Generation of ROS (reactive oxygen species) by *Chattonella marina* as a possible factor responsible for the fish-killing mechanism

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In Japan, HABs due to *C. marina* and *H. akashiwo* often cause mass mortality of aquacultured fish, especially yellowtail

Recent *Chattonella* red tide

Chattonella **is known to be highly toxic to fish, especially yellowtail. Recent** ***Chattonella*** **red tide seems to be involved in the mass mortality of shellfish**

Proposed toxic factors of *Chattonella*

I. Toxins: hemolytic toxin, polyunsaturated fatty acids, neurotoxins (brevetoxins)
II. Mucus substances
III. Reactive oxygen species (ROS)
IV. Still unknown toxins

Ruptured *Chattonella* cell suspension has no toxic effects

Major Questions

(1) Is *C. marina* really producing ROS?

(2) How about other raphidophytes?

(3) Why is *C. marina* producing ROS?

(4) Is ROS involved in fish-killing by *C. marina*?

(5) What is the mechanism of ROS production in *C. marina*?

(6) What is the ROS-mediated toxic mechanism of *C. marina*?

(7) Are there any other possible toxic factors?

Is *C. marina* really producing ROS?

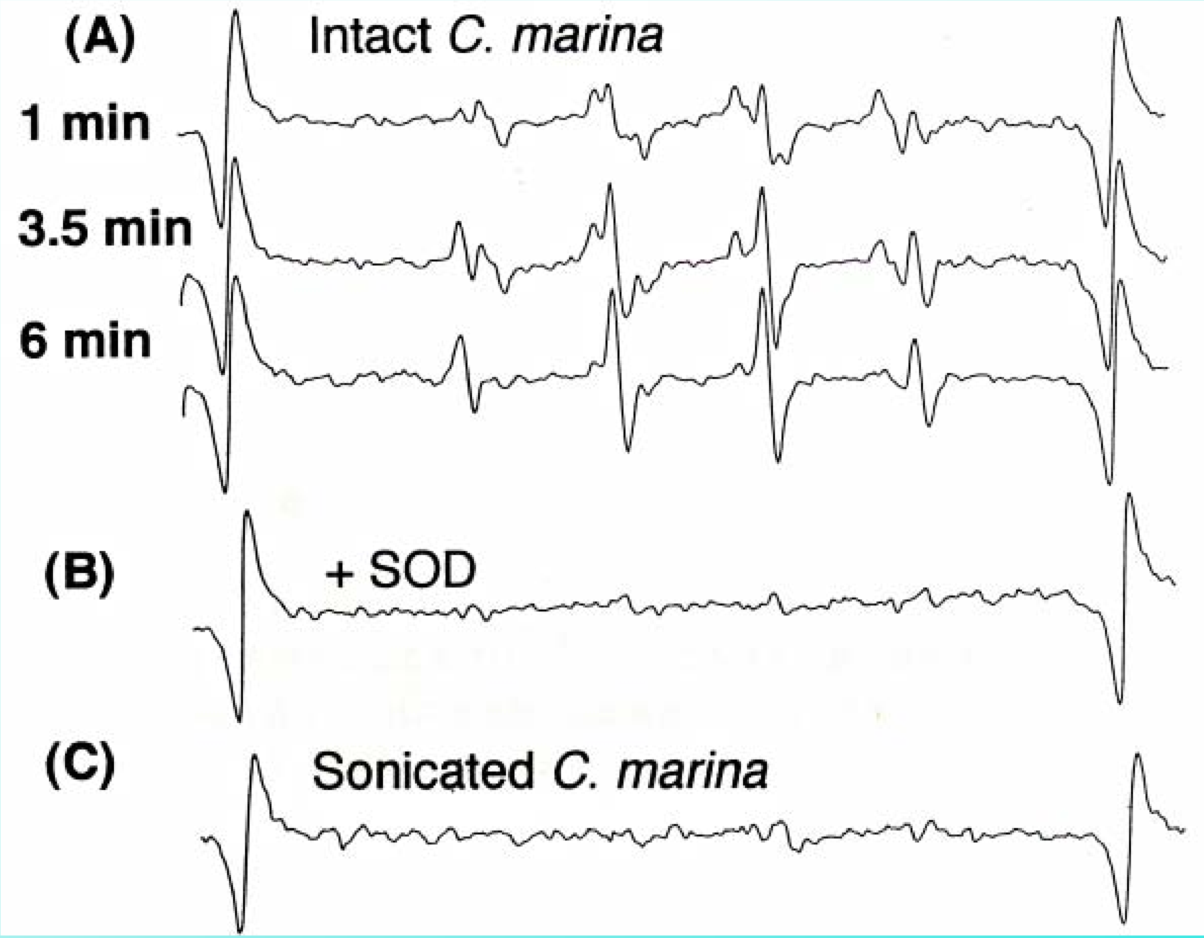
Detection of ROS in *C. marina* cell suspension by various assay methods

Cytochrome c reduction assay: +

Chemiluminescence assay: +

ESR spectroscopic assay (the most reliable method): +

Detection of ROS in *C. marina* cell suspension by ESR spectroscopy



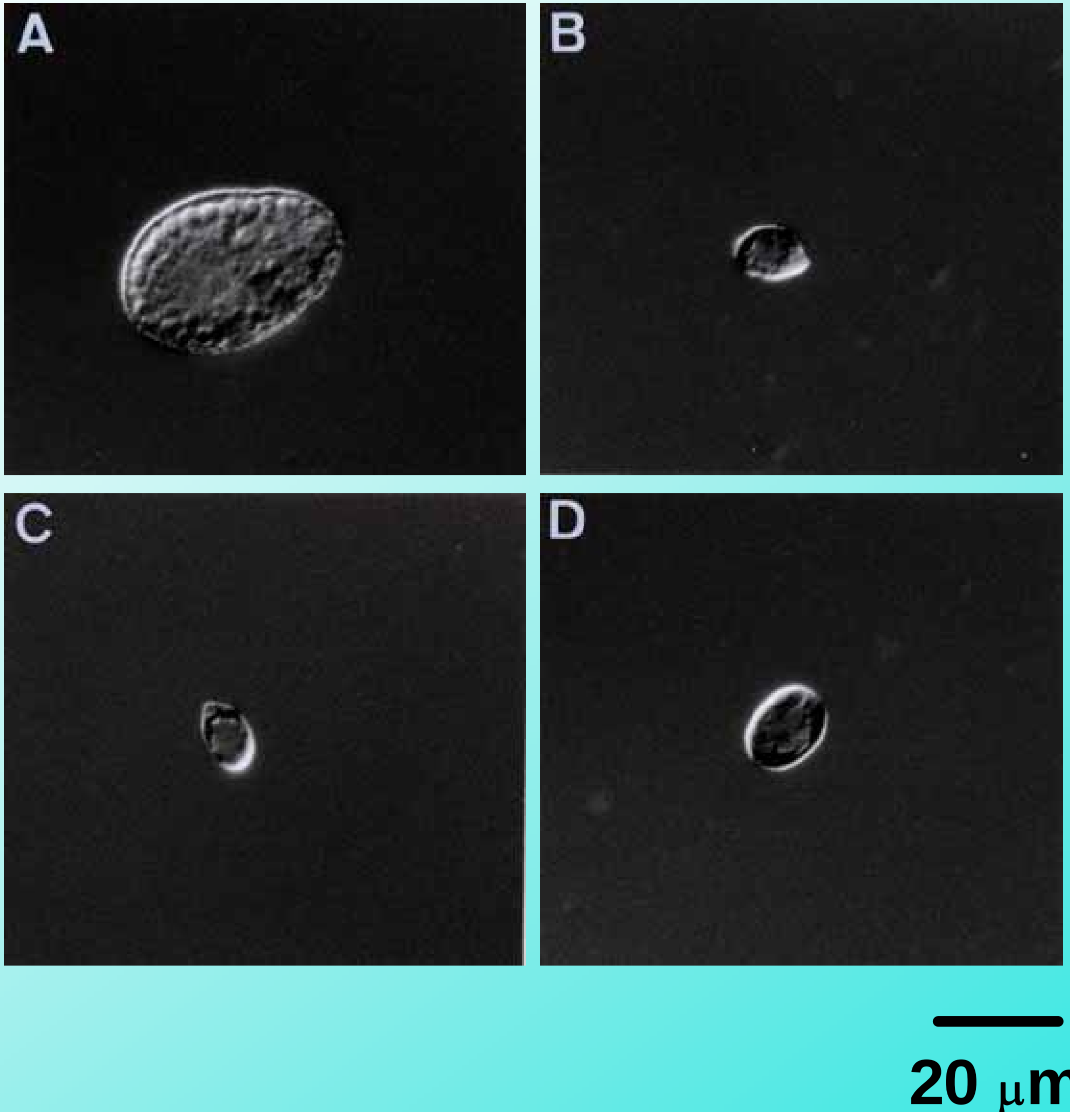
***C. marina* continuously produces ROS (mainly superoxide)**

Ruptured cell cannot produce ROS anymore, meaning that intact live condition is essential for the effective ROS generation

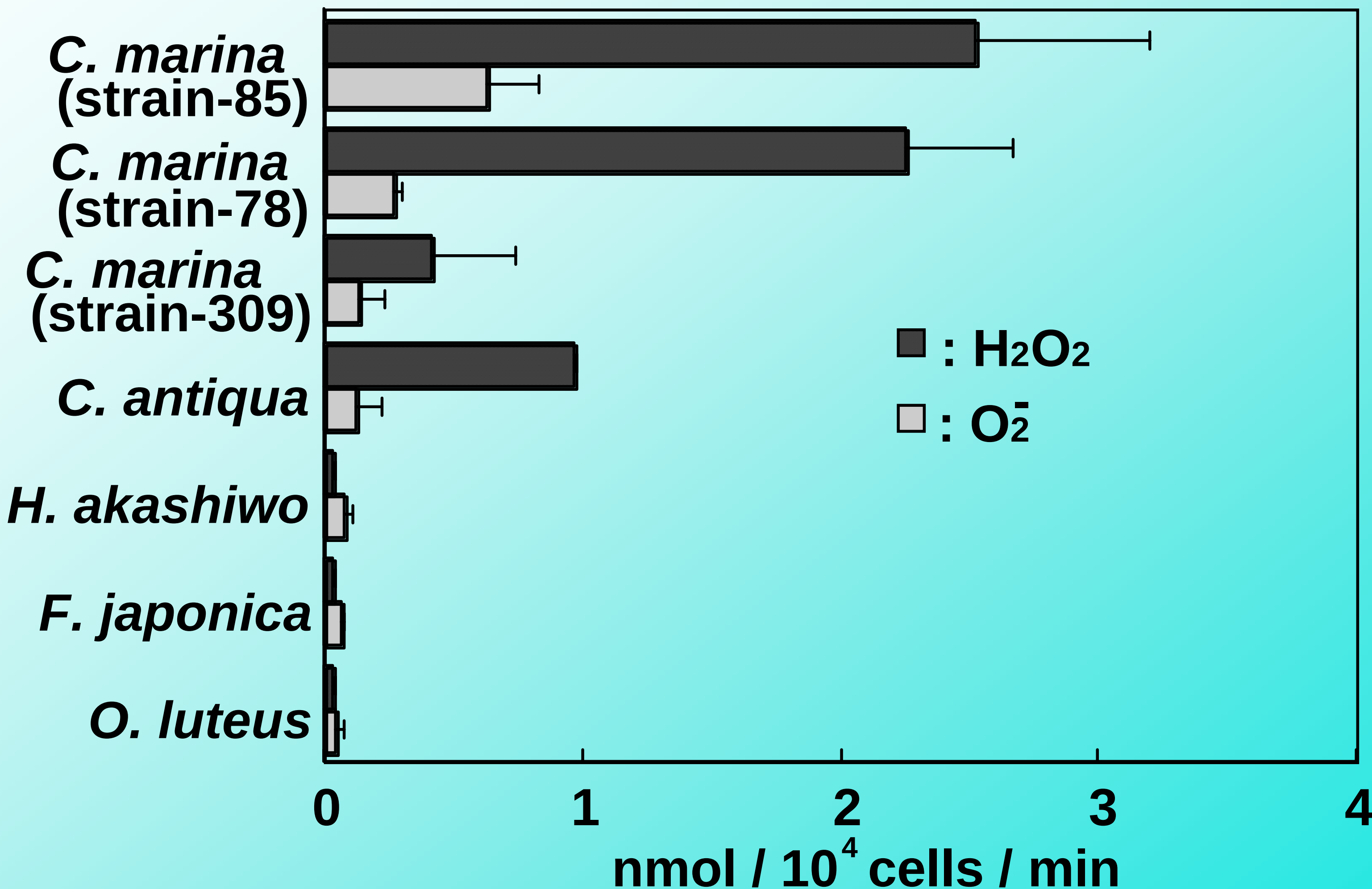
How about other raphidophytes?

Is ROS production a common feature among raphidophytes?

