COMPARISON OF DIFFERENT FLUOROMETRIC METHODS FOR BALLAST WATER TESTS

LIVE/DEAD STATUS OF PHYTOPLANKTON SCIENTIST CHALLENGE

ADMINISTRATORS DILEMMA

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MEA-nl is an independent research and test facility

Content

- Application of plant-pigments as tool in BWMS tests
- Phytoplankton (basics, pigments, photosynthesis)
- Potential and limitations of fluorometry
  - Biomass/cell number
  - Viability
- Different BWM technologies (chemical. UV)
- Conclusions & Recommendations

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Basic Information II

(researches, stakeholders, administration)

- Different instruments to measure phytoplankton biomass (fluorescence) and/or viability (photosynthetic efficiency)

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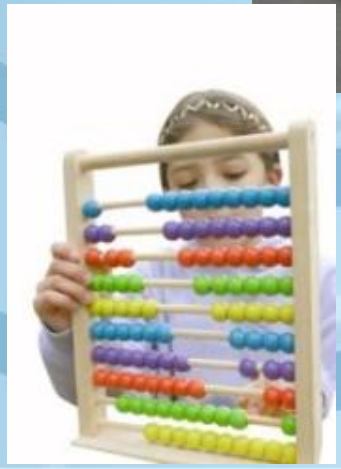
Basic Information

(researches, stakeholders, administration)

- Plant-pigments; energy producers in phytoplankton
  - Proteins capable of converting light into energy
  - Between 5 to 15% of total cell biomass
  - Energy $CO_2 + H_2O \Rightarrow$ organic material + O_2
  - Many different pigments (color to algae)
  - (few) fluorescence signals

- Unique fluorescence properties
- Biomass (presence absence)
- Viability (life/dead)
- Variety of analytical methods available
- Easy to use
- Non-destructive method
- No-chemicals required
- Fast and reliable
- Small to large instruments, in-line



Basic Information
(researches, stakeholders, administration)

- Plant-pigments; energy producers in phytoplankton
  - Chlorophyll (dominant pigment)
  - Measured chemically but also its fluorescence

- Light photochemistry
  heat (3%)
  fluorescence (variable amount F_v)
    - Minimum (F_0) and maximum (F_m) amount
- Quantum efficiency of photochemistry

  - Proteins capable of converting light into energy
  - Energy $CO_2 + H_2O$ organic material + O_2

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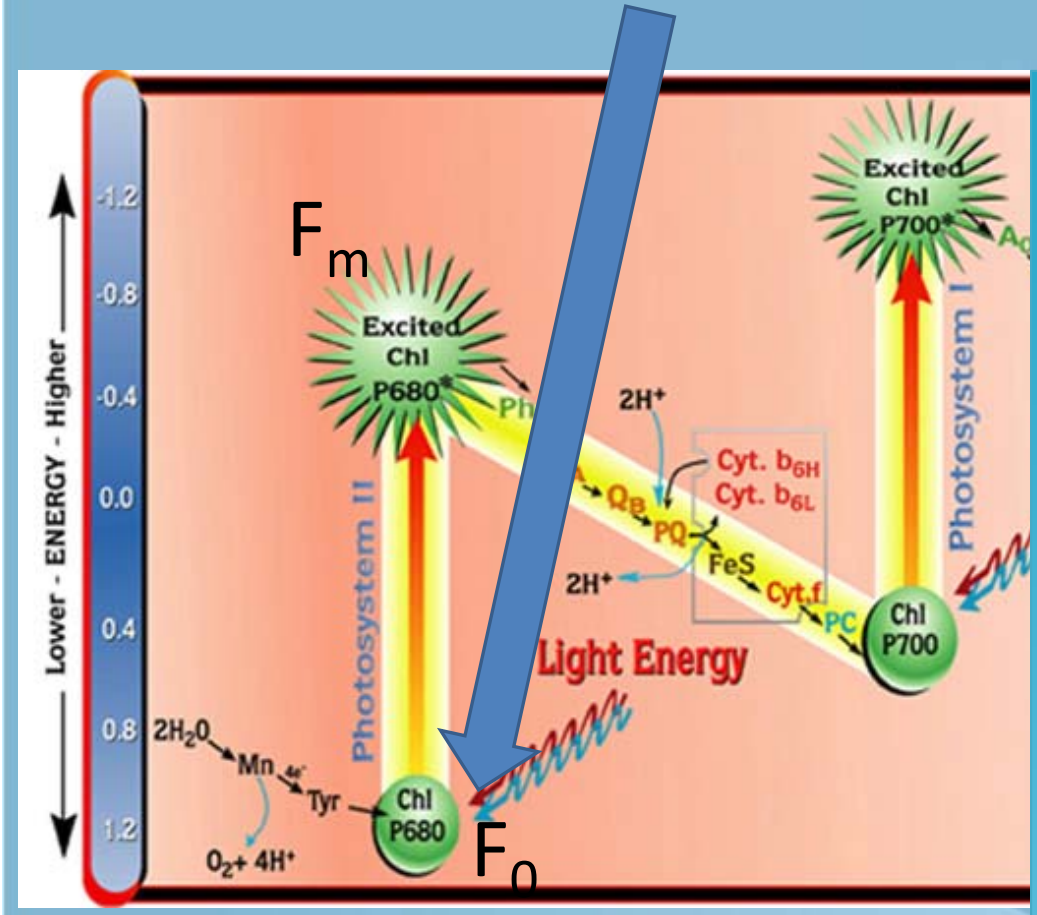
Basic Information
(researches, stakeholders, administration)

F0 can be used to estimate phytoplankton <u>biomass</u>



F0 minimum
Fm maximum
Fv variable Fm-F0

F0 minimum

Calibration of fluorometers

Detection limits (factors of importance)

- Detection limit cells/ ml
- Detection limit cell volume
- Chlorophyll per volume

