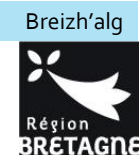




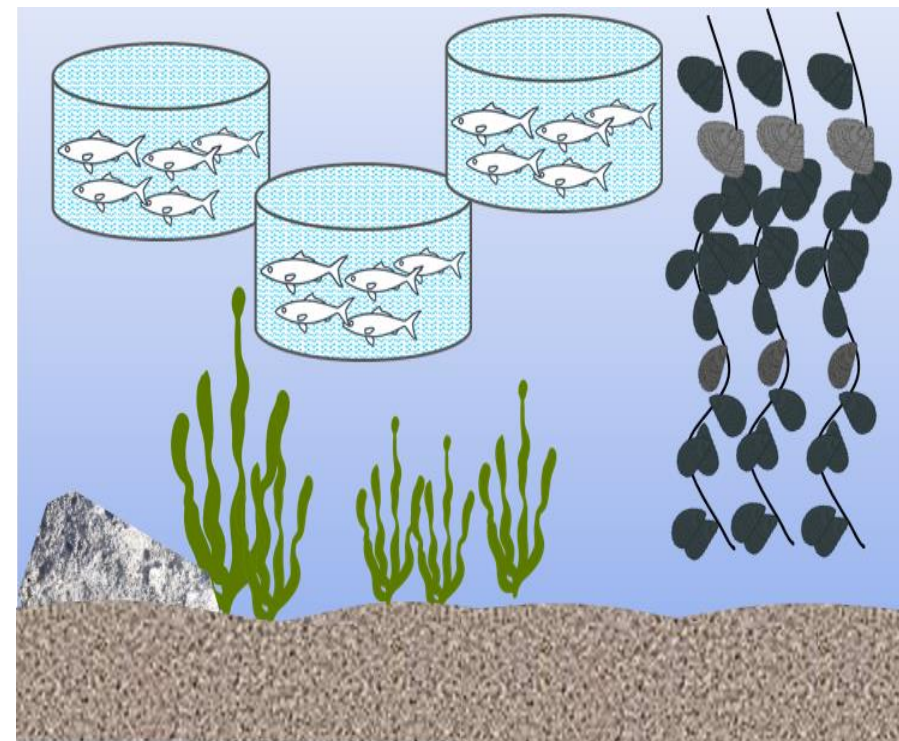
# INTEGRATED CULTIVATION OF EUROPEAN SEA BASS *Dicentrarchus labrax* AND *Ulva* sp. IN RECIRCULATING AQUACULTURE SYSTEMS

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# Integrated Multi-Trophic Aquaculture (IMTA)

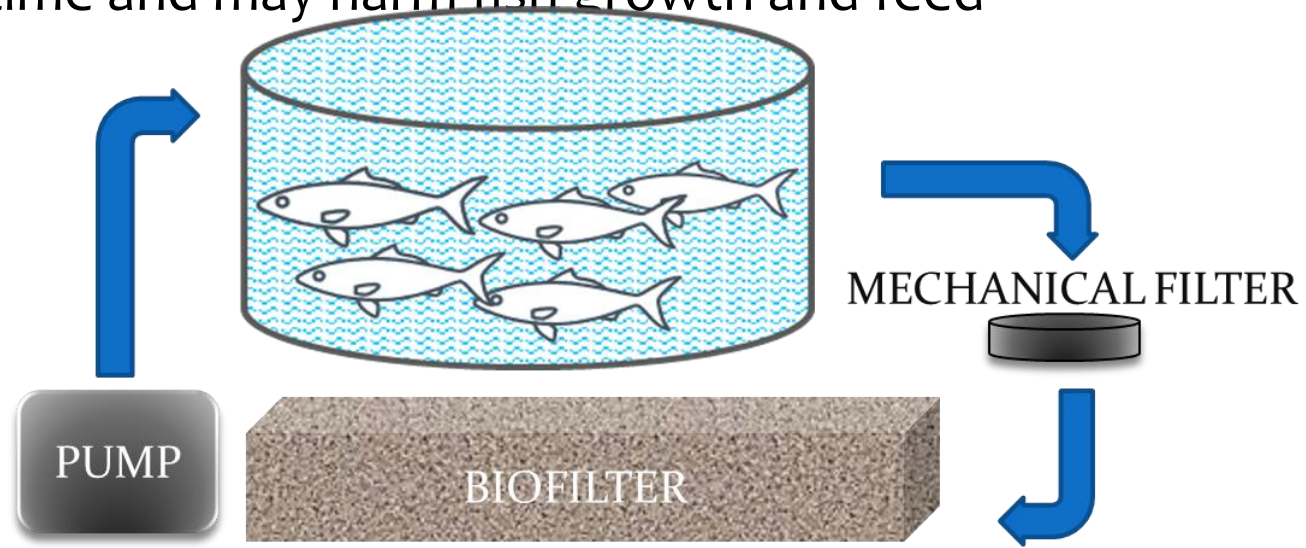
- IMTA provides the by-products, including waste, from generating (fed) aquatic species as inputs (fertilizers, food) for cleaning (extractive) species.
- IMTA uses multiple species from different trophic levels for reducing wastes and costs (through recycling of wastes) while increasing total productivity (in weight and in value) with respect to feed input and pollution output.
- In Greece Aquaculture occurs in sea cages and IMTA (fish & molluscs) is currently only on experimental stage, in order environmental effects to be minimized and total production to be increased.



