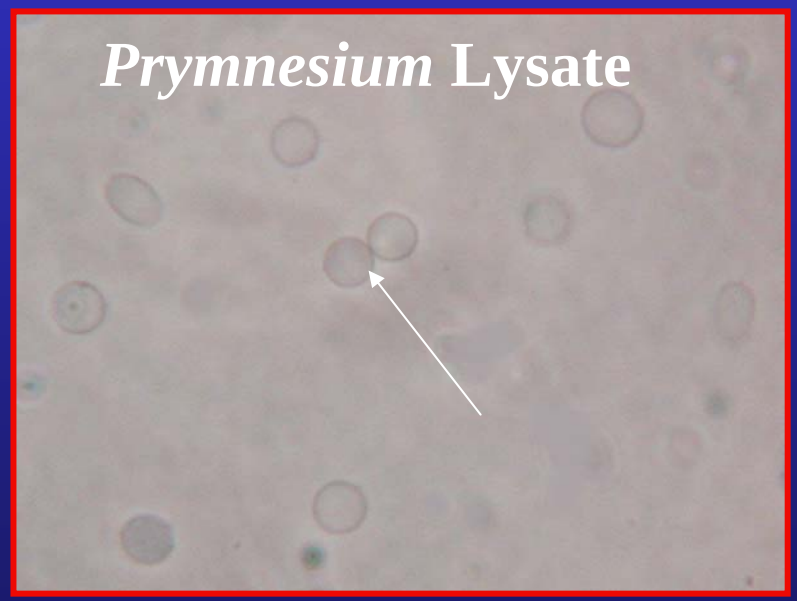
- **All grew well in 32 ppt**
- **Texas *Prymnesium* grew rapidly and had the highest numbers/ml**
  - **Maintained cell numbers between .5 – 1.25 million cells/mL at the end of the experiments**
- **Strain 995 grew best at 32 ppt but growth decreased correspondingly with lower salinities**
- **Texas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

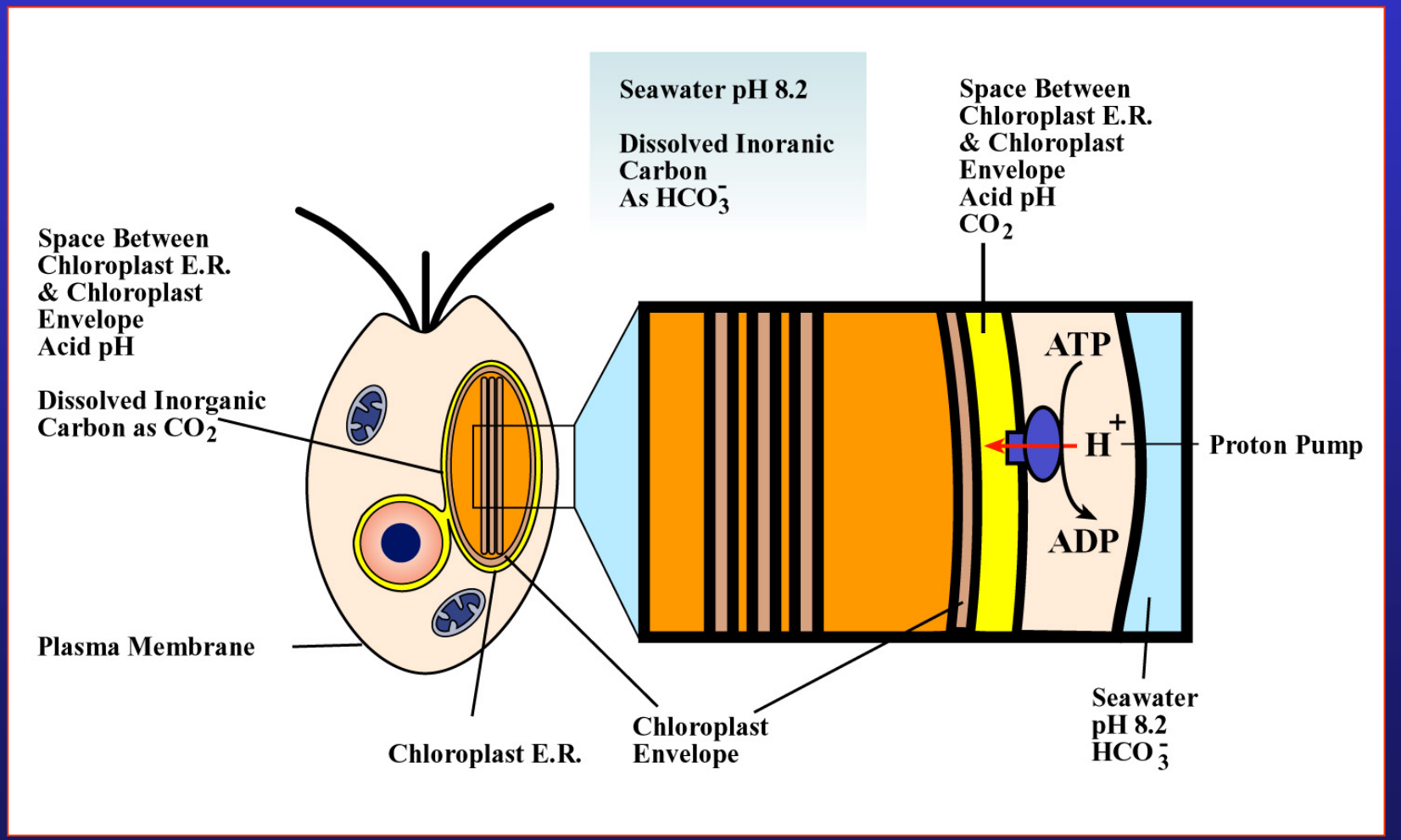
- **Several phytoplankton grown with *Prymnesium***
- **Observed daily with inverted light microscopy & transmision electron microscopy (1000's of cells)**
- **No evidence of mixotropy in any of the three strains used in this study**