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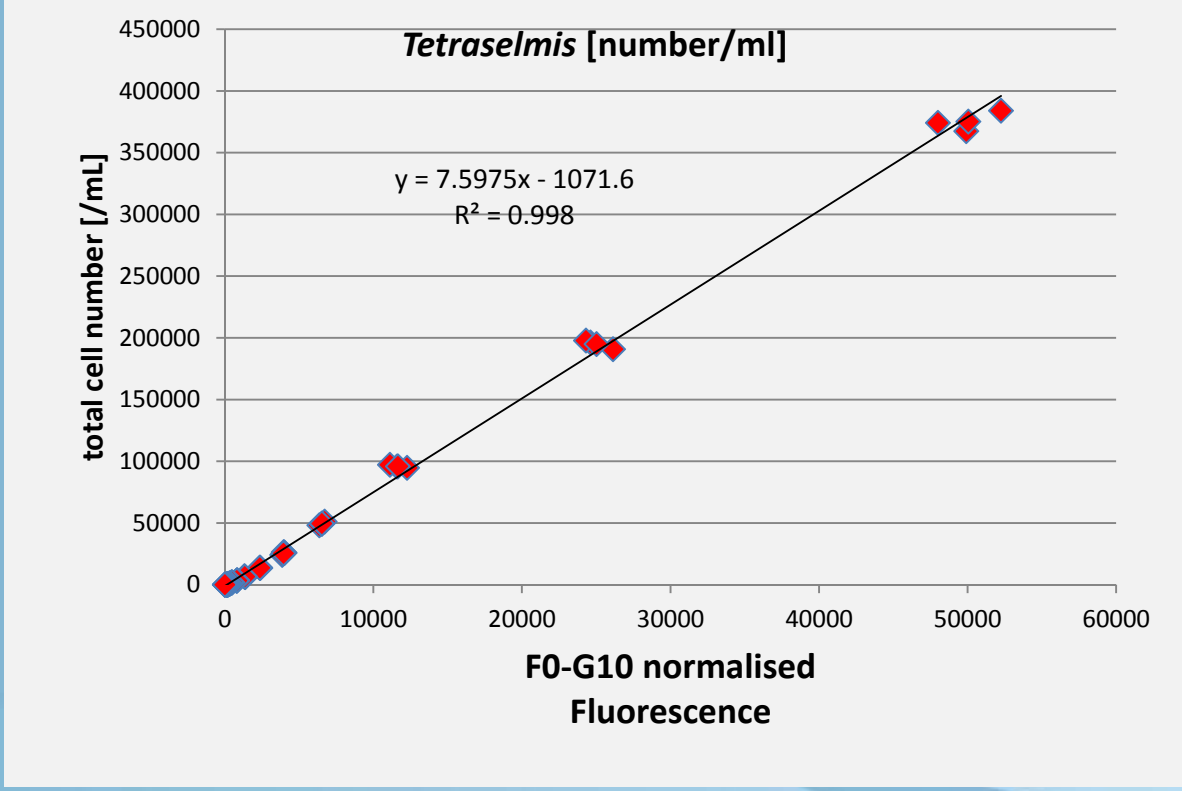
Basic Information

Calibration of fluorometers against known standards)

F0 biomass versus cell number (*Tetraselmis*), 10 micron
Lower detection limit 26.9 cells/mL WALZ water PAM
Detection limit of fluorescence ~ 0.1 µg chlorophyll /L



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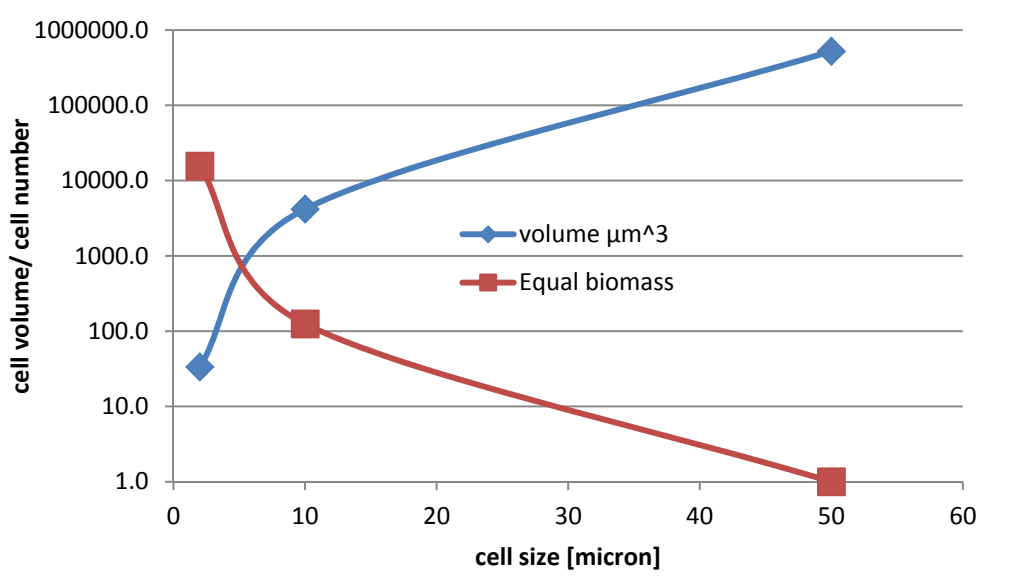
Basic Information

Calibration of fluorometers against known standards

- IMO relevant size class 10 – 50 micron (5 fold variation)
- Plant-pigments varies with the cell volume (µm vs µm^3)
  - 10 – 50 micron (125 fold in volume/chlorophyll) !!!!

conversion size to volume			4/3*3.14* (size/2)^3
size µm	radius µm	volume µm^3	Equal biomass
2	1	33.5	15625
10	5	4187	125
50	25	523333	1
14	7	11488	45.55



