Aquaphotomics for understanding water functionality

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Aim

To investigate **water functionality**.

What do we mean by "water functionality"?

In general water is vital for life.

But one water is not equal to another water...

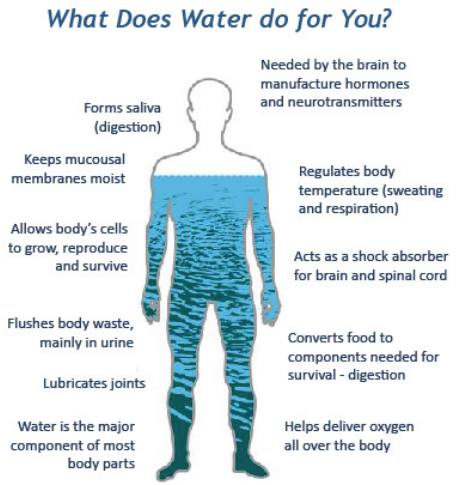
So our question is:

What does a specific water **do** for health, agriculture, etc.? What is it's **function**?

Water functionality

Does a certain type of water result in faster growth, taller produce, higher immunity/resistance, longer shelflife, higher diversity, etc.

Does a certain type of water give us higher immunity/resistance, is it good for specific applications, e.g. for curing a certain disease, should we drink it for a short or long period, etc.



Waters on the market

Spring /natural waters with different mineral composition, water ‘structure’, claimed functions, such as health benefits, and more.

‘Treated’ waters with claimed functions, such as health benefits and agricultural benefits.

Need for...

...understanding **differences** in functionality between waters

...providing more **details** than vague terms such as ‘water structure’, ‘information’, ‘energy’, ‘health’, ‘vitality’, etc.

...**informing** consumers, **enabling** them to make informed choices what water to use at what time and in what situation

...**informing** water companies about specific properties, how long it holds, when to bottle and how to store their water

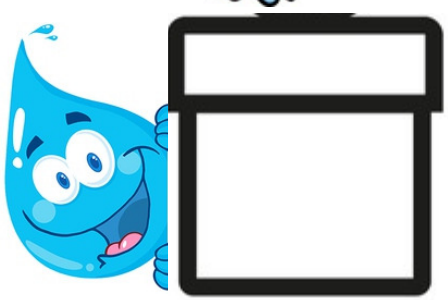
What I will present...

- Case study ‘Efibia treated’* water
- Testing water functionality in the field
- Aquaphotomics water measurements to understand water functionality
- Preliminary results to identify spectral features related to water functionality
- Future goals and requirements

**Since 2016 Efibia changed name to ESENÇA*

Example: ‘Efibia treated’* water

Tap Water (Control)



Black Box

Efibia Water

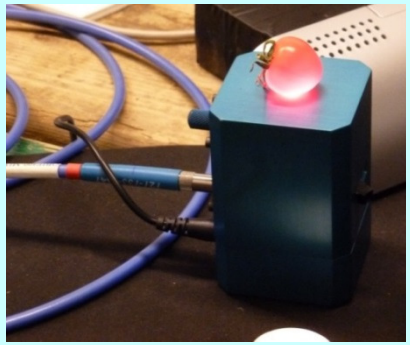
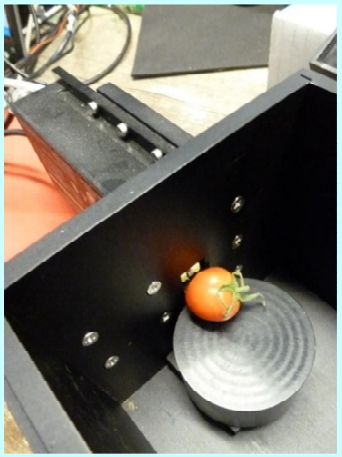
**Since 2016 Efibia changed name to ESENÇA*

Investigating function of ‘Efibia treated’ water

5 years biological experiments*





Time consuming, external factors influence results, season, weather, sunlight, soil condition, diversity within the species

**ViaLight Research, the Netherlands*

Understanding water functionality using Aquaphotomics

Spectra of biological systems (fruits, vegetables, leaves, etc.) grown with treated water.