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?

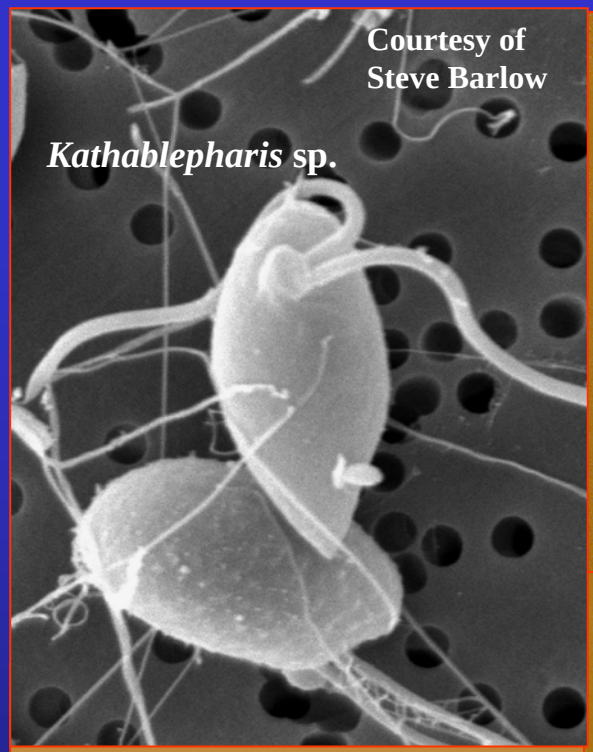


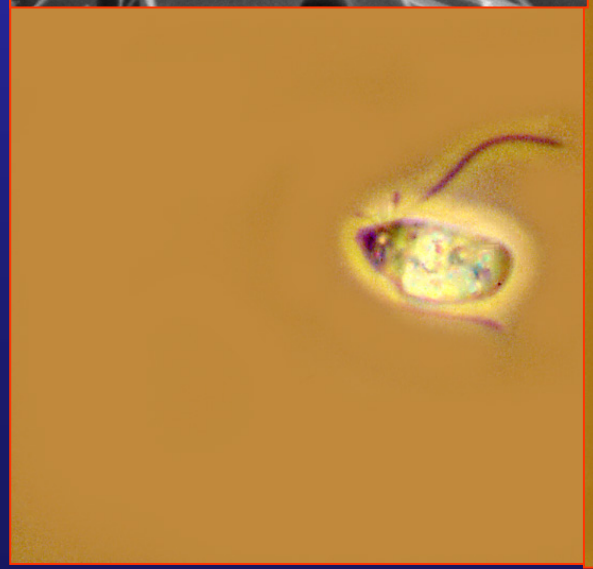
Adaptive Advantage of Chloroplast Endoplasmic Reticulum – Carbon Dioxide Utilization?