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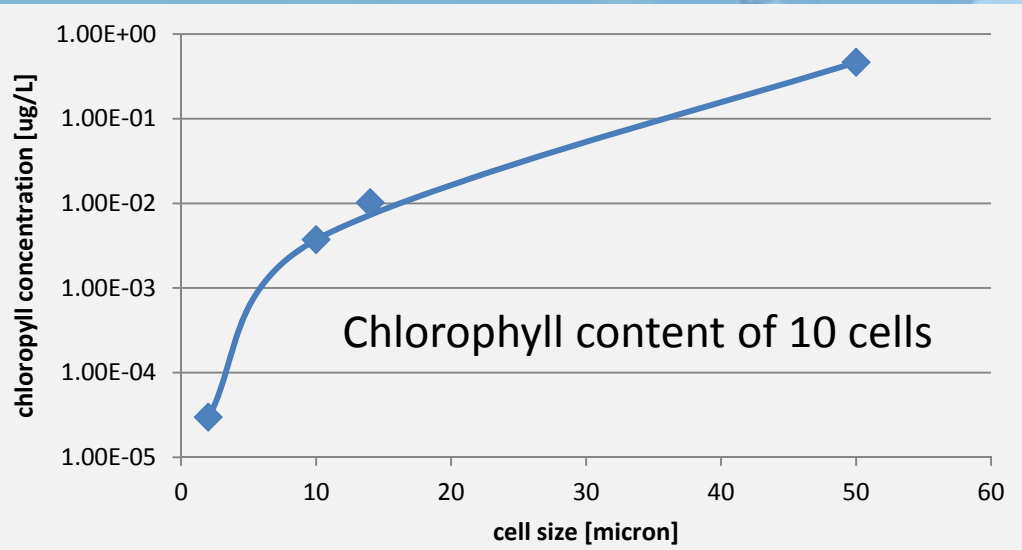
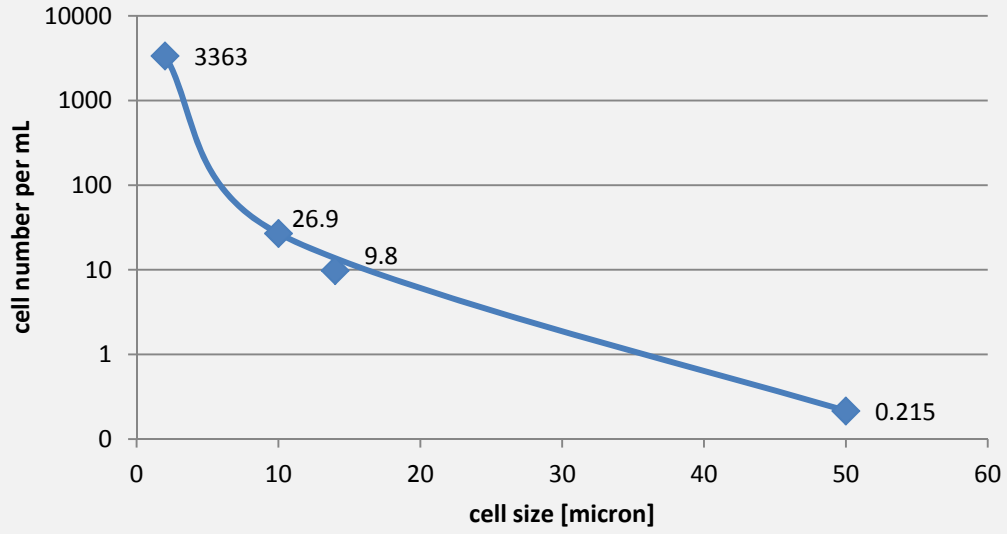
Basic Information

Calibration of fluorometers against known standards

chlor/cell	**3.717**	**pg/cell**			
				pg/10 cells	µg/L
cell number	cell size	conversion volume		chlor/cell	total chlor/L
10	2	0.0080		0.297398	2.97E-04
10	10	1		37.17	0.0372
10	50	125		4647	4.6468
10	14	2.744		102	0.1020





Cell number required to reach detection limit of 0.1 µg/L

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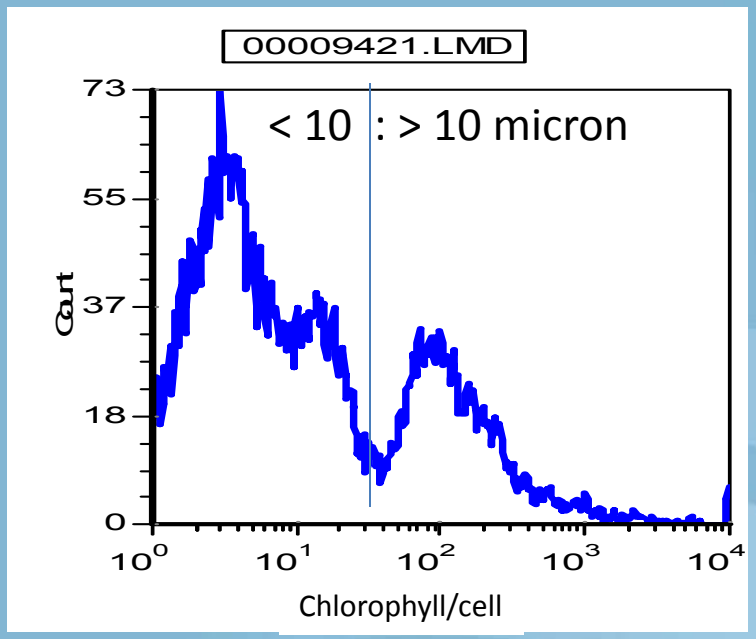
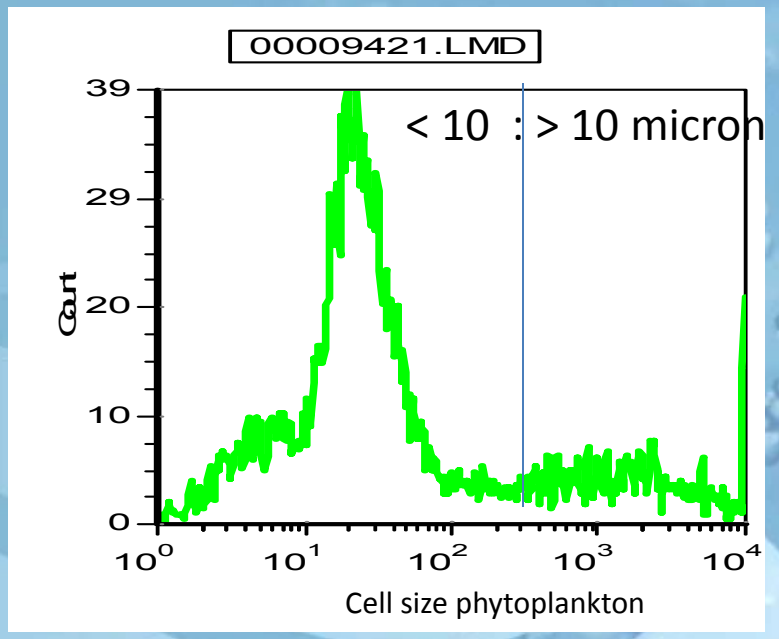
Basic Information
The real world

