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A novel way to verify the ozone dosing in the field

Aikaterini Spiliotopoulou[1 2], Richard Martin[3], Henrik R. Andersen[1]

[1]Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet 115, 2800 Kongens Lyngby, Denmark
[2] OxyGuard International A/S, Farum Gydevej 64, 3520 Farum, Denmark
[3] Water ApS, Farum Gydevej 64, 3520 Farum, Denmark

Introduction

Ozonation as an additional treatment step has become a widely accepted water polishing technology (Roselund, 1975; Colberg et al., 1977; Owsley, 1991; Cryer, 1992). The water in low exchange recirculating aquaculture systems (RAS) is heavily loaded by organic and inorganic compounds (Bullock et al., 1997; Davidson et al., 2011), where proteins, ammonia and heavy metals are the most pronounced (Davidson et al., 2011). As water recirculates, those compounds are accumulated in high concentrations, creating toxic conditions for aquatic organisms, leading to system failure (Bullock et al., 1997; Davidson et al., 2011).

When ozone is applied to RAS, kills bacteria (Bullock et al., 1997; Davidson et al., 2011; Summerfelt et al., 1997; Powell et al., 2015), removes natural dissolved organic matter (DOM), increases redox level, stabilizes oxygen concentration, and accelerates protein degradation, while it increases water clarity and UV transparency (Davidson et al., 2011), improving coagulation, filtration (Antoniou & Andersen, 2012) and nitrification processes.

However, in a non-meticulously designed system, residual ozone with longer lifetime, will reach the culture tanks causing significant harm to cultured specie (Bullock et al., 1997; Davidson et al., 2011). The risk to lose fish due to overdosing and the high ozonation cost in case of generators malfunction are limiting parameters and contribute to a reluctance by the aquaculture industry to use ozone. Therefore, ozone should be properly delivered, efficiently dissolved and accurately controlled to ensure that it is completely consumed before returning to the culture tanks.

Residual ozone in water is determined by expensive (Accuvac® test kit, Hach Lange) or complicated colorimetric methods (Bader & Hoigné, 1981). It can also be indirectly determined with the traditional oxidation/reduction potential (ORP) sensors which are expensive, having slow response and limited accuracy (Bullock et al., 1997). Fluorescence spectroscopy is a promising technology for both off and on-line monitoring in water treatment applications (Reynolds & Ahmad, 1997).

Fluorescence is able to determine fast and accurately (Hudson et al., 2007; Henderson et al., 2009) DOM in wastewater effluents (Carstea et al., 2016), drinking water (Cumberland et al., 2012), fresh water (Baker, 2001) seawater (Coble, 1996) and RASs (Hambly et al., 2015). Additionally, total organic carbon (TOC) (Carstea, et al., 2016), biological oxygen demand (BOD) (Hudson et al., 2008), phosphate, nitrogen-based compounds (Baker & Inverarity, 2004) and microbial abundances (Cumberland, et al., 2012) can be identified, which are key parameters for the sustainability of a RAS. Hambly et al. (2015) support, that fluorescence is an upcoming real-time monitoring technique to monitor OM in RAS and therefore optimize the holistic RAS management. According to Hambly et al. (2015), the DOC and the feed are proportionally correlated, while fluorescence intensity enhancement was observed with increased feed input.

Ozone is a well-established technology in multiple application having undeniable benefits towards water quality. The most obvious effect of ozone addition in organic loaded water samples is the decolorization. Therefore, an investigation of the possibility to combine the fluorescence OM determination and the bleaching effect of ozone in OM in order to determine the ozone dose will be

conducted. The fluorescent properties of aquatic DOM, its high reactivity towards ozone and the risk of residual ozone presence in culture tanks, lead to investigate the possibility of fluorescence to measure indirectly the residual ozone into water in correlation with the extinction of the oxidized by ozone DOM. The present study attempts to determine the ozone demand and dose in water by fluorescence spectroscopy, utilizing the natural fluorescence stemming from proteins, which are contained into RAS. The principle that the method relies on, derives from the relationship between fluorescence intensities and DOM degradation by ozone.

Methods

*Water samples.*Water samples were collected from 2 fish farms, an experimental facility and 2 aquariums, Den Blå Planet (public aquarium) and the aquarium in Tivoli (amusement park), all situated in Denmark, and used for experiments the following day.

Ozone delivered to water. The experimental set-up for the ozonation was based on a 20 g/h ozone generator from O3-Technology AB (Vellinge, Sweden) which was supplied with dry oxygen gas. Ozone concentration was determined by the indigo method (Bader & Hoigné, 1981) measured at 600 nm with a spectrophotometer (Hach Lange).

Ozone analysis. Water samples were spiked with a volume of ozone stock solution as described in Hansen et al. (2016). Ozone dose was determined by adding the same amount of ozone as in the sample, in acidified MilliQ water bottles, containing phosphate buffer and a sufficient amount of potassium indigotrisulphonate. Afterwards, the absorbance was measured at 600nm and compared to the blank.

Fluorescence. The intensity was determined by a fluorimeter (Cary Eclipse, Varian). The composition of RAS water samples in terms of DOM was further analyzed, utilizing a fluorimeter, measured in predetermined excitation/emission wavelength pairs (Table 1) provided by literature (Hudson et al., 2007). Samples were transferred in a quartz cuvette and subjected to further analysis.

Table 1: Excitation/Emission wavelength pair for fluorophores based on Hudson et al., 2007.

Fluorophore type	**Fluorophore name** (Coble, 1996)	**Excitation/Emission wavelength (nm)**
Protein-like (Tyrosine)	B	231/315
Protein-like (Tryptophan)	T	231/360
Humic-like	A	249/450
Protein-like (Tyrosine)	B	275/310
Protein-like (Tryptophan)	T	275/340
Humic-like	C	335/450

Experiments

Water from RAS was subjected to ozonation, in order to investigate the correlation between fluorescence indices and DOM degradation. Experiments were conducted in a laboratory. Different ozone doses were delivered to water samples, and then the fluorescence degradation was measured. The ozone doses varied from 0-14 mg/L. After ozonation, the samples were stored at 15^{o}C for an hour. In each experimental batch, one sample was not spiked with ozone to provide reference value (blank), however was subjected to the same experimental conditions as the rest of the samples e.g. retention time and temperature. Obtained data were analyzed using MS Excel and Prism Graph Pad.

Results and discussion

The water comes from a raceway trout model farm receiving water from a stream, equipped with simple water treatment technology such as airlifts, mechanical and biological filters.