# Sea bass and *Ulva* indoor seawater RAS with biofilter

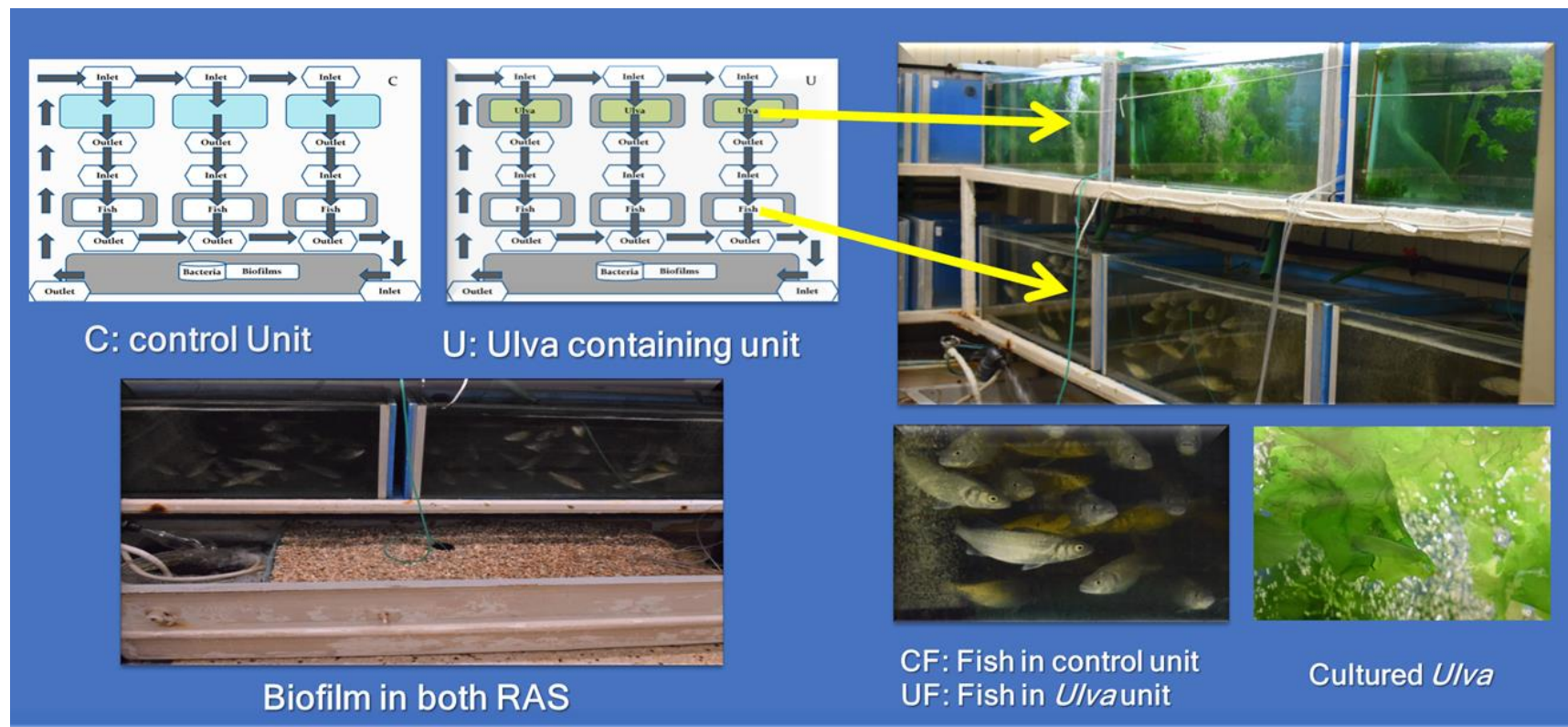


- One potential way to reduce environmental impact, is the increased use of land-based (closed recirculating aquaculture systems -RAS) systems with bacteria biofilters
- In RAS, nitrate accumulates over time and may harm fish growth and feed intake
- Seaweed filtration has the potential to convert N-rich effluents into valuable biomass
- However, the combination of denitrifying biofilters with algal biofilters can be complex and costly



*The cultivation of sea bass and Ulva in IMTA-RAS is a case study of the project IMTA-EFFECT (Integrated MultiTrophic Aquaculture for EFFiciency and EnvironmenTal ConservaTion).*

In this study, *Ulva* sp. and European sea bass (*Dictrarchus labrax*) were co-cultured in RAS, in order to assess the potential of *Ulva* sp. to improve water quality and possible effect on the growth of sea bass.



**C: control Unit**      **U: Ulva containing unit**

**CF: Fish in control unit**      **UF: Fish in *Ulva* unit**

**Biofilm in both RAS**      **Cultured *Ulva***

# The tanks (12) were separated in 4 groups



## Control-RAS (6)

## Ulva-RAS (6)



 CW: water tanks without *Ulva*



 UW: water tanks with *Ulva*



 CF: fish tanks in control unit

 UF: fish tanks in *Ulva* RAS



# GENERAL EXPERIMENTAL PROCEDURES

- The experiment lasted 12 weeks.
- Fifty fish were placed in each tank
- Fish were manually fed to satiation twice a day.