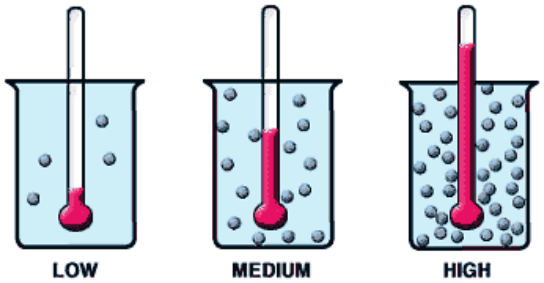
&

Spectra of treated water under various perturbations.

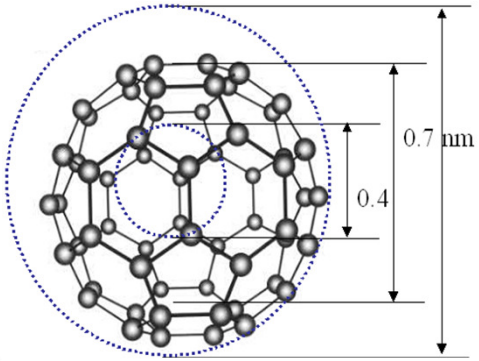
Light  Water

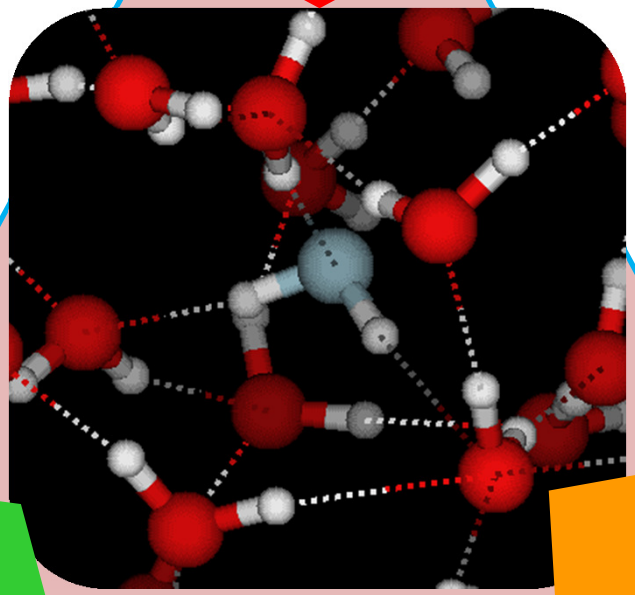
To find water bands in the spectra related to water functionality

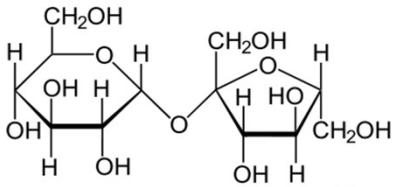
Water absorption spectrum changes in ***bio systems*** under various perturbations

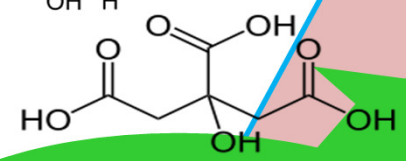


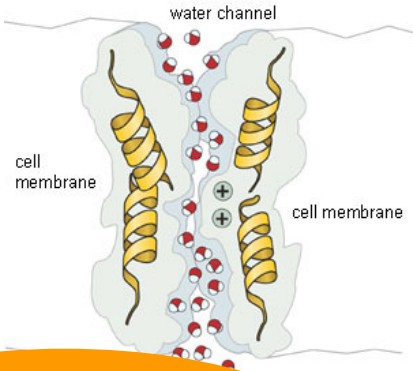
Physical











Chemical

Biological

Courtesy R. Tsenkova

Experimental design
Keep in mind...

Water is **continuously influenced** by its environment (great property! Water=mirror) -> however well controlled experiments required

Claimed function of the water, e.g.:

- **'stable' properties** (e.g. holding 'immune booster' property for a long time), or
- **'rhythmically changing'** properties (e.g. following natural cycles).

Experimental design and interpretation of results depends on question/functionality.