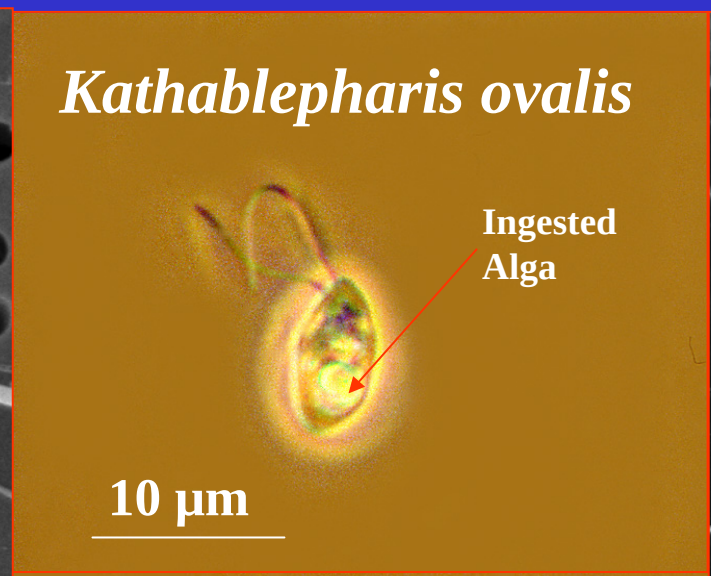
- **Dissolved Inorganic Carbon (DIC) availability might promote rapid growth of *Prymnesium***
- **Chloroplast Endoplasmic Reticulum might provide a ready source of carbon dioxide for the Calvin Cycle**
- **Would impart an adaptive advantage over algae that lack a CER**

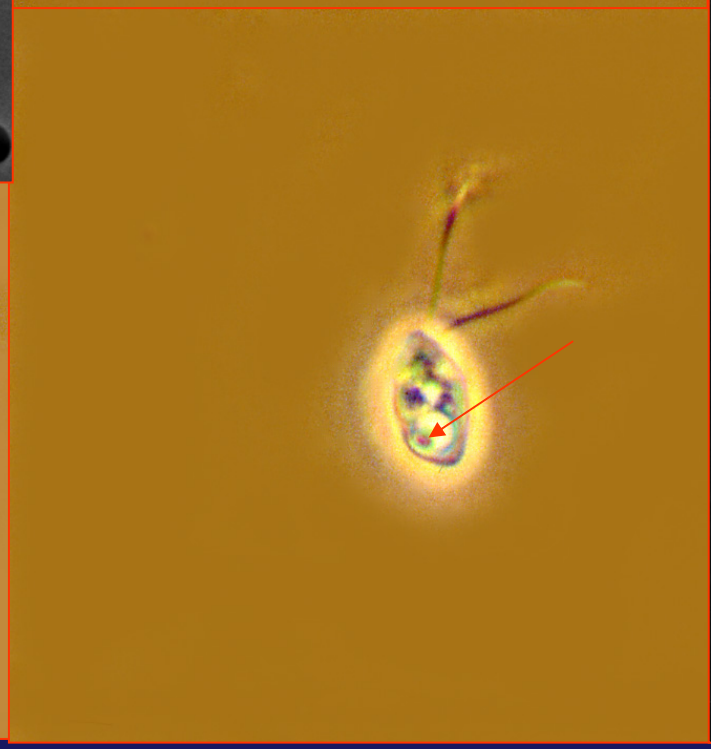
Biocontrol of *Prymnesium*?

- **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.