- D.O., pH, salinity and temperature were measured every day (except week-end)
- Temperature: 23,0 °C and Salinity: 34,4 psu
- Water samples were taken twice a week.
- Statistical analyses were performed using the Statgraphics software, and one way ANOVA.



# DIFFERENCES BETWEEN TWO TRIALS

Dietary fish oil was replaced by a mixture of vegetable oils (50% rapeseed oil: 50% palm oil).

Dietary fish oil from sardines

- Initial body weight: 35,42 ± 0,476 g)
- Initial fish density: 12,05 kg/m<sup>3</sup>
- Ulva was renewed every two weeks
- *Ulva* density: 0,97 kg/m<sup>2</sup>
- Light intensity (on surface of *Ulva* tanks): 450 lux

- Initial body weight : 21,6 ± 0,15 g)
- Initial fish density: 7,36 kg/m<sup>3</sup>
- Ulva was renewed every week
- Ulva density: 0,71 kg/m<sup>2</sup>
- Light intensity (on surface of *Ulva* tanks): 758 lux



## Water quality parameters

- Ammonia nitrogen was determined by the indophenol blue method
- Nitrate nitrogen was determined by Brucine method (EPA 352.1)
- Nitrite nitrogen was determined by Griess reaction method
- Orthophosphate-Inorganic phosphorus was measured with the ascorbic acid method (EPA 365.3)

## Proximate composition

- Moisture was determined by the AOAC 950.46 method
- Ash was determined by the AOAC 938.08 method
- Protein and N content was determined by Kjeldahl digestion according to AOAC Official Methods 981.10 & 2001.11
- Fat was determined by Soxhlet extraction, according to AOAC Official method 991.36