‘Efibia treated’ water

Hypotheses/Claims:

- Better resistance, ‘healthy’
- Stable, better self-regulation

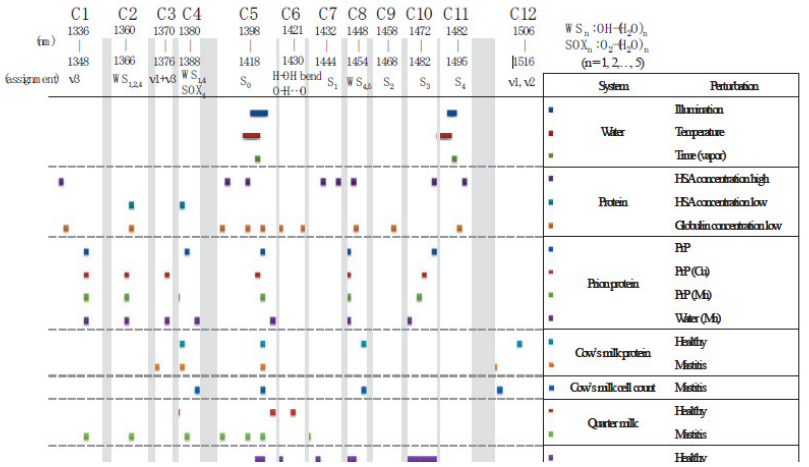
Need to:

- Find specific absorbance bands (water absorbance pattern WAP)
- Show repeatability of results

Compare aquagrams of:

- Tap water (Control)
- Water after treatment (Efibia)

Table 1. Water matrix co-ordinates (WAMACS): characteristic water absorbance bands with a high contribution in respective spectral models developed under various perturbations: concentrations, temperature, illumination, disease, damage, host molecules in the structure or in solution, particles size.[40,43,50,66,68,72–74]



Aquaphotomics experiments

- Measurement protocol
- Measurement set up
- Water handling protocol
- Aquagram calculation
- Interpretation of results

Measurement protocol - Perturbations

Probe/tickle/challenge/perturb water

- Light is usually not enough to bring out small differences between waters.
- Add an extra perturbation
  - To stimulate water to show more of itself, to bring out smaller differences
  - To overrule external factors influencing the experiment (such as weather)
- E.g. temperature, electromagnetic radiation, other molecules, and more.

Perturbations for ‘Efibia treated’ water

- Halogen light is used to acquire NIR absorbance spectrum in range 1300-1600nm (1st overtone region)
- Temperature changes are used to stimulate the water.

Measurement protocol - Repetitions

- In general: At least three repetitions
- For testing ‘Efibia treated’ water: More repetitions
  - Multiple tappings of Efibia should have the same ‘functionality’, i.e. show the same water absorbance bands
  - Older tap water should give same results, showing stability over time

Repetitions for ‘Efibia treated’ water

Exp. #	Experiment Date	Water tapping Date
1	09-04-2015	07-04-2015
2	10-04-2015	07-04-2015
3	23-04-2015	07-04-2015
4	24-04-2015	07-04-2015
5	20-05-2015	18-05-2015
6	21-05-2015	18-05-2015
7	27-05-2015	18-05-2015
8	28-05-2015	18-05-2015
9	15-06-2015	09-09-2014
10	16-06-2015	09-09-2014

Control for:

- Time of day effects:

One test is two consecutive days: one day Control in the morning and Efibia in the afternoon, other day the other way around.

- Moon phase effects:

Repeat test in other moon phases.

- Seasonal effects:

Repeat test in other season.

- Tapping date/bottling effects:

Samples from the same tapping day, same bottle, different bottle same day, different bottle other tapping day, etc.

Measurement set up

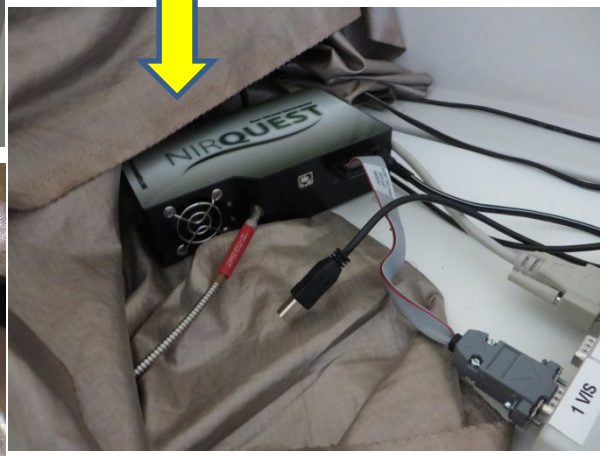
Always log:
Room temperature
Room humidity
Operator