The degradation kinetics of chromophores and fluorophores in the investigated samples suggest one-phase decay (Figure 1). Humic-like fluorescence (green and orange lines) was half when approximately 5 mg O_3/ L was dosed (Figure 1). Spiking with the same ozone dose (5 mg O_3/ L)

the already low intensity protein-like fluorescent OM (red, blue, brown and black lines) was almost extinct (Figure 1), as it has been previously observed in Świetlik & Sikorska (2004). It has been reported that humic-like substances when subjected to ozonation either increased in intensity or remained stable, while for protein-like, a decrease in intensity was typical (Henderson et al., 2009). The fact that the humic-like fluorescence is easier to detect than the protein-like fluorescence, makes the humic-like fluorescence the most promising for the future industrial application (Li et al., 2016). Additionally based on our findings, it can be concluded that for RAS, relatively low ozone does are sufficient to increase water transparency.

High ozone doses up to 14 mg O_3/ L were spiked to investigate fluorescence behavior and if it will eventually be completely removed. The addition of 14 mg O_3/ L, reduces significantly fluorescence intensity but is not able to oxidize it completely. More specifically, the fluorescence (both humic and protein-like) in RAS, has a reduction ranging from 90% to 97.7% (Figure 1).

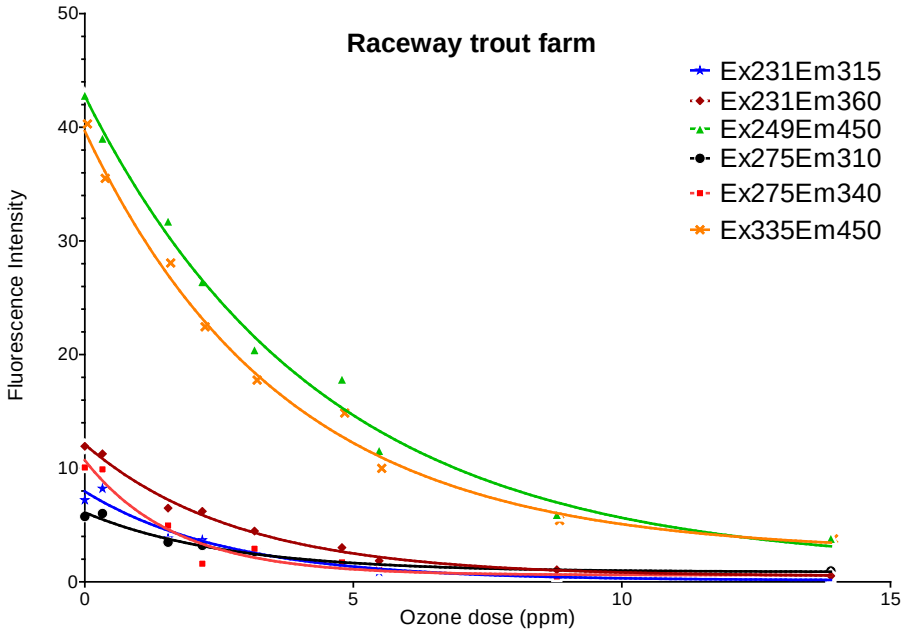

Figure 1: Water characterisation based on fluorescence-like matrix.

Conclusions

Fluorescence spectroscopic has great potential to be used as a monitoring tool in RAS because of the great sensitivity and selectivity towards OM, fluorophores and consequently ozone, especially in low ranges (0-5mg O_3/ L). The present work suggests a technique which can be further developed in order to manufacture accurate, low-cost, real-time measurement sensors to define dissolved ozone into water.

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Technical University of Denmark

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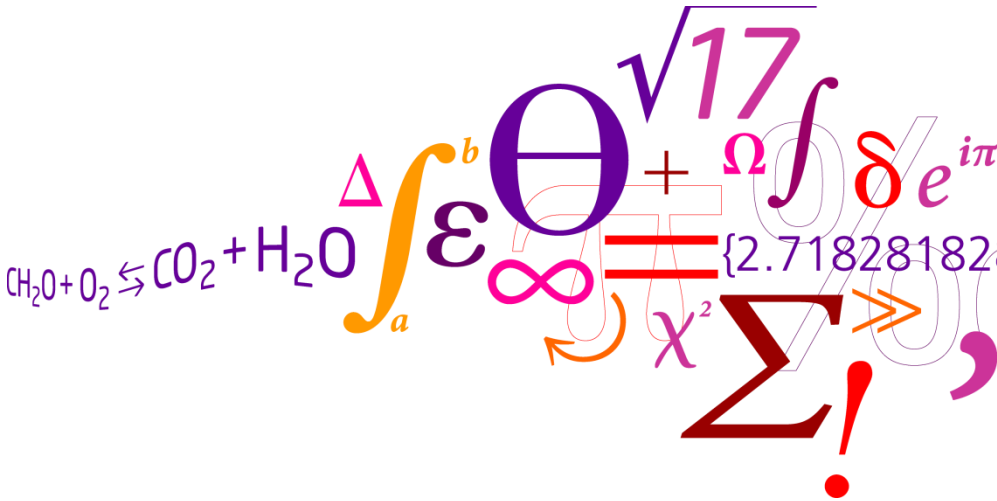
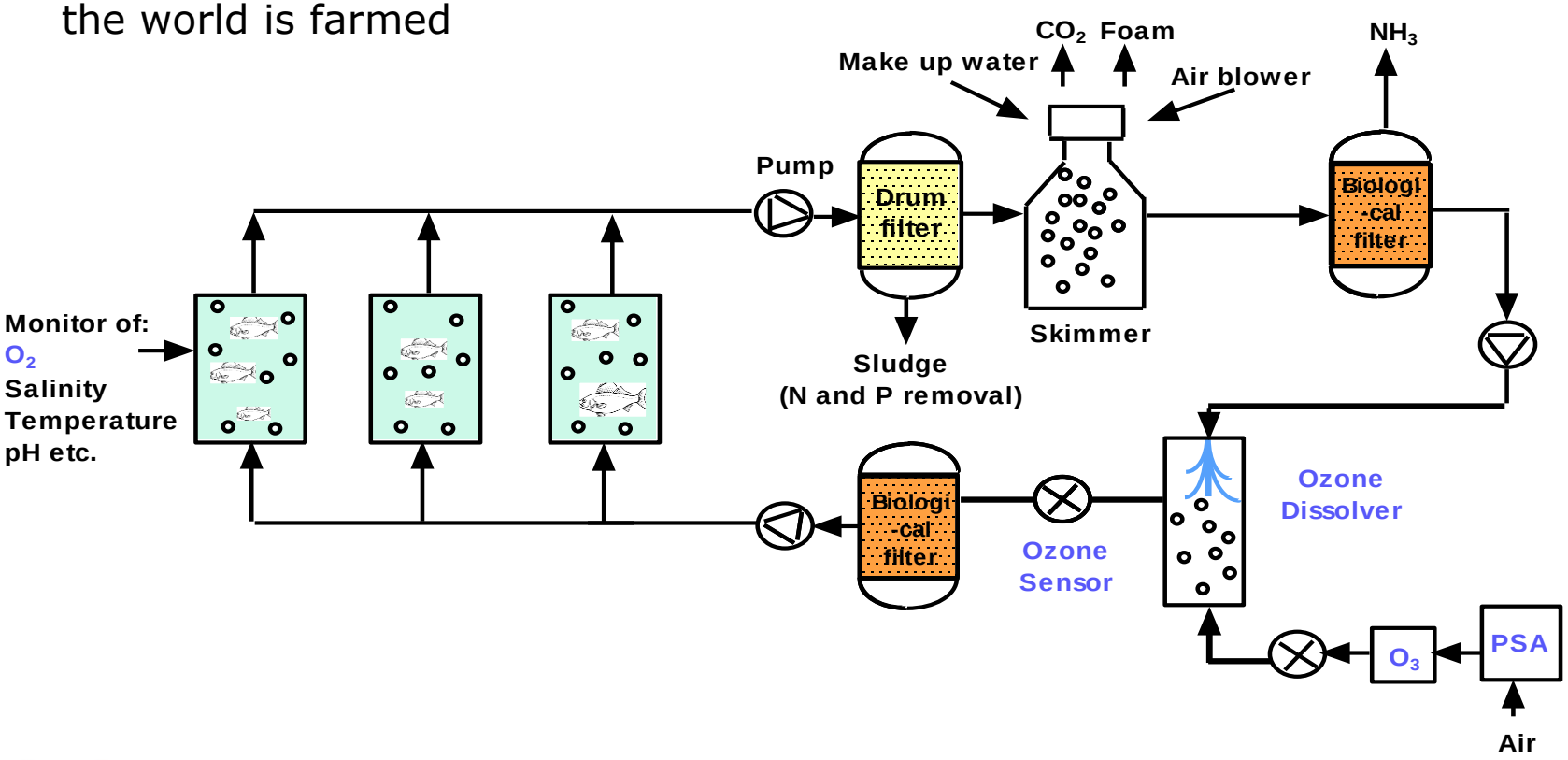
[1]Department of Environmental Engineering, Technical University of Denmark
[2] OxyGuard International A/S
[3] Water ApS