# GROWTH PARAMETERS

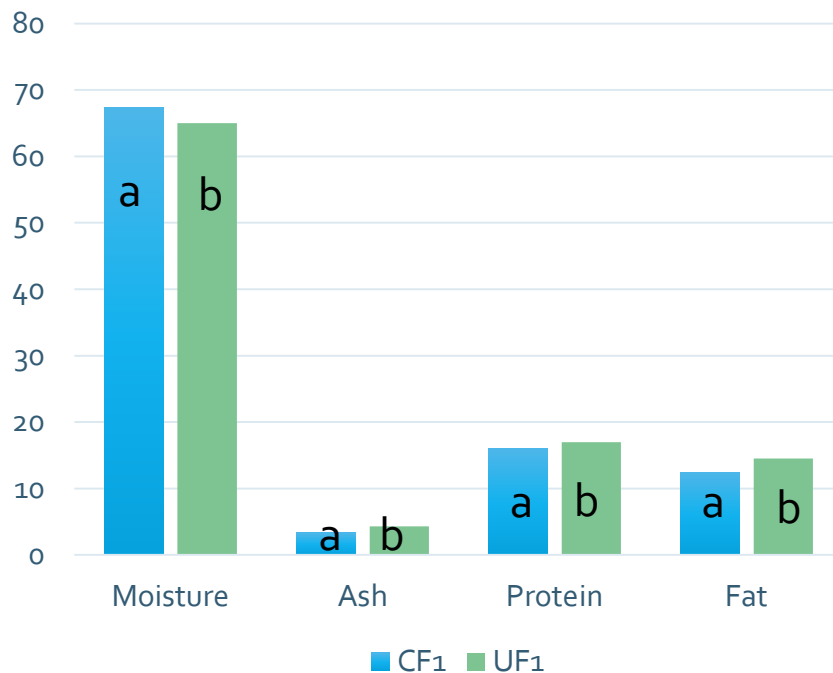
1ST TRIAL	CONTROL	ULVA	P-VALUE
CONDITION FACTOR (K)	1,21±0,008 <sup>a</sup>	1,23±0,007 <sup>b</sup>	P<0,05
BWI	135,5±4,51	137,5±1,49	NS
SGR	1,02±0,021	1,03±0,007	NS
FCR	1,06±0,033	1,10±0,017	NS
FEED CONSUMPTION	51,01±0,186 <sup>a</sup>	53,43±0,193 <sup>b</sup>	P<0,001
MORTALITY (%)	4,66±3,712	4,00±1,155	NS

2ND TRIAL	CONTROL	ULVA	P-VALUE
CONDITION FACTOR (K)	1,29±0,005 <sup>a</sup>	1,31±0,005 <sup>b</sup>	P<0,05
BWI	193,14 ± 4,935	184,52 ± 3,084	NS
SGR	1,26 ± 0,019	1,23 ± 0,012	NS
FCR	1,36 ± 0.015	1,39 ± 0.010	NS
FEED CONSUMPTION	56,74 ± 0,879 <sup>b</sup>	55,07 ± 0,765 <sup>a</sup>	P<0,001
MORTALITY (%)	0	0	NS