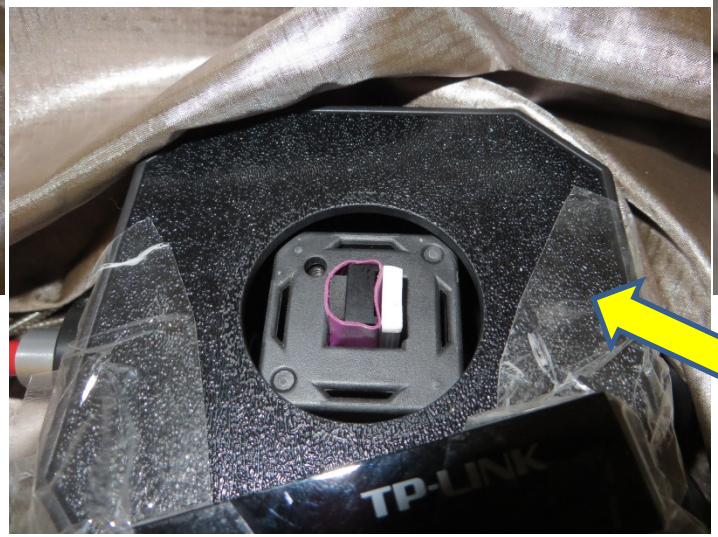
Fiber based NIR absorption spectroscopy set up

Halogen lamp

Silver lined sheet to cover set up for reducing EM disturbances.

NIR spectrometer



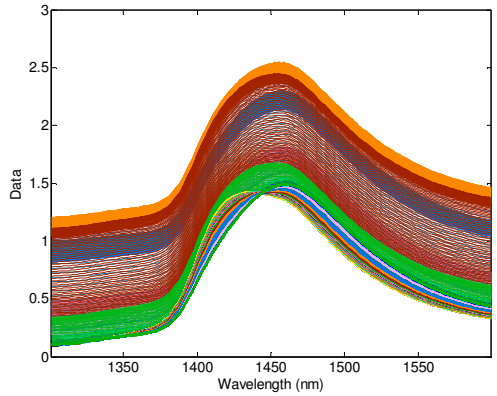


Temperature controlled cuvette holder (1mm cuvette inside)

Water handling protocol

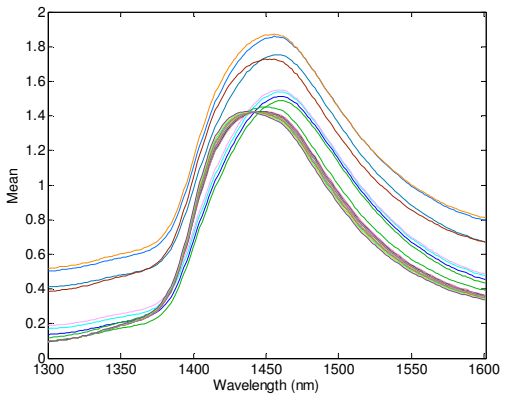
- Store waters separately in similar conditions
  in EM shielded containers.
- Before measuring, water is filtered with 0,2um filter
- Filling and handling cuvette:
  - Rinse cuvette 3x with distilled water (DW) (400 µl)
  - Rinse pipette tip 3x with DW and 2x with sample water (SW)
  - Rinse cuvette 2x with SW and 3rd time fill it with SW
  - Rinse cuvette cap 1x with DW and 2x with SW (make it dry before putting it on cuvette with a clean tissue)
  - Put cap on cuvette
  - Weigh cuvette before and after measuring (to check for evaporation)
  - Always use same cuvette for all samples (due to slight differences in cuvettes)

Aquagram calculation



Raw spectra 1300-1600nm

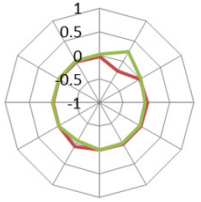
- Spectra should be of good quality.
- We look at differences of 10^{-4} nm.
- Spectrometer should have high accuracy and large optical density
- Measurement set up should be very stable.