Water
make it simple and let it work

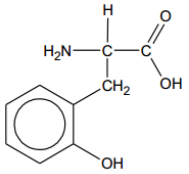
Recirculating Aquaculture System (RAS)

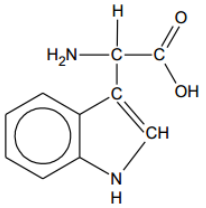
- 16% of animal derived protein is from fish
- More than 2,6 billion people get more than 20% of their protein intake from fish
- A few years ago: more than 60% of the fish consumed around the world is farmed

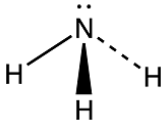


RAS implications

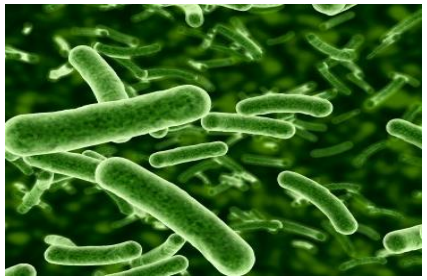
- Low exchange RAS (90% or more of water is recycled)
- Accumulation of:
  - Dissolved organic mater (DOM)
  - Micro-particles
  - Dissolved N-compounds (e.g ammonia)
  - Heavy metals
  - Microbial abundancies
- Potentially leading to:
  - Suboptimal conditions



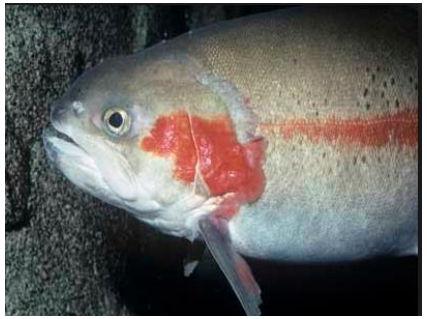


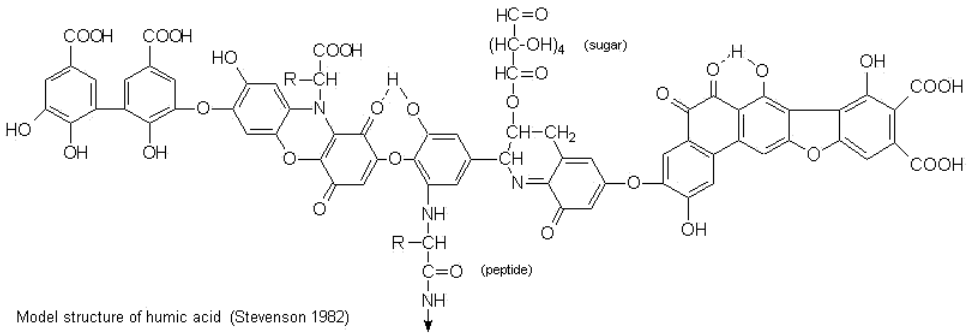


Cd^{2+} Cu^{2+}



Hg





Model structure of humic acid (Stevenson 1982)

Pb^{2+} As^{3+}

Water
make it simple and let it work

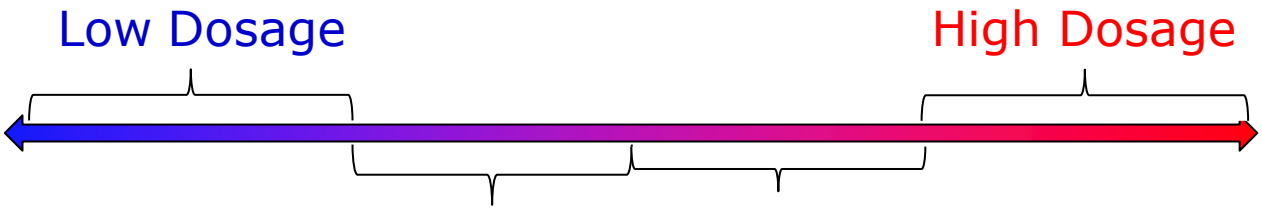
Dual Functions of Ozone

- Oxidation
  - Strong oxidizing agent
    - Rapid reactions
    - Removal of natural DOM
    - Acceleration of protein degradation
    - Increased water clarity and UV transparency
    - Improve
      - coagulation
      - filtration and
      - nitrification processes.
- Disinfection
  - Efficient against
    - Bacteria
    - Viruses
    - Parasite

Challenges

- Ozone overdose
  - **Never present in culture tank**
  - Significant harm to cultured species
    - > 0.01 mg/L
- In case of saltwater system:
  - Hypobromous acid formation
    - toxic
- Reluctance to use ozone due to:
  - Risk of losing fish
  - Cost

Need for an operational method to **monitor the ozone demand** in the water phase!!!