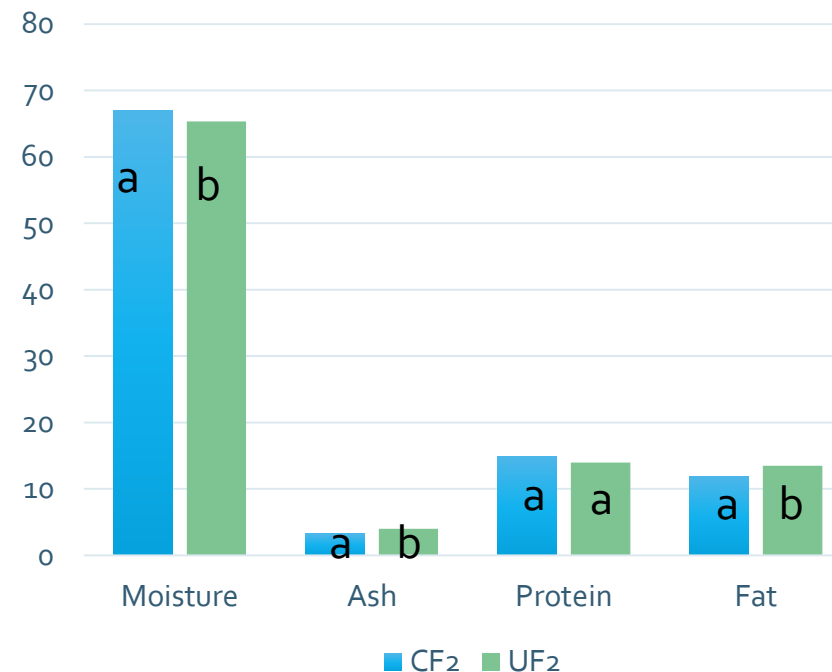




## TRIAL 1



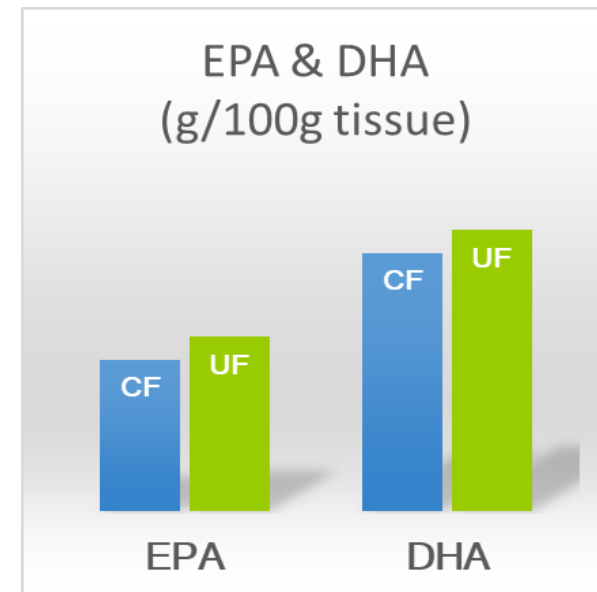
## TRIAL 2



# Fatty acid profile of sea bass fed VO-based diet

## Content (% total lipids)

	CF	UF
Saturated	25,38±0,305 <sup>b</sup>	25,09±0,205 <sup>a</sup>
Monounsaturated	47,64±0,494 <sup>a</sup>	48,15±0,266 <sup>b</sup>
Polyunsaturated	26,87±0,341 <sup>a</sup>	26,71±0,257 <sup>a</sup>
n-3 Polyunsaturated	11,81±0,358 <sup>b</sup>	11,44±0,324 <sup>a</sup>
n-6 Polyunsaturated	14,23±0,258 <sup>a</sup>	14,47±0,270 <sup>b</sup>
n-3/n-6	0,83±0,036 <sup>b</sup>	0,79±0,035 <sup>a</sup>