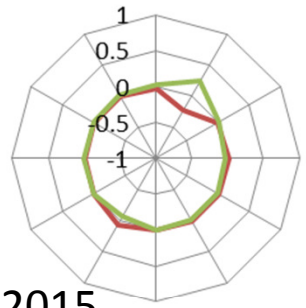
Average spectra per temperature perturbation step

Preprocess spectra (Smoothing and EMSC)

$(\text{Aquagram Control})_\lambda =$
$((\text{AVG Control})_\lambda - (\text{AVG all})_\lambda) / (\text{SD all})_\lambda$
$(\text{Aquagram Efibia})_\lambda =$
$((\text{AVG Efibia})_\lambda - (\text{AVG all})_\lambda) / (\text{SD all})_\lambda$



Aquagrams ‘Efibia treated’ water

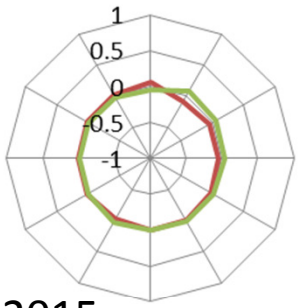


09-04-2015

07-04-2015

Control

Efibia

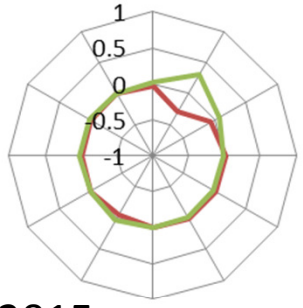


10-04-2015

07-04-2015

Control

Efibia

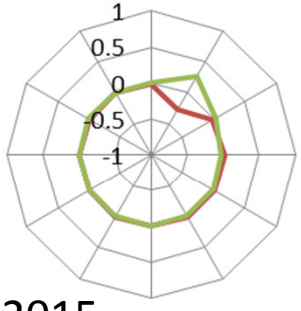


23-04-2015

07-04-2015

Control

Efibia

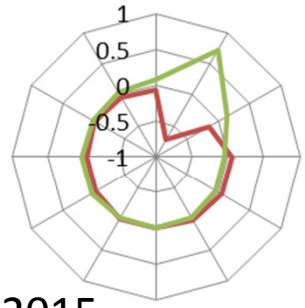


24-04-2015

07-04-2015

Control

Efibia

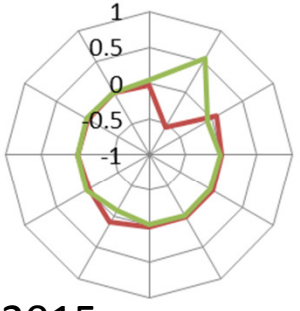


20-05-2015

18-05-2015

Control

Efibia

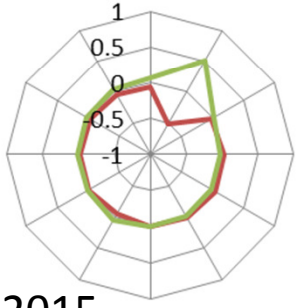


21-05-2015

18-05-2015

Control

Efibia

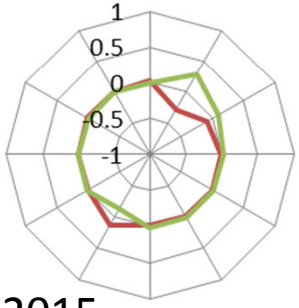


27-05-2015

18-05-2015

Control

Efibia

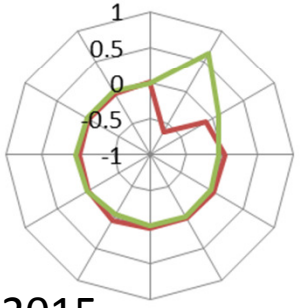


28-05-2015

18-05-2015

Control

Efibia

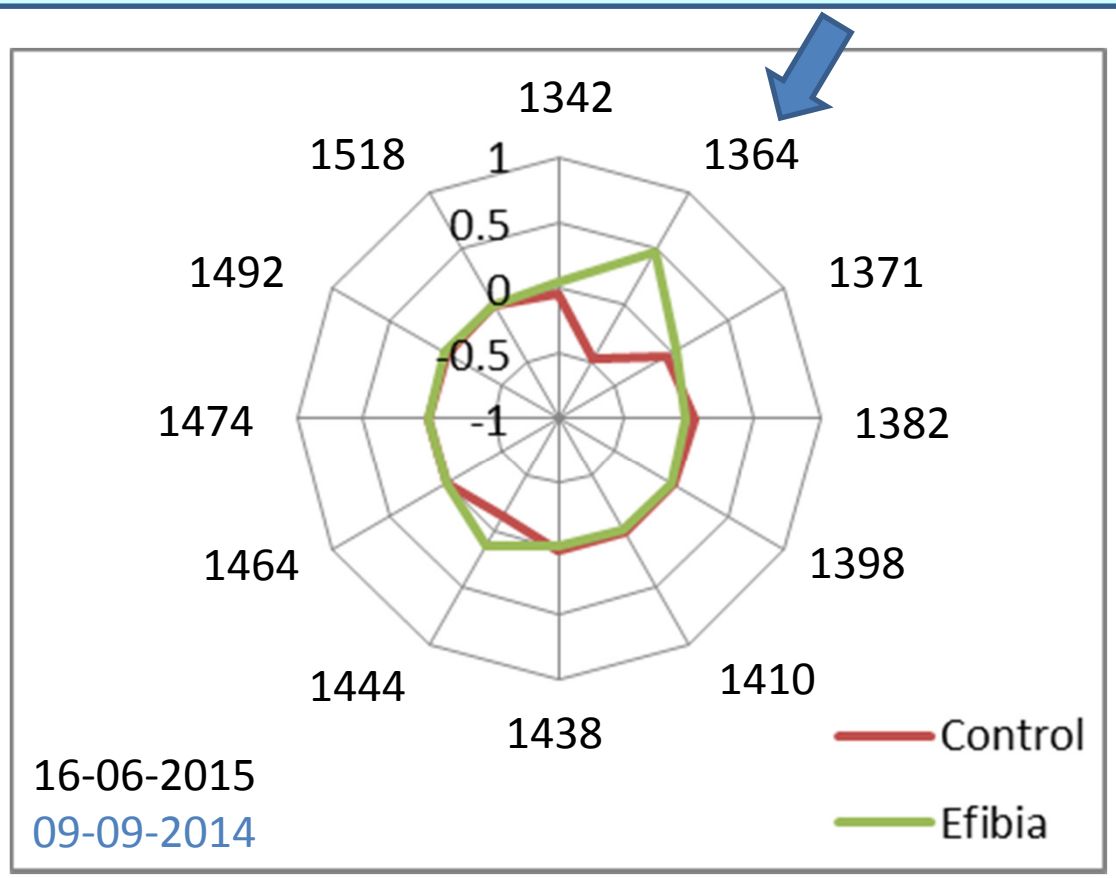


15-06-2015

09-09-2014

Control

Efibia



$(\text{Aquagram Control})_\lambda$	$= ((\text{AVG Control})_\lambda$	$- (\text{AVG all})_\lambda)/ (\text{SD all})_\lambda$
$(\text{Aquagram Efibia})_\lambda$	$= ((\text{AVG Efibia})_\lambda$	$- (\text{AVG all})_\lambda)/ (\text{SD all})_\lambda$