- Size class distribution (flow cytometry)





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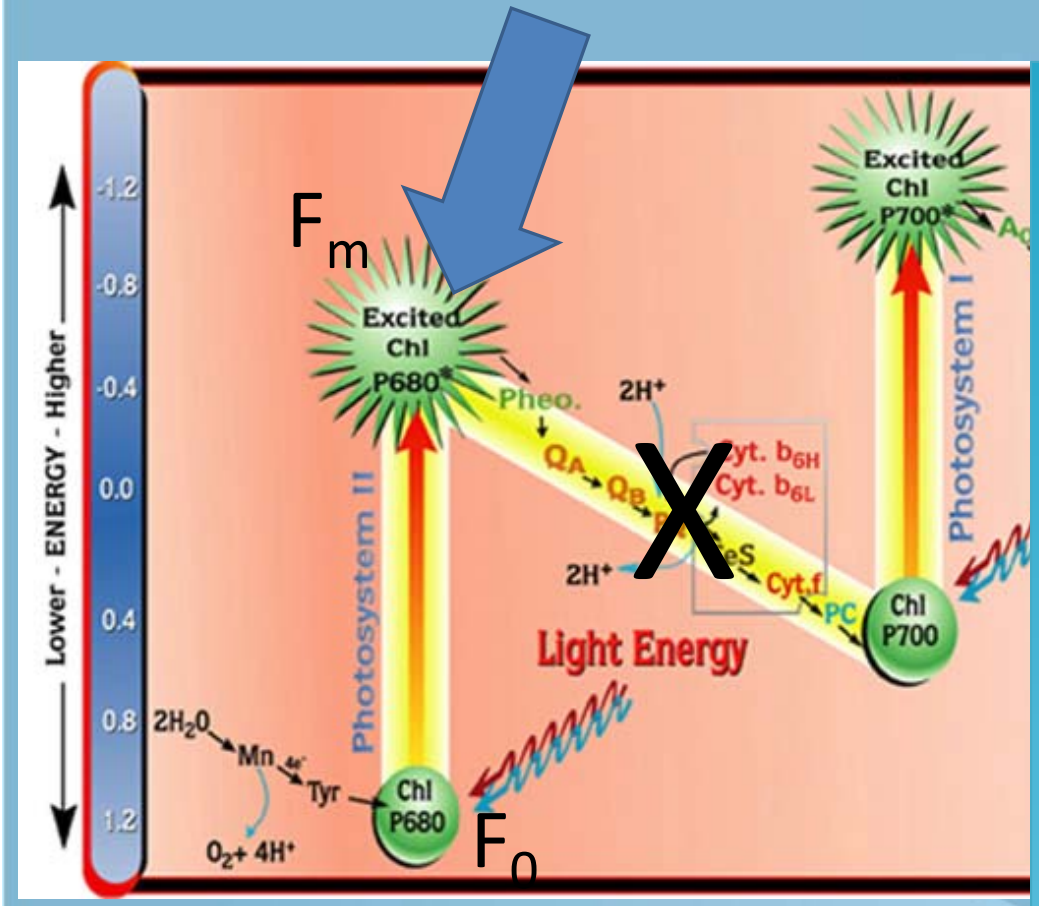
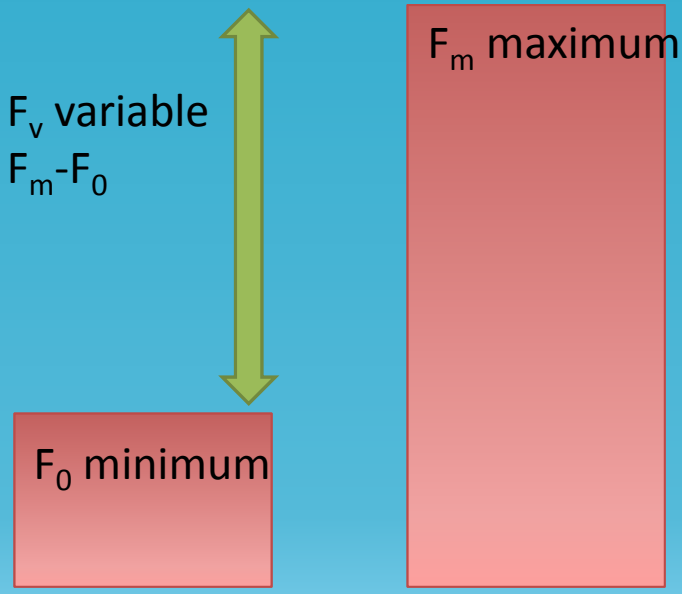
Basic Information Viability

Fm can be induced chemically: add DCMU (dichloromethylurea/Diuron, herbicide) --- electron transport is blocked, fluorescence is maximized =Fm