Traditional residual ozone determination

- Dissolved (actual) ozone into water
  - Off-line colorimetric method (e.g. DPD, indigo trisulfonate)
    - Spectrophotometer
      - complicated method
    - Test kits
      - expensive
  - Online measurement
    - Potentiometric principle probe
      - quite expensive
    - Oxidation potential reduction (OPR)
      - cheap
      - do not measure ozone
      - non specific (cannot distinguish e.g. O_3 from Cl_2)
      - risk of failure when exposed to high ozone concentration

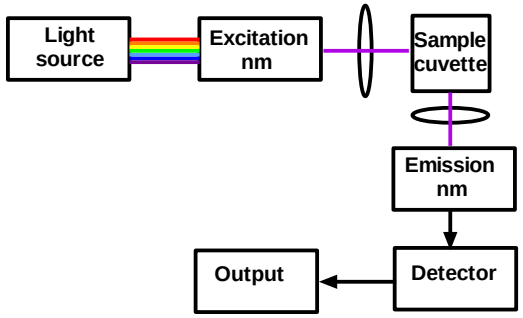
Delivered Ozone determination

We propose a new method to determine how much ozone dosage is added into water

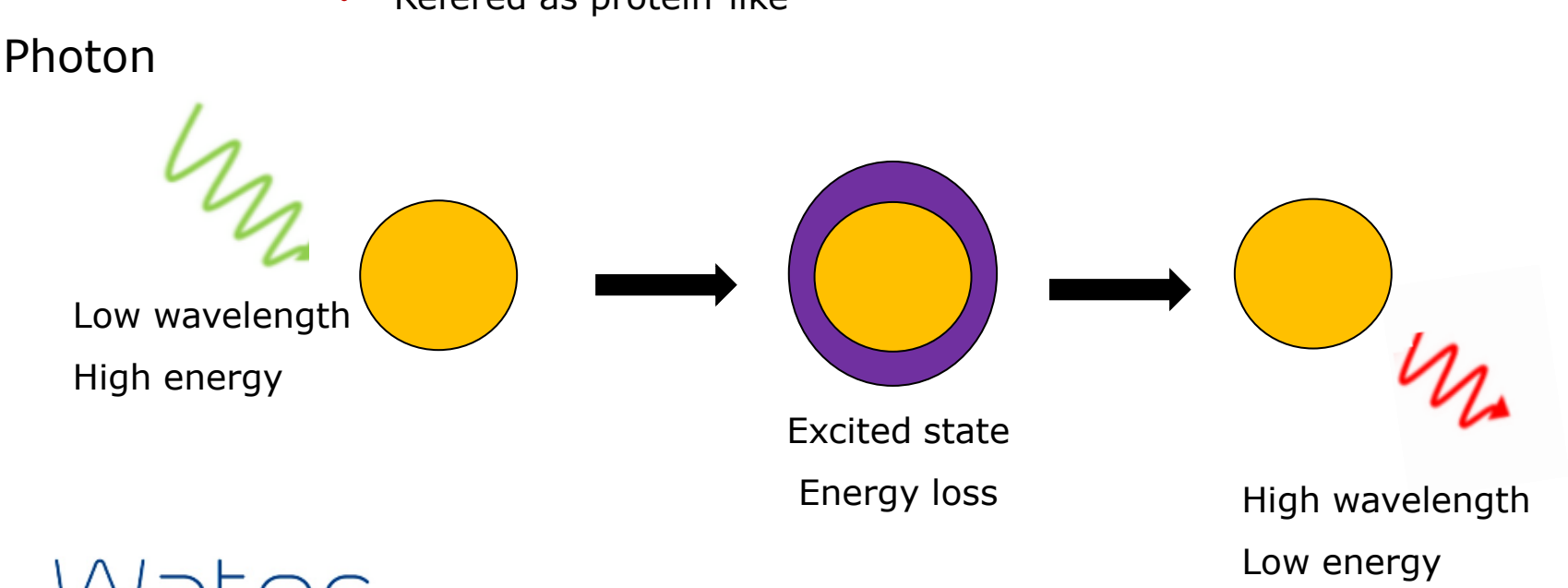
- Fluorescence
  - Based on natural fluorescence of DOM
    - rapid detection
    - precise characterization of DOM composition
  - Tested in wastewater, river water, seawater, etc.
  - **Never used to control ozone in aquaculture until now**

Fluorescence

- DOM contains:
  - Chromophores (absorb light)
  - Fluorophores (re-emit light)
    - Humic substaces (plant origin)
      - Refered as humic-like
    - Amino acids (proteins)
      - Refered as protein-like



(Fluorescence principle)



Fluorescence transitions

- Based on fluorescence transitions published in an wastewater overview paper (Hudson et al., 2007)
  - To characterized micro-pollutants in waste water
- We use the same wavelength pairs

Fluorophore type	Excitation/Emission wavelength (nm)
Protein-like (Tyrosine-like)	231/315
Protein-like (Tryptophan-like)	231/360
Humic-like	249/450
Protein-like (Tyrosine-like)	275/310
Protein-like (Tryptophan-like)	275/340
Humic-like	335/450

Our Aim

- Does naturally fluorescent DOM exist in RAS?
- Is the natural fluorescence in RAS reacting with ozone?
- How could this knowledge be implemented in real life applications?

Water
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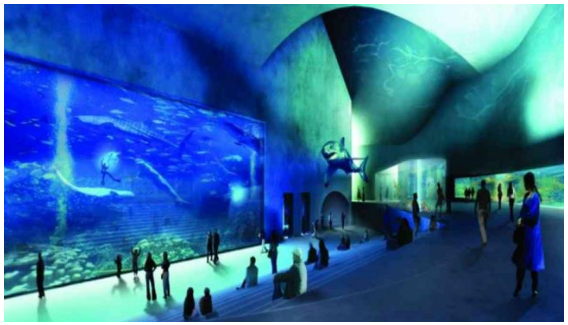
Sampling sites

