

# Importance of inputs from scientists to effective implementation of ballast water management convention

Yasuwo Fukuyo

The University of Tokyo

Katsumi Yoshida

Laboratory of Aquatic Science Consultant

Shin-ichi Hanayama

Ocean Policy Research Foundation

# International Convention for the Control and Management of Ships' Ballast water and Sediment

Diplomatic Conference for Adoption: February 9-13, 2004  
after 14 yrs. Of complex negotiation

Marine Environment Protection Committee (MEPC) is now  
making 13 Guidelines referred to the Convention for  
uniform application of the conference, e.g.

G8: Guideline for Approval of Ballast Water Management  
Systems,

G9: Procedure for Approval of Ballast Water Management  
Systems that Make Use of Active Substance

Some of them have been adopted, but the others will be  
completed at MEPC 55 (October 2006)

# Purpose of the Convention

(one paragraph in the preamble of the Convention)

**RESOLVED** to prevent, minimize and ultimately

Current serious problem

Lack of a management system to meet the requirement of the Convention

Therefore scientific inputs are eagerly waited for several difficulties.

Signed by 8 countries (Argentina, Australia, Brazil, Finland, Maldives, Netherlands, Spain, Syria ) as of September 1, 2005

.....still far from the entry into force

Measures that all ships must comply are either:

D-1: Ballast water exchange

95 % volumetric exchange of BW or  
(rem: this method may become invalid after 1916)

D-2: Ballast water treatment to reduce organisms at discharge

1. Less than **10 viable organisms per cubic metre** greater than or equal to 50 micrometres in minimum dimension  
Less than **10 viable organisms per millilitres** less than 50 micrometres and greater than or equal to 10 micrometres in minimum dimension;
2. Indicator microbes, as a human health standard:
  - .1 Toxicogenic *Vibrio cholerae* (01 and 0139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gram (wet weight) of zooplankton samples;
  - .2 *Escherichia coli* less than 250 cfu per 100 milliliters;
  - .3 **Intestinal Enterococci** less than 100 cfu per 100 milliliters

## Difficulties in uniform and proper application of the standards

1. Interpretation of the required condition in the Convention:  
measurement of viability,  
measurement of minimum dimension (MD)  
especially for colony forming species
2. Measurement of the number of organisms in BW, especially  
the following wide range individual numbers:  
0-9 individuals (MD  $\geq$  50) in 1 m<sup>3</sup>  
>10<sup>5</sup> individuals (MD  $\geq$  50) in 1 m<sup>3</sup>  
0-9 individuals (50 > MD  $\geq$  10) in 1 ml  
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3. Analysis of sample from land-based tests must be completed  
within 6 hours after sampling.

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# Minimum dimension

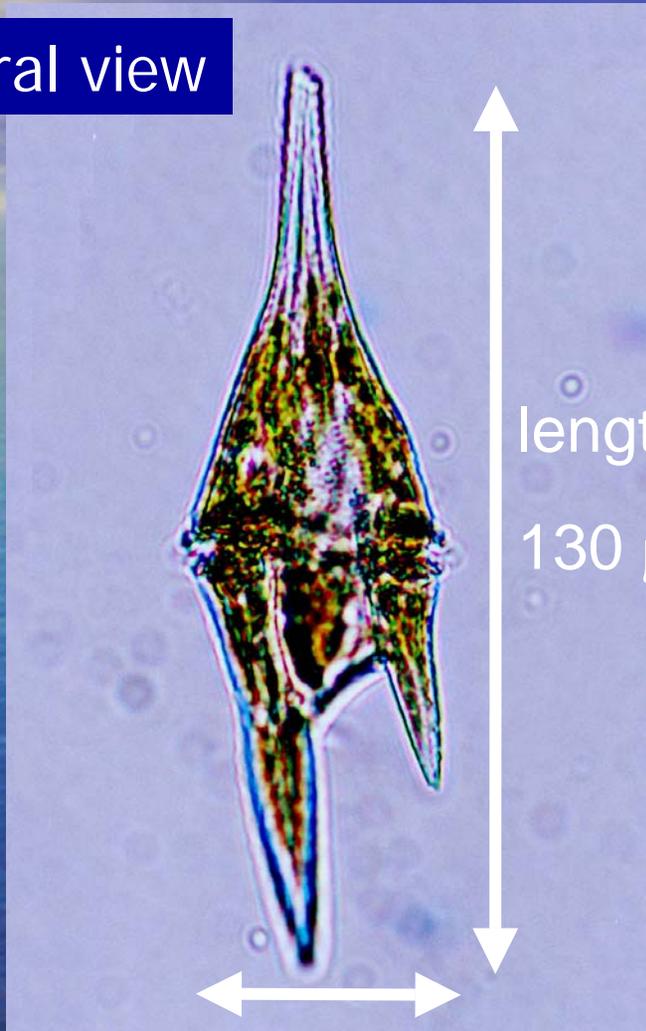
Shortest size of measurement  
in three dimensions



1. Minimum dimension of the example A is “Depth 2 cm”.
2. Minimum dimensions of solitary cells and group of cells must be measured in different way.