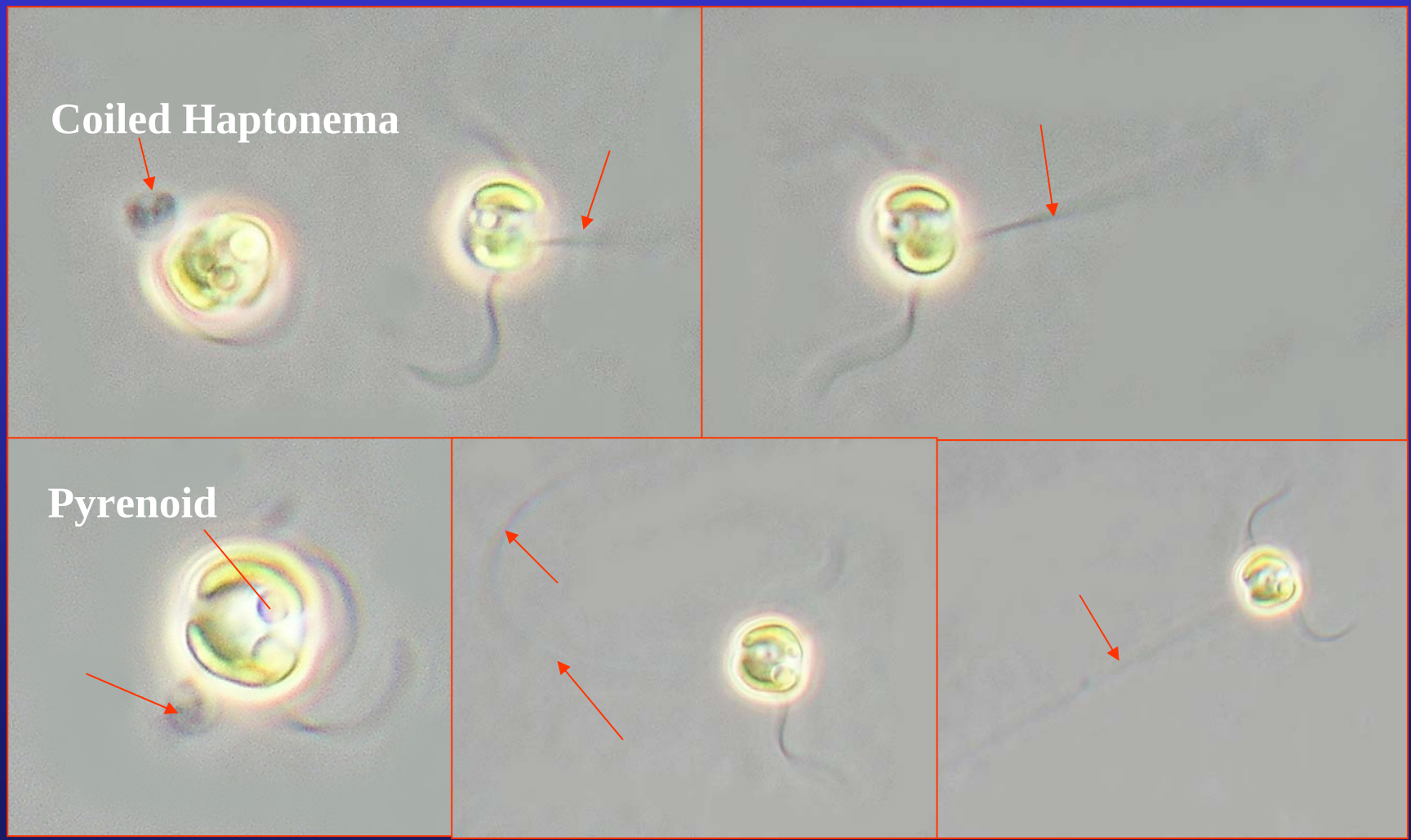



Kathablepharis ovalis
Ingested
Alga
10 µm




10 µm

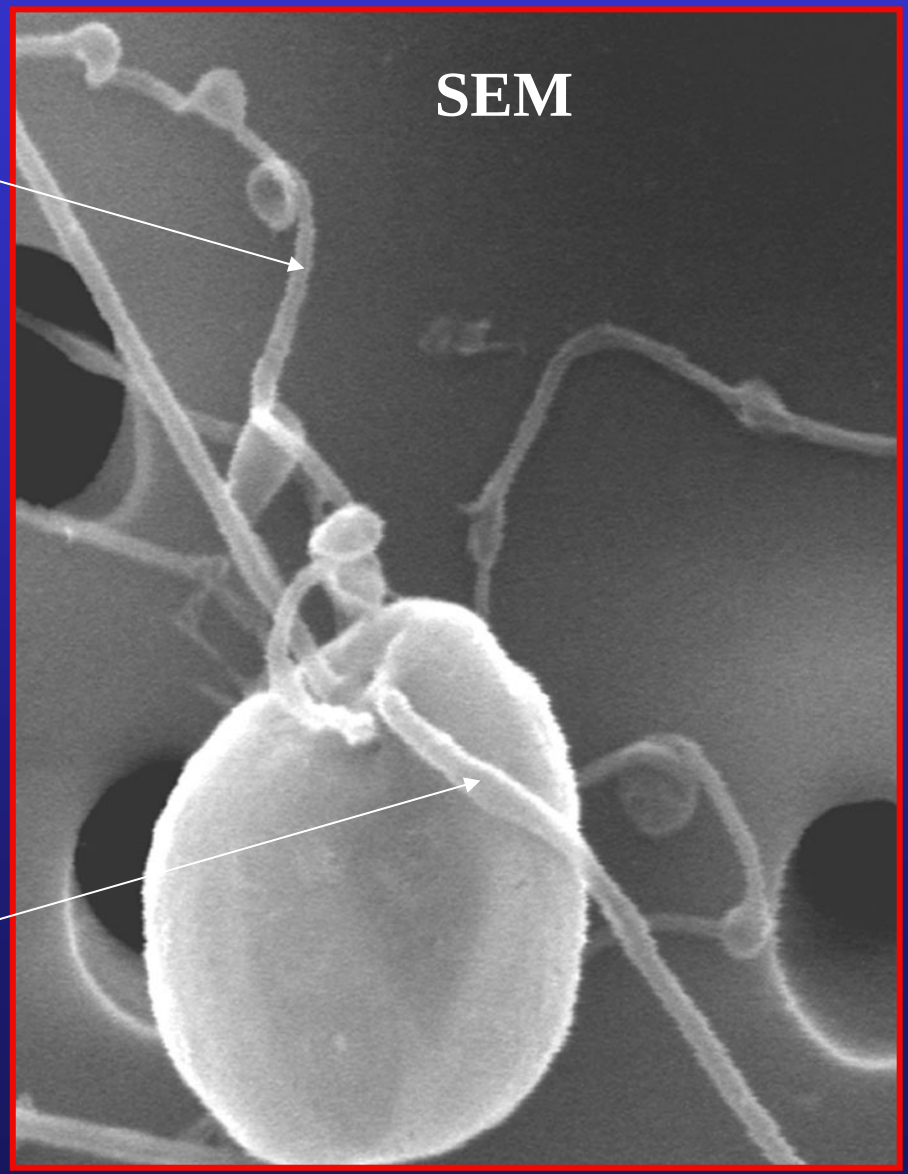
Chrysochromulina Haptonema