F_0 minimum
F_m maximum
F_v variable F_m-F_0



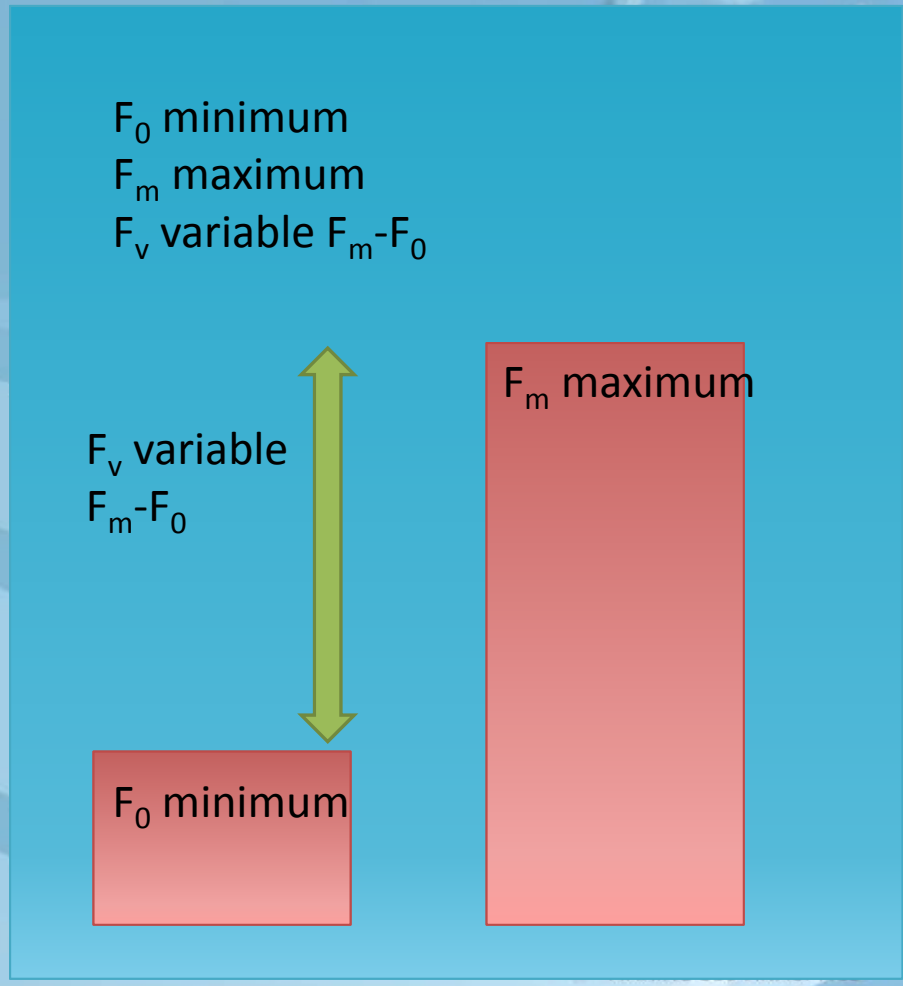
Basic Information Viability

F_m can be induced chemically using fancy manipulation of excitation and emission light (Pulse Amplitude Modulation)

Photosynthetic efficiency (normalised for biomass)

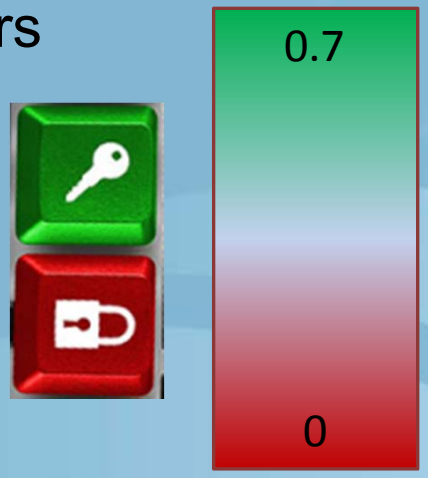
$F_v/F_m = (F_0 - F_m)/F_m$

Varies between 0 ~ 0.7



Basic Information Viability

- Fv/Fm affected by:
- Light, temp, salinity
- Nutrients (N, P, CO_2)
- Trace metals (Fe)
- Viruses
- Internal stressors
- Chemicals
- UV-light



Photosynthetic efficiency (normalised for biomass)

$F_v/F_m = (F_0 - F_m)/F_m$

Varies between 0 ~ 0.7

- Loss in photosynthetic efficiency
- Terminal process/ dead of the phytoplankton cell if Fv/Fm < 0.15

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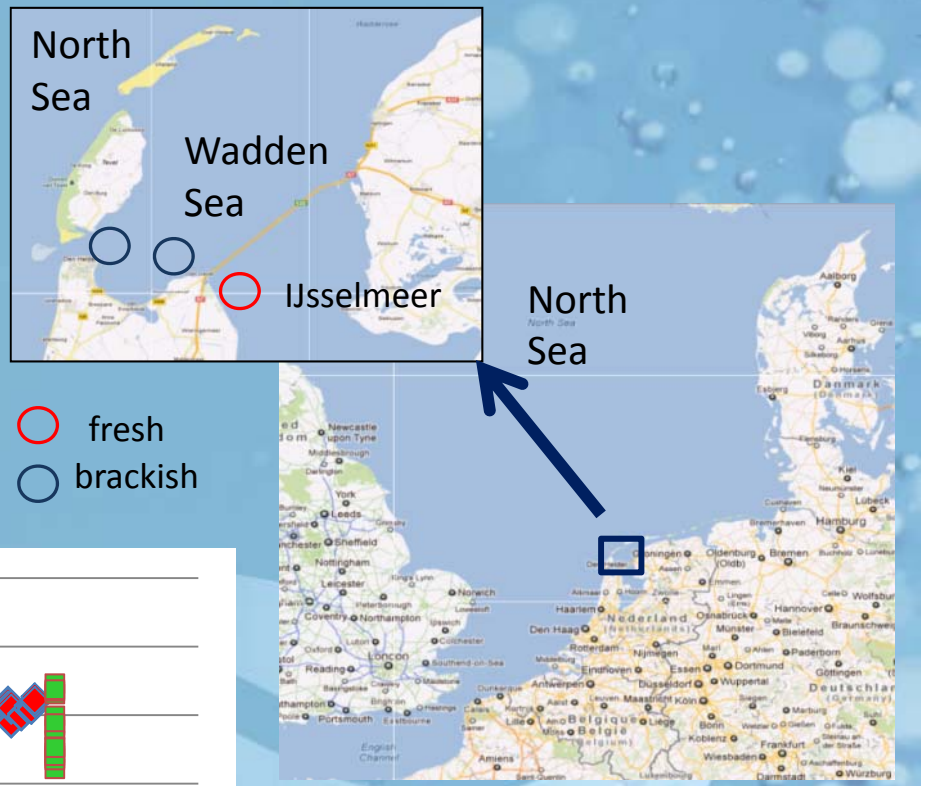
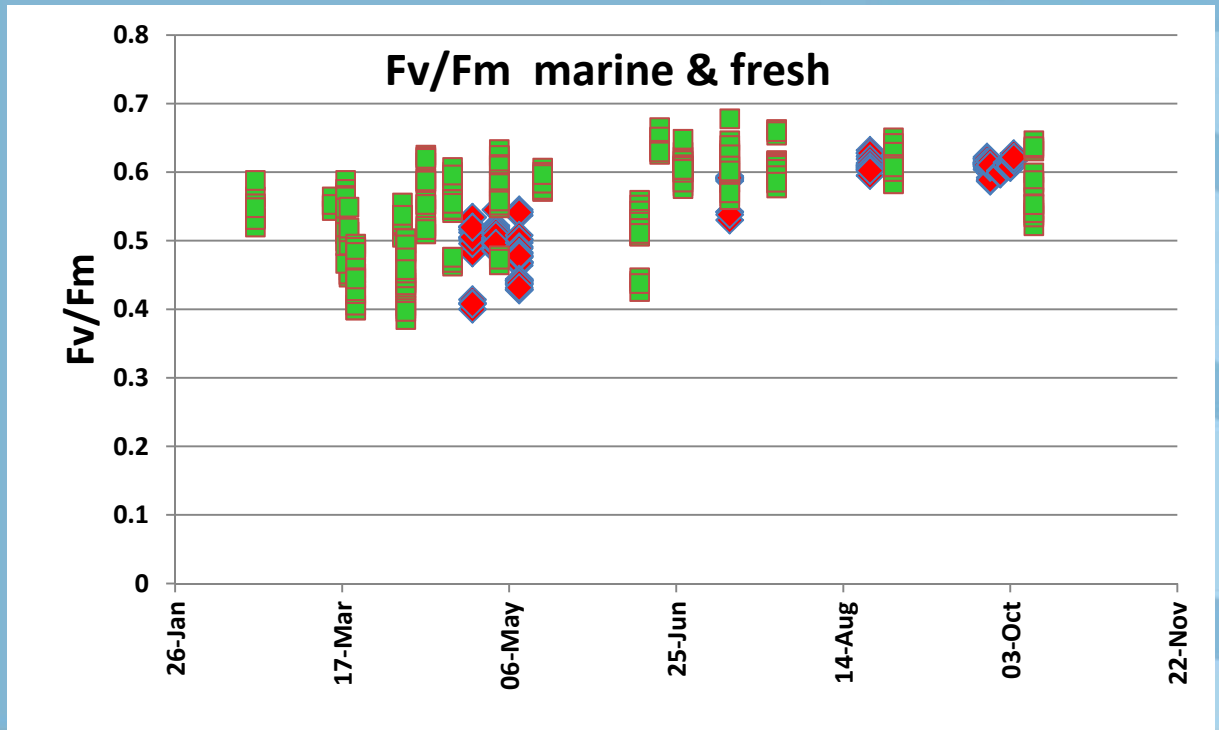
Variations of the real world