Model trout farm



Tivoli

Eel fish farm

Pilot scale RAS

The Blue Planet

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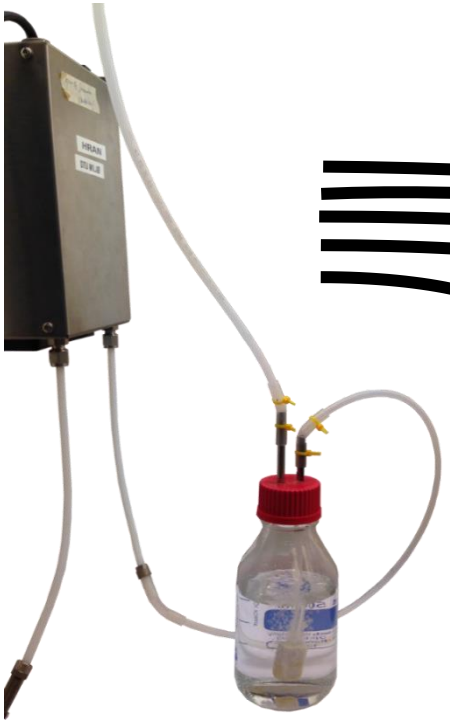
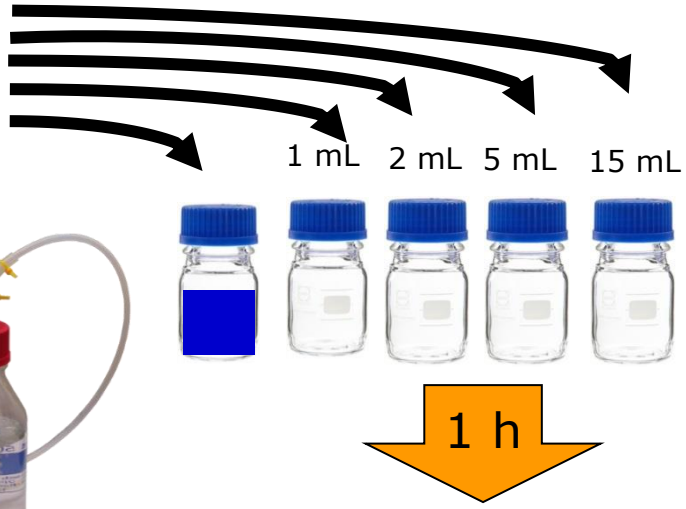
Experimental setup-lab scale