*Ulva* was collected from the Saronic gulf




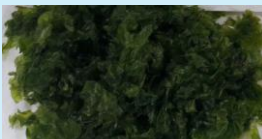


Sorted and thoroughly cleaned with seawater and weighted after drying

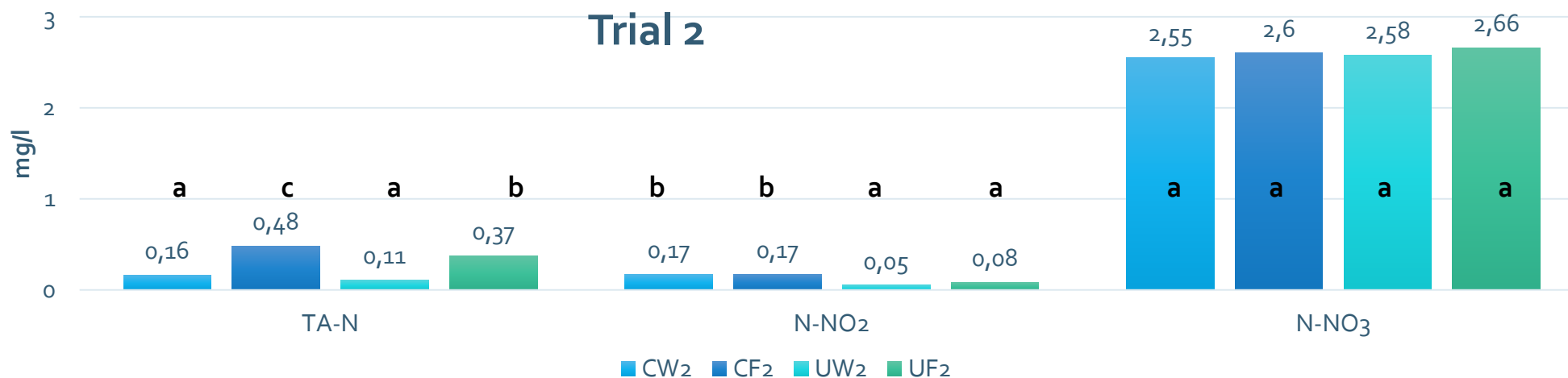
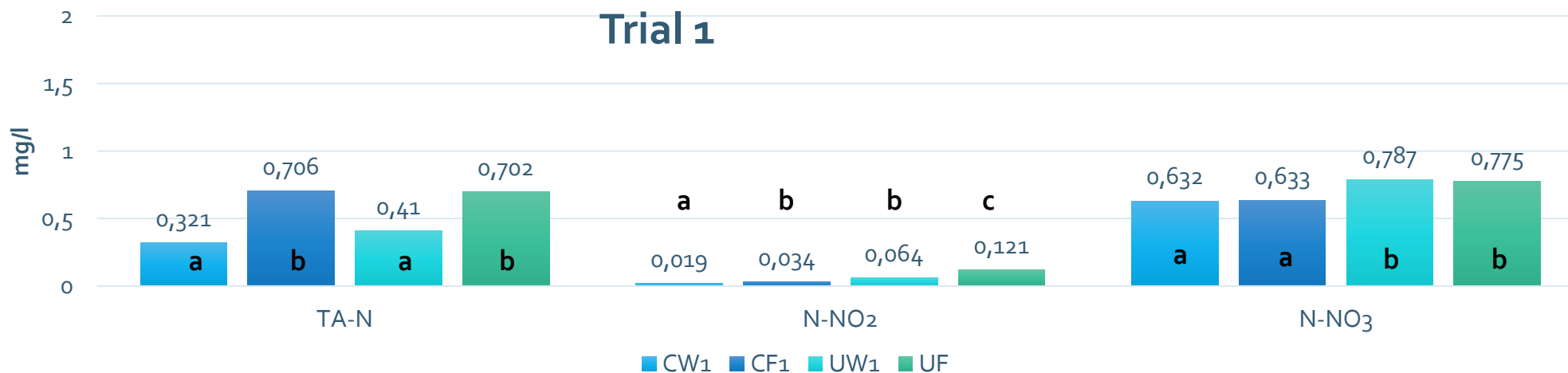


In the upper level tanks (UW), unattached thalli of *Ulva* were kept suspended by air diffusers situated at the bottom of the tanks