C2 : **1360-1366 (1364)nm : OH-$(H_2O)_1$, OH-$(H_2O)_2$, OH-$(H_2O)_4$**

1360nm 1st overtone free OH stretch **OH-$(H_2O)_4$** - 3675cm^{-1} (Robertson et al, 2003)

1366nm 1st overtone free OH stretch **OH-$(H_2O)_2$** - 3660cm^{-1} (Robertson et al, 2003)

In all experiments ‘**Efibia** treated’ water showed a different reaction compared to Control at **1364 nm**.

The 1360-1366 nm range (water band C2) has been assigned to the **‘solvation shell’**.

The reproducible higher absorption for ‘Efibia treated’ water in this band, is an indication that it has different functional effect compared to Control water, related to solvation/solubility.

The fact that nine month old water showed similar results, tells us that ‘Efibia treatment’ property remains **stable over a longer period**.

Further interpretation of aquagram

General	'Efibia treated' water
Water absorbance pattern (WAP)	Main difference at 1364nm
Assignment to water molecular structure and bonds between molecules, references to literature (overtones of fundamental frequencies)	Solvation shell "Organized" water around molecules, free protons and electrons available for reactions outside this shell <other interpretations?>
Finally what does it mean for function of water? Reference data of water used in biological system or aqueous system is required	So, what did we find in biological tests? Can we relate 'solvation shell ' differences to what we see experimentally in agriculture?