- Challenge water
- (Marsdiep, Wadden Sea)
- Lake IJssel

Overall; healthy phytoplankton in challenge water





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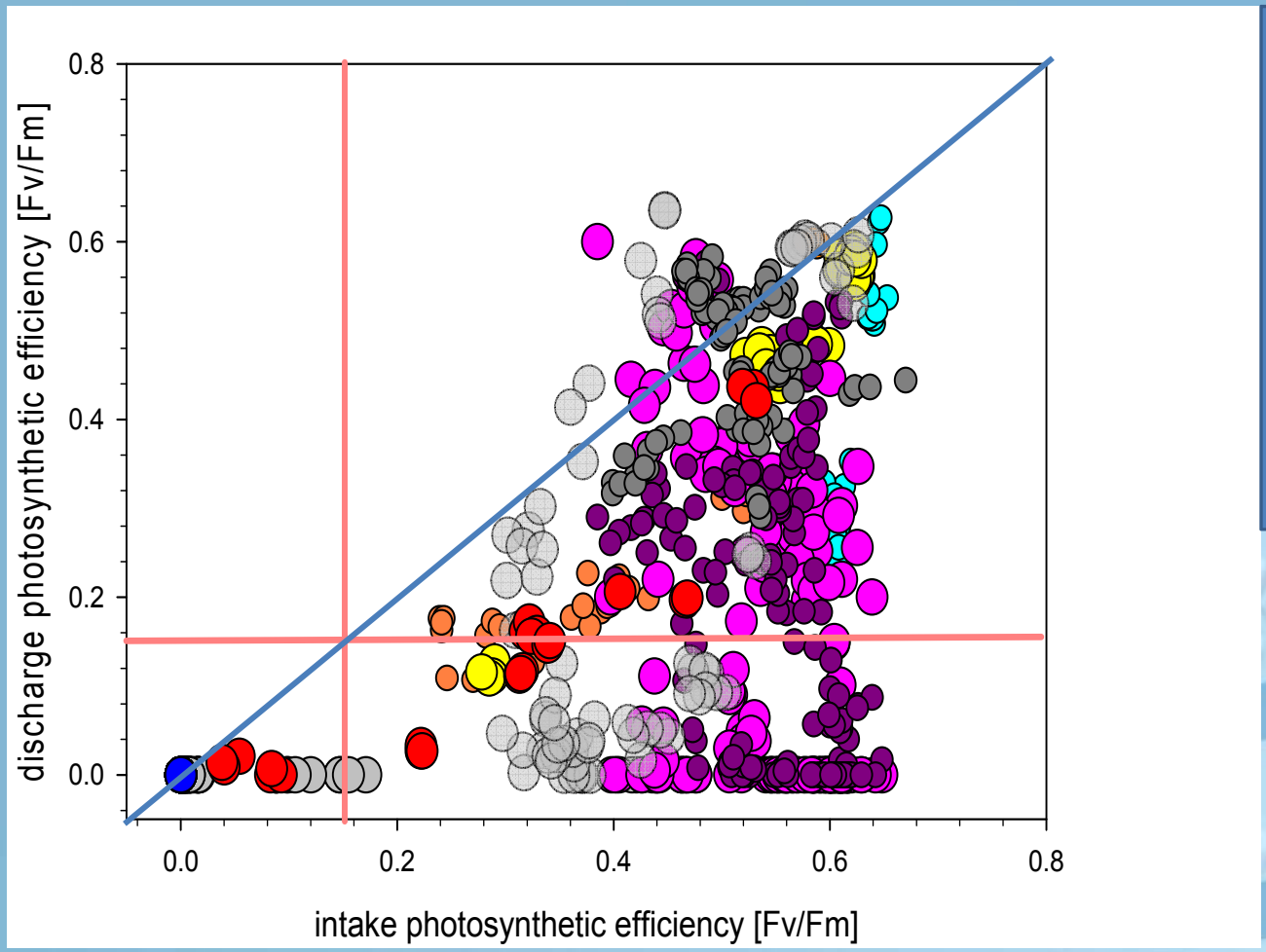
Viability; reality a mess?