# ULVA proximate composition

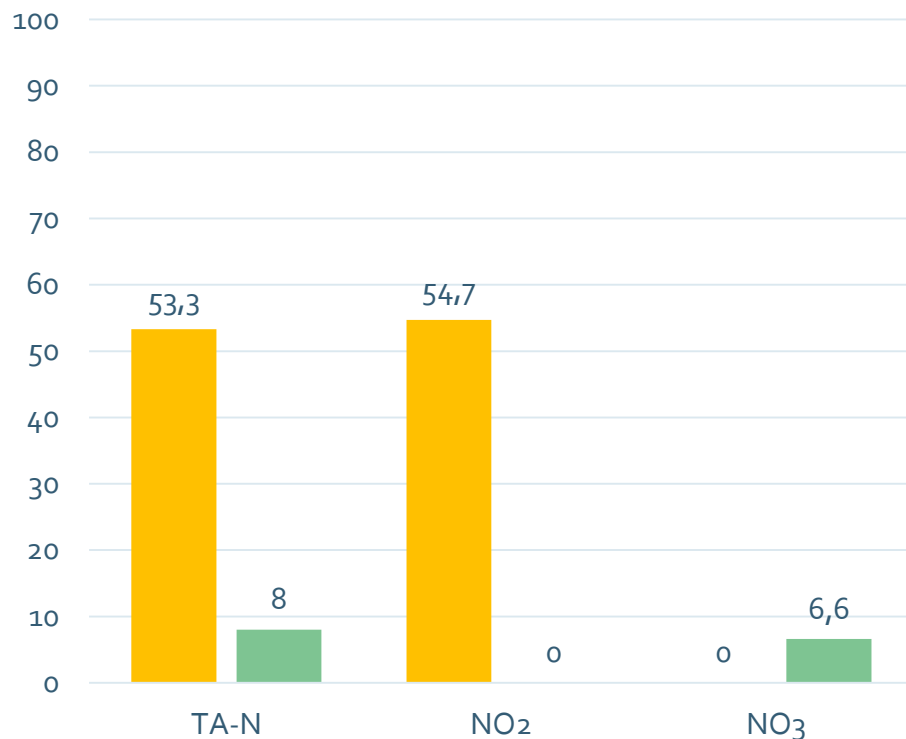
Trial 1		Ash	Fat	Protein	Carbohydrate <sup>b</sup>	Nitrogen
		(% dry weight)	(% dry weight)	(% dry weight)	(% dry weight)	(% dry weight)
Fresh Ulva		26.82±0.739	0.98±0.090	8.58±0.117	63.09±1.541	1.56±0.023
2 weeks cultivated Ulva		30.62±0.848	0.95±0.094	14.92±0.199	52.40±1.393	2.69±0.042
p-value		*	NS	*	*	*
Trial 2		Ash	Fat	Protein	Carbohydrate	Nitrogen
		(% dry weight)	(% dry weight)	(% dry weight)	(% dry weight)	(% dry weight)
Fresh Ulva		31.58±0,723	0.85±0.096	12,47±0.493	58.24±1.971	2.00±0.189
1 week cultivated Ulva		31,63±0.524	0.83±0.083	17.94±1.184	49.07±1.354	2.87±0.079
p-value		NS	NS	*	*	*





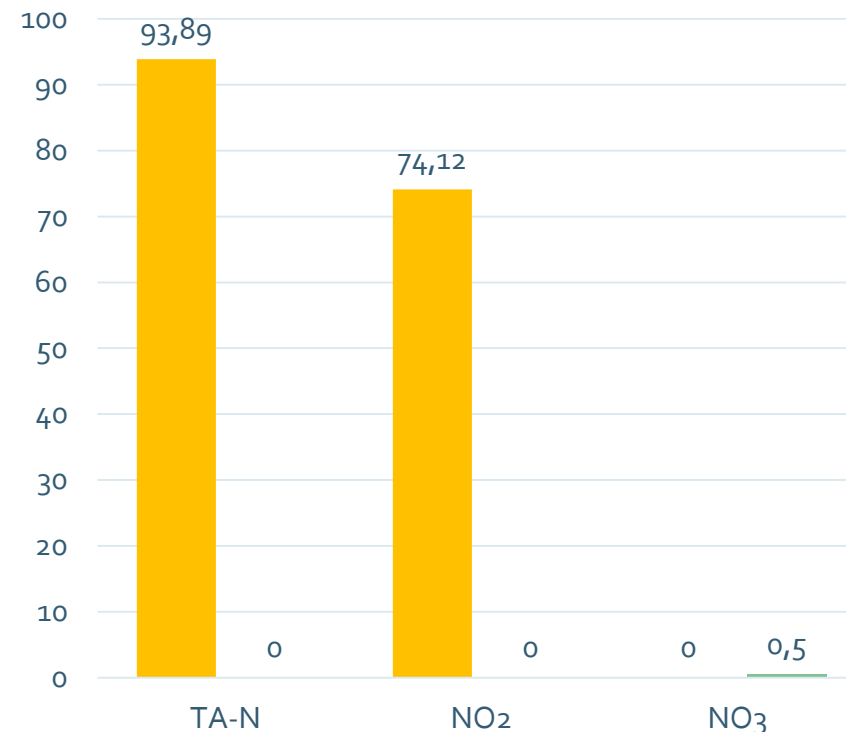
# N-removal by biofilters

## Trial 1



■ Bacteria 1 (gravel bed) ■ ULVA 1