Biological tests

Differences between Efibia and Control water, **not every time repeatable**, showing that Efibia water **might** have a function:

- Germination rate Efibia Asian salad seems to be higher
- Efibia Asian Salad seem more resistant to water stress (3/4 exp, 30 plants each)
- Efibia Saxa bean roots seem to have more rhyzobia (showing better synergy with soil microbes)
- Efibia wheat seedlings seem to grow taller under light stress (4/6 exp, 10 seeds each)
- And many more tests performed...

More testing required, not concluding anything yet, still work to be done!

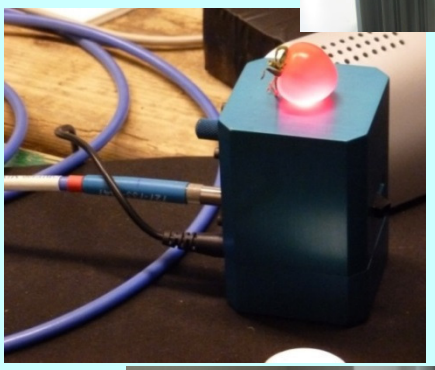
Vegetable spectra

NIR spectra for 4 years. Both in the field as well as in the lab.

External factors/variables influence results (year, temperature, humidity, location, diversity, farmer, etc.).

We did find differences between Efibia grown and Control grown vegetables.

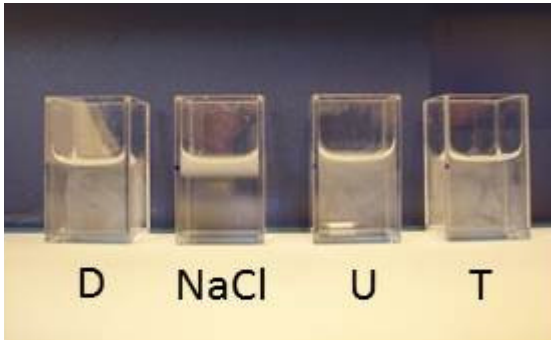
However, more spectra required to be able to come to conclusions.

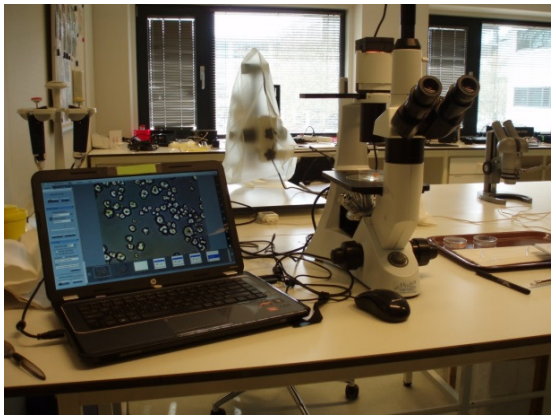


Other tests

We performed more tests to compare ‘Efibia treated’ and Control water, such as:

- Diffusion of substances in water
- Probiotic growth in water
- Diffusion of water on filter paper
- Exclusion zone size with Nafion
- pH
- Redox
- Conductivity
- External measurements:
  - Nutrient contents vegetables
  - Soil composition tests





OUR AQUAPHOTOMICS EXPERIMENT WAS MOST REPRODUCIBLE!

Water functionality analysis
Goals & Future

- Understanding and ‘water-marking’ waters
- Helping suppliers by performing aquaphotomics measurements, to:
  - Understand functionality of their water
  - Measure stability/dynamics of their water
  - Develop best storage means of their water
  - Give them an independent ‘functionality water-mark’ with aquagram/WAP
- Informing consumers objectively
- Building up fundamental knowledge about water molecular structure, water dynamics, water stability, water functionality, ‘information holding capacity’, and more...

Water functionality analysis Requirements

- Build up a large database
- Educate/share knowledge
- Standardize protocols
- Standardize analysis
- Collaborate with labs worldwide
- Work with multidisciplinary teams: (quantum) physicists, chemists, biologists, dataminers, and other experts for interpretation of water functionality
- Acquire good experimental reference data

Thank you very much for your attention.

&

Looking forward to

worldwide aquaphotomics collaborations.

Take care of your bodily water!