all data of BWM systems

Photosynthetic activity at intake (t=0) and after holding period of 5 days (discharge; t5)



Every BW treatment method gives other results

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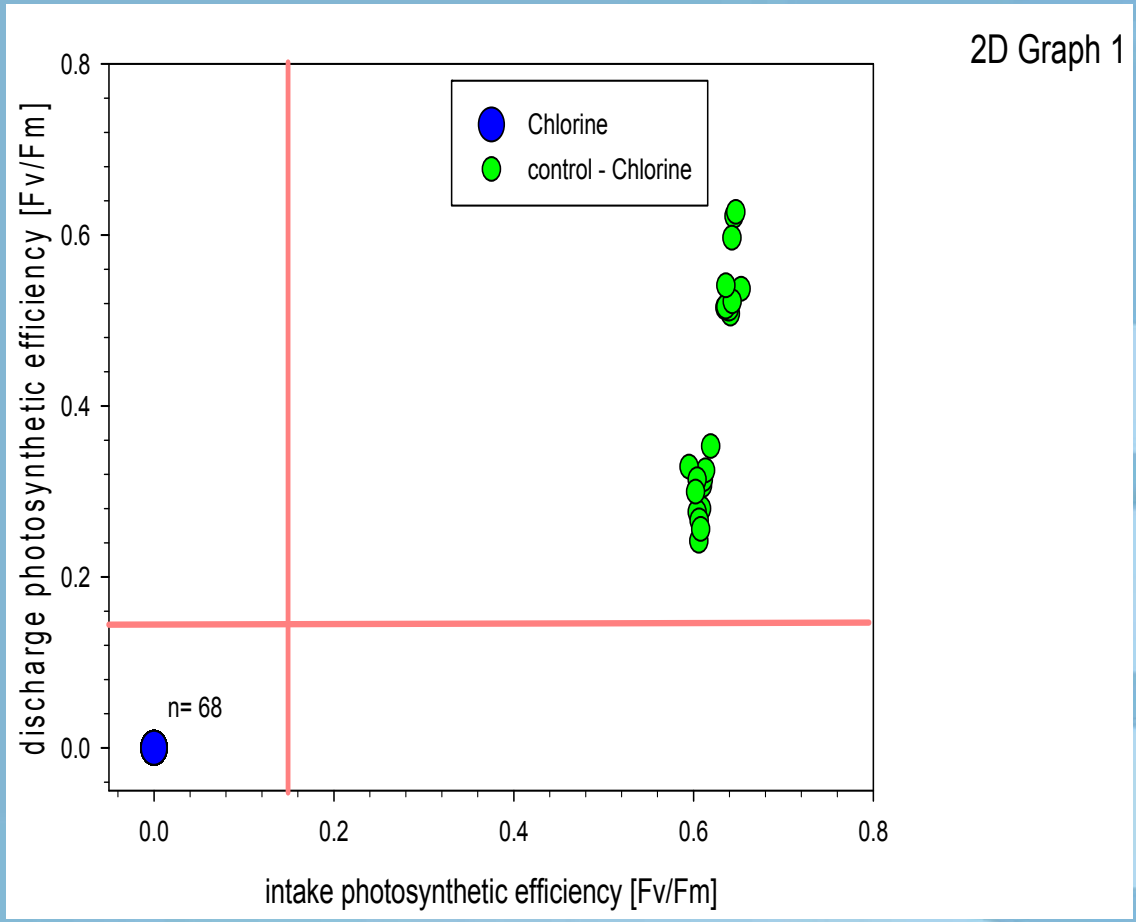
Impact of BWM systems - chlorination

- Photosynthetic activity at intake (t=0) and after holding period of 5 days (discharge; t5)



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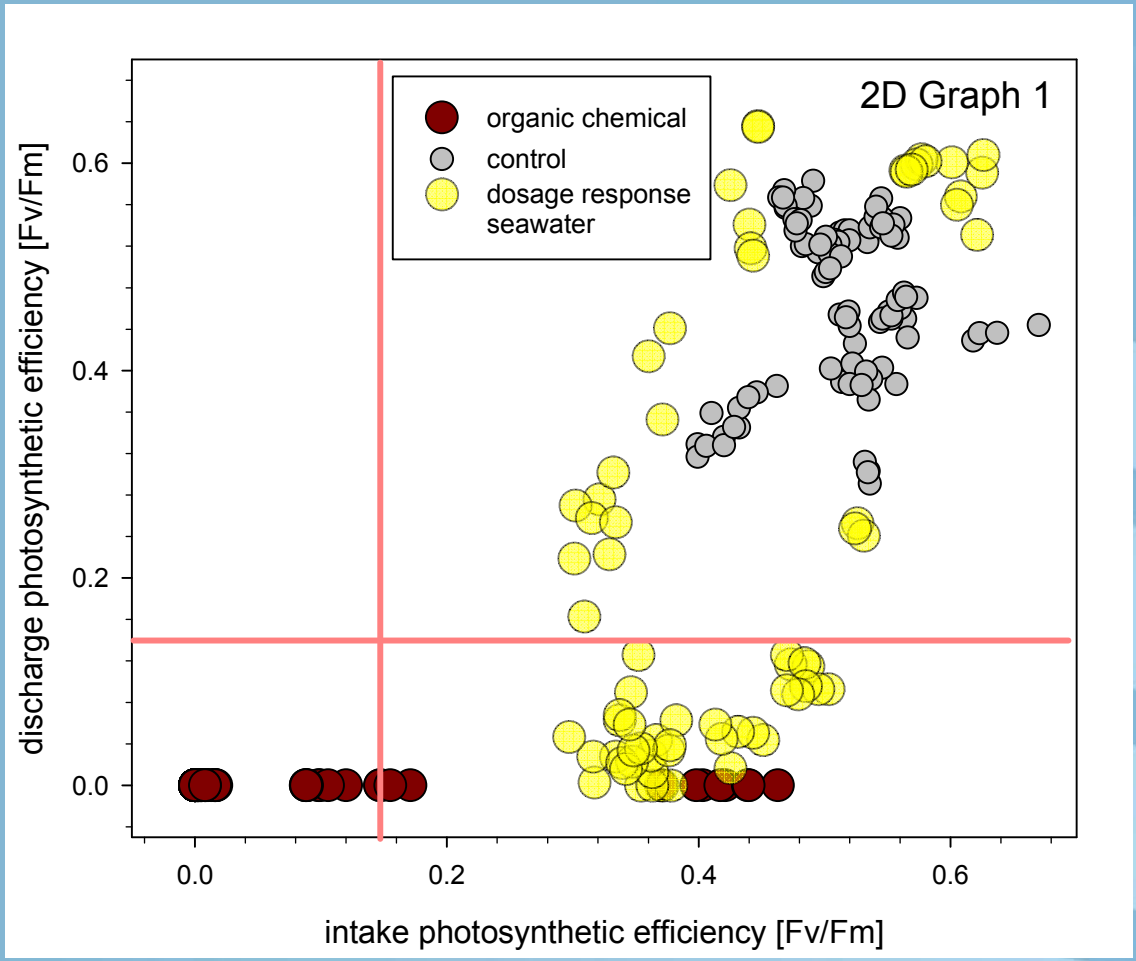
Impact of BWM Systems - Organic disinfectant