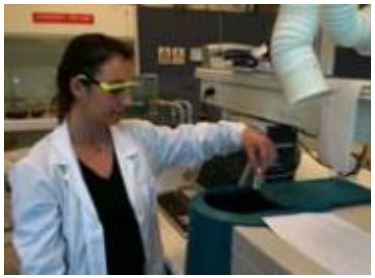
Stock solution of ozone



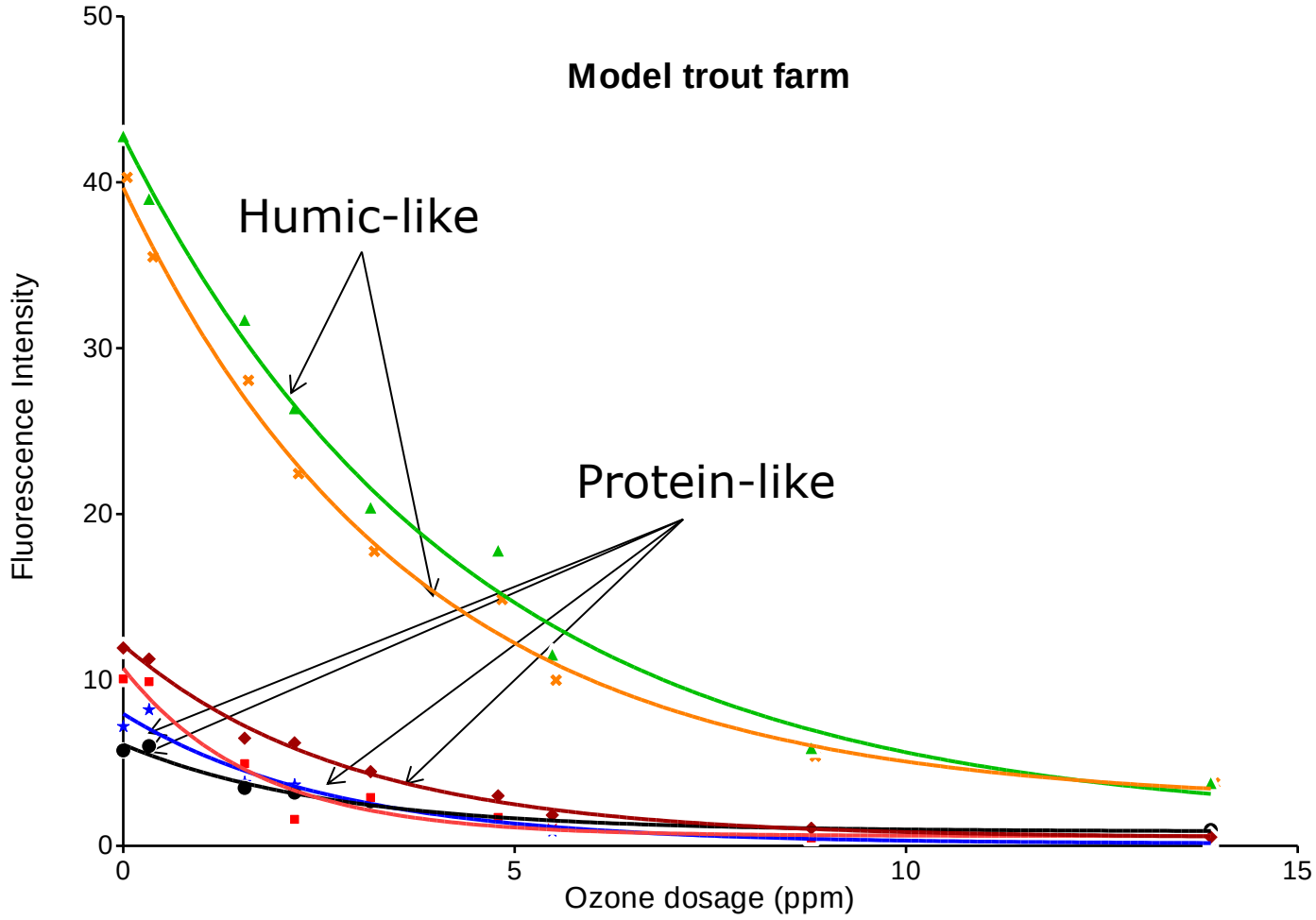
Fluorescence analysis

Water characterization

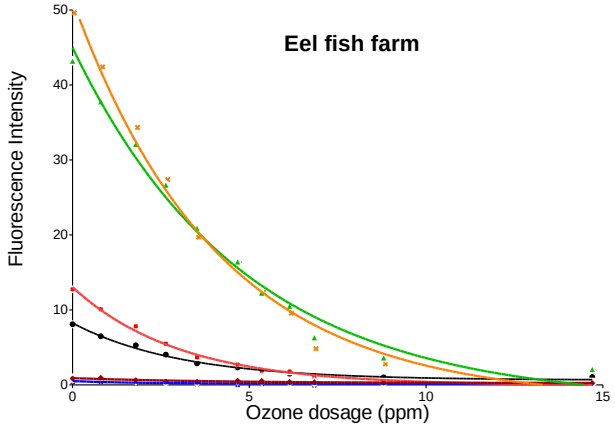
- Ozone doses
  - 0 to 20 mg O_3/L

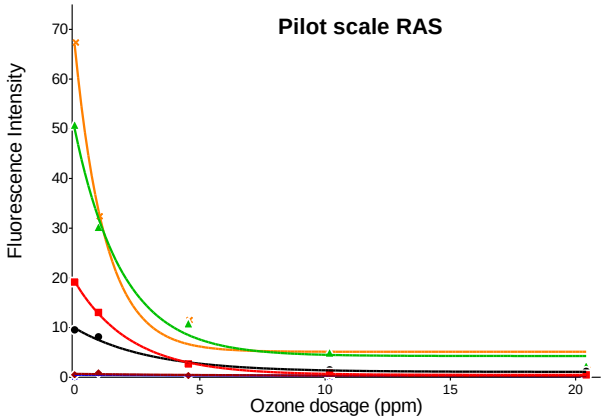


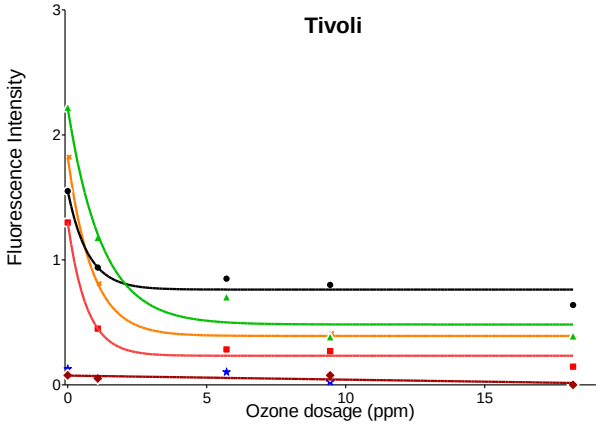
Water characterization based on fluorescence

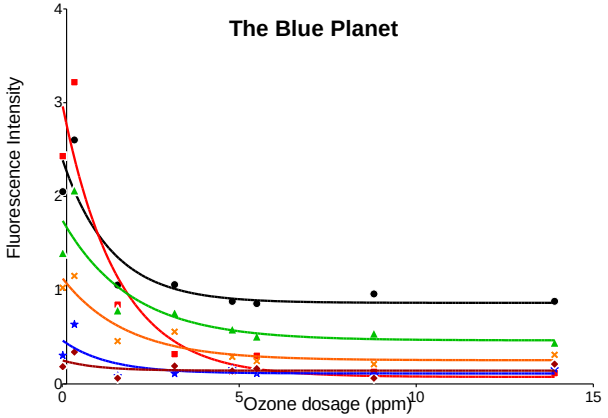


Fluorescence profile in different water samples









- Fish-farms: humic-like fluorescence dominates
- Aquariums: more diverse fluorescence
- High ozone sensitivity in low concentrations

Water
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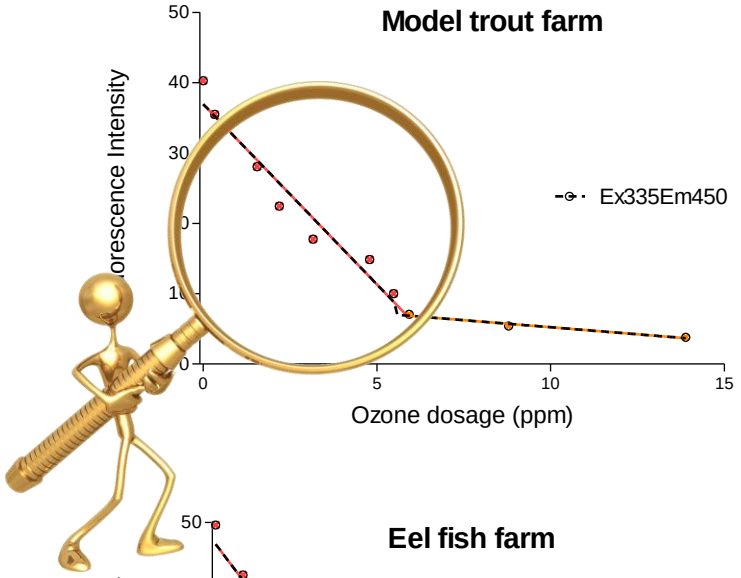
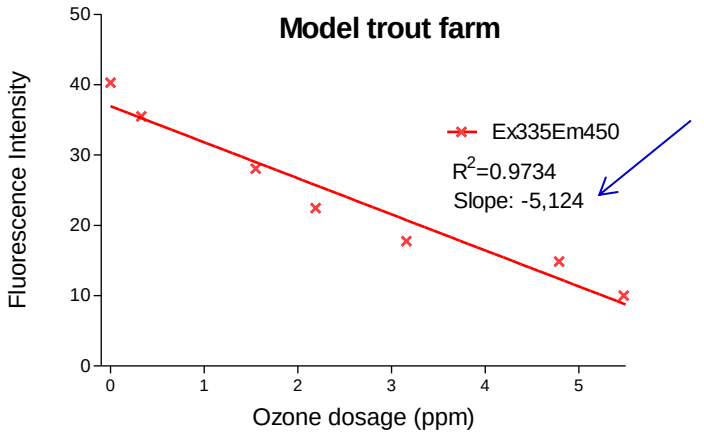
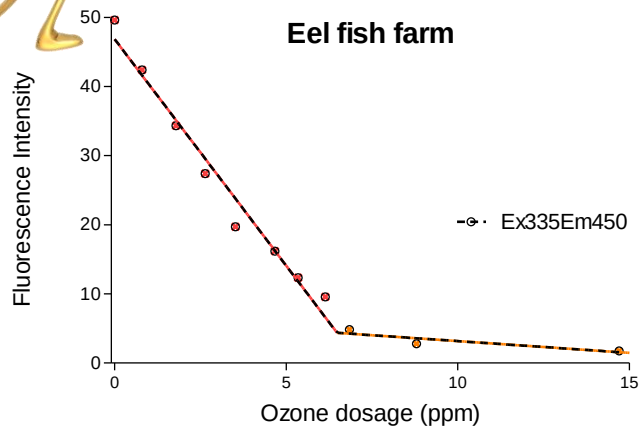
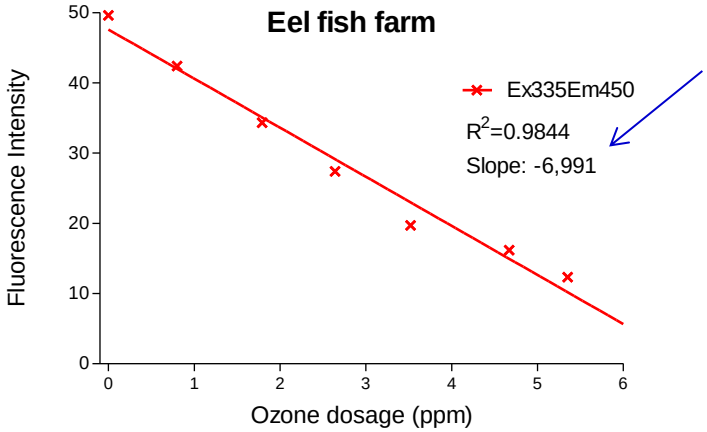
Humic-like fluorescence calibration curve