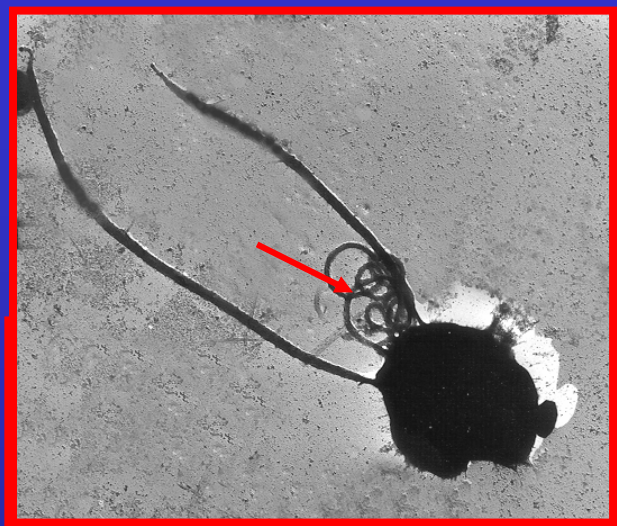
Chrysochromulina parva

Long, uncoiled Haptonema

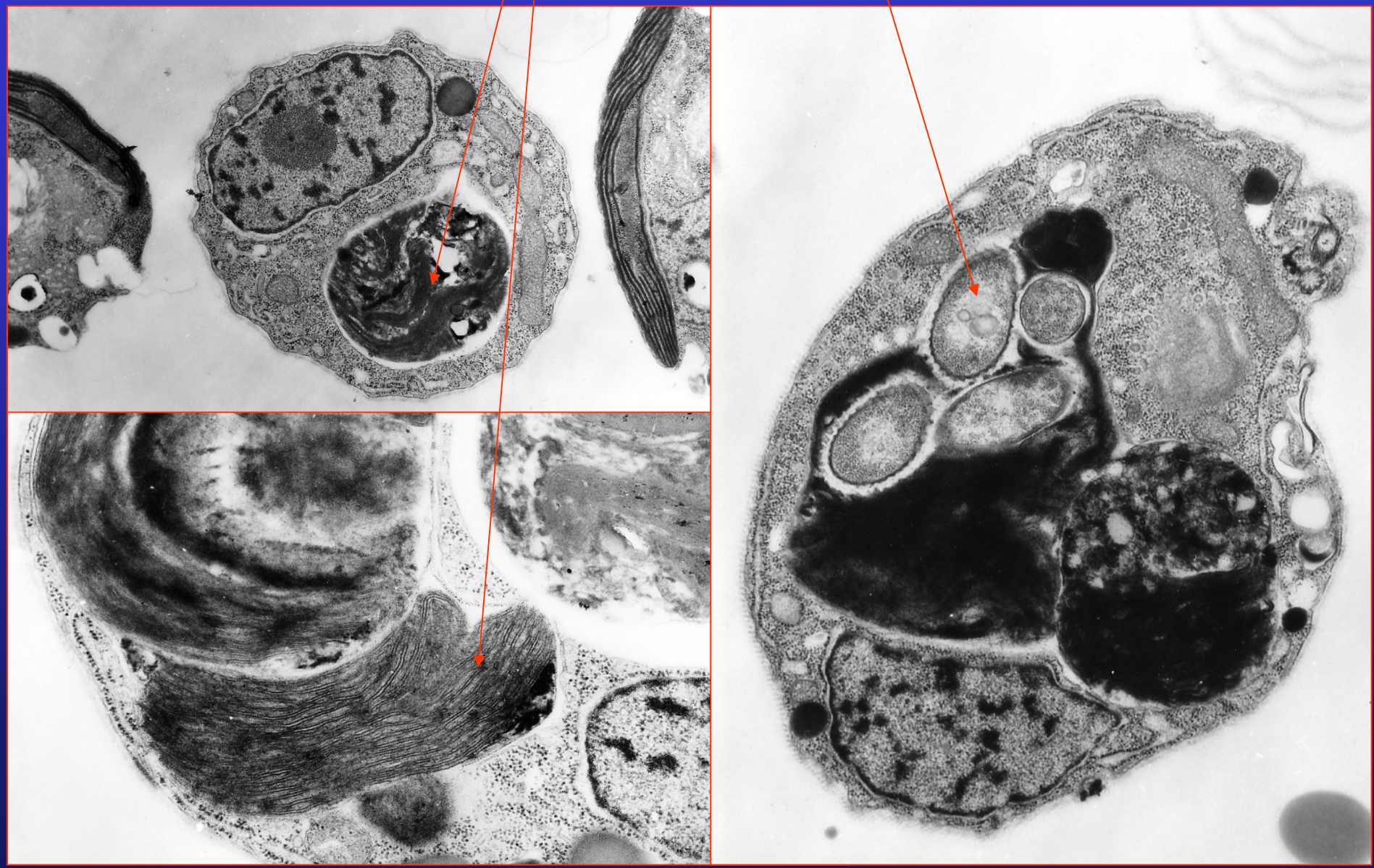
Flagellum



Chrysochromulina parva



Ingested *Chrysochromulina* & some bacteria in *Kathablepharis*



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

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


THE END