# Solitary cell (simple shape)

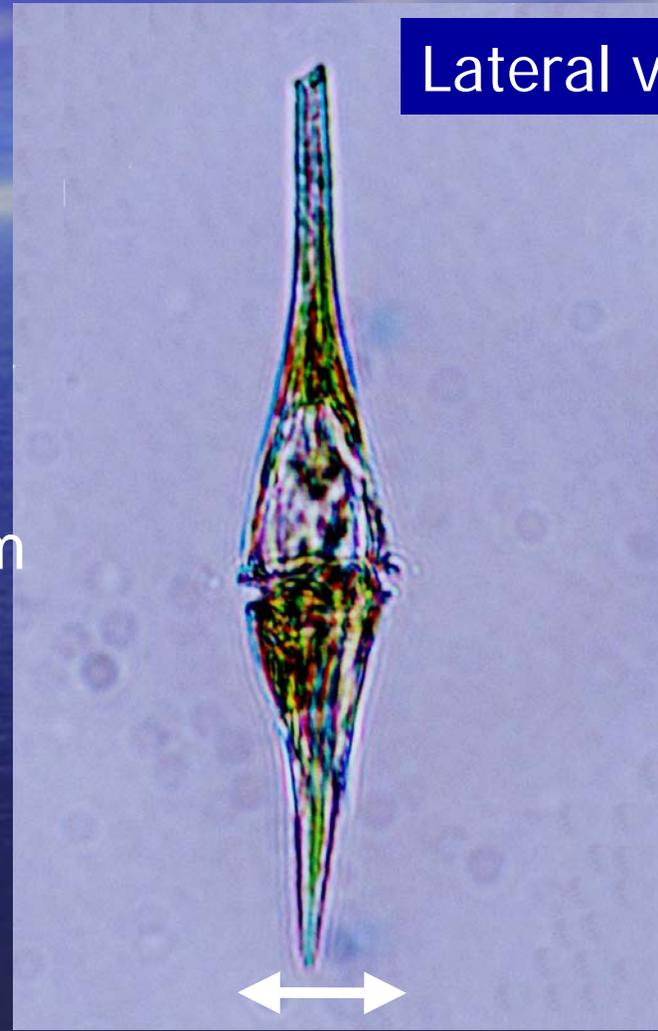
Ventral view



width 35  $\mu\text{m}$

length  
130  $\mu\text{m}$

Lateral view

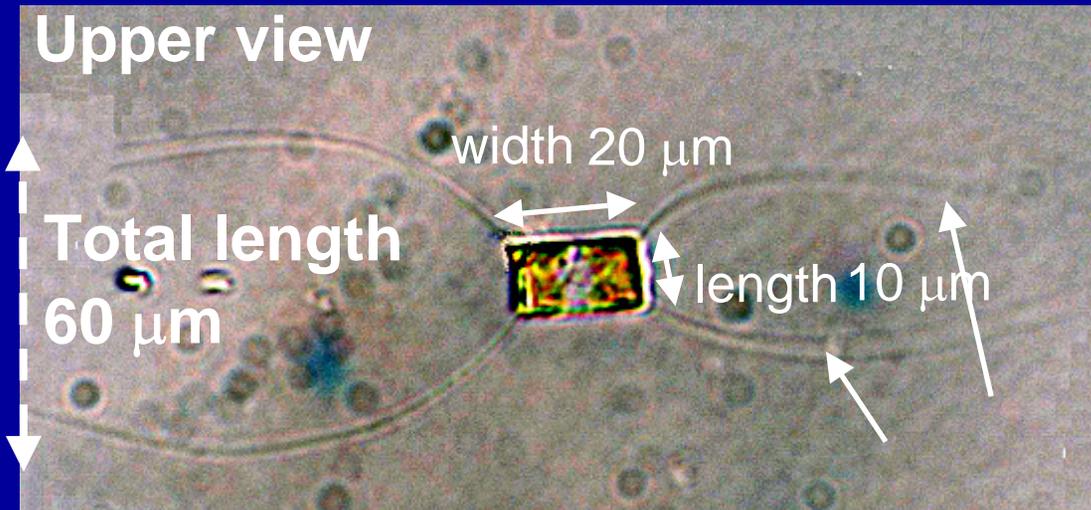


depth 25  $\mu\text{m}$

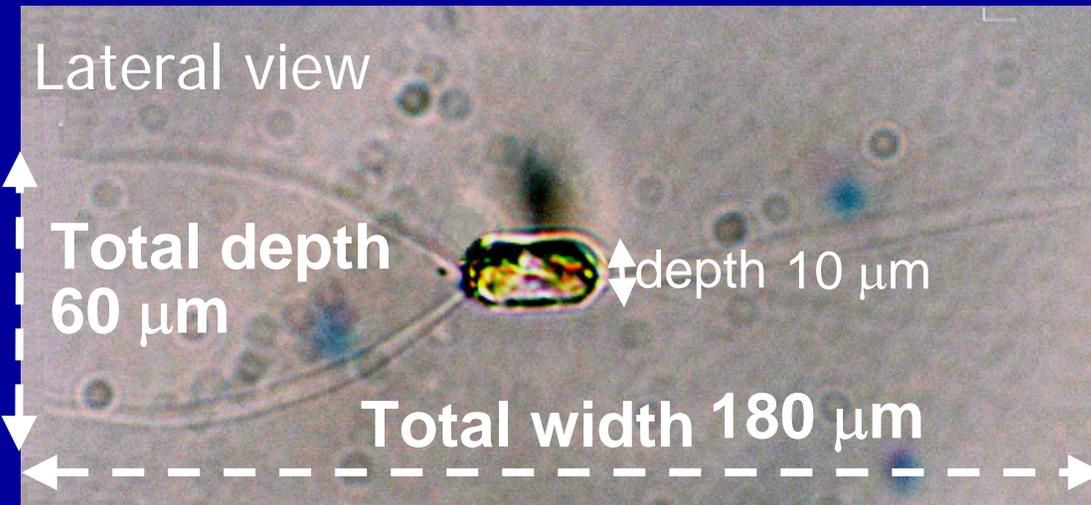
minimum dimension

# Cell solitary (cell with spines)

Upper view



Lateral view



If spines are included,  
minimum dimension is  
total length: 60 μm

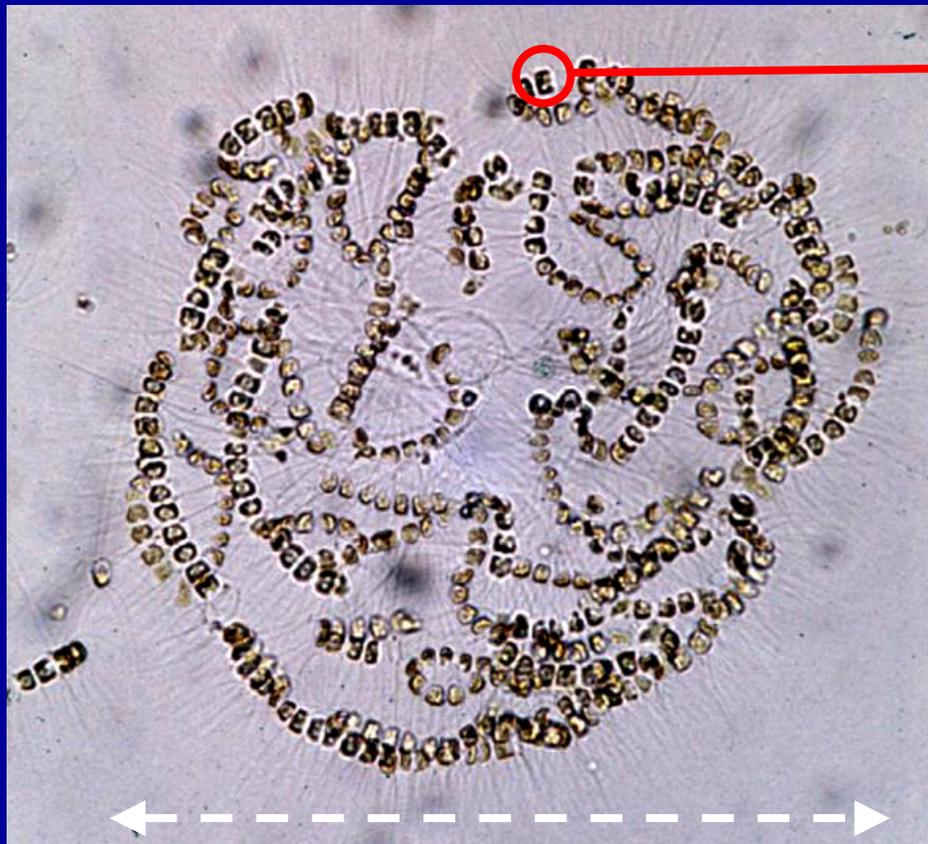
→ L group

If not included,  
minimum dimension is  
depth: 10 μm

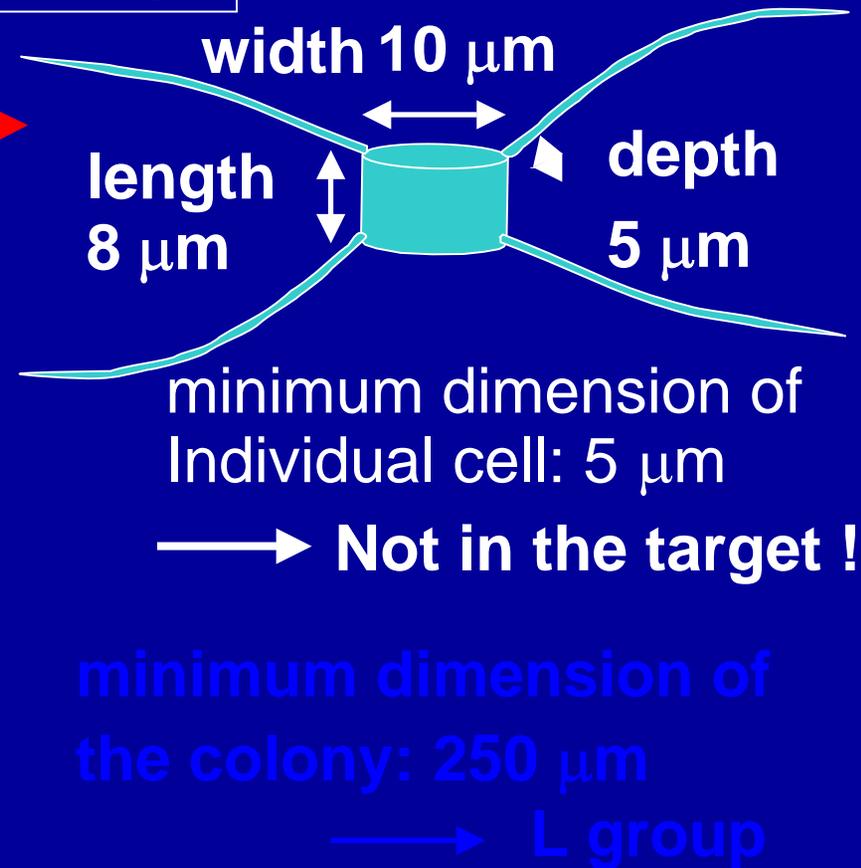
→ S group

Spines are often broken, and then the minimum dimension varies. Therefore spines shall not be included at measurement.

## Cells forming a group (case 2)



250 μm



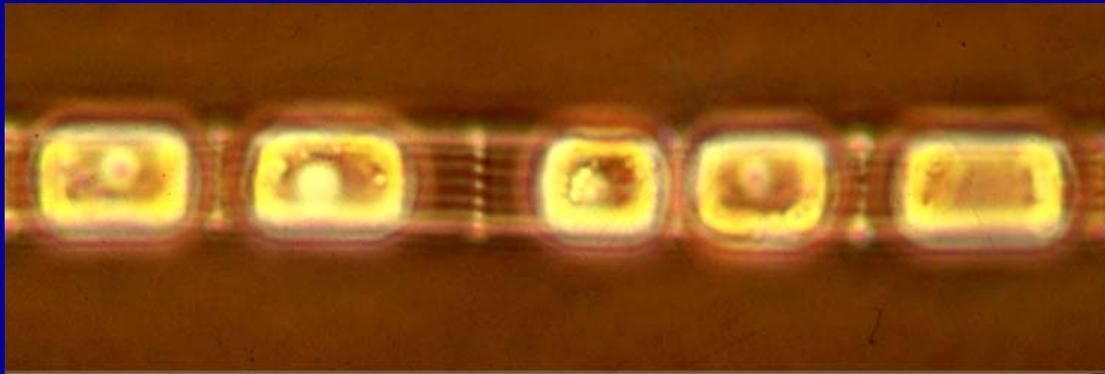
Not in the target !

minimum dimension of the colony: 250 μm

L group

Number of cells or number of colony, which shall we count ?  
Japan recommend to **recognize a colony as one organism**, regardless the number of individual cells

## Cells forming a group (case 1)



about 10 micrometer

→ **S Group ??**



smaller than 10 micrometer

→ Not in the groups



larger than 10 micrometer

→ **S Group**

Three photographs show a same species having different minimum dimension. Inclusion or exclusion in the S groups is depending on an individual cell or group.

# Judgment of viability

9.39 **Determining viability** of a certain individual can be done through live/dead judgement by

- 1) **morphological change** (shape and colour of cells),
- 2) **mobility**,
- 3) **staining using dyes** and
- 4) **regrowth experiment**.

- • • viability of organisms shall be evaluated using at least one/two different criteria among the four.