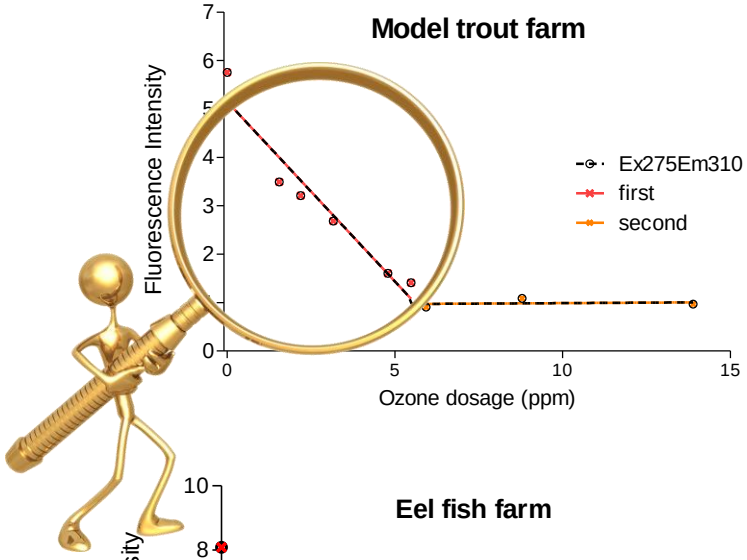


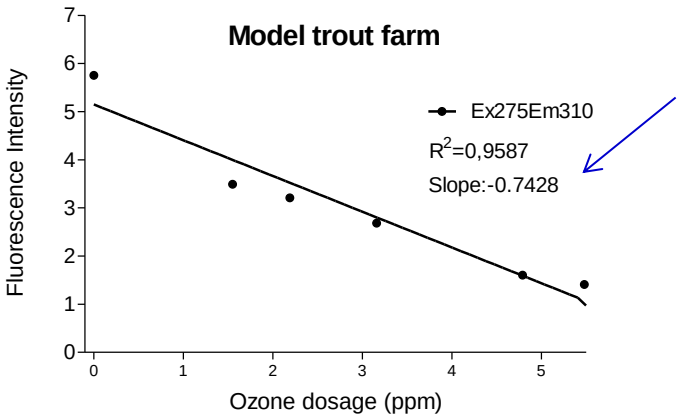


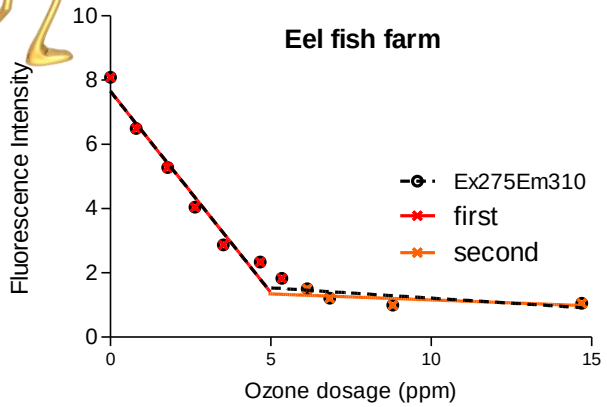


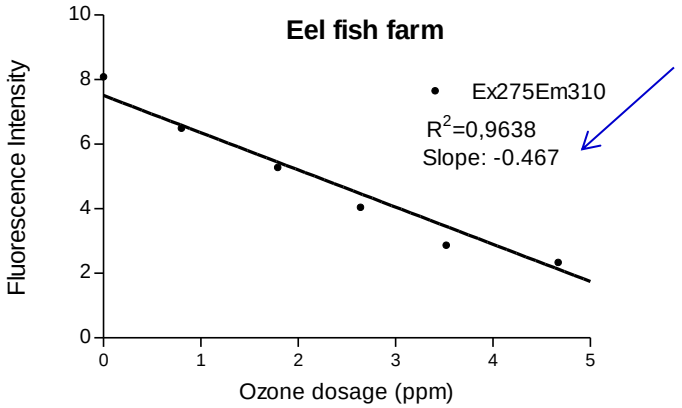
- Slopes among samples varied

Protein-like fluorescence calibration curve







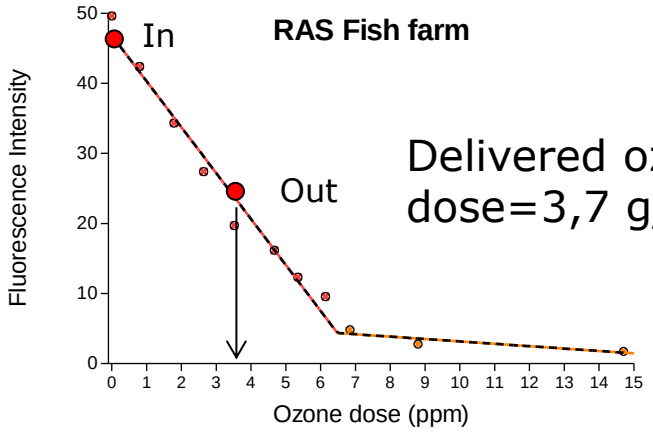


- Slopes among samples varied
  - Other OM contained in water are competing fluorescence
- Unlike to have a universal sensor controlling ozone into water