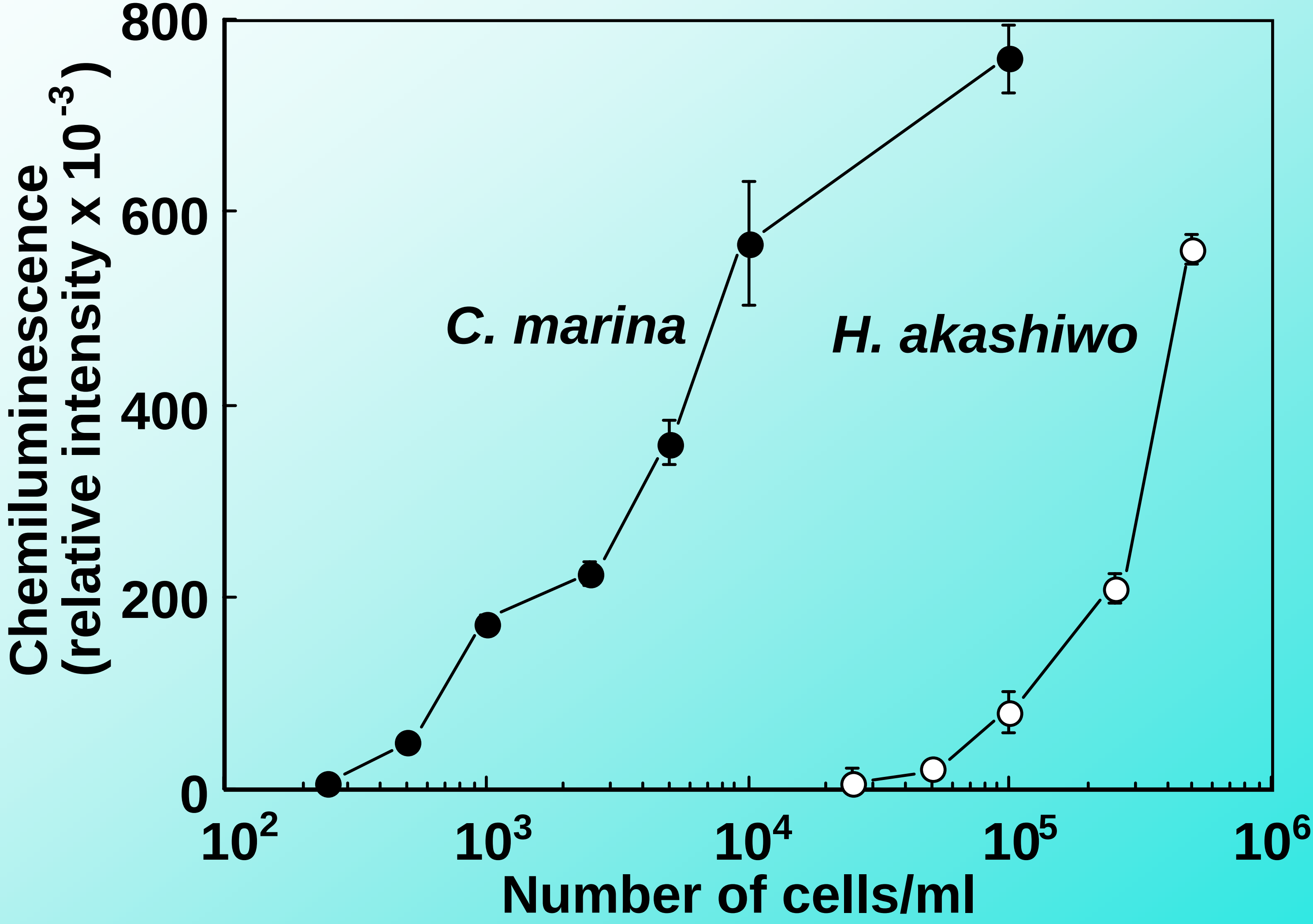
(A) *C. marina*, (B) *O. luteus*, (C) *H. akashiwo*, and (D) *F. japonica*



ROS generation by raphidophycean flagellates



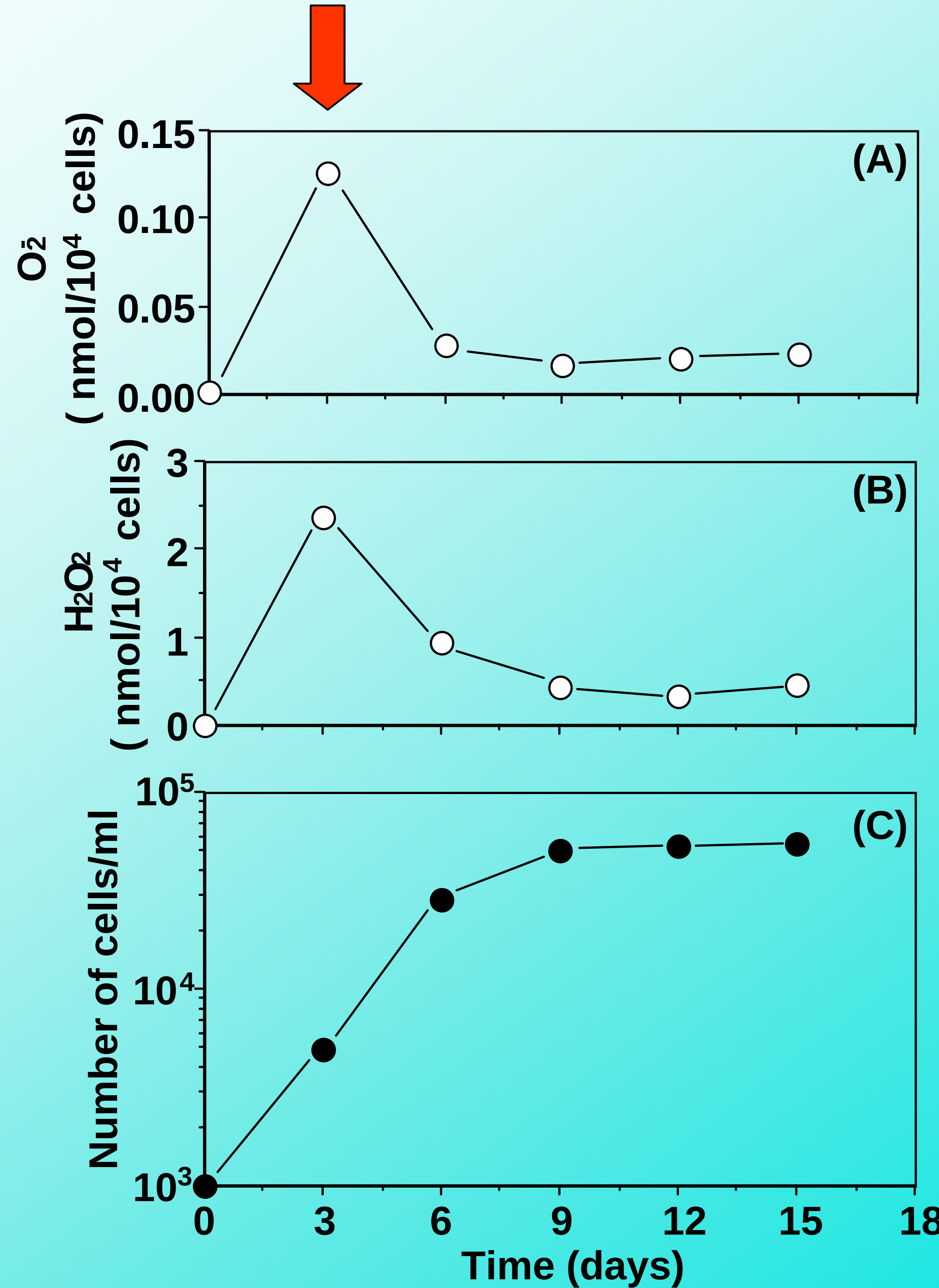
Cell density dependent ROS generation by *C. marina* and *H. akashiwo*



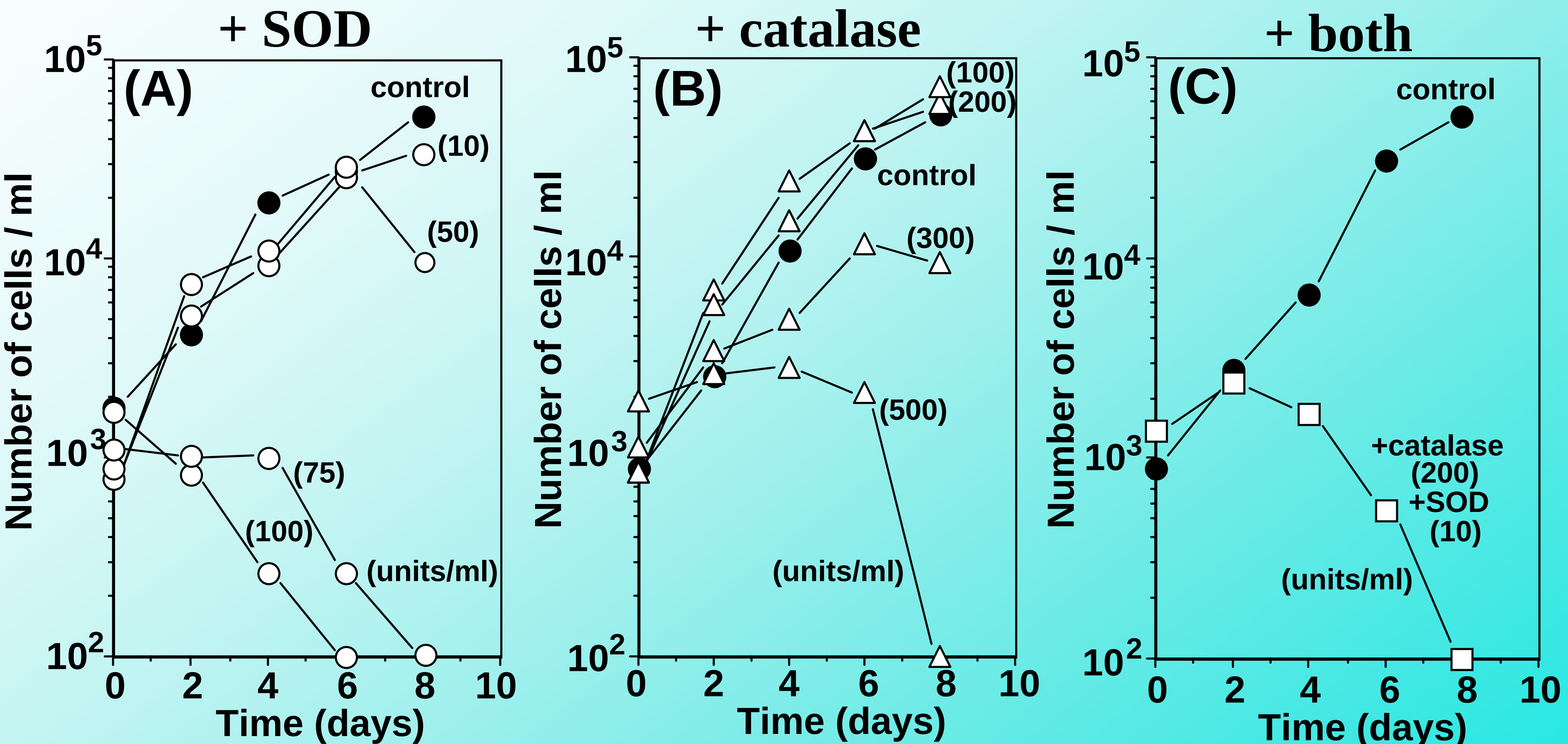
ROS levels in raphidophytes are quite different, even in *C. marina*, different ROS levels are observed among the strains

Why is *C. marina* producing ROS?

The generation of ROS became the highest during the exponential growth phase



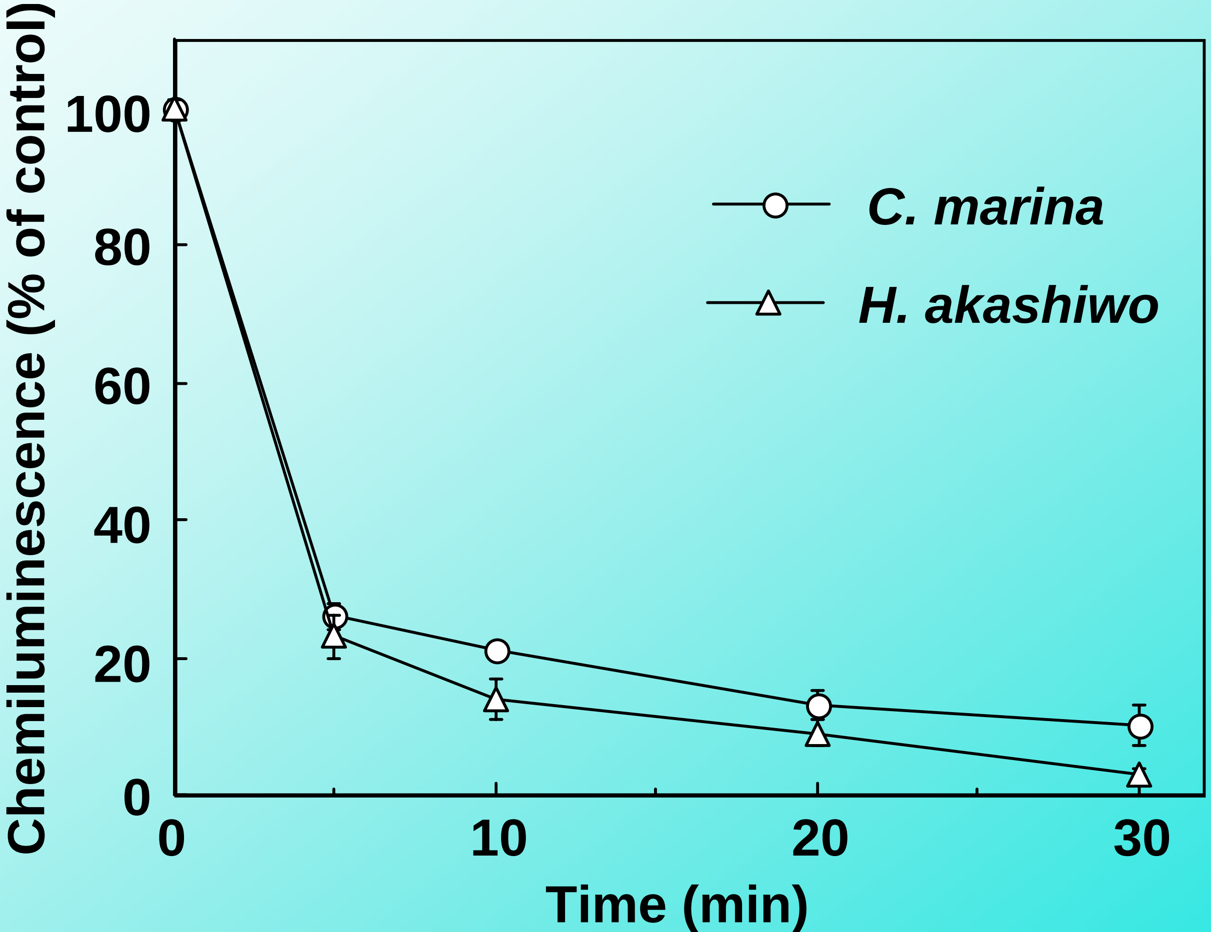
The growth of *C. marina* was inhibited by SOD and catalase



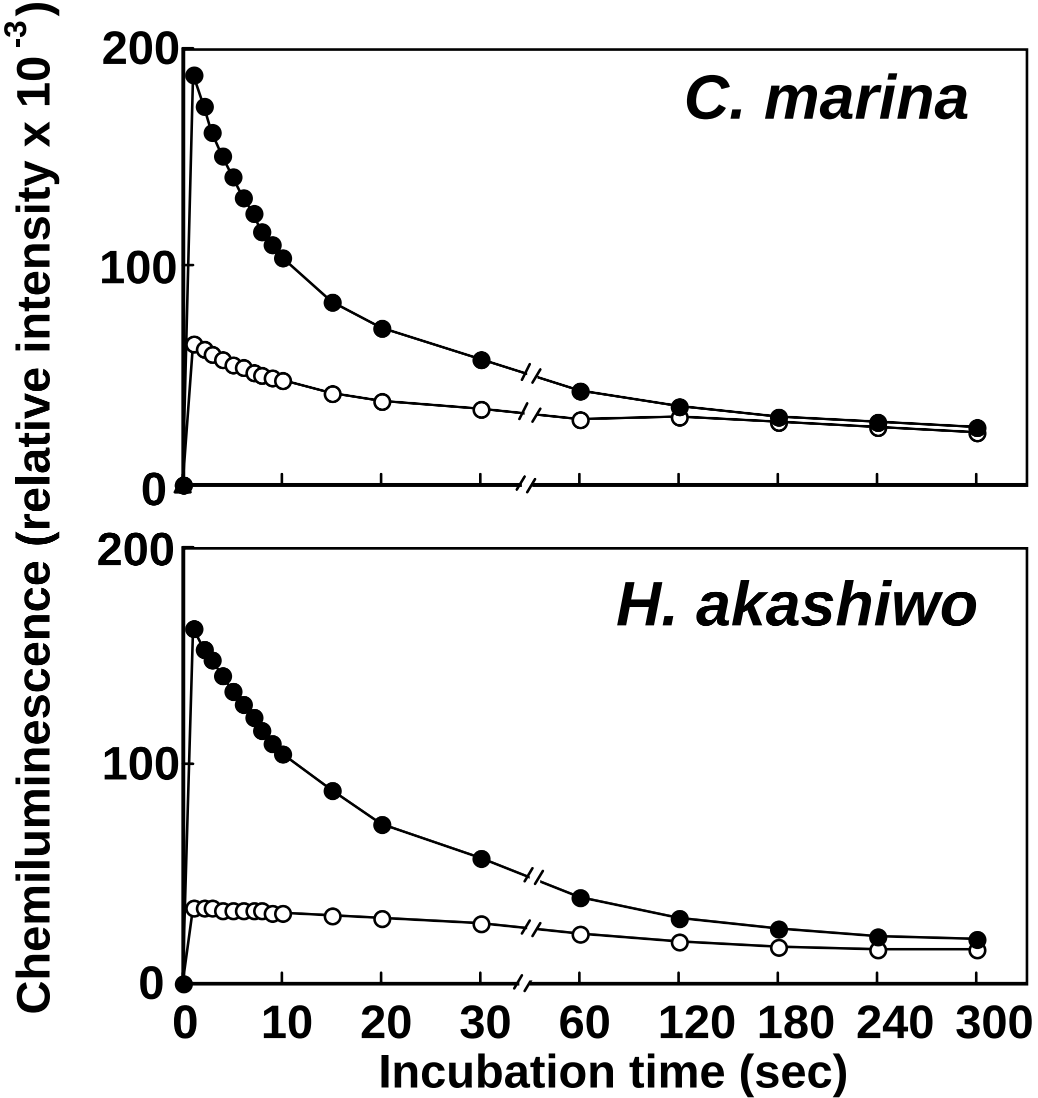
ROS generation by *C. marina* is deeply linked with the metabolic potential and may play an essential role in the own survival rather than defense purpose

What is the mechanism of ROS production in *C. marina*?

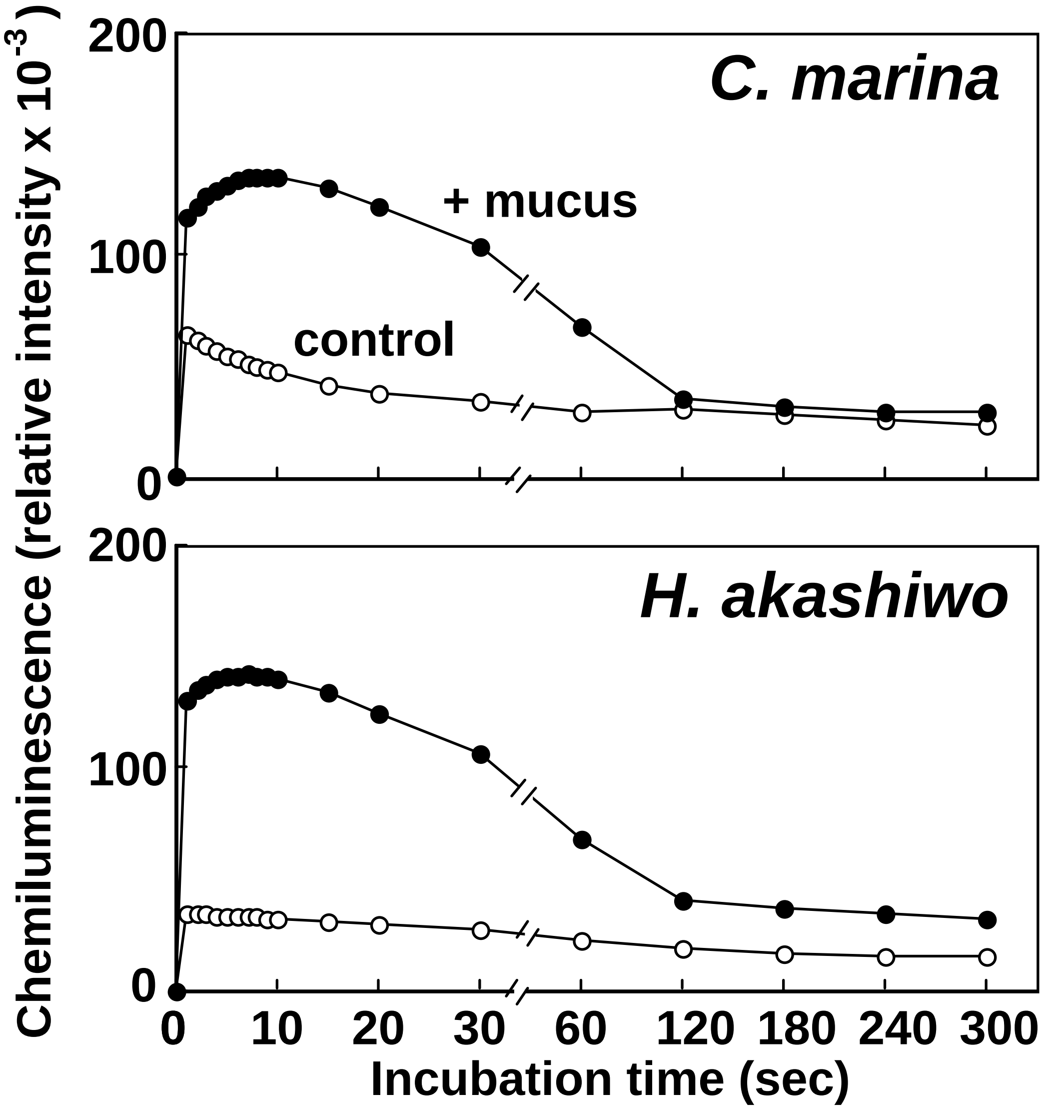
Protease (proteinase K) inhibits ROS generation by *C. marina* and *H. akashiwo*



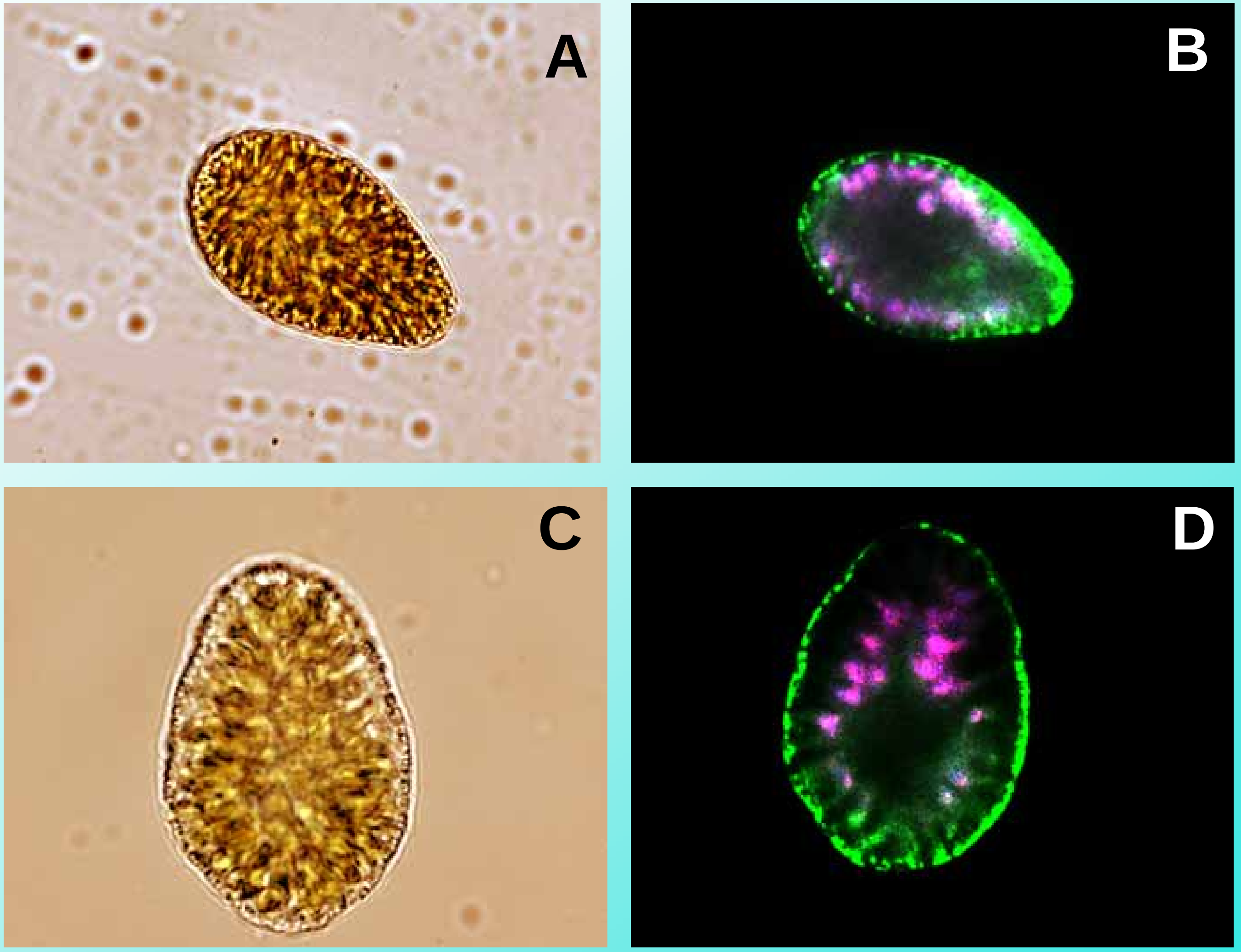
Lectin (Con A) stimulates *C. marina* and *H. akashiwo* to induce increased ROS generation



Gill mucus from yellowtail stimulates *C. marina* and *H. akashiwo* to produce increased levels of ROS



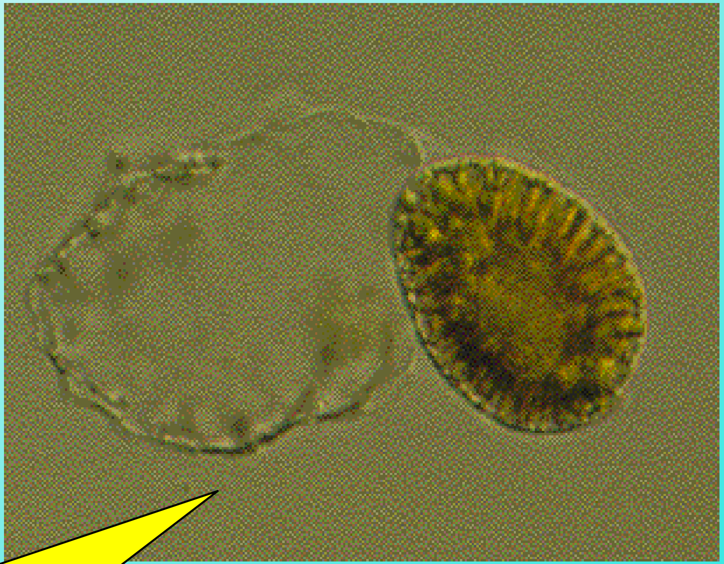
Fluorescence microscopic observation of *C. marina* (A, B) and *C. ovata* (C, D) after incubation with MCLA as a specific fluorescent probe for superoxide



Cell surface structure of *Chattonella*: Glycocalyx

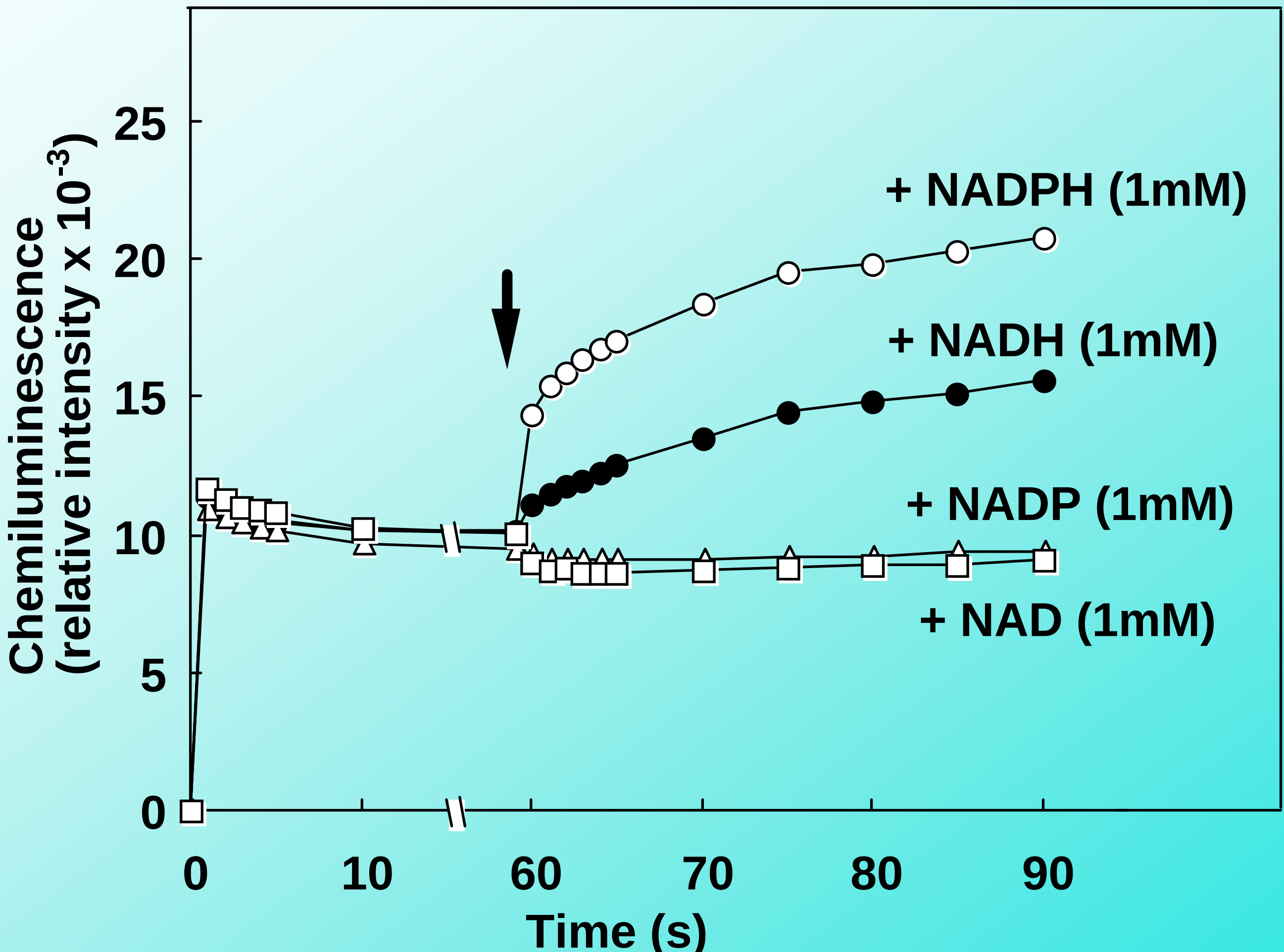
Normal condition

Physical stimulation: vigorous agitation or ultrasonic



Discharged glycocalyx

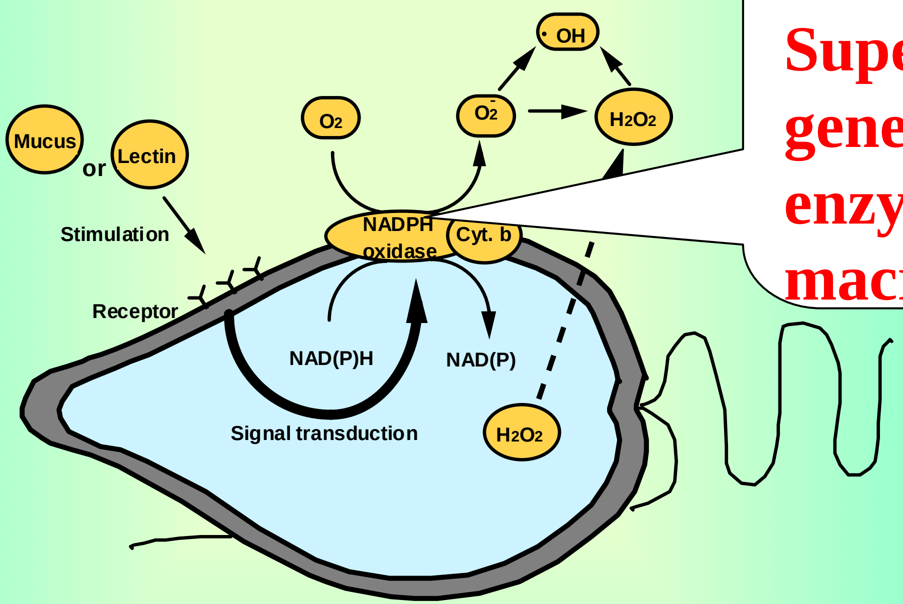
Presence of NADPH-dependent ROS generation system in Glycocalyx



Enzymatic system located on the cell surface of *C. marina* is responsible for ROS generation, and the activity can be increased by the stimulation of lectin or gill tissue mucus

Superoxide generating enzymatic system on *Chattonella* cell surface

Generation systems of reactive oxygen species (ROS) in *C. marina*



Similar to Superoxide generating enzyme in macrophage

What is the ROS-mediated toxic mechanism of *C. marina*?

Glycocalyx, cell surface structure of *C. marina*

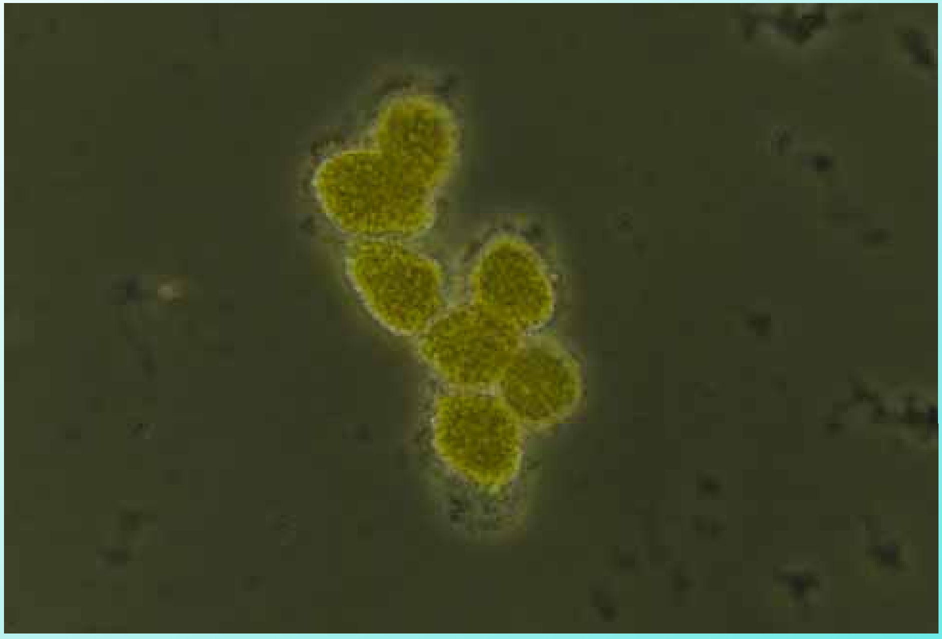
Location of ROS generation system in *C. marina*

Involvement of glycocalyx in the fish-killing mechanism

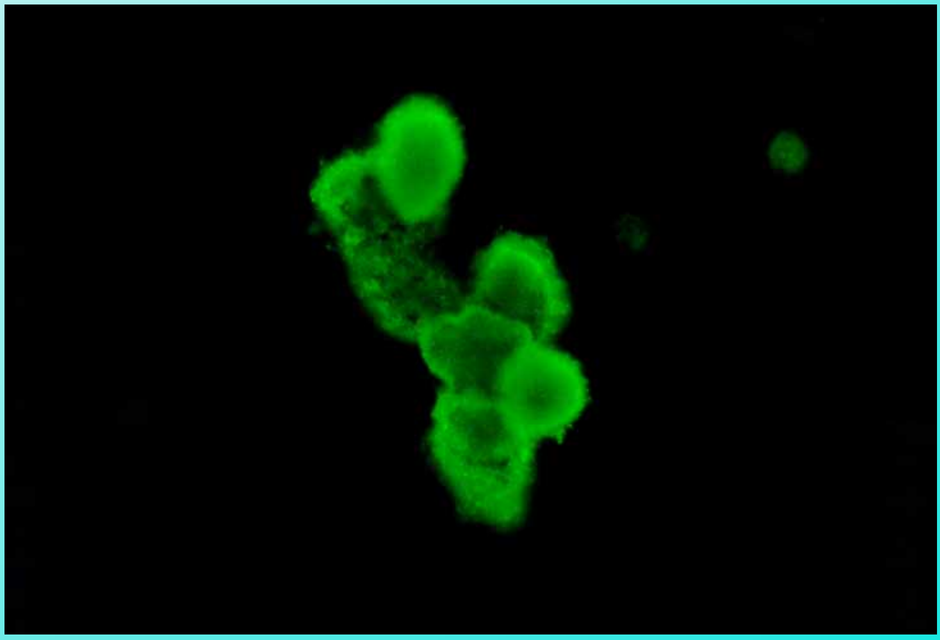
Glycocalyx can easily discharged from *C. marina* under the physical or chemical stimulations

Indirect immunofluorescence staining of *C. marina* with anti-glycicalyx antiserum and FITC-labeled secondary antibody

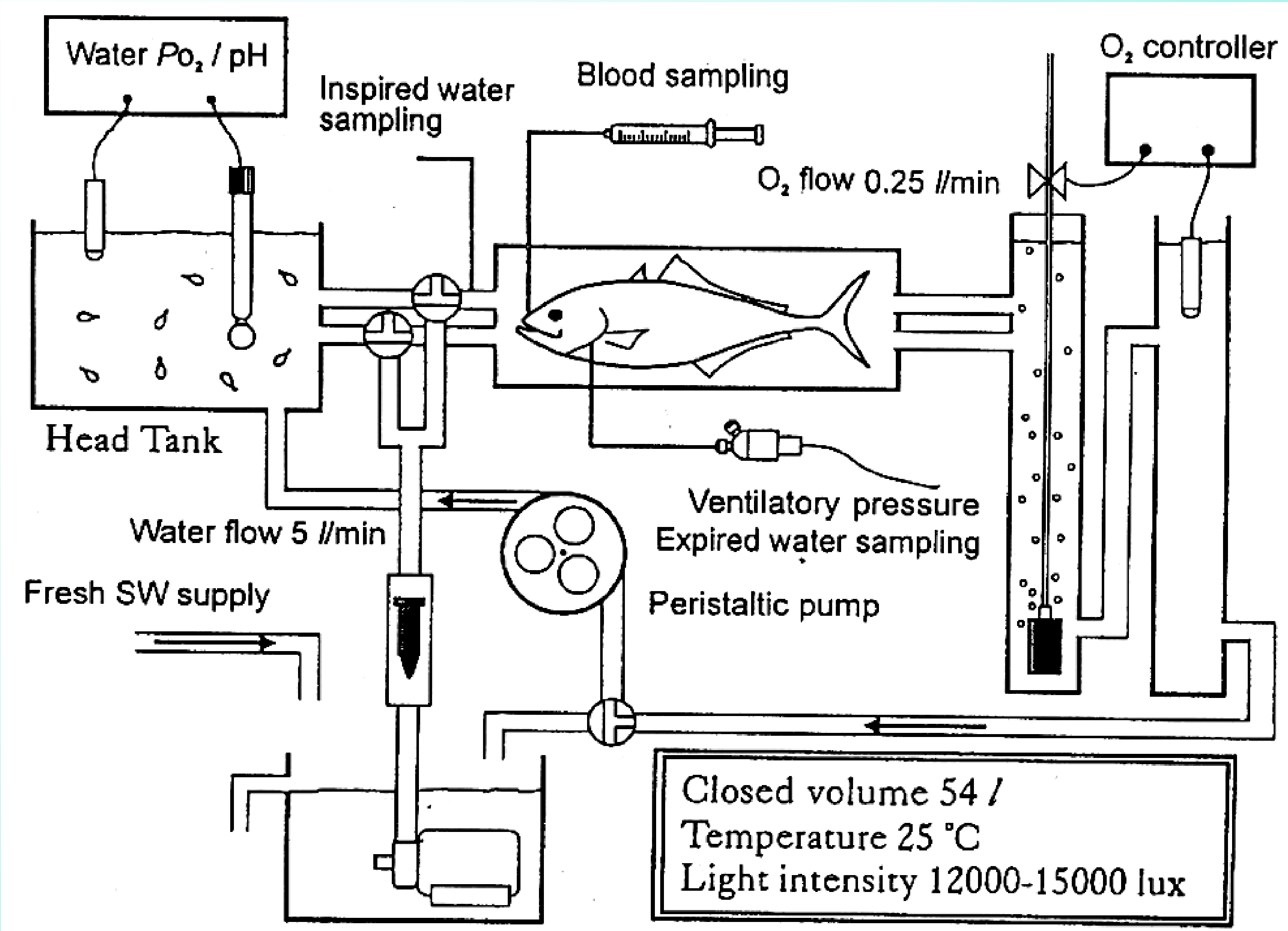
Phase-contrast



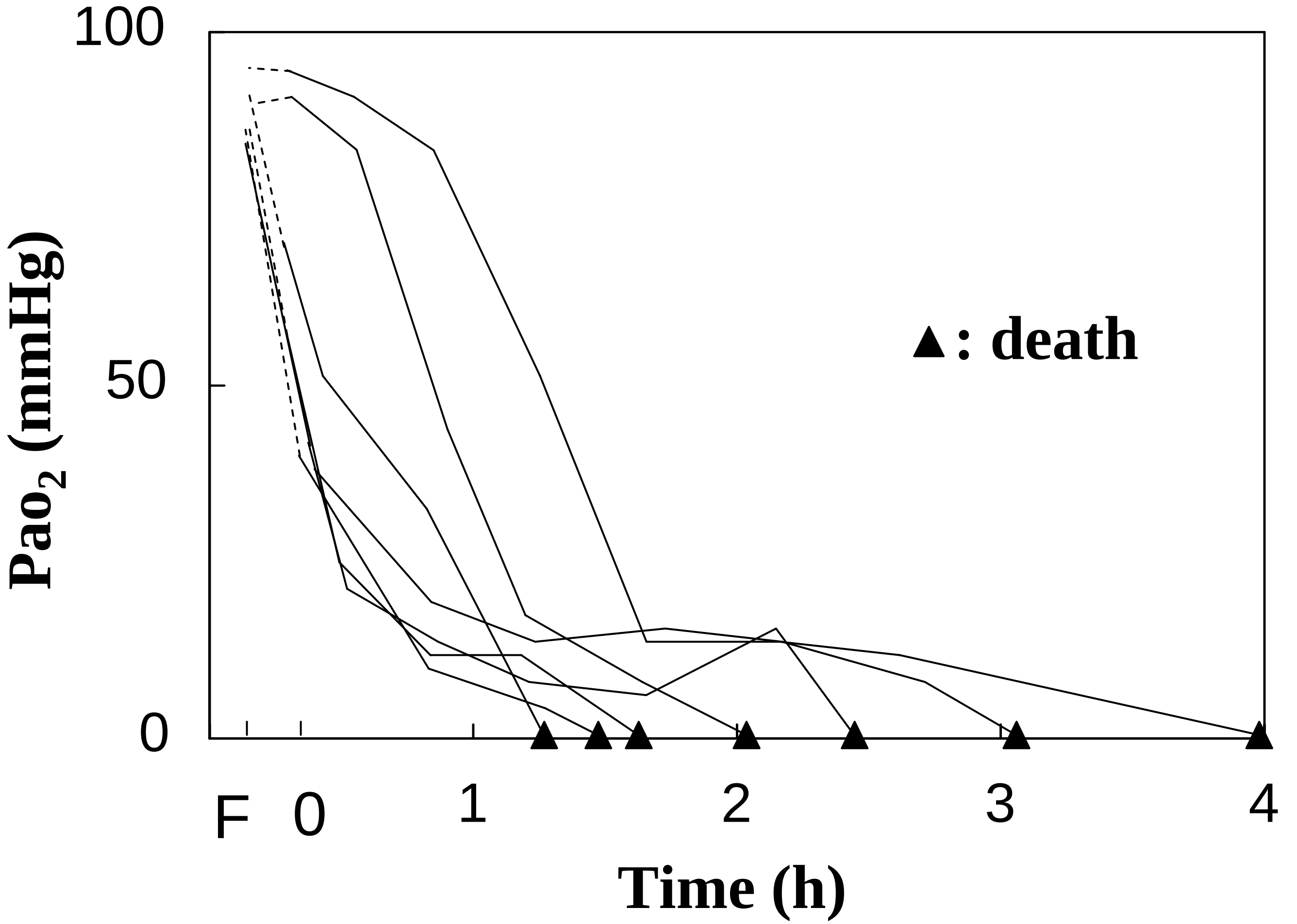
Fluorescence



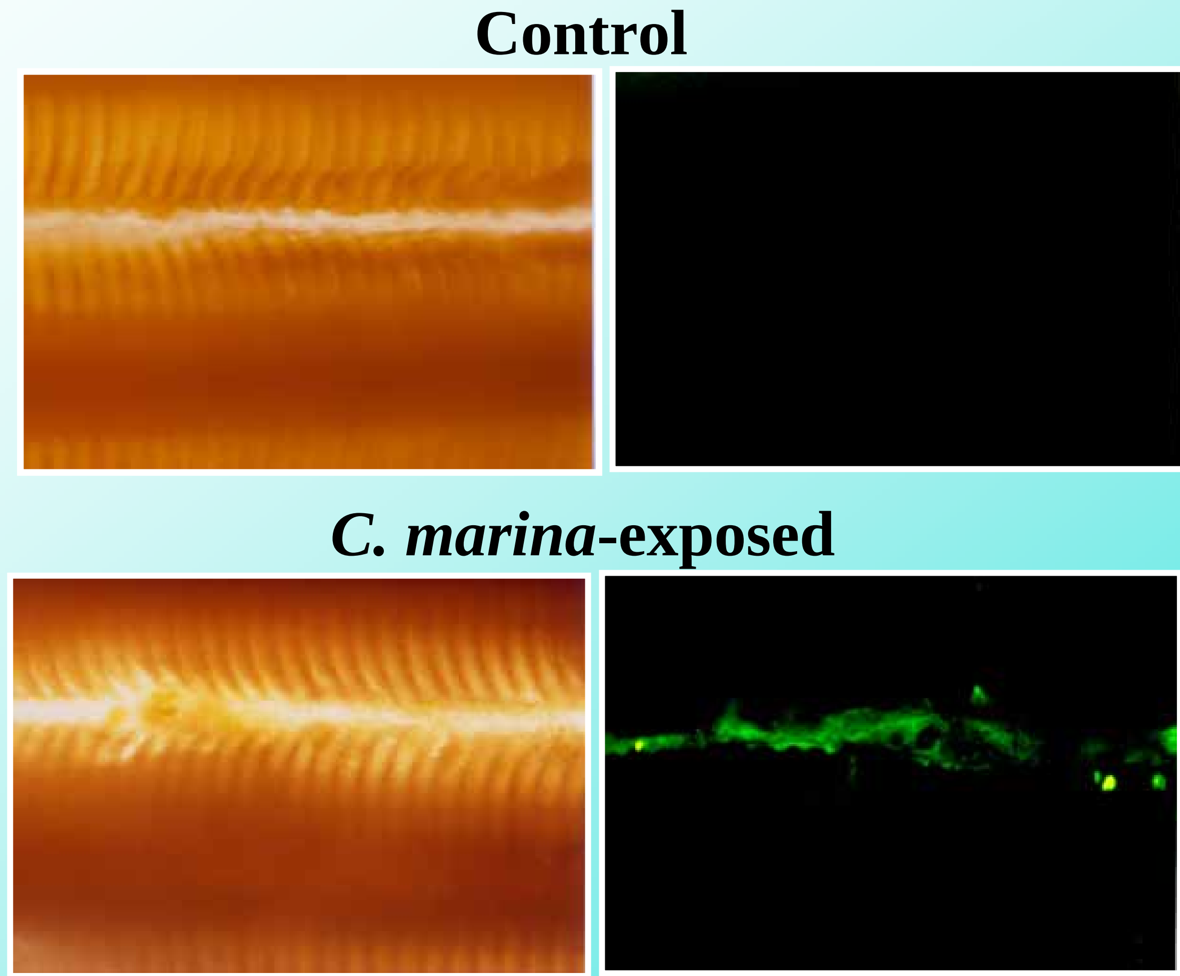
The system for monitoring blood oxygen pressure of yellowtail after exposure to *C. marina* (from Dr. Ishimatsu)



Changes in oxygen partial pressure of arterial blood (Pao_2) in yellowtail exposured to *C. marina* (from Dr. Ishimatsu)

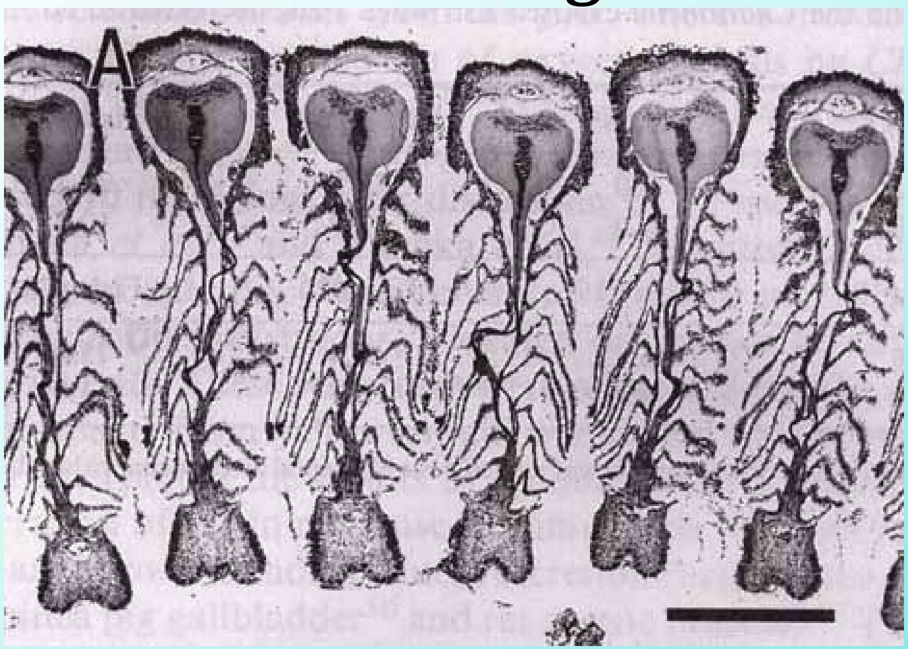


Indirect immuno-fluorescence staining of gill tissues of control and *C. marina*-exposed yellowtails with anti-glycocalyx antiserum and FITC-labeled secondary antibody

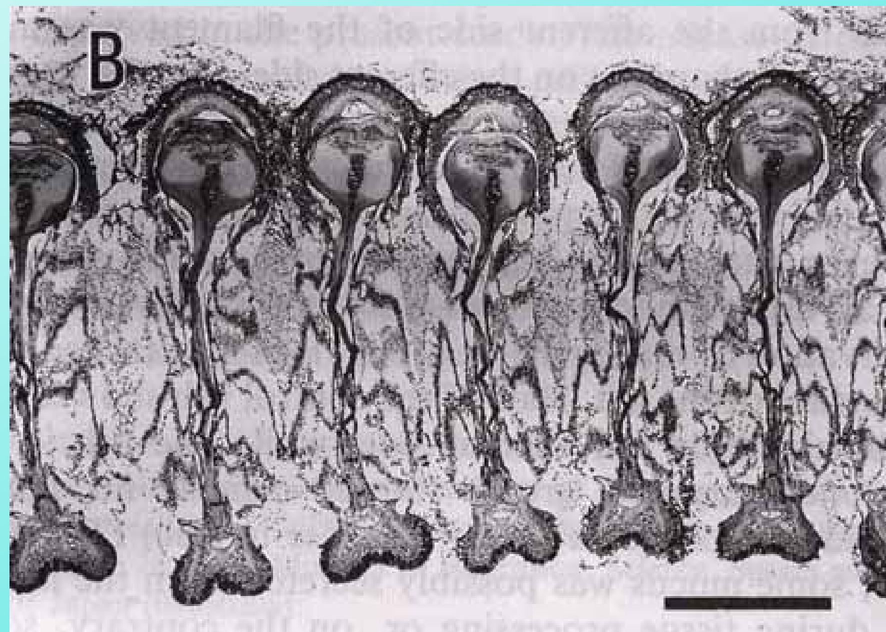


Gill tissue damage after exposure to *C. marina*

Normal gill



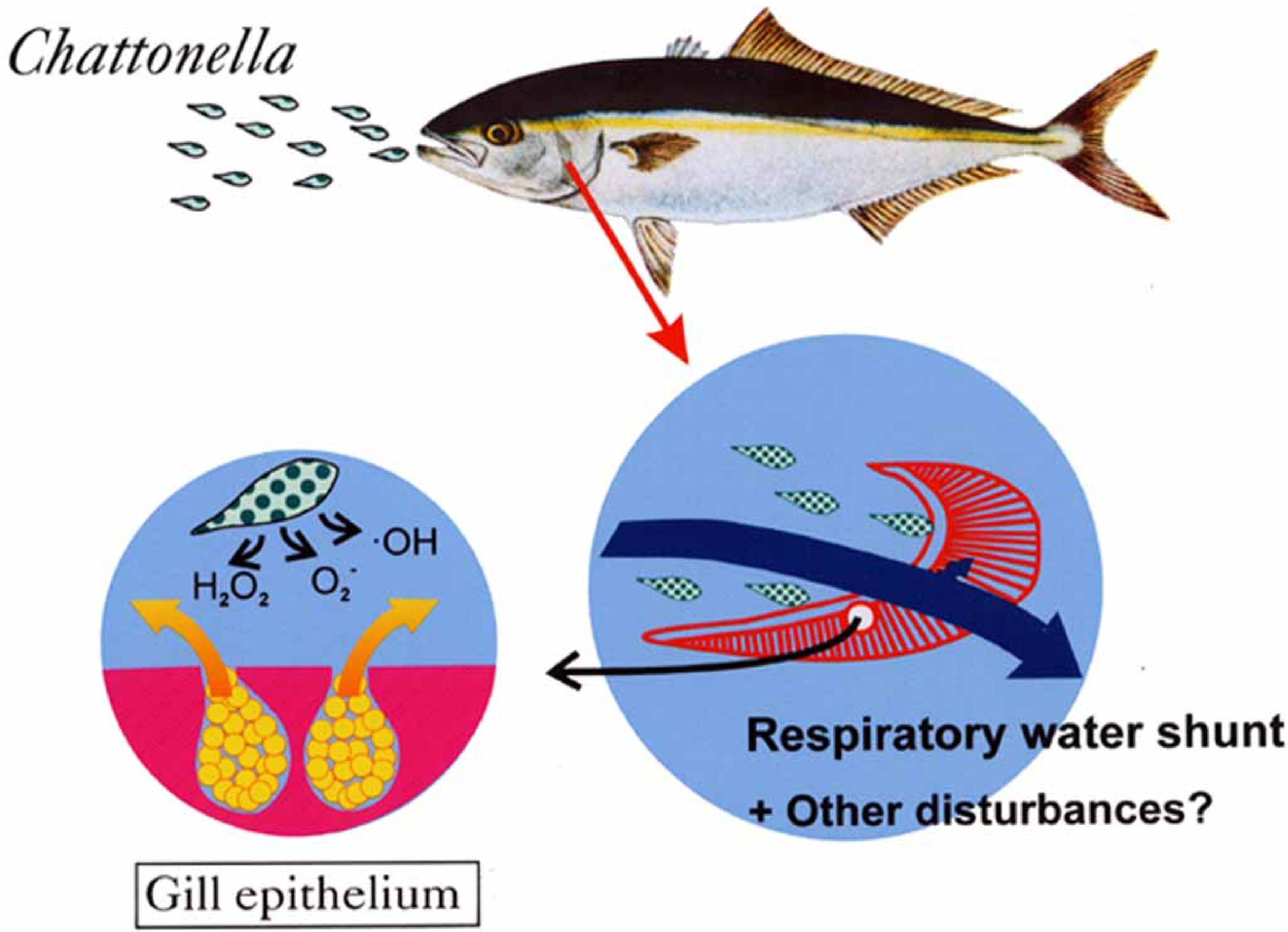
Gill after exposure to *C. marina*



Provided by Dr. Ishimatsu

The earliest physiological changes observed in yellowtail after exposure to *C. marina* is the decrease in arterial oxygen partial pressure.

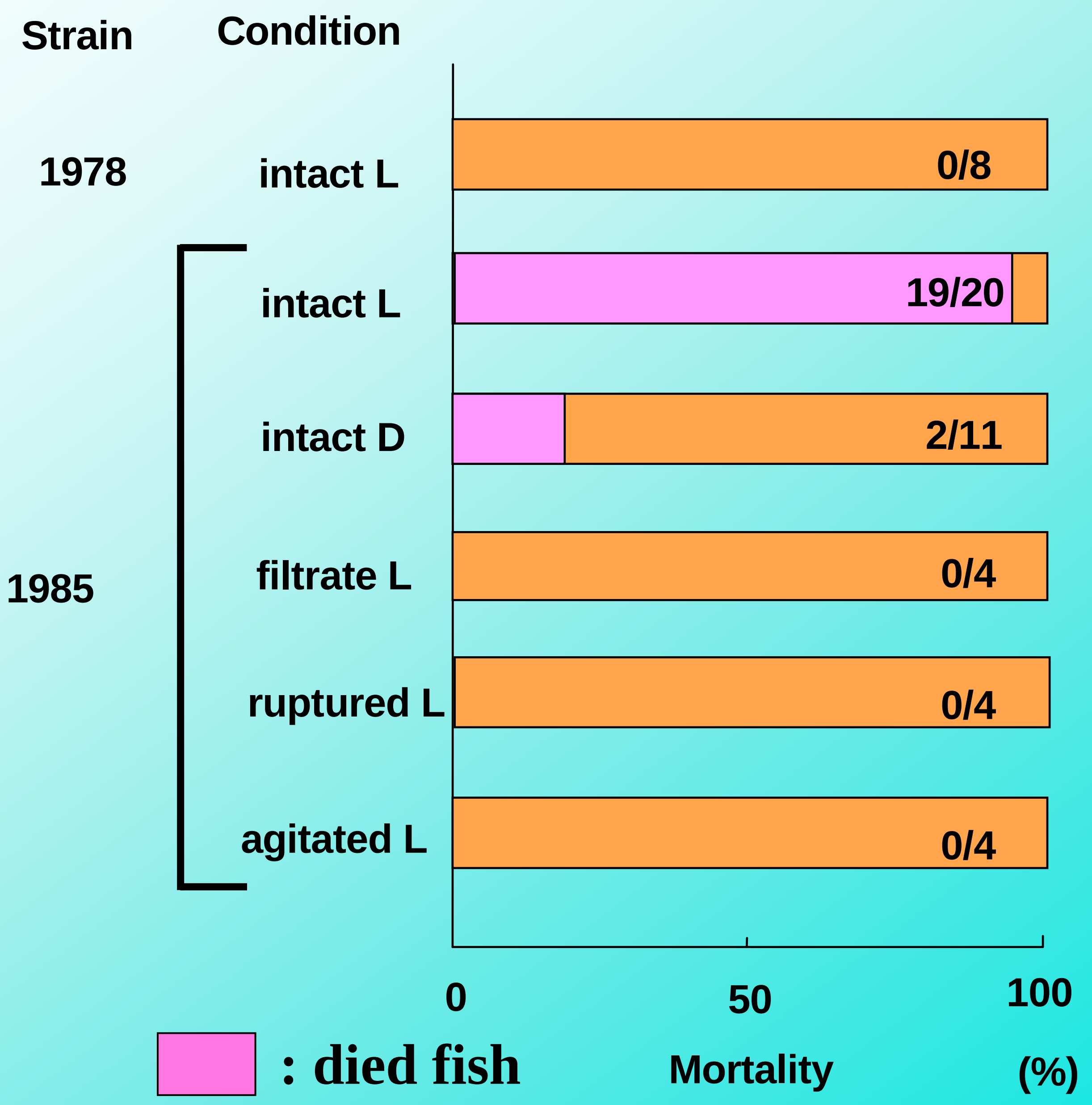
The primary target of *C. marina* is gill.



Chattonella
·OH
H_2O_2
O_2^-
Respiratory water shunt
+ Other disturbances?
Gill epithelium

What is the evidence for the involvement of ROS in gill tissue damage and eventual fish-killing by *C. marina*?

Fish (yellowtail) mortality within 8 h of exposure to *C. marina*.



Strain 1978 : low ROS generation

Strain 1985 : High ROS generation

Processes leading to fish death by *C. mrina*

ROS-mediated gill tissue damage (gill surface might be covered with glycocalyx discharged from *C. marina*)

↓

Continuous ROS generation by glycocalyx might induce over secretion of mucus substances on gill tissue

↓

Dysfunction of gill

↓

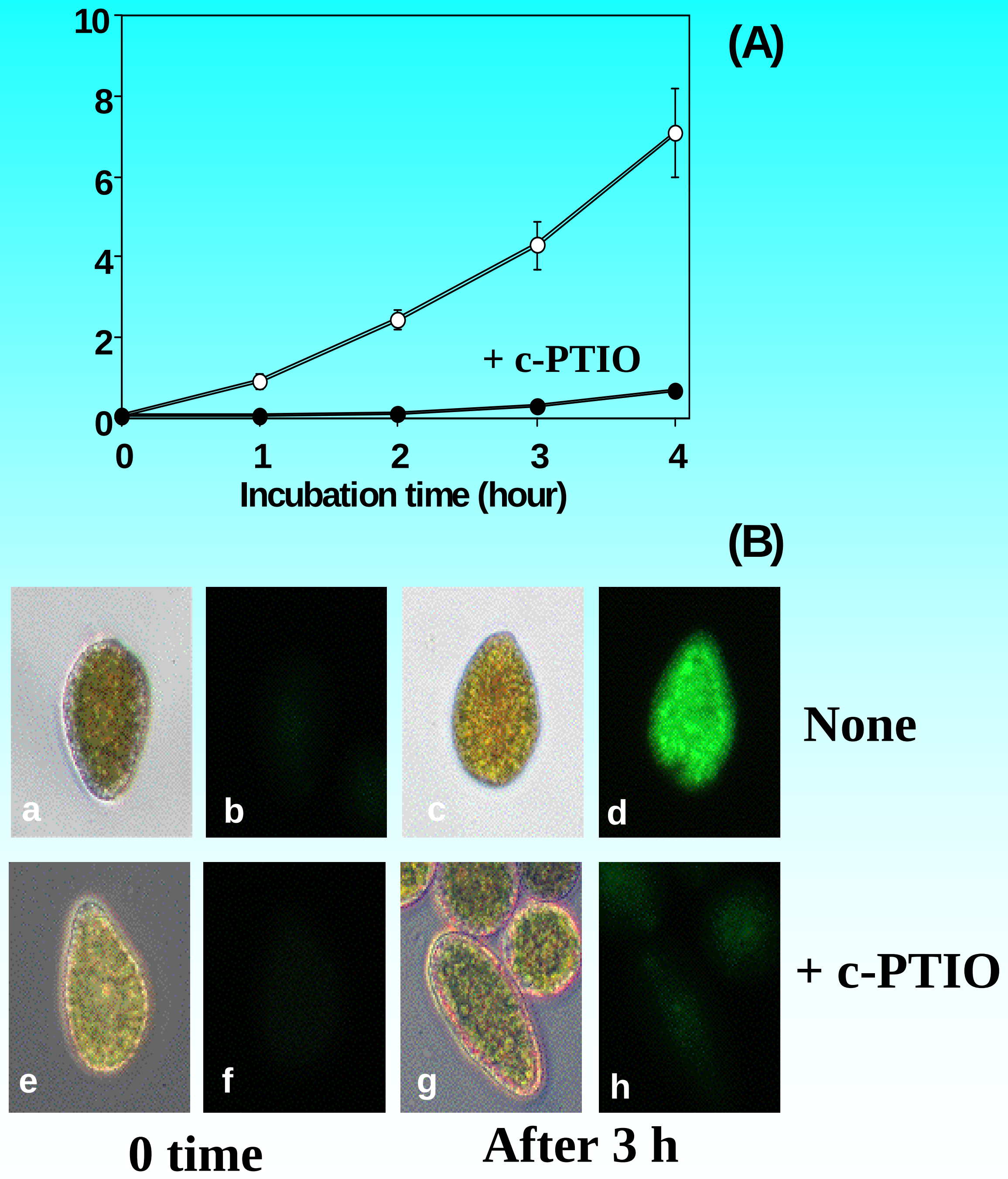
Decrease in the arterial oxygen partial pressure

↓

Suffocation

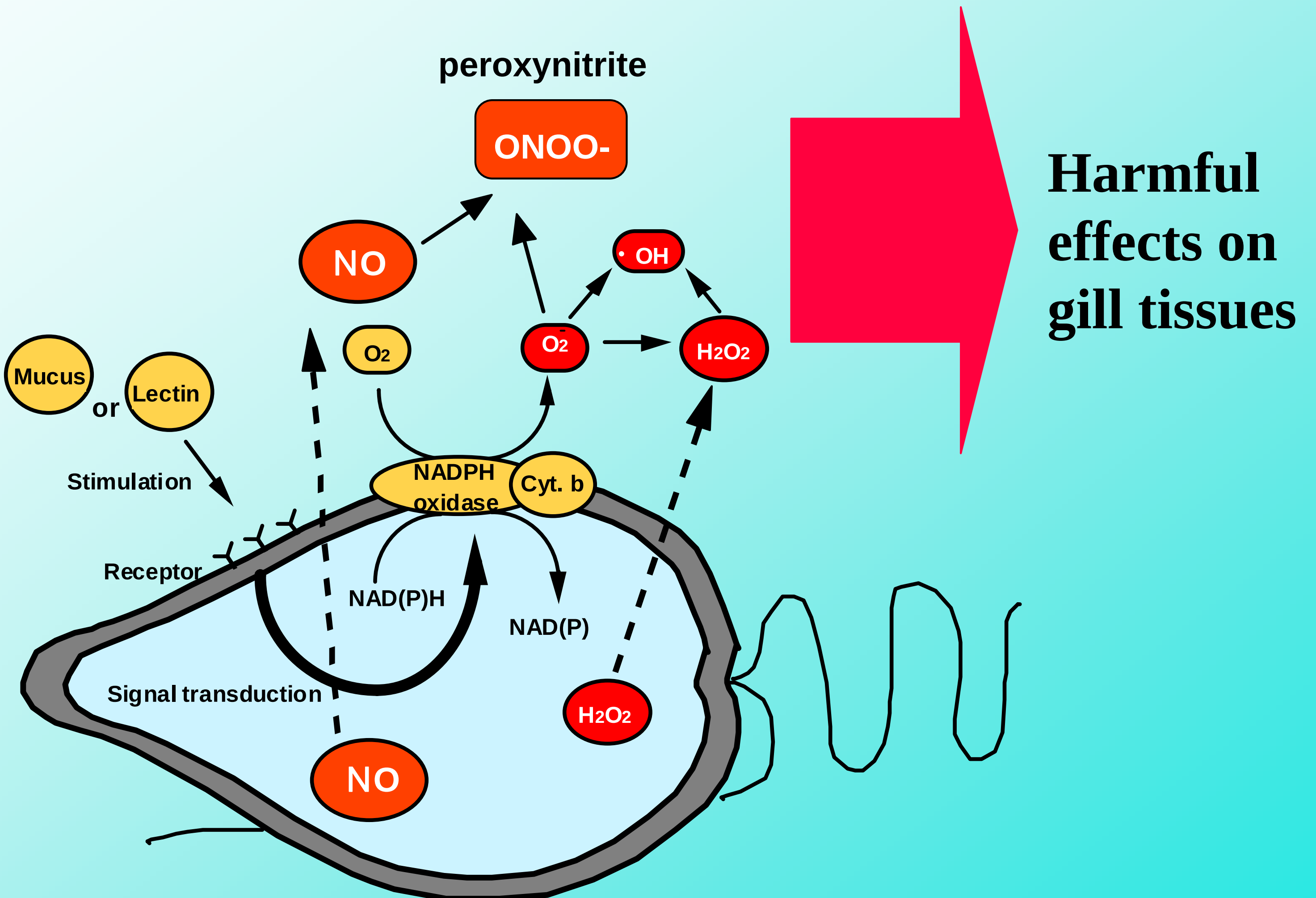
Are there any other possible toxic factors?

In addition to ROS such as H_2O_2, O_2^-, and •OH radical, *C. marina* produces nitric oxide (NO)

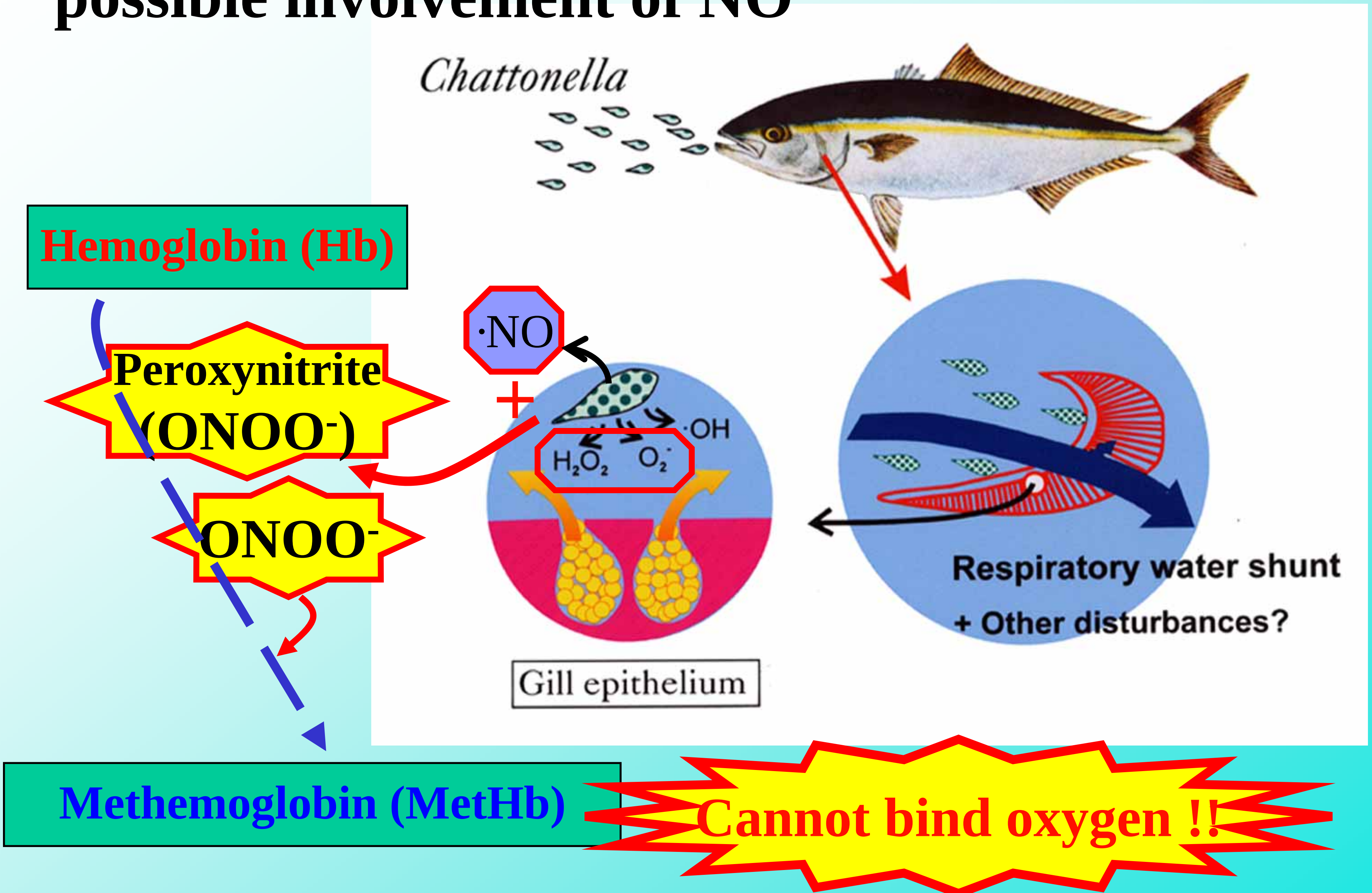


Nitric oxide (NO) production by *C. marina*. (A) Kinetics of NO production detected by NO-specific fluorescence probe (DAF-FM DA). (B) Fluorescence observation of *C. marina* in the presence or absence of specific NO scavenger, carboxy-PTIO

Generation of reactive oxygen species in *Chattonella marina*

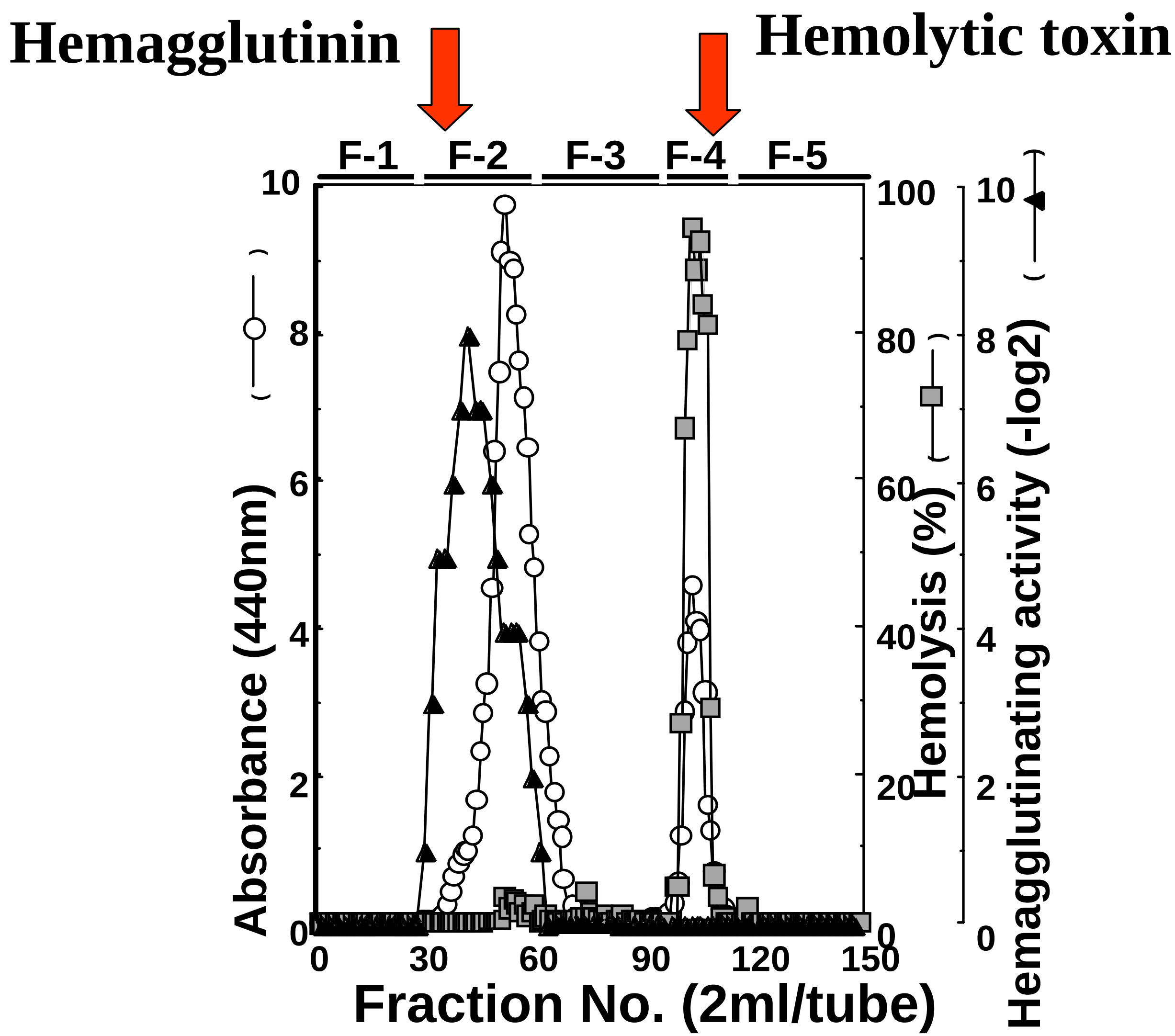


ROS-mediated toxic mechanism of *C. marina*; possible involvement of NO

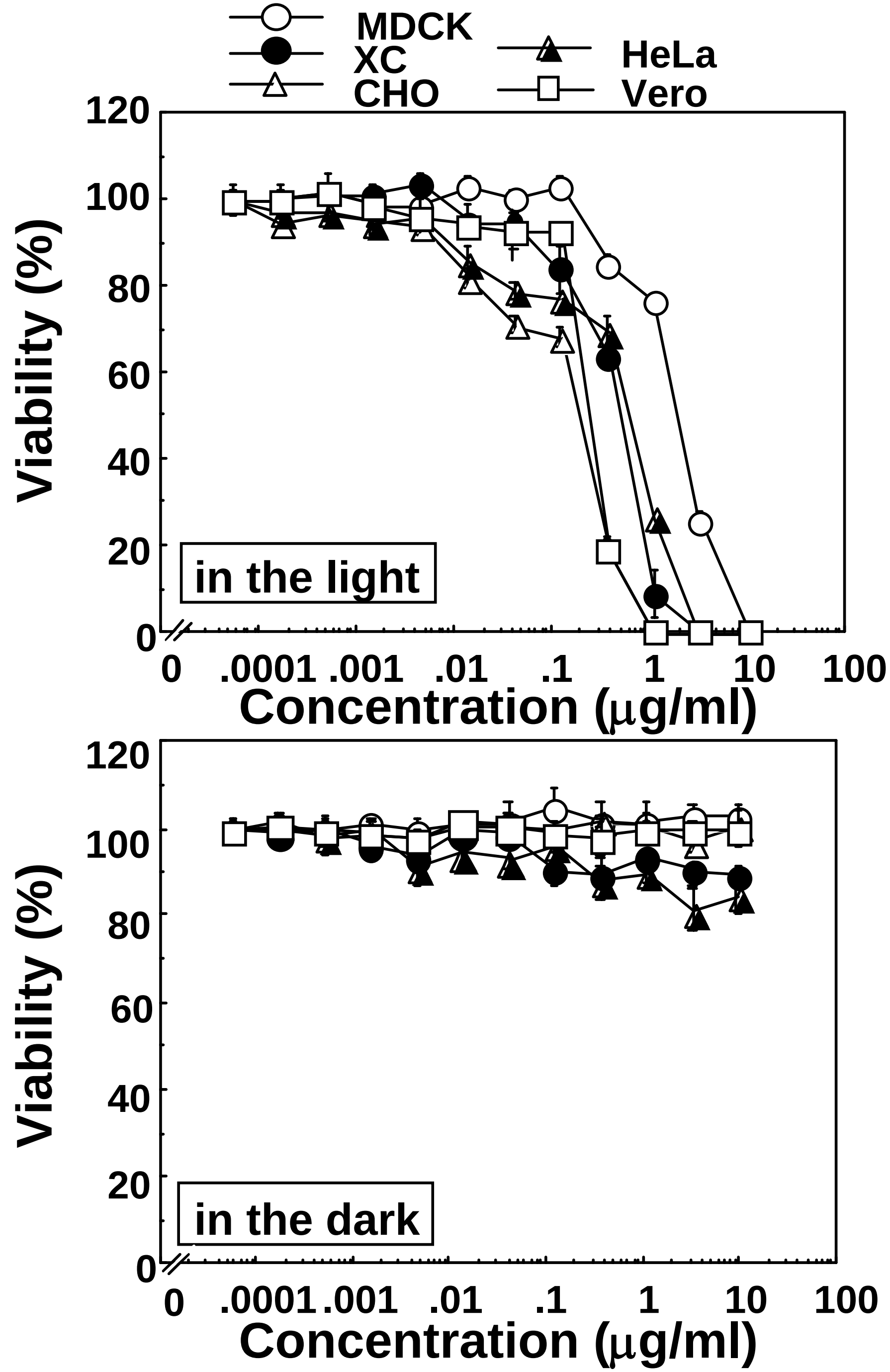


Hemolytic toxins in *C. marina*

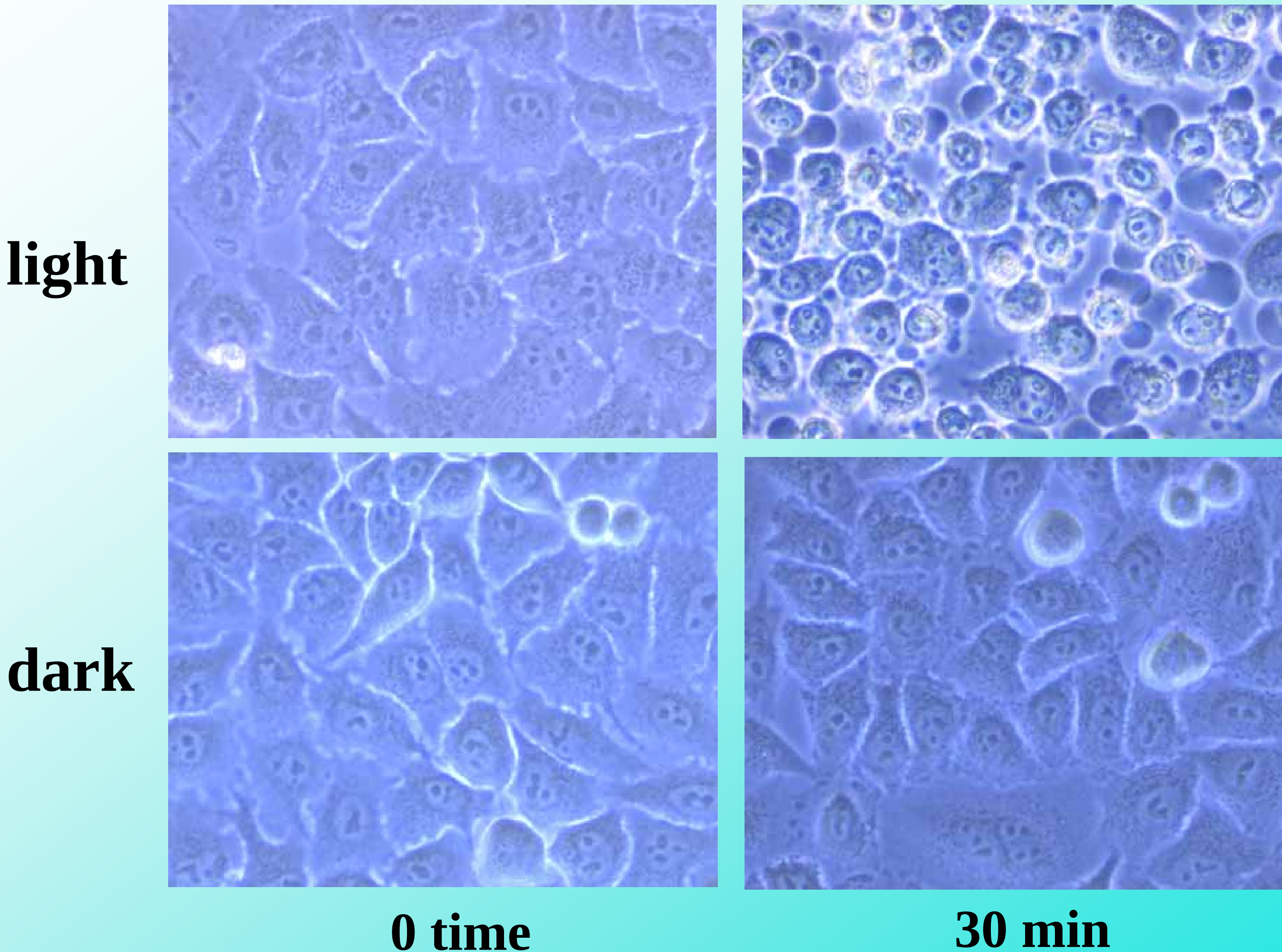
Hemolytic and hemagglutinating activities in the methanol extract of *C. marina*



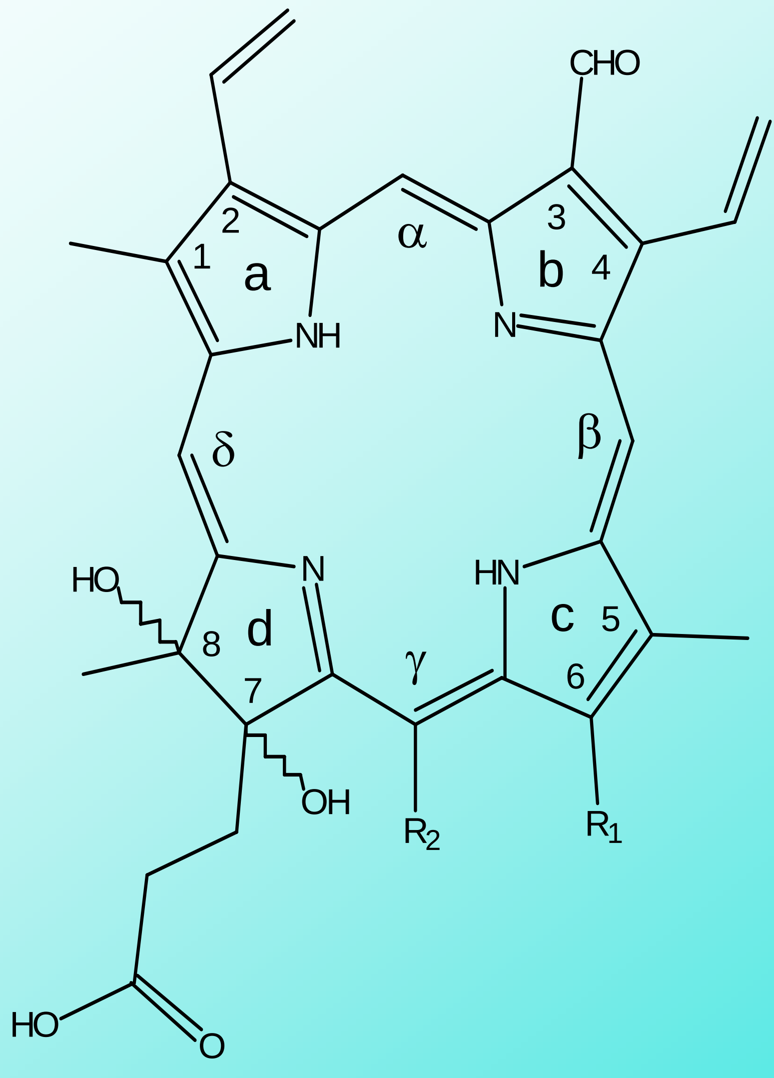
Hemolytic toxin from *C. marina* shows light-dependent cytotoxicity on various cell lines



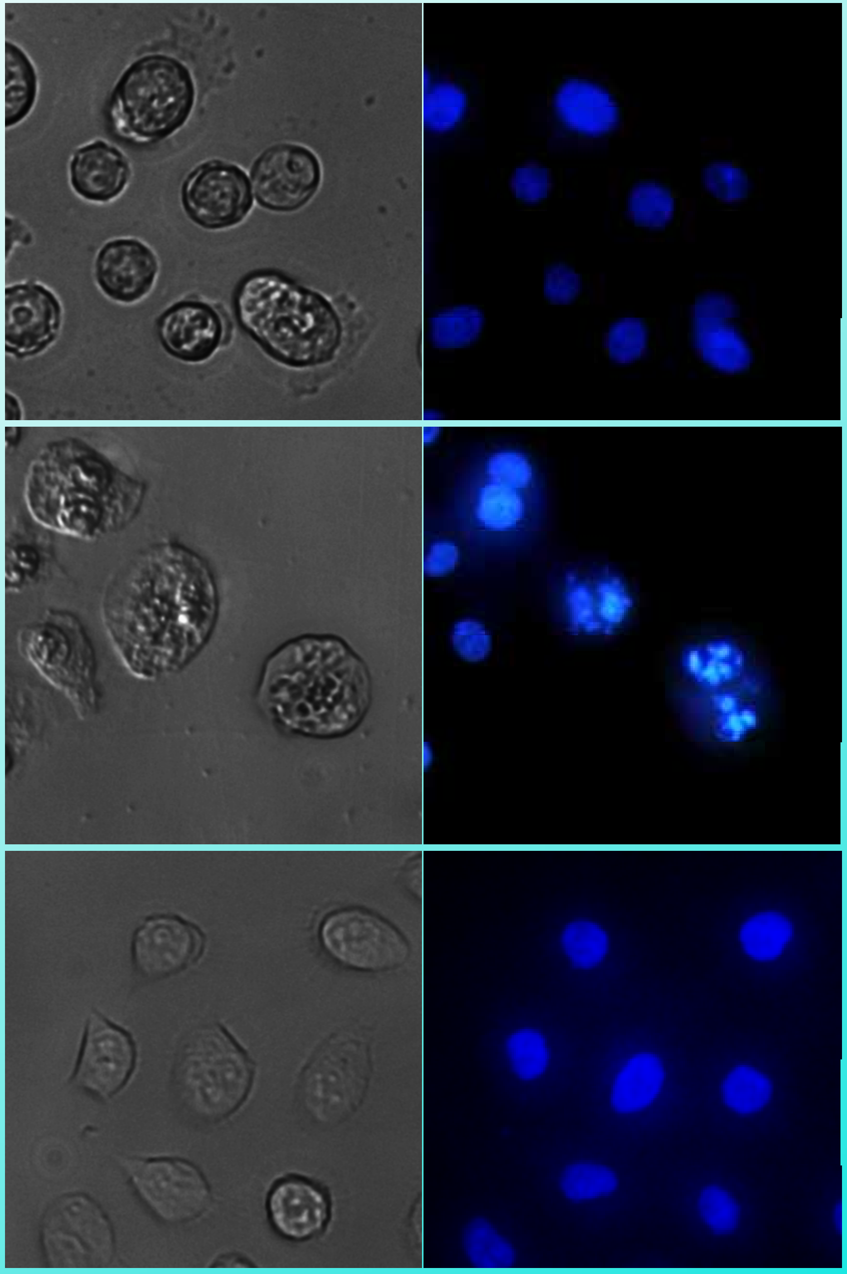
Homolytic toxin induced light-dependent morphlogical changes in HeLa cells



Light-dependent hemolytic toxin isolated from *C. marina* may be a porphyrin derivative



Hemolytic toxin isolated from *C. marina* induces necrosis in HeLa cells



+ hemolytic toxin from *C. marina*

+ pheophorbide

control

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