# Plankton group (Live specimen)

group of cells

pollen

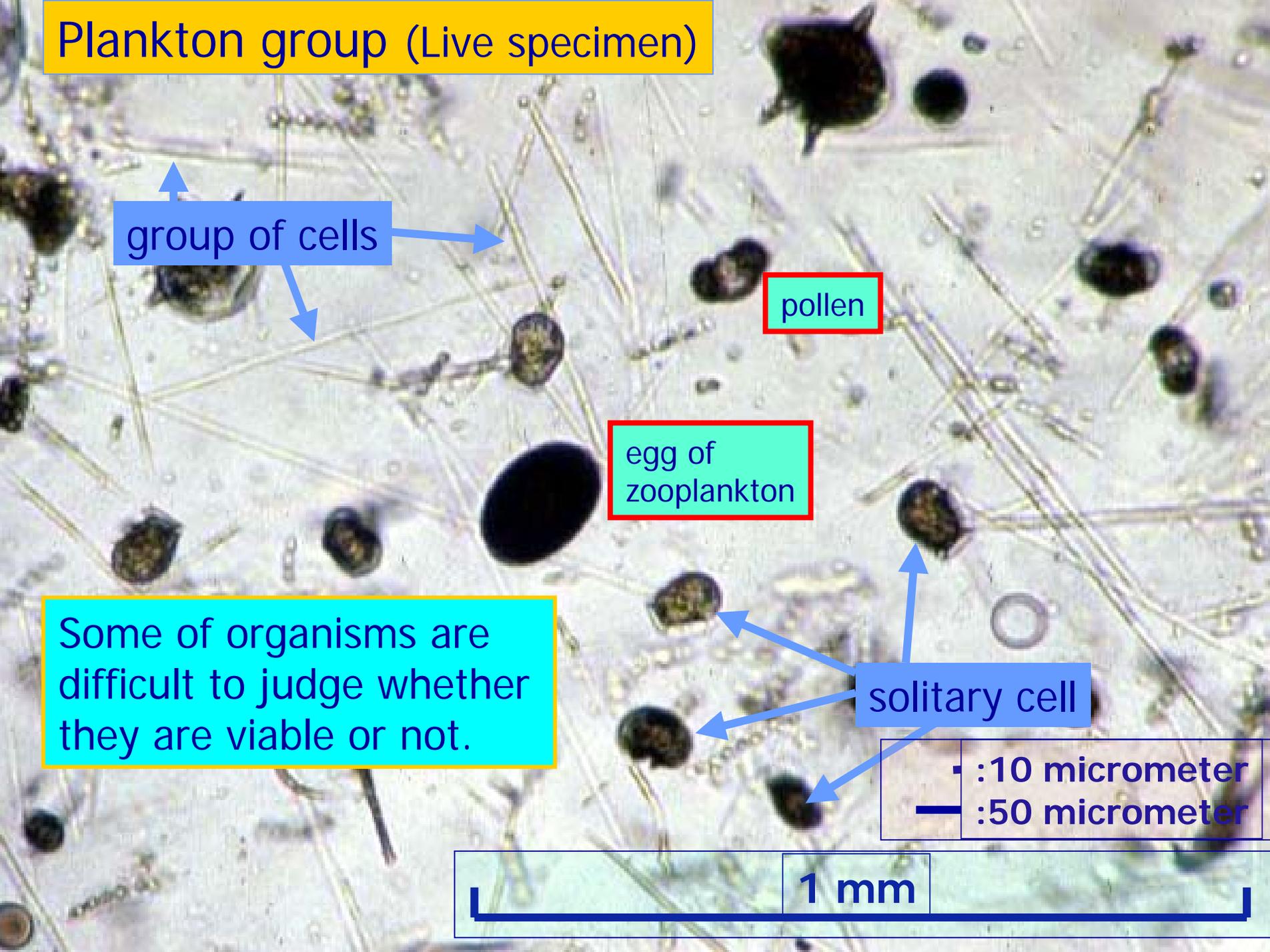
egg of zooplankton

Some of organisms are difficult to judge whether they are viable or not.