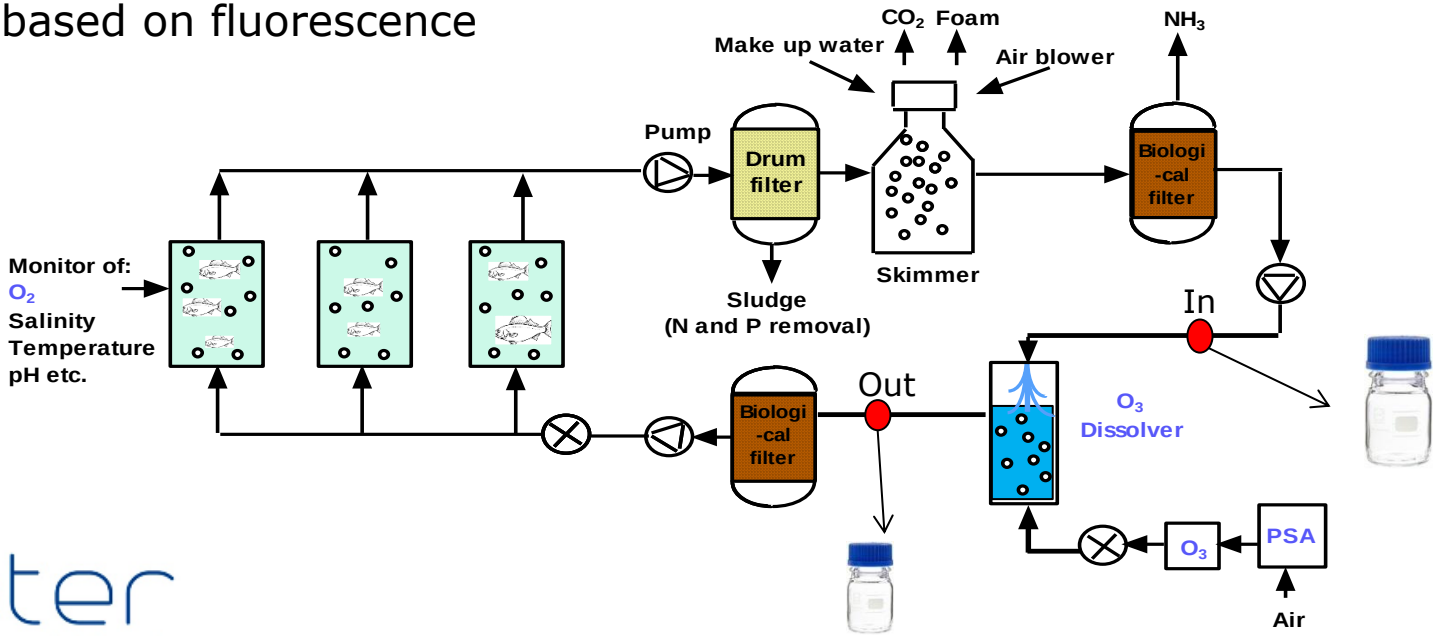
Water
make it simple and let it work

Application #1: Determination of delivered ozone dose

- Does the generator deliver the ozone dose that the specifications promise?
- Validation of ozone generator
- Without sensor installation
- How does it work?
  - Grab samples before and after
  - Calibration curve in the lab based on fluorescence



Delivered ozone dose=3,7 g/m^3



Water
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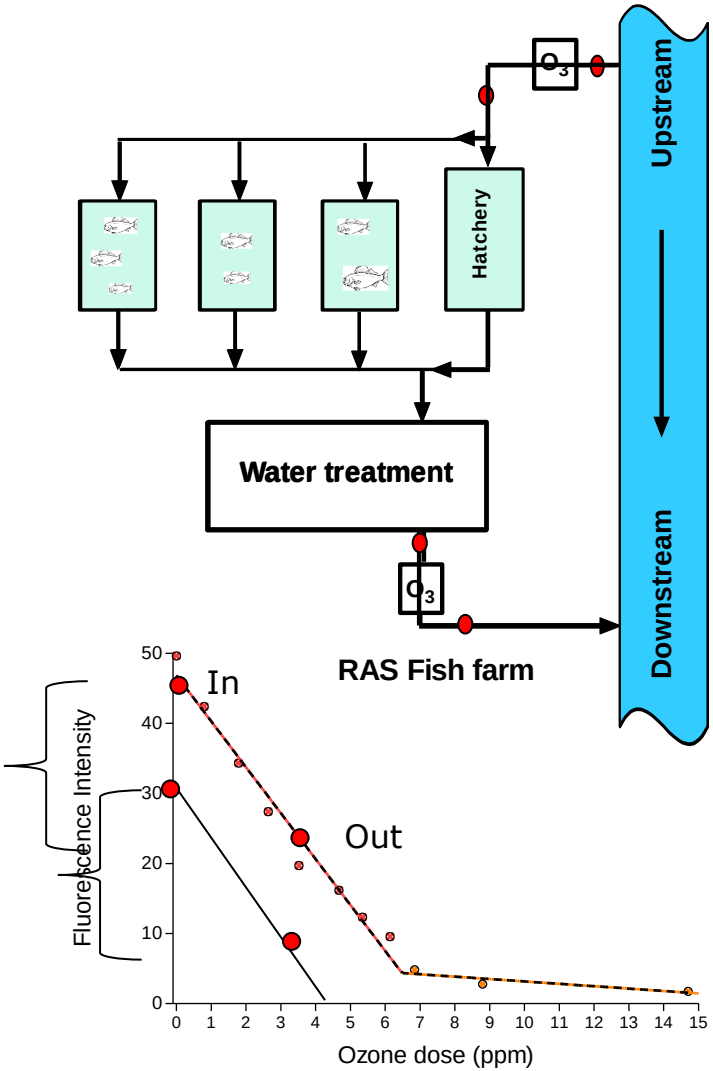
Application #2: On-line control of flow through systems

- Ozone dosage is based on:
  - Sensor in the inlet
    - Evaluate water quality via fluorescence
    - Based on it ozone dosage is determined
  - Sensor in the outlet
    - Adjustment of the ozone dose
    - Ensure water quality suitable to be discharged in the recipient.

Fluorescence in the inlet might alter but their difference should be the same

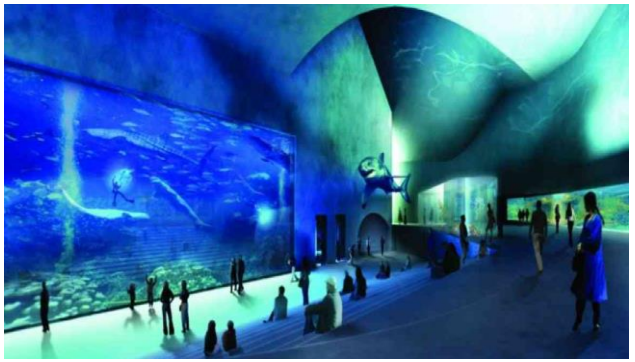
e.g. dilution due to rainfall



Take-home message

- Fluorescent DOM does exist in aquaculture water
- Fluorescence is highly sensitive to ozone mostly in low ranges (0-5 mg O_3/L)
- Fluorescence can be used as:
  - Off-line control verifying ozone dosage and evaluating ozone generator leading to a more robust operation
  - On-line sensor in flow through system controlling ozone dose by keeping fluorescence within predetermined ranges

Acknowledgements



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Thank you for the attention !!!

Water

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