- Photosynthetic activity at intake (t=0) and after holding period of 5 days (discharge; t5)



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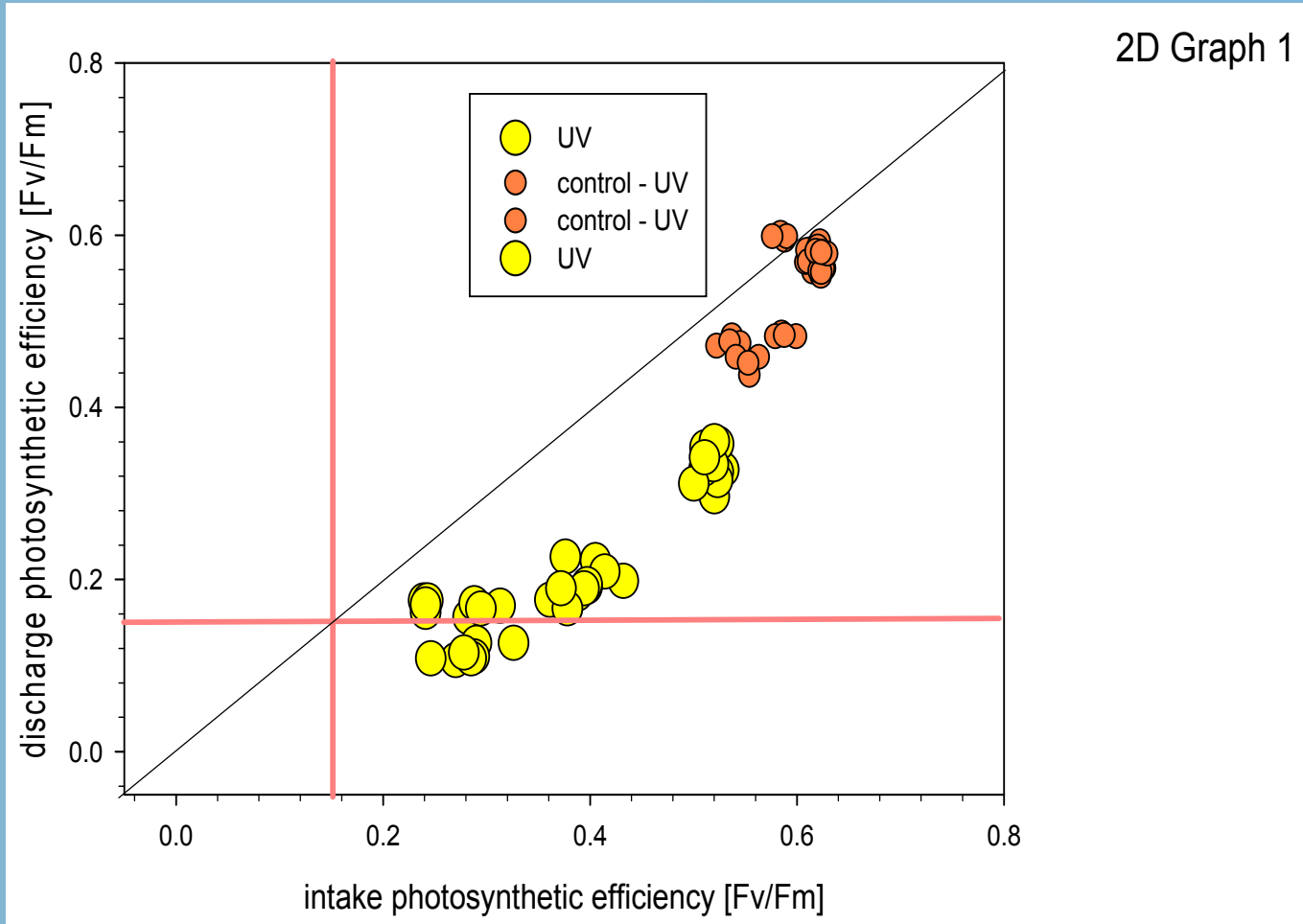
Impact of BWM systems - UV

- Photosynthetic activity at intake (t=0) and after holding period of 5 days (discharge; t5)
- Delayed effect of second UV-treatment at discharge



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Summary advantages disadvantages

- Fluorometry powerful tool in addressing phytoplankton
- On-line sensors can assist in detecting BWMS performance
- Conversion into actual numbers is not that straightforward
- Viability is affected by presence of IMO not relevant phytoplankton (< 10 micron)
- Rapid development of innovative (handheld) tools
- Phytoplankton is not the only relevant group of organisms in 10 – 50 micron size class (micro-zooplankton)
- Familiarization with this technology and its limitation

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Conclusions & recommendations

- Focus of research and improvement should be on BWM System differences, cell size/volume ...etc etc
- using sample concentration or more sensitive instruments

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STILL CONFUSED BUT AT A HIGHER (FLUORESCENT) LEVEL

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