solitary cell

- :10 micrometer  
— :50 micrometer

1 mm



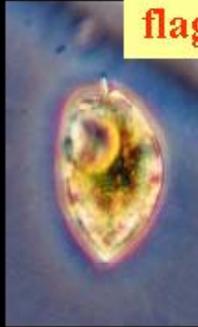
# Morphological change

## Phytoplankton

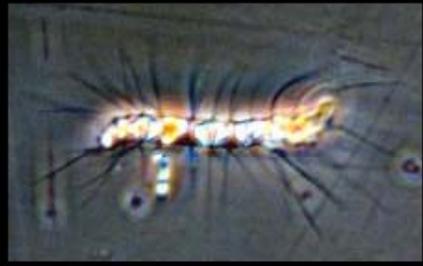
Normal



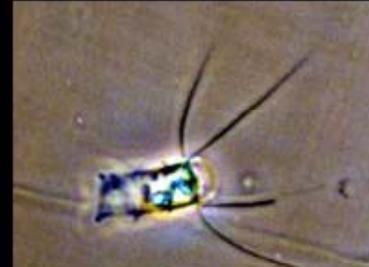
flagella lost



Cell walls were destroyed



Chloroplast were bleached



Protoplasm lost almost

Damaged cells

## Zooplankton

Normal



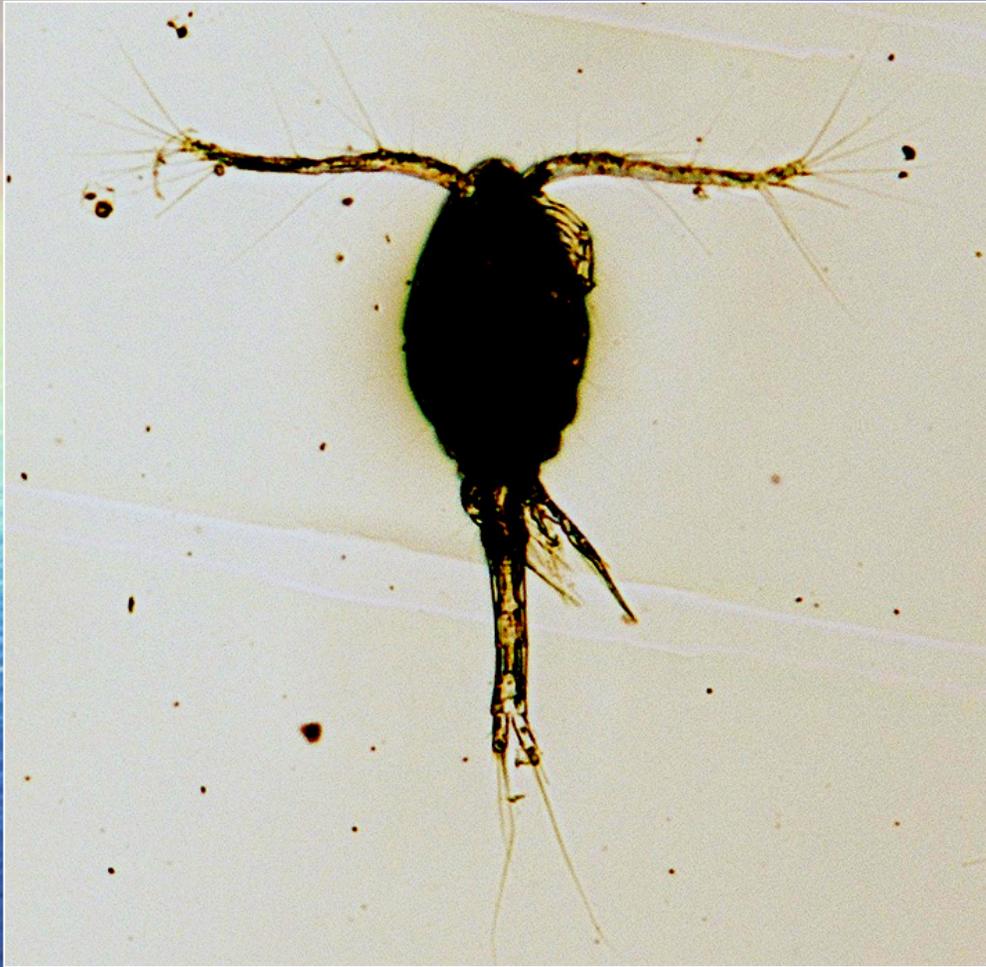
Antenna and abdomen were cut



Body was destroyed completely

Damaged individuals

# Morphological change- loss of appendages

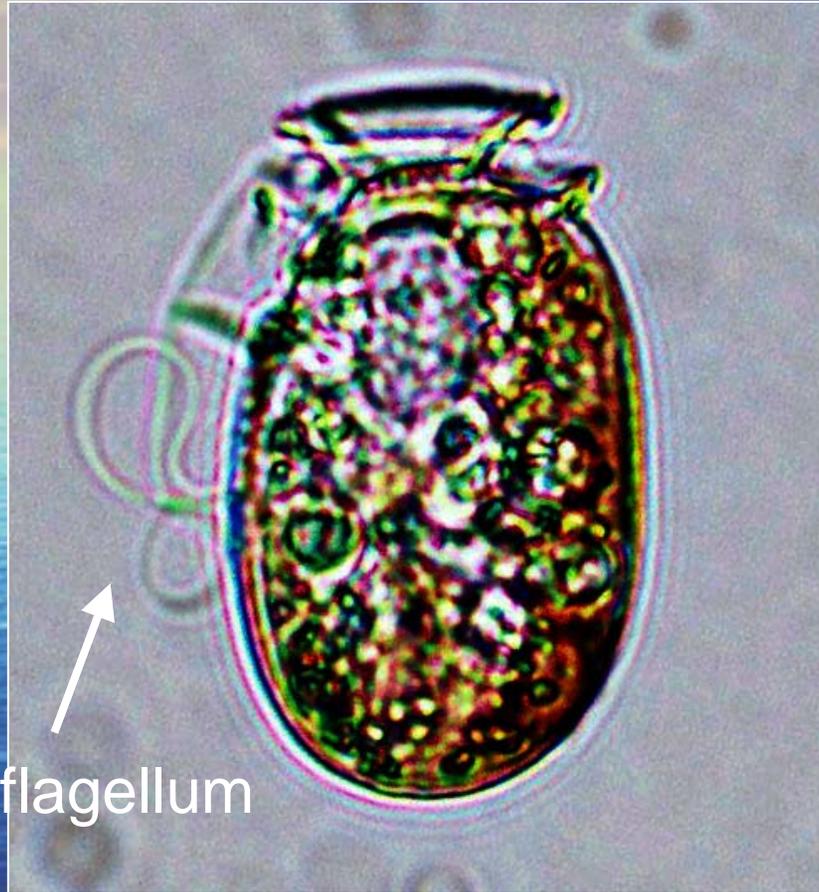


**viable**



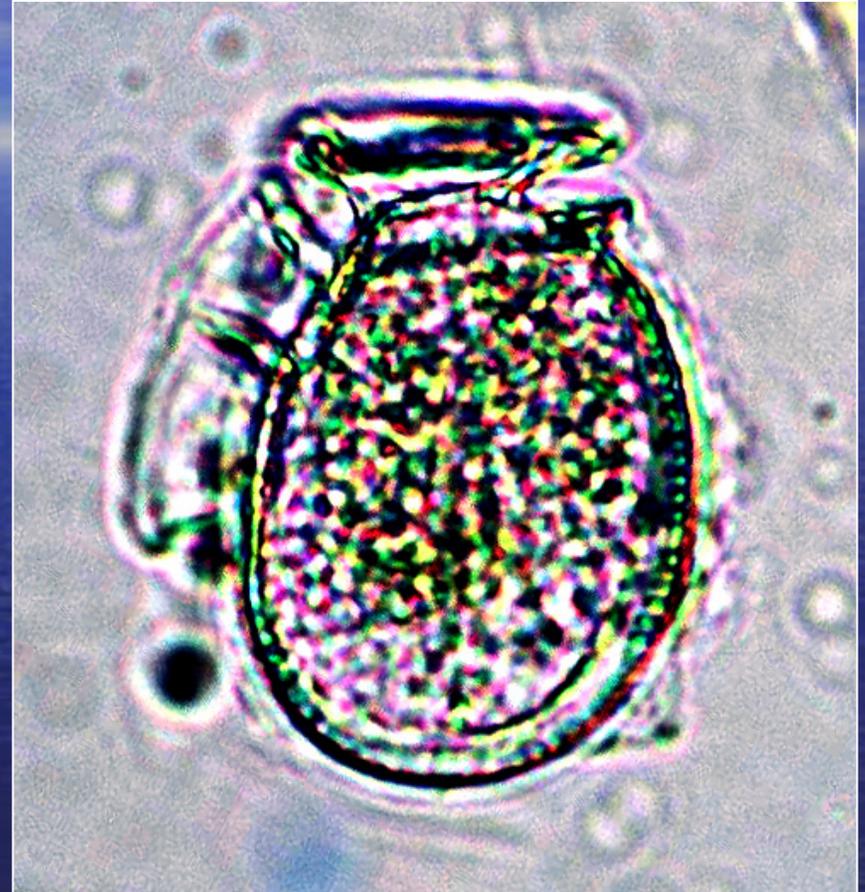
**dead**

# Morphological change- loss of flagella



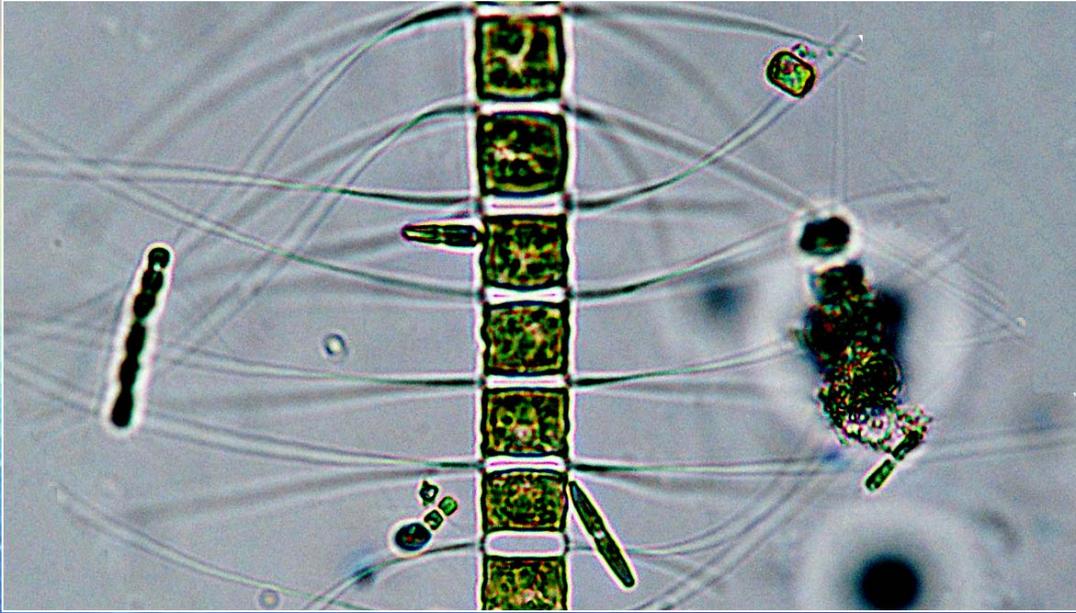
flagellum

**viable**



**dead**

# Morphological change- loss of chloroplasts

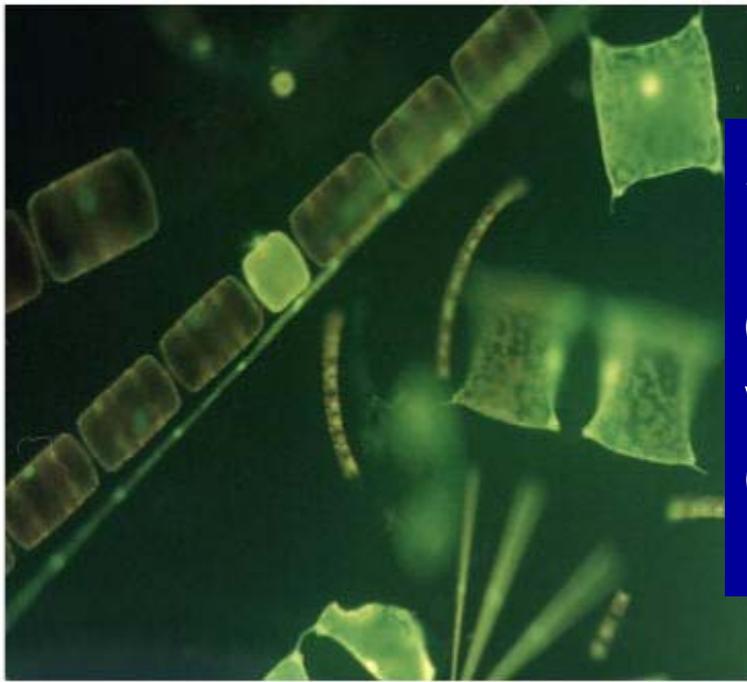
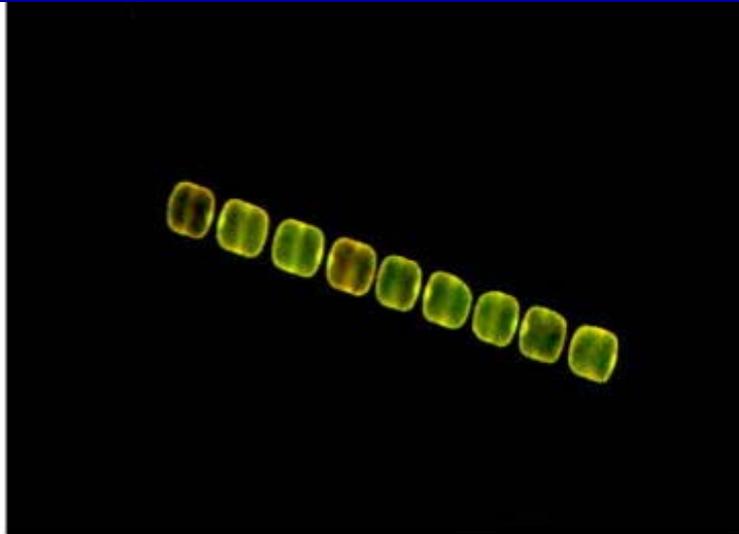
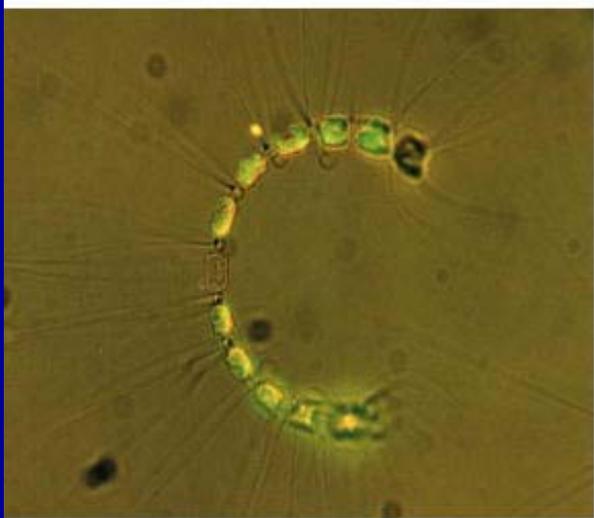


**viable**



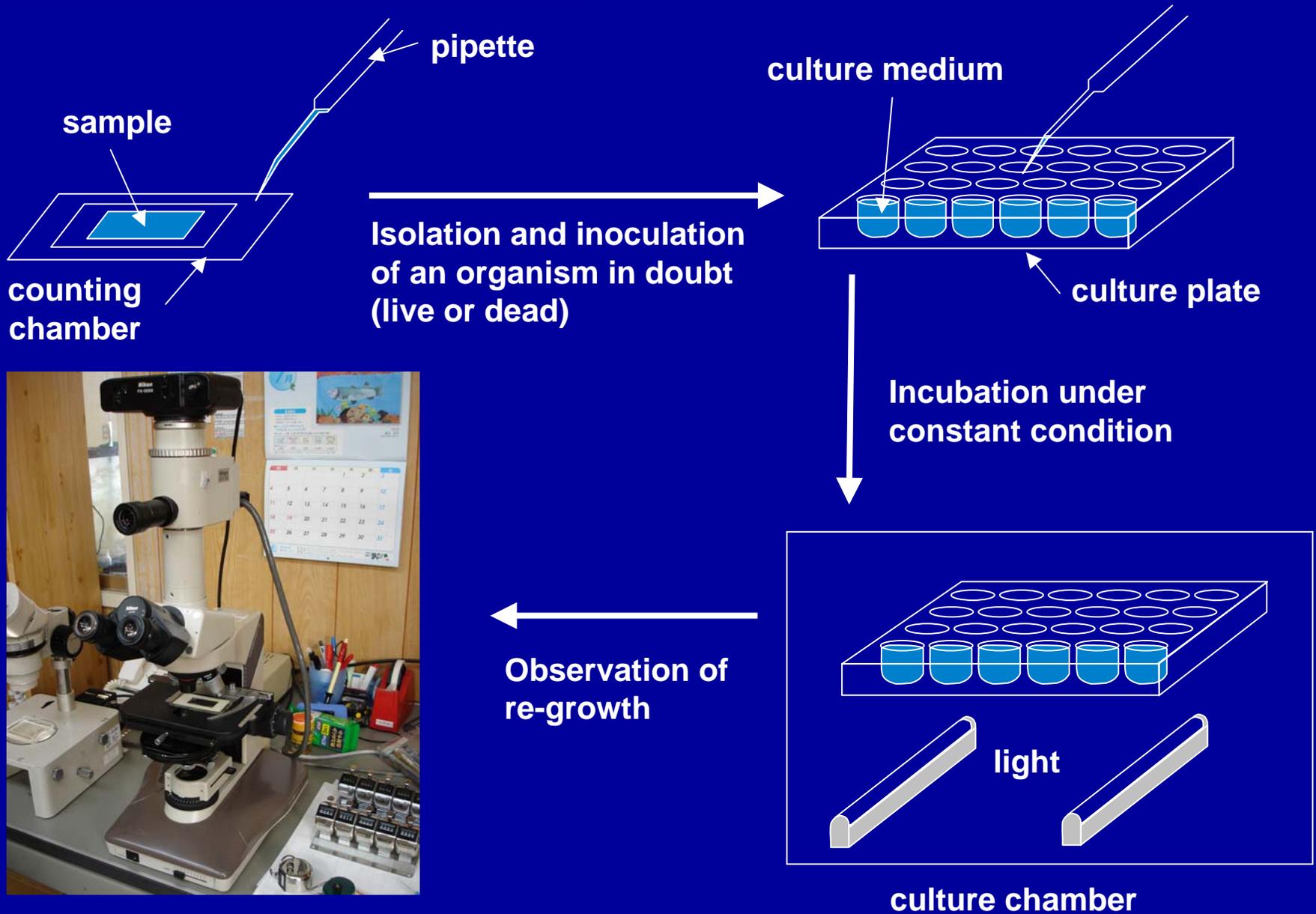
**dead**

# Staining using dyes such as Fluorescein diacetate (FDA)



Staining using FDA shows living cells with yellowish color. The strength of color varies depending on activity of an enzyme, and therefore it indicates vital power.

# Re-growth experiment



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# Step 1

## Sample collection

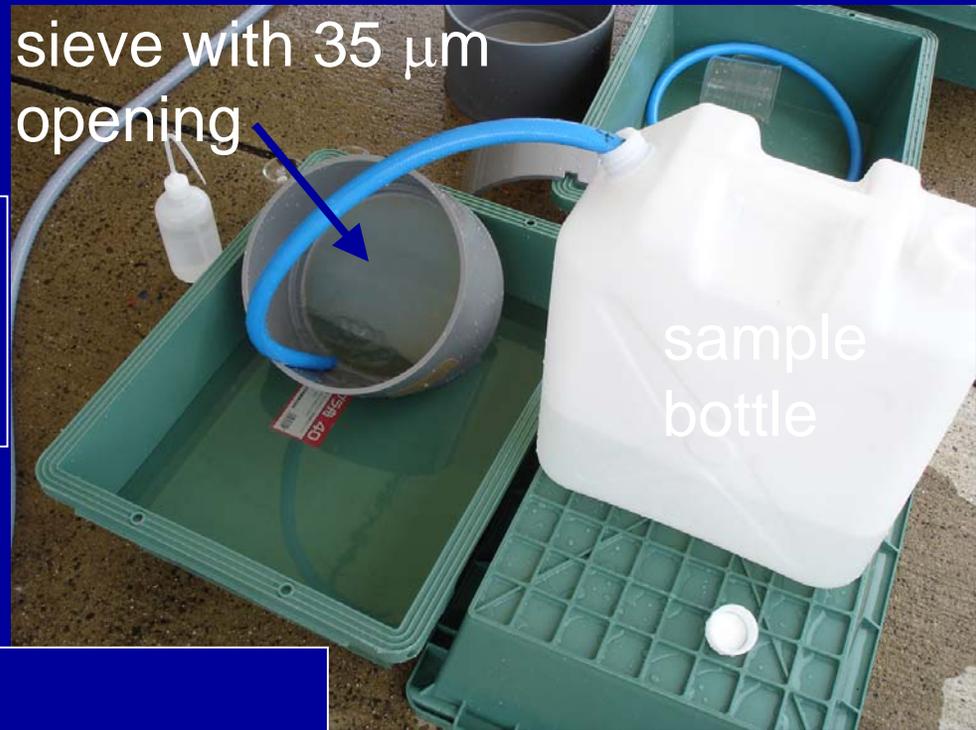
1 m<sup>3</sup> for MD>50 plankton  
1 liter for 50>MD>10 pl.



Sample concentration to  
less than 10 liter for MD>50 plankton  
less than 100 ml for 50>MD>10 pl.



Concentrate denser, if  
possible, at laboratory



**Step 2**



**sieve**

concentrate denser  
at laboratory



**filtered  
sea water**

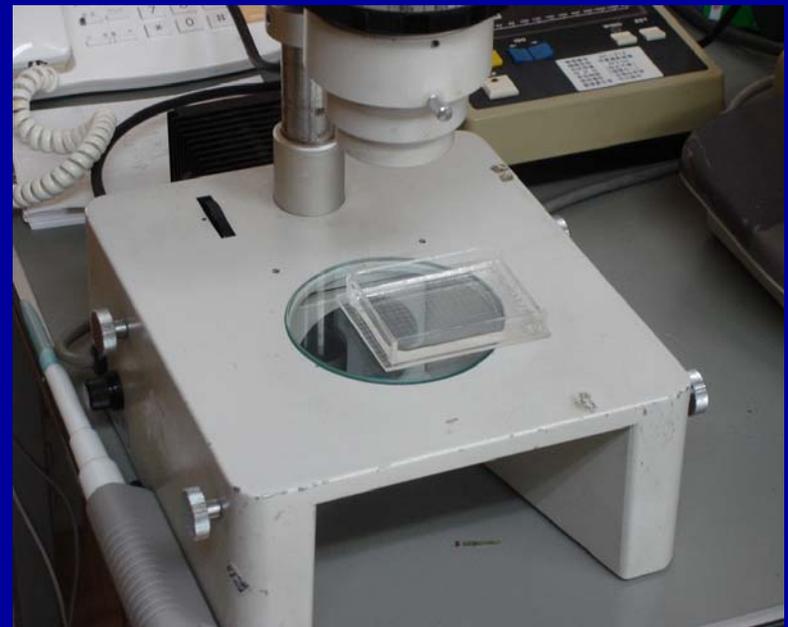
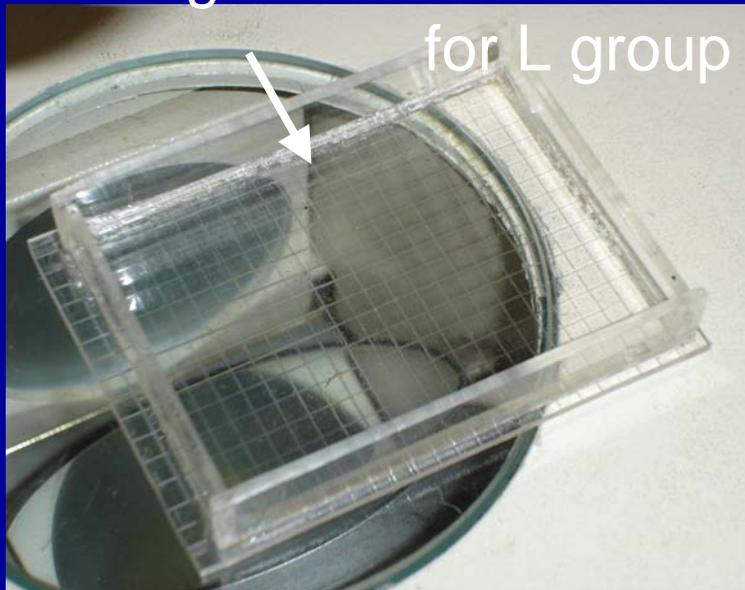
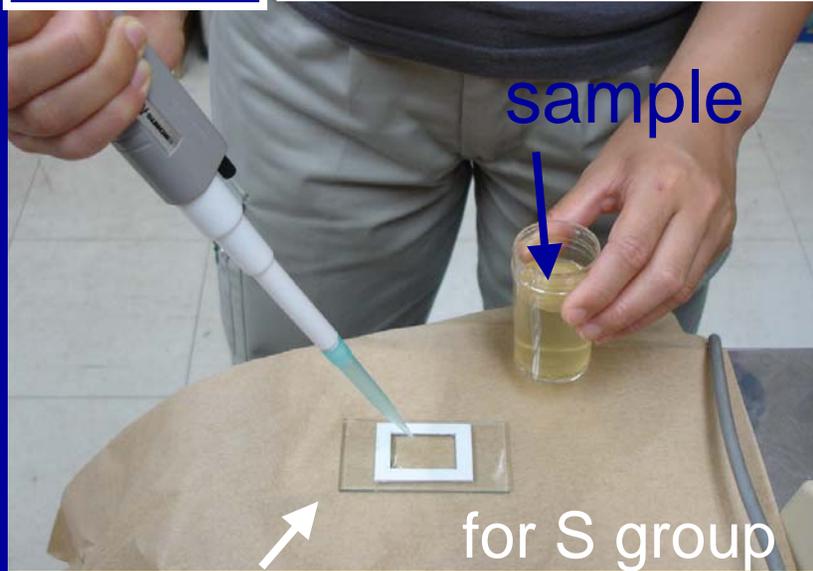
adjust volume of conc.  
sample using filtered SW



