



**Programmed Cell Death in
Trichodesmium: Implications for Blooms
at Sea**

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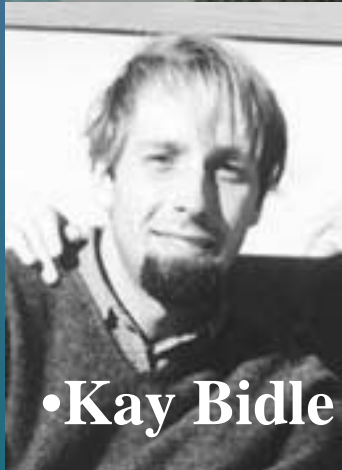
Co-authors.....

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Institute of
Marine and
Coastal Sciences,
Rutgers
University, USA

•Kay Bidle



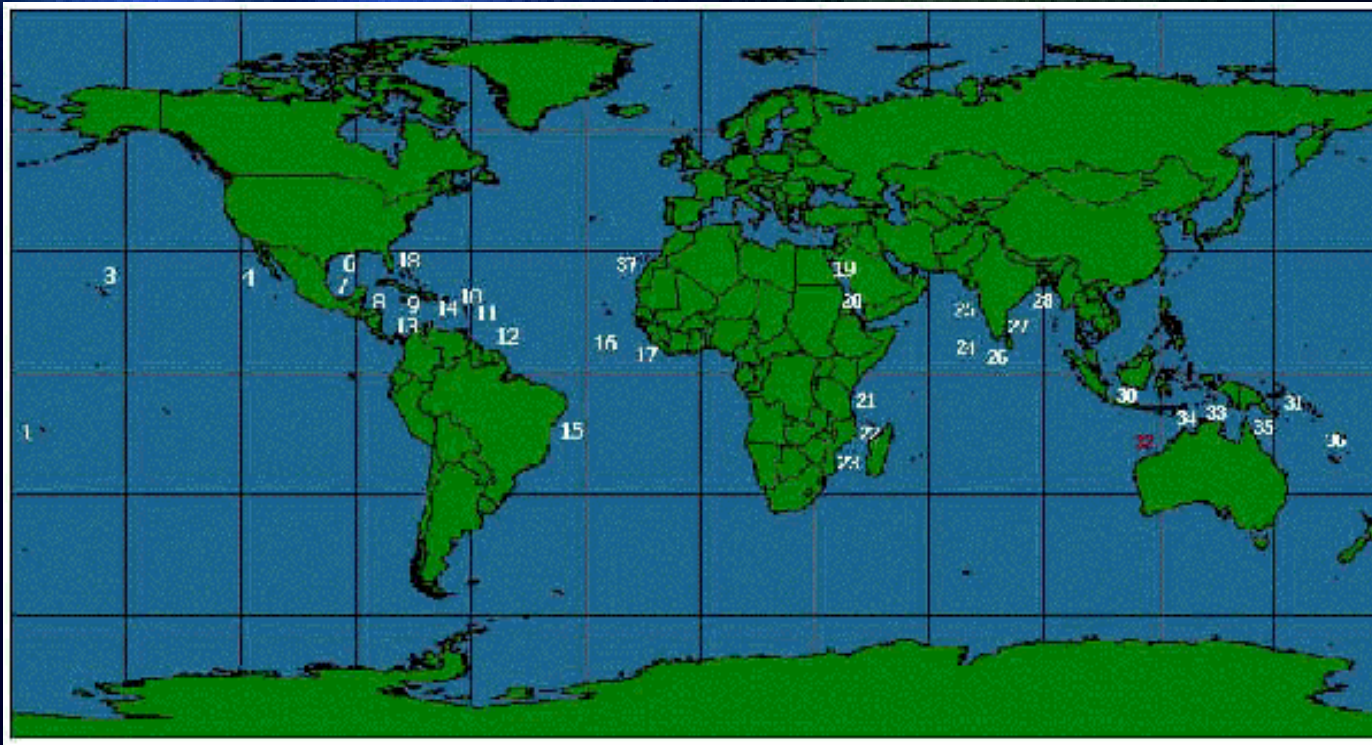
Paul Falkowski



Xavier Mari
IRD – New Caledonia

Trichodesmium spp.

Major pelagic diazotroph



http://www.usc.edu/dept/LAS/biosci/tricho/trichohome/World_Tricho.htm

Trichodesmium spp.

- Contribution of *Trichodesmium* to:
- Global N₂ fixation – 36% (Gallon 2001)
- Marine N₂ fixation - 50-90% (Capone)



Demise of *Trichodesmium* Blooms ?

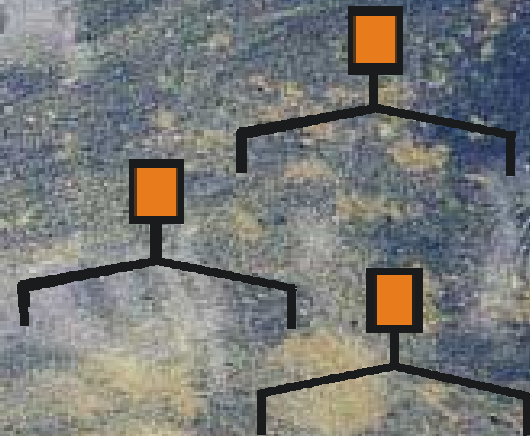


Photo: JM O'neil



*Grazing by
harpacticoid
copepods:
Macrosetella*

(O'neil, J.Plankton Res. 1998).

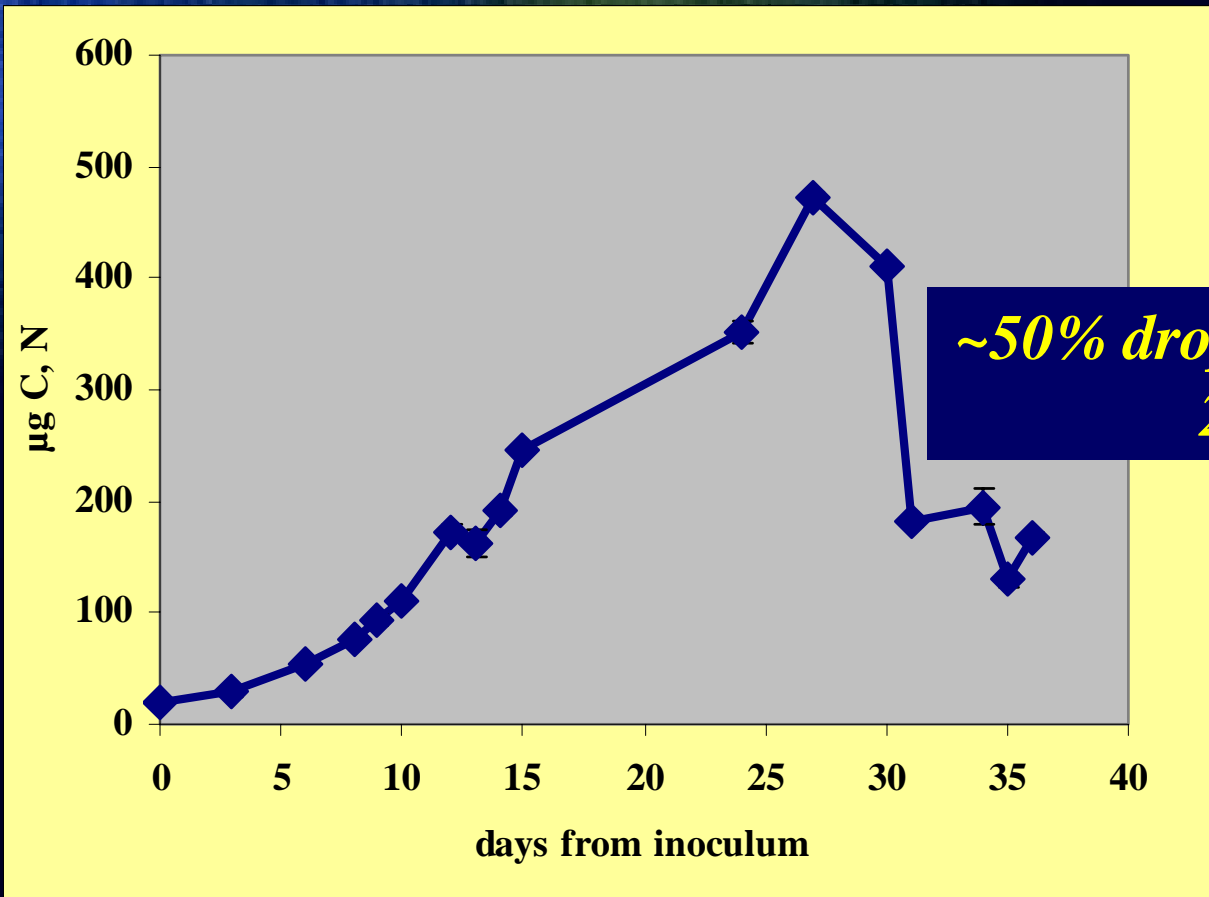


*Bacteriophage
Infection and Lysis
(Ohki, 1999)*



BUT:

***Trichodesmium* often displays rapid biomass crashes without presence of grazers or viruses**

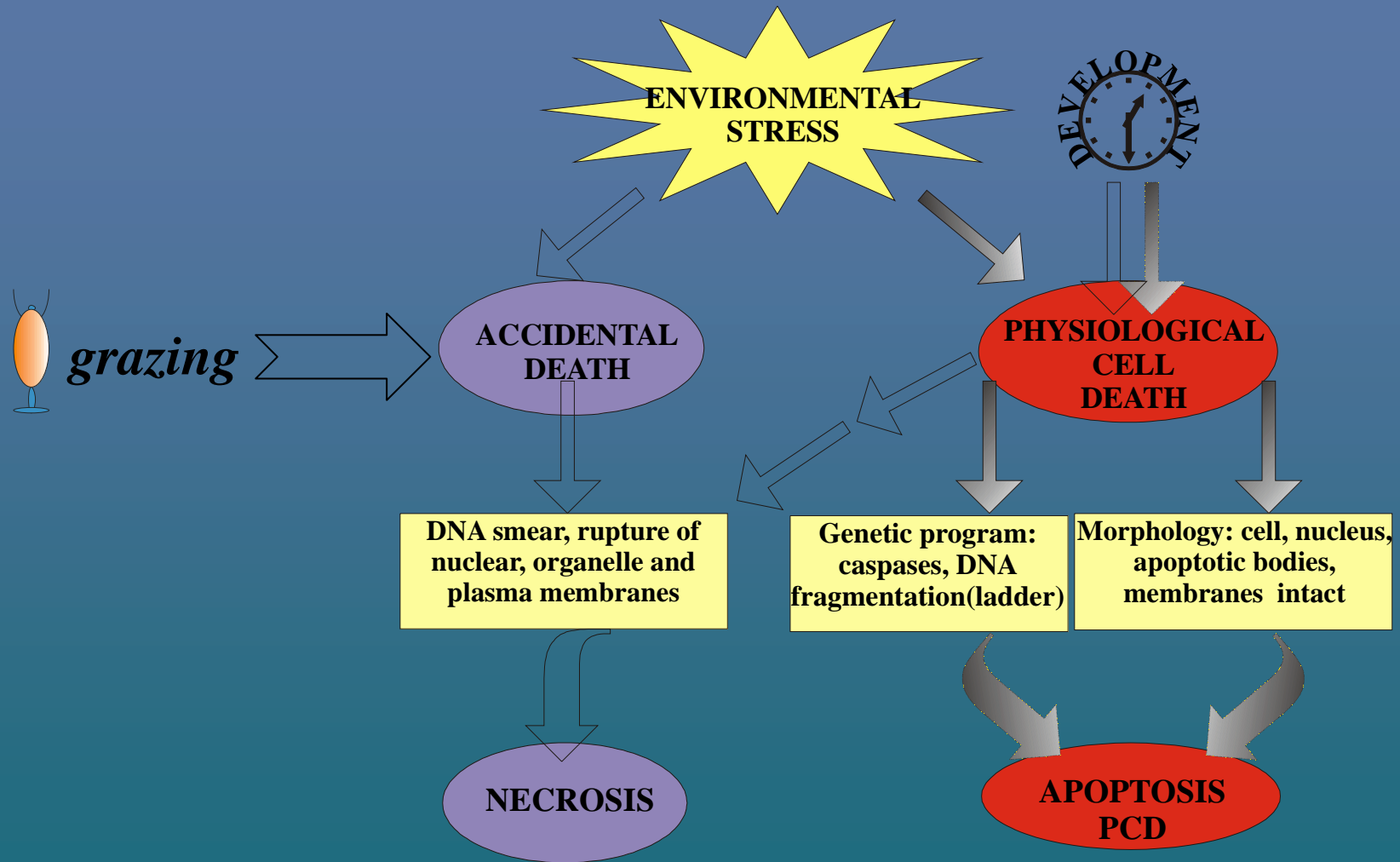


~50% drop in biomass in 2 days

WHY??



Pathways of death....

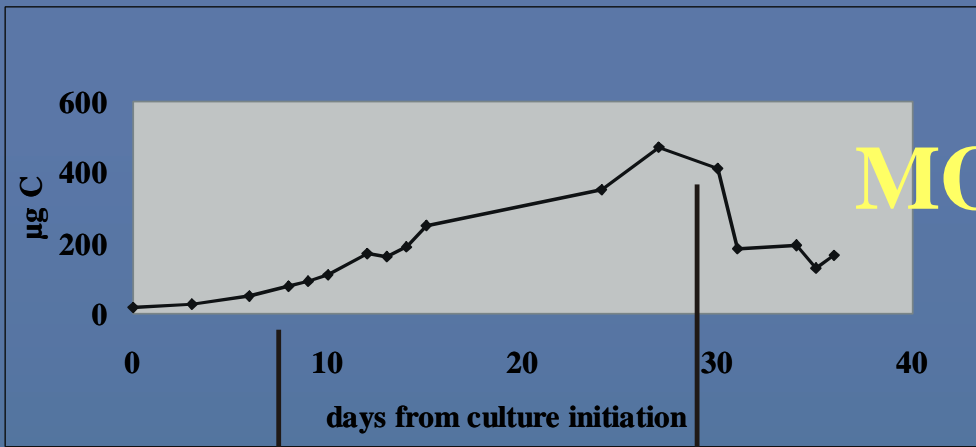


OBJECTIVE

- **DOES AN APOPTOTIC, AUTOCATALYTIC PROGRAMMED CELL DEATH (PCD) EXIST AND OPERATE IN *TRICHODESMIUM* ?**

APOPTOTIC, PCD CHARACTERISTICS

- ***MORPHOLOGICAL***
- ***SYNTHESIS and ACTIVITY OF
PROTEASES and NUCLEASES***
- ***GENETIC POTENTIAL***



MORPHOLOGICAL EVIDENCE:

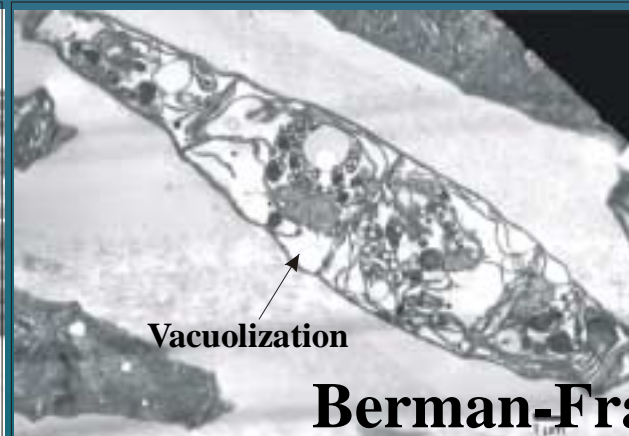
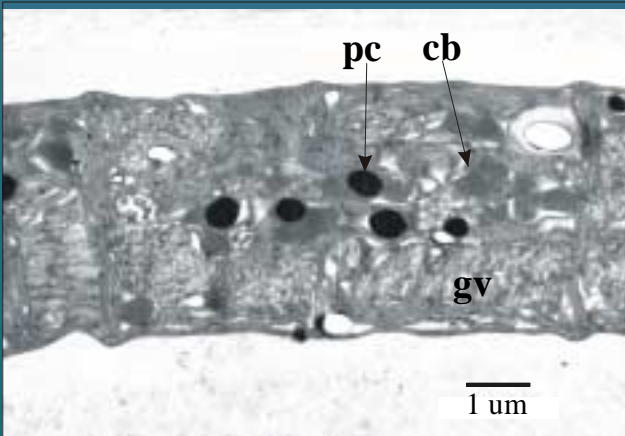
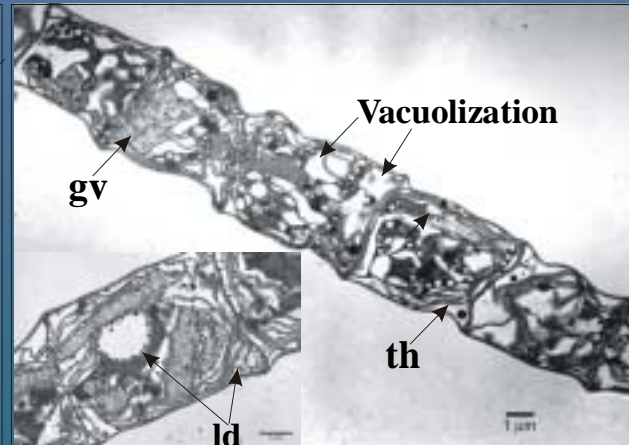
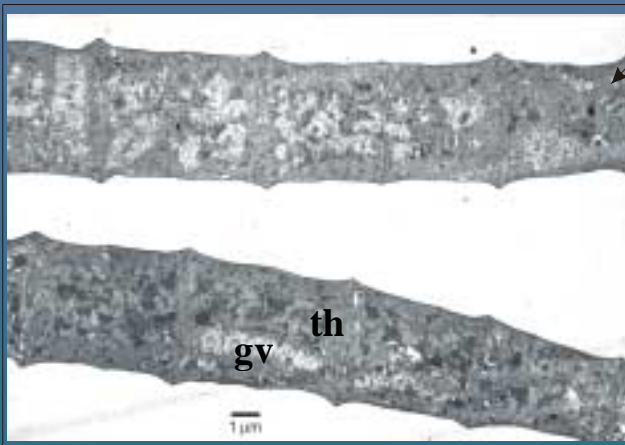
- Membranes intact

- Increased vacuolization.

- Less phycocyanin, carboxysomes, thylakoids.

- lipid droplets.

- Breaks between cells.



MORPHOLOGICAL EVIDENCE:

Membrane impermeable stains

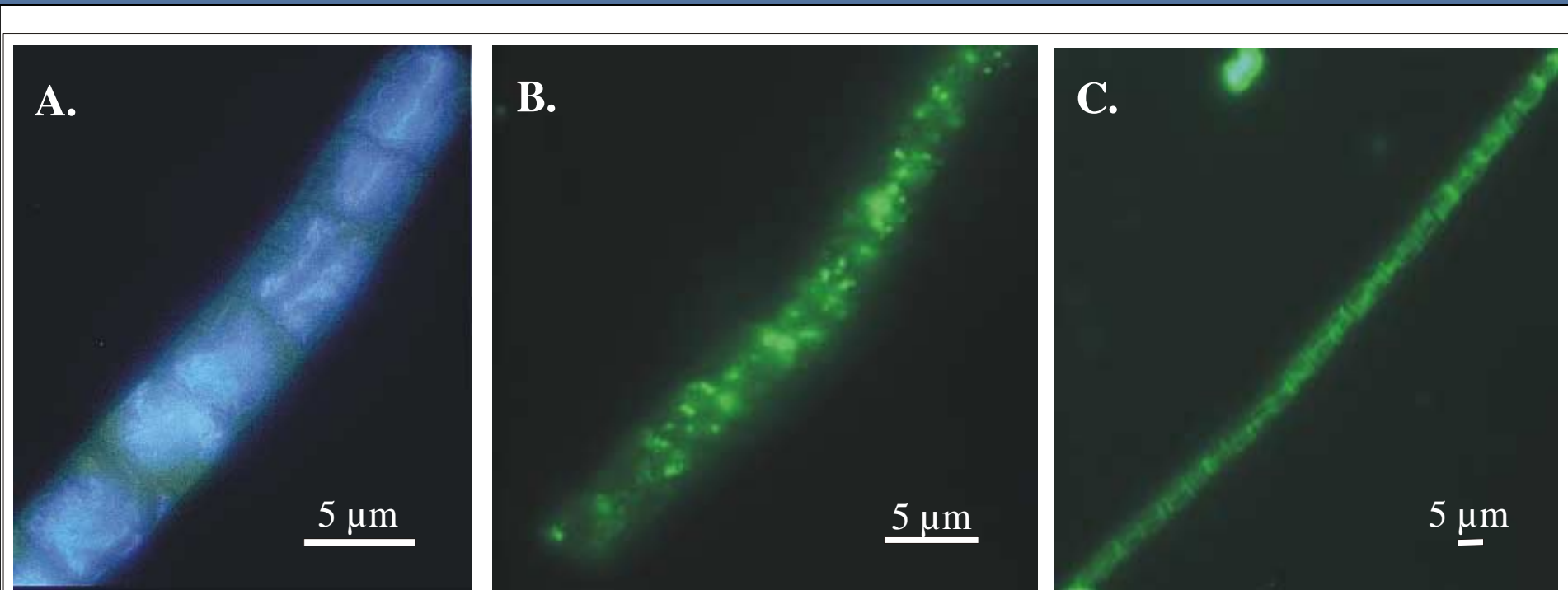
SYTOX-green



Cells under oxidative stress - APOPTOTIC

DNA fragmentation

Terminal Deoxynucleotidyl Transferase dUTP Nick End (TUNEL) labeling



Healthy cells
– Intact DNA

3 h after
H₂O₂ spike –

Negative
control

Berman-Frank et al. L&O 2004

BIOCHEMICAL EVIDENCE:

CASPASES and APOPTOSIS

- **Caspases** (cysteine aspartate-specific proteases) contain conserved QACXG pentapeptide with cysteine in the active site.
- **Central role in initiation and induction of apoptosis** - Caspase cascade: initiators, executioners (Effector).
 - NO activation of caspases found in NECROTIC death!
- **Caspase 3**: main executioner caspase. Activation essential for DNA fragmentation, chromatin condensation and plasma membrane blebbing.

BIOCHEMICAL EVIDENCE:

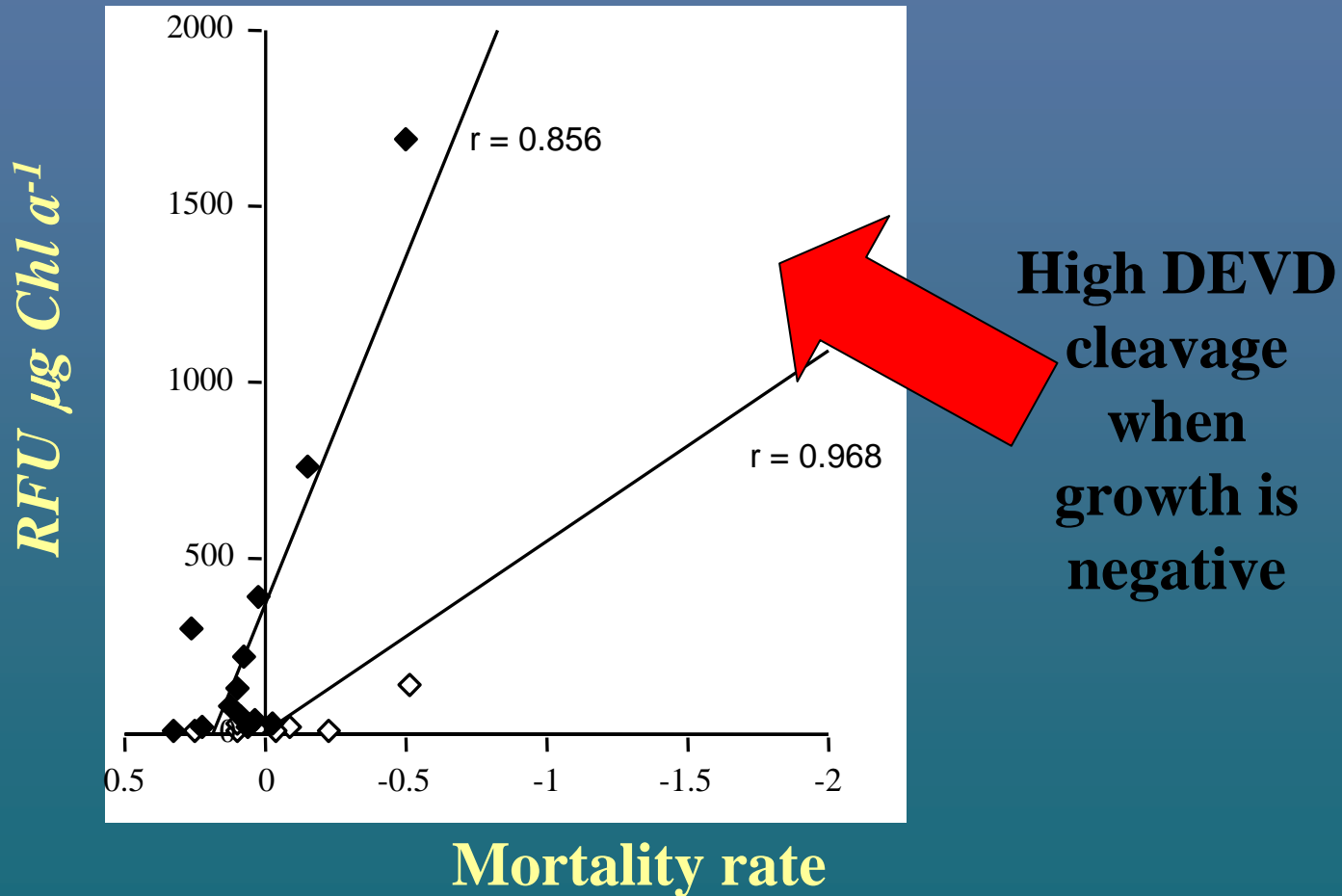
Increase of caspase-3 immunoactivity during
Trichodesmium sp. IMS101 bloom
progression.



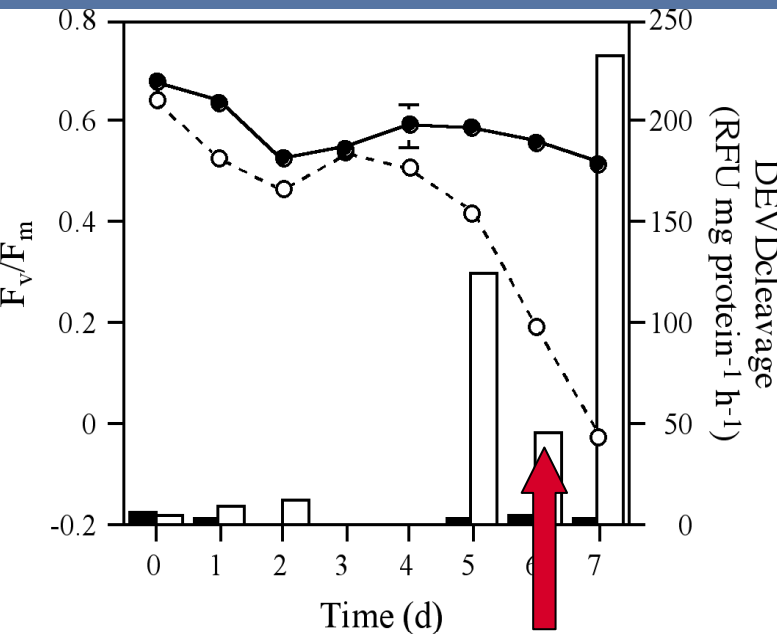
Bloom decline

Whole cell protein extracts were challenged with polyclonal antibodies to recombinant human caspase 3

Dependence of DEVD cleavage on physiological status of *Trichodesmium* sp. IMS101 .



Induction of nucleases with nutrient stress



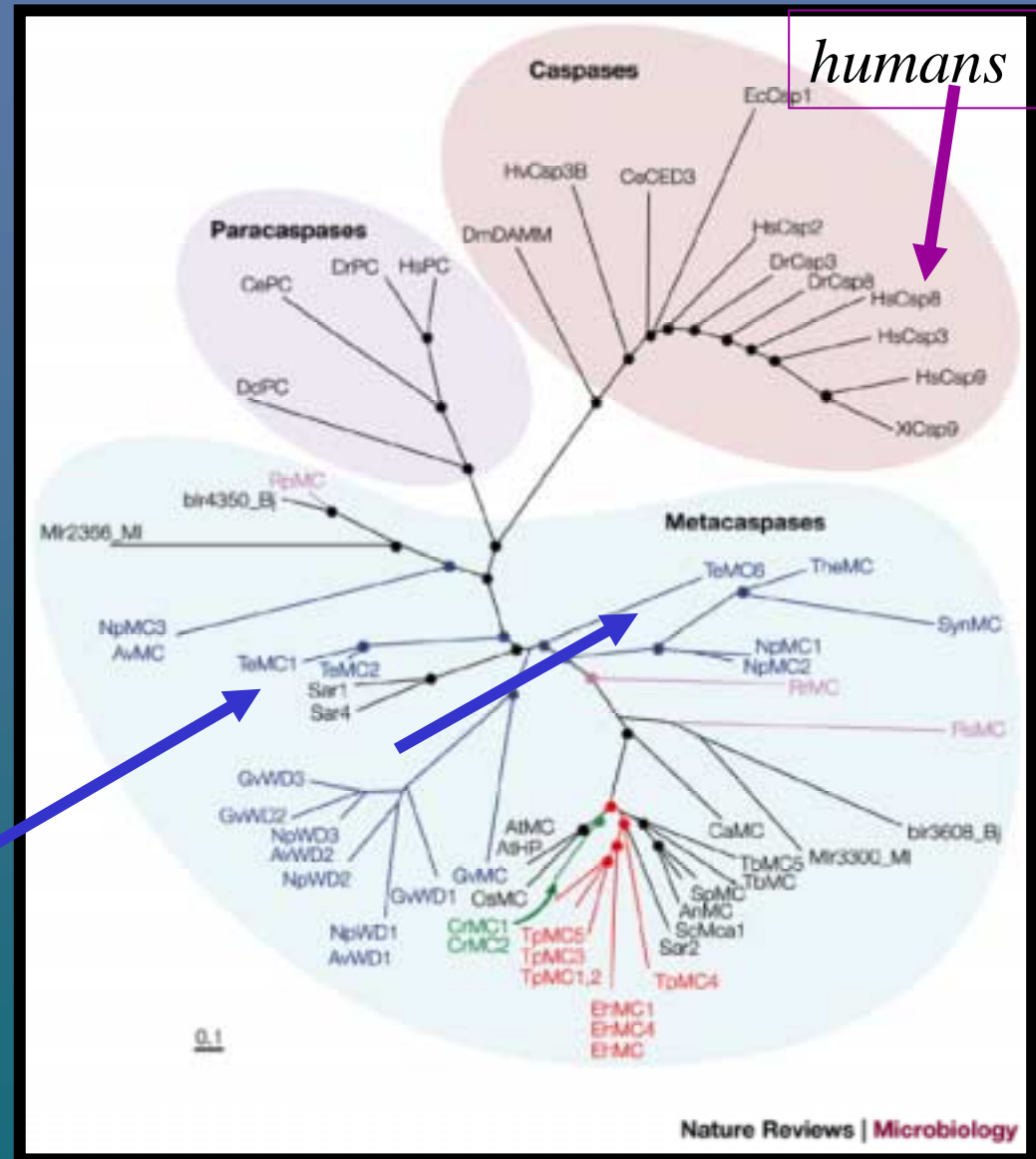
Genetic potential.

- **METACASPASES**
- Caspase-like proteins identified in plants, fungi, unicellular protozoa and multiple bacterial species including *Synechocystis* and *Anabaena* (Uren *et al.*, *Molecular Cell* **6**, 961-967)

Metacaspases overview

multiple protein sequence
alignments –
(Bidle & Falkowski 2004)

Trichodesmium



Key sequence similarities to yeast metacaspase Mca-1, a caspase-like enzyme that mediates PCD in aging cells .

```
ZP_00070972 1 ----MRIBALVVGINEHVFEPG-----LNLKAPVKDAEAIAEMLEKYCNFHVQGLP----
ZP_00073861 1 MDKKNLRQALVVGINRYPLLLKKKKLGDNLNKAAPKDAEAIAANILEKYCKFRIQRLPSLPS
ZP_00074337 1 --MTNQNIATITIGVQVEYEFLLTP-----LKAYAANDAKKMRDFLLDEADEDDVFYLS---
ZP_00073436 1 ----MTNKAITIGVQKYQFFSP-----LKAYAANDAEKMRNFLLDEAGEDEVLYYS---
ZP_00075051 139 -QPIKNRWAFLVGVNRYNDPGVH----K-LKFCVKDVQKLEKKLKELCYTMVCLHD----
ZP_00073782 1 -----YAVVIGVGDDLPIITI-----D-DATAIANVIGDLSRCAYP-----
ZP_00073655 344 ----SHNYALLIGVGDYKYPDWS-----LPVTVKDVQAISKSLTNPDLCSYID-----
ZP_00071249 29 ----MERRRALLIGITIFGEGLDN-----FSPTLEDVQVMKKCLEKCKGFEVIQ-----
YeastMCA-1 154 --TGRRKALLIGINYYIGSKNQ-----LRGCINDAHNIFNFLTNGYGYSSDD-----
```

histidine- and cysteine-containing catalytic diad found in true caspases (p20 subunit), paracaspases, metacaspases

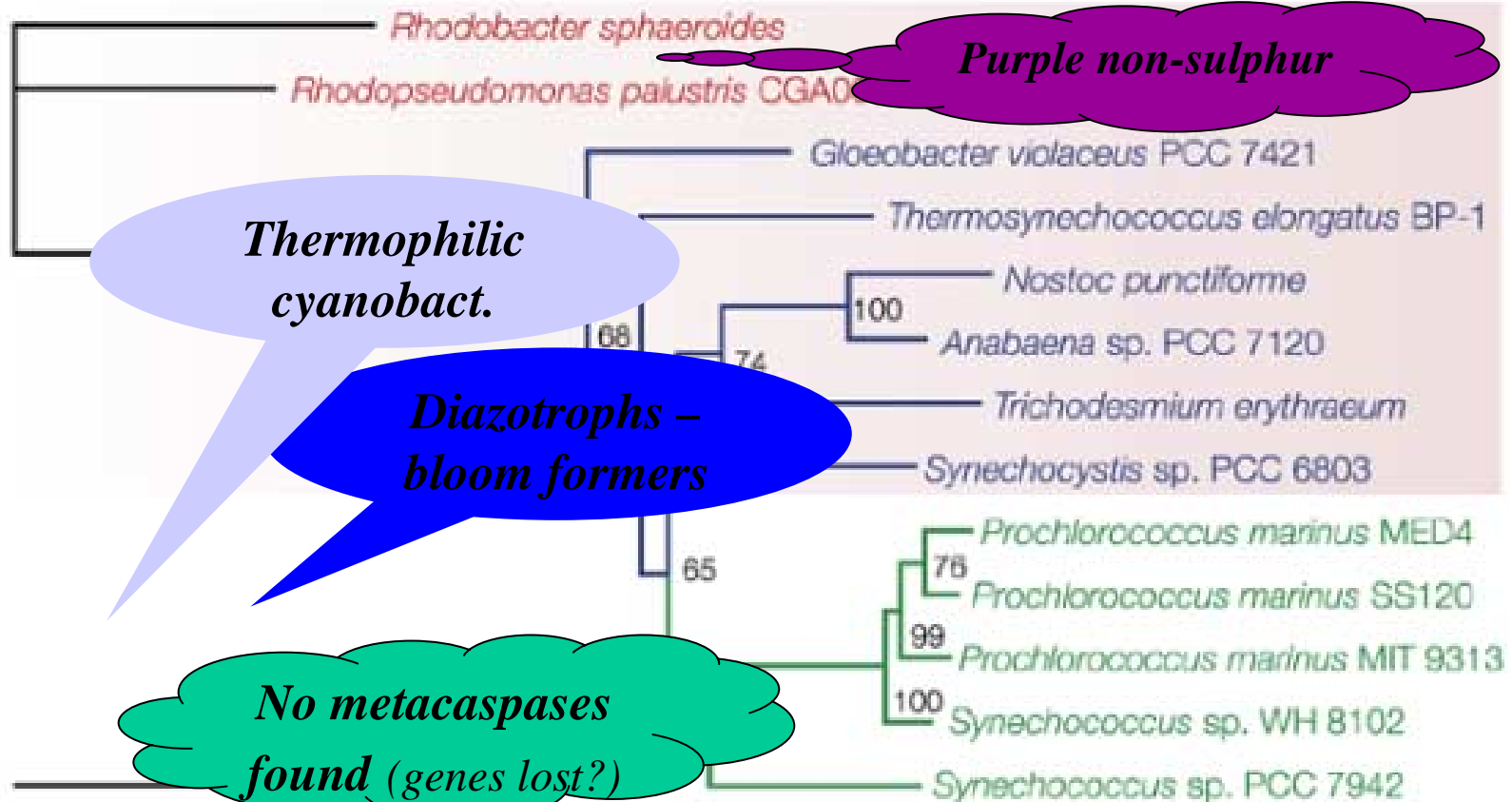
```
ZP_00070972 48 --KDYDITGRERFISDGFVYRKNLKIKVSNLFNPISKNEIPDVALFFFAHGCFVTTTEGGV
ZP_00073861 61 LPKNYDQEGTERFDPKGGVKINELQEAIIINLFKPRKKNETPDVALFFFAHGGYVDEKGDII
ZP_00074337 49 -----DNSPKINGASTRPTRSRIELVLEDEVKKLKSLK-TGDNLWFFFSGHCHRENNN--
ZP_00073436 47 -----DYSPHINGDYTRPTRSNLEFLLENQFKEPFMG-IGDNWFFFSGHGGLRENG--
ZP_00075051 189 -----DLESLSR---RFPIHNNIEAELINLCNMVDVD---DILLVYFACHGCKI
ZP_00073782 35 -----TDQVRLLTG-EKANRANVLSALSWSLAETTQKD---DTAIVYFSGHGII
ZP_00073655 388 -----DENHLRLLCNEQATKQNILNNINWLQEQAKND-PEATILVYYSHGWLDRKST--
ZP_00071249 74 -----E-----ENLDRKDMEYAINKFFYESQKS---DTLLIYVSSHGITDAEG--
YeastMCA-1 198 ----IVILTDDQNDLVRVPTRANMIRAMQWLIVKDAQPN---DSLFIHYSGHGGQTEDDLDC
```



```
ZP_00070972 106 REGFLVTSADV---QLKRDYIGISLSWLKDLLRQSPVKQIIVWLDCCFGGELLNSQ---
ZP_00073861 121 REGFLATSEAA---HLSENVYGISLNLKRLQDSPVQEQIIVWLDCCFSGEFLNFDRE-
ZP_00074337 100 -IDYLPIDG---HSNVERSGISVDYIIQQLQKSGADNIVLILDA CRNKSDGGKGG--
ZP_00073436 97 -IDYLPVLDG---YKNVQKSGISVNYIIQQLQKCGADNIVLILDA CRDEGDARRGK-
ZP_00075051 235 QPMLIVRDTR---LPTIEKTGLSLADIKKSMHSSKARRLVLTLLDACHMGEVETGRGID-
ZP_00073782 82 -YYLMPYGYN---LENLEGTAIRGETFTECLRAIKTKLLVLLDCCHAGGQADPKGII
ZP_00073655 439 EKYVLIIPHDTSPIKQKTQKTALPATDFNNALQEISAQKLLVIIDSCHAQGMATAKE--
ZP_00071249 113 -TFYIATSHTSYDRDTEIITGATEASYFHRQIKNCTSNSQVWLLDLCYSCAFVVRGYSK-
YeastMCA-1 251 DEEDGMDDVIYPVDFETQGPIDDEMHDIMVKPLQQGVRLTALFDSCHSGTVLDDLPT-
```

16S rRNA – Potential metacaspase inheritance

(Bidle & Falkowski 2004. Nature Rev. Microbiol.)



Conserved PCD domains detected in *Trichodesmium*

Domain description	Domain function	<i>T. erythraeum</i> protein sequence
DegQ trypsin-like serine protease HtrA-like protease	Posttranslational modification, protein turnover, chaperones	ZP_00072870 ZP_00073979 ZP_00071776
Predicted NTPase (NACHT family)	Signal transduction	ZP_00073901 ZP_00073386 ZP_00071486
AP-ATPase, NB-ARC domain	Shared by plant resistance genes and regulators of cell death in animals	ZP_00072978
WD-40 domain	Eukaryotic proteins Adaptor/regulatory in signal transd., pre-mRNA processing	ZP_00070972 ZP_00073861

Evidence for PCD in *Trichodesmium*:

- **MORPHOLOGICAL:** SYTOX, TEM, TUNEL
- **PRESENCE OF PCD COMPONENTS:** metacaspase similarity, DEATH Domains
- **SYNTHESIS AND ACTIVITY OF CASPASE-LIKE PROTEINS:**
 - A. Westerns – human caspase-3
 - B. Activity increased with stress and age of culture
 - C. Activity inhibited by caspase-3 inhibitors

From Lab To Sea.....



- **Project DIAPAZON –**
- **New Caledonia**

Objective

- **DOES PCD OCCUR IN NATURAL POPULATIONS UNDER ENVIRONMENTAL STRESS?**



Looking for *Tricho*....



Le vent chasse les *Tricho*....



Plan A:
GONE WITH THE WIND....

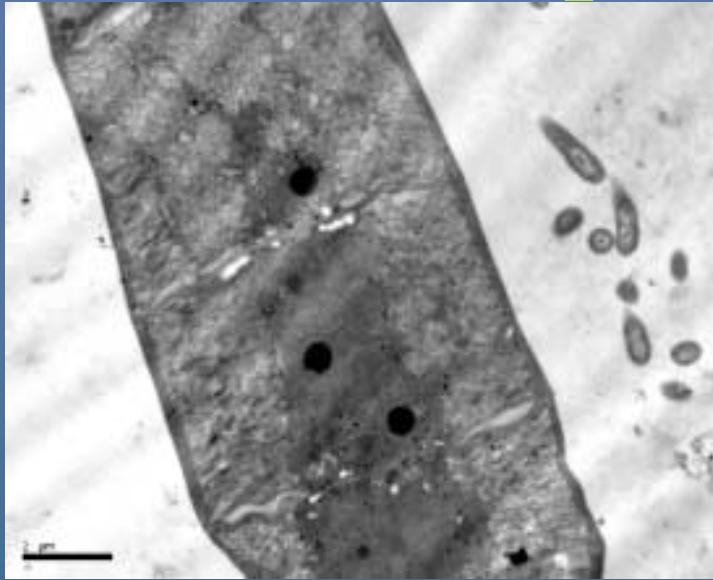
Plan B: Simulations!



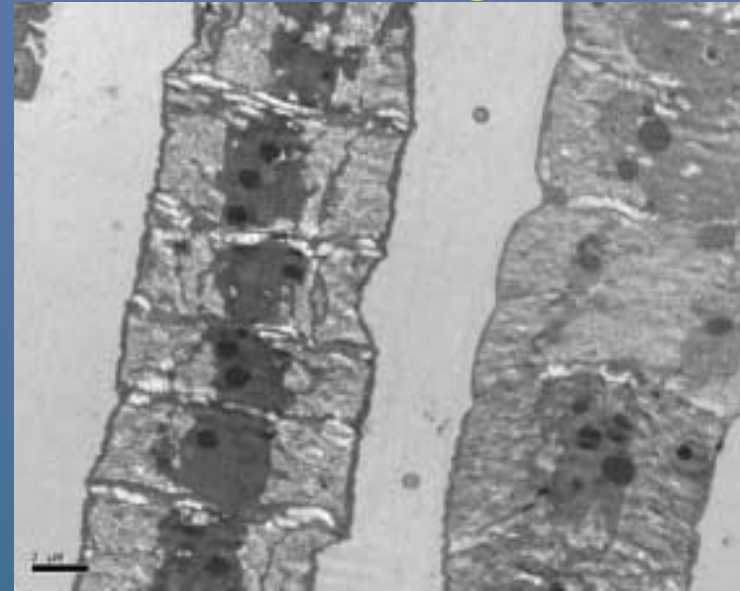
The Noumea experiments



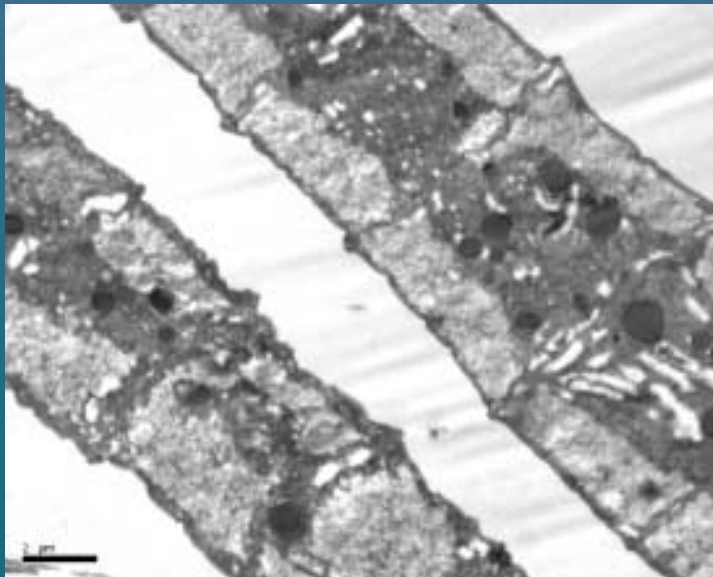
Morphological Changes



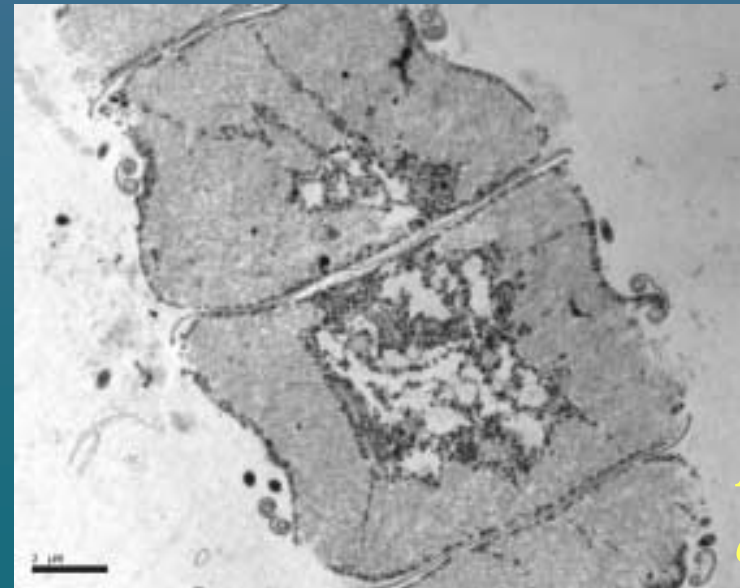
3 h



8 h



23 h



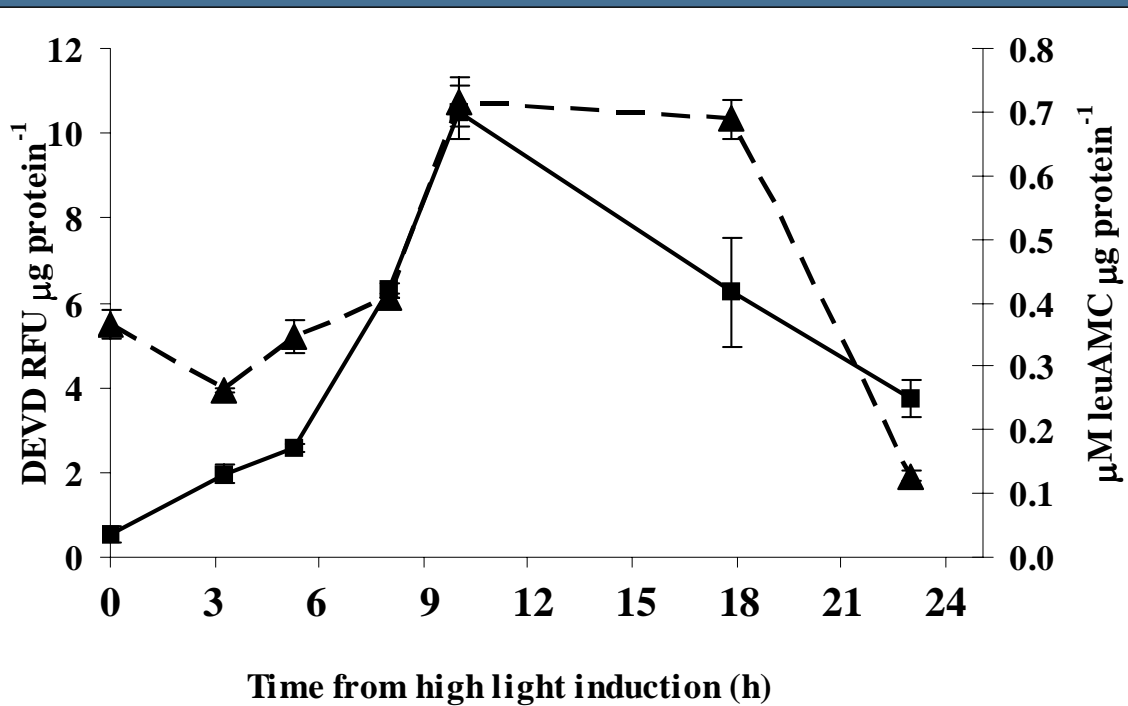
*Bottom
of flask*

DEVD and General Proteolytic Activity

A

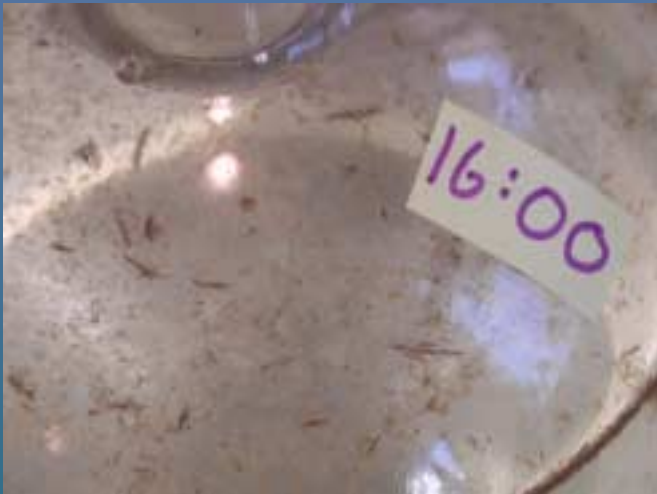


iii



OXIDATIVE STRESS INDUCES APOPTOSIS

(Chandra et al. Free Rad. Biol Med. 2000)



*Buoyant
trichomes*

$0.3 \pm 0.22 \text{ RFU } \mu\text{g protein}^{-1}$
range (0.04 – 0.7)

DEVD
ACTIVITY
T. erythraeum



*Sinking
trichomes*

$17.1 \pm 0.22 \text{ RFU } \mu\text{g protein}^{-1}$



**PCD and the Fate of *Trichodesmium*
Blooms ?**

Transparent exopolymeric particles (TEP) PRODUCTION AND BLOOM DECLINE

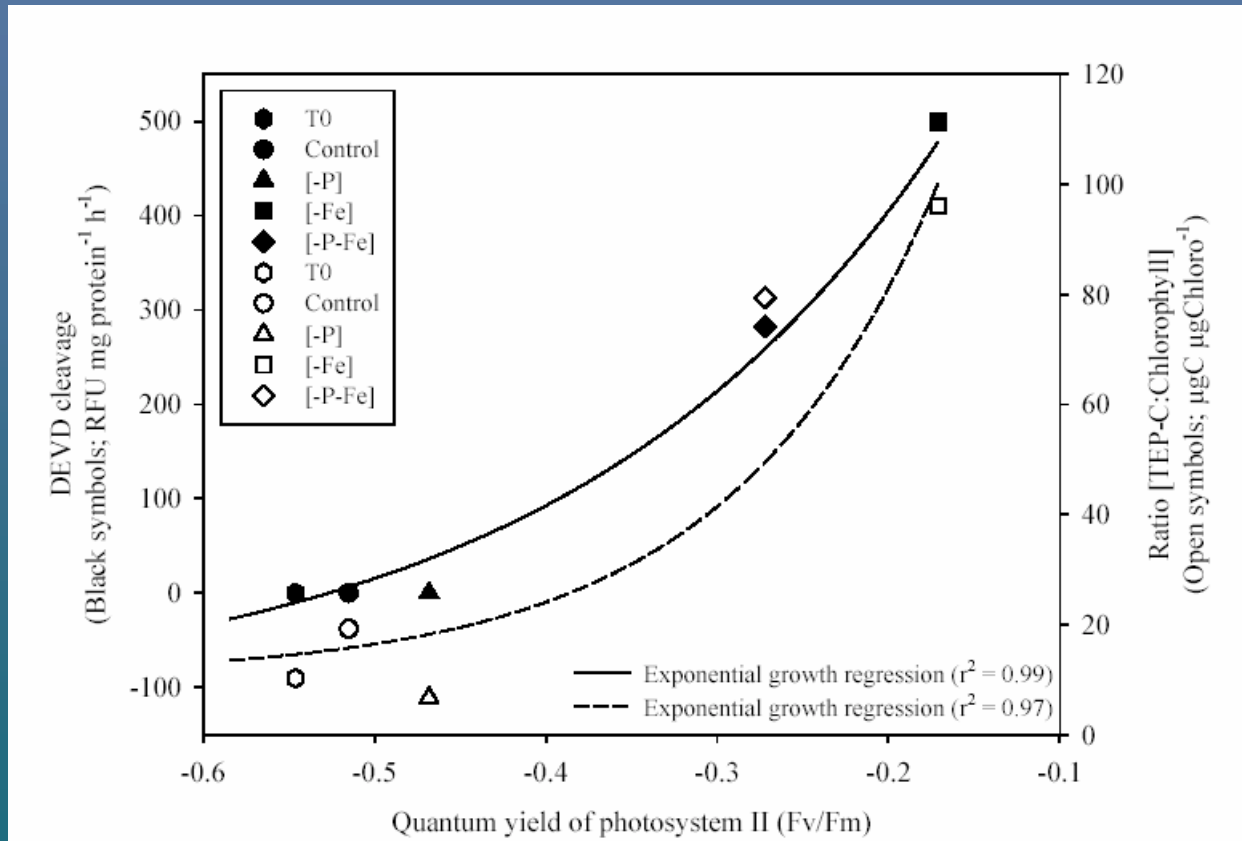
- Source of organic matter for sedimentation and biogeochemical recycling.
- Sorption sites for dissolved organic and inorganic matter.
- Attachment sites for bacteria.
- Food source for microphageous protozoans, for particle grazers such as copepods or for passive filter feeders such as tunicates,
- TEP can coagulate with other particles into marine snow aggregates, and enhance the vertical flux of major elements, and of adsorbed trace elements.

Hypothesis

- The production of TEP by *Trichodesmium* is positively coupled to the induction of a PCD pathway.

Coupling of TEP production with caspase activity and physiological state

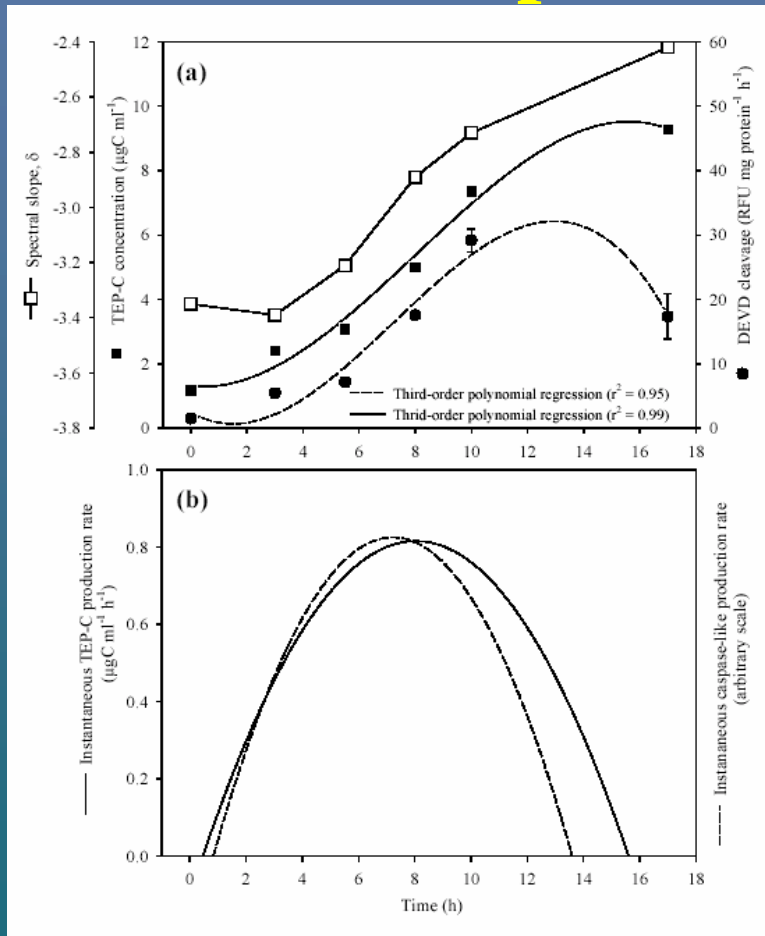
DEVD cleavage



TEP-C: ChlorophyllII

Fv/Fm

In natural and laboratory populations – coupling between PCD induction and TEP production



With PCD induction:

1. Increase in caspase activity
2. Increase in TEP-C
3. Increase in size of TEP particles

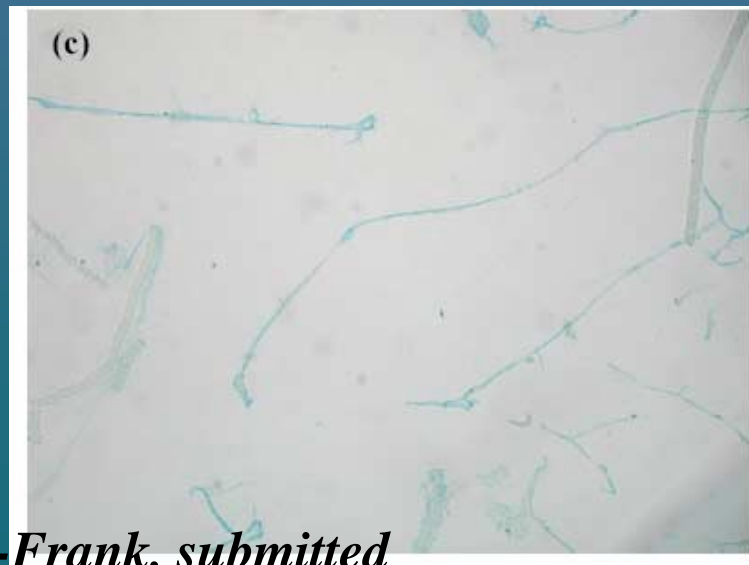
TEP production in *Trichodesmium*



2-4 h

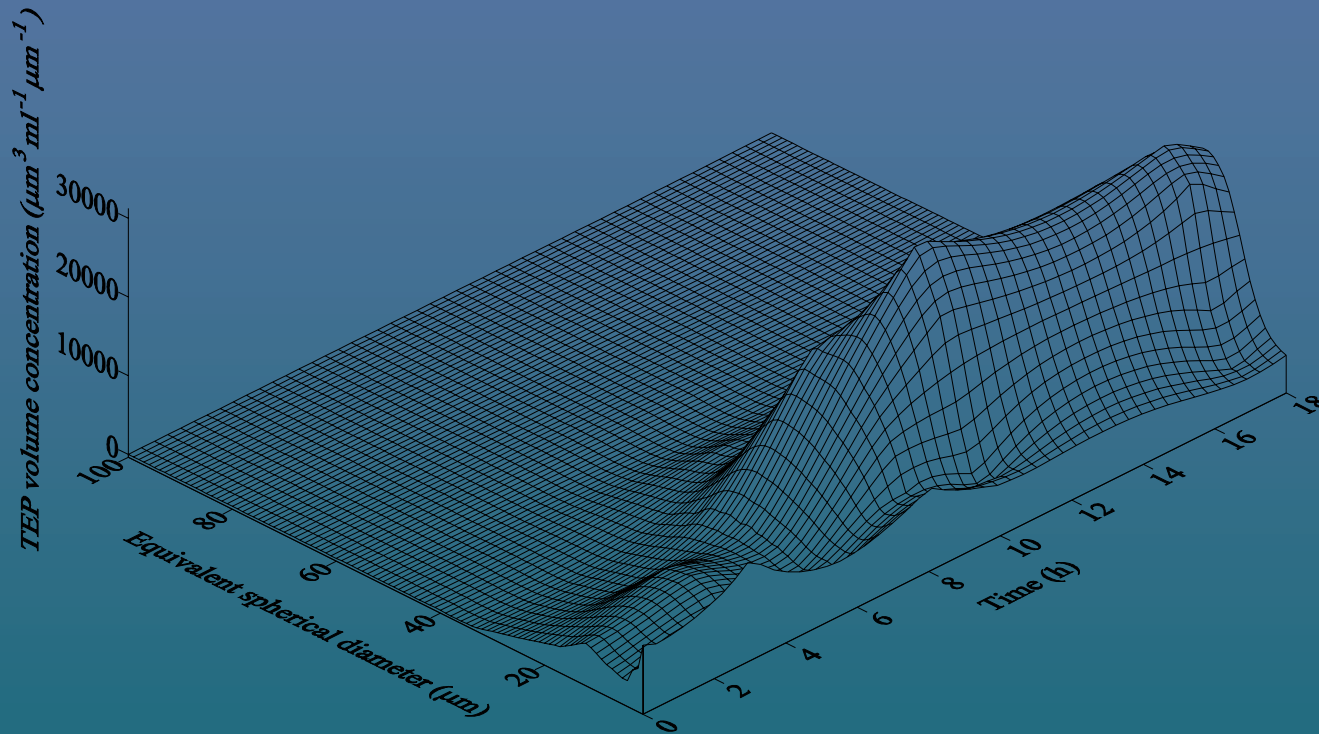


6-10 h



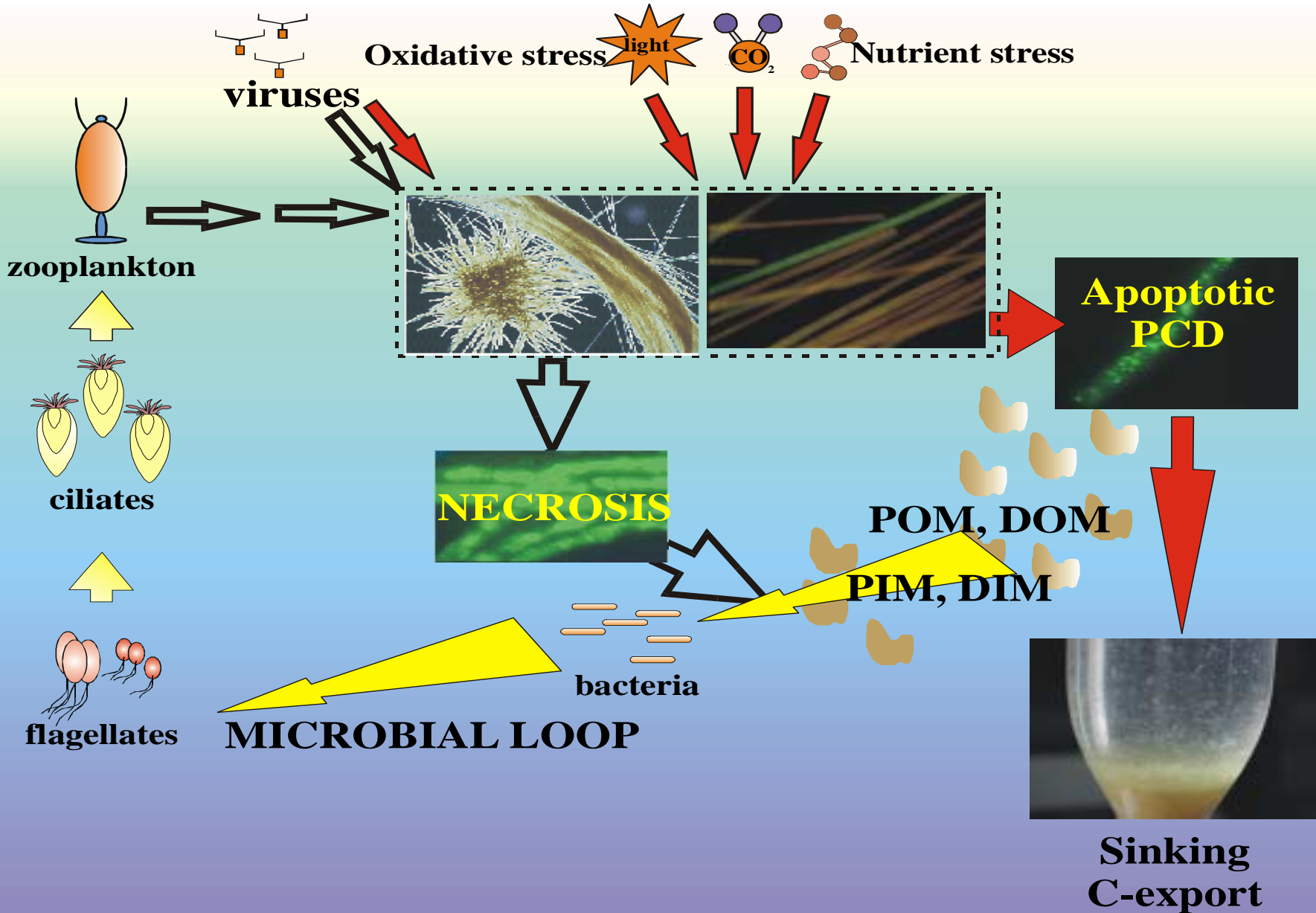
14-16 h

Change in TEP production with time



Mari, Haramaty, Berman-Frank. submitted

Implications: Bloom Mortality Pathways



Fate of *Trichodesmium* Blooms ?



Still looking!!!

Required research!!!
Follow a natural bloom development
and crash (preferably in a nice,
tropical paradise...).



Merci!!



- *C. DUPOUY,*
- *A. LE BOUTEILLER,*
- *THE WHOLE TEAM OF THE DIAPAZON PROGRAM, IRD-NOUVELLE CALEDONIE*
- *V. STAROVOYTOV: TEM analysis, RUTGERS U.*