伊万里 040701 コト0-167  
スリット 0.3mm 高尾  
PR: 10~50um, 処理済

伊万里 040701 コト  
スリット 0.3mm 高尾  
PR: >50um, 処理済

# Step 3 count organisms under microscopes



Lack of experts who can measure minimum dimension and judge viability of various groups of organisms is current situation.

Therefore, use of new sophisticated machines such as flow-cytometer and flowcam are highly expected.

But accuracy to low concentration of organisms (1-9 individuals in  $1\text{m}^3$ ) is always high barrier.

Confirmation of viability, and observation of minimum dimension is also difficult for the machines.

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3. Analysis of sample from land-based tests must be completed  
within 6 hours after sampling. This requirement is impossible  
for bacteria analysis because of necessary incubation time.

4. Preparation of land-based test condition
  - 400 m<sup>3</sup> test water with organisms ( $50 > MD \geq 10$ ): 10<sup>3</sup> ind./ml
  - 400 m<sup>3</sup> test water with organisms ( $MD \geq 50$ ): 10<sup>5</sup> ind./m<sup>3</sup>
5. Confirming safety of chemicals used in the treatment
  - to ship crew at the storage
  - to environment at the discharge of BW
6. For detection of pathogenic bacteria using agar plate methods, special attention for human safety is necessary.
  - Vibrio cholerae* (O1 and O139),
  - Escherichia coli*,
  - Intestinal *Enterococci*

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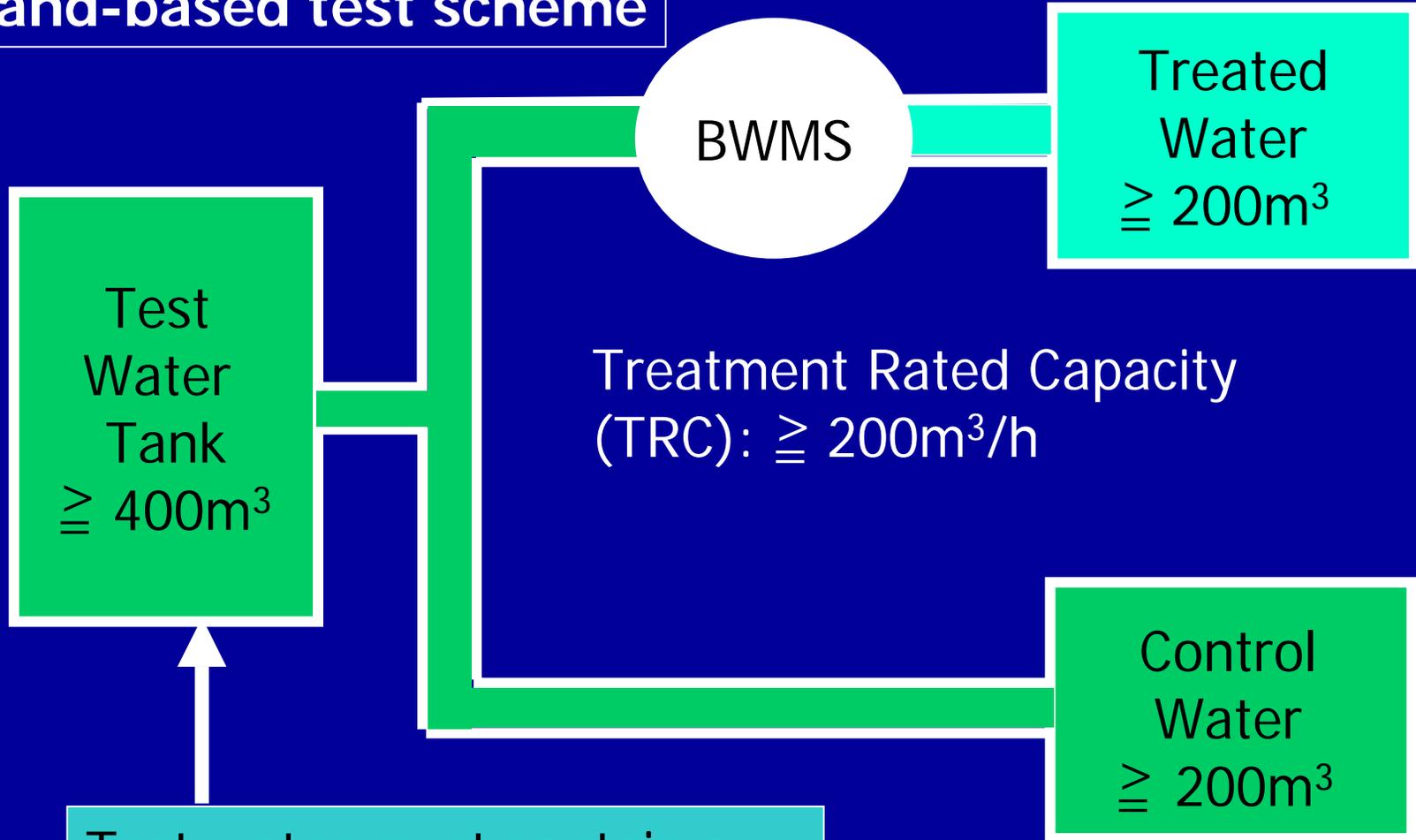
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*Vibrio cholerae* (O1 and O139),

*Escherichia coli*,

Intestinal *Enterococci*

## Land-based test scheme



Test water must contain a certain amount of organisms for evaluation of the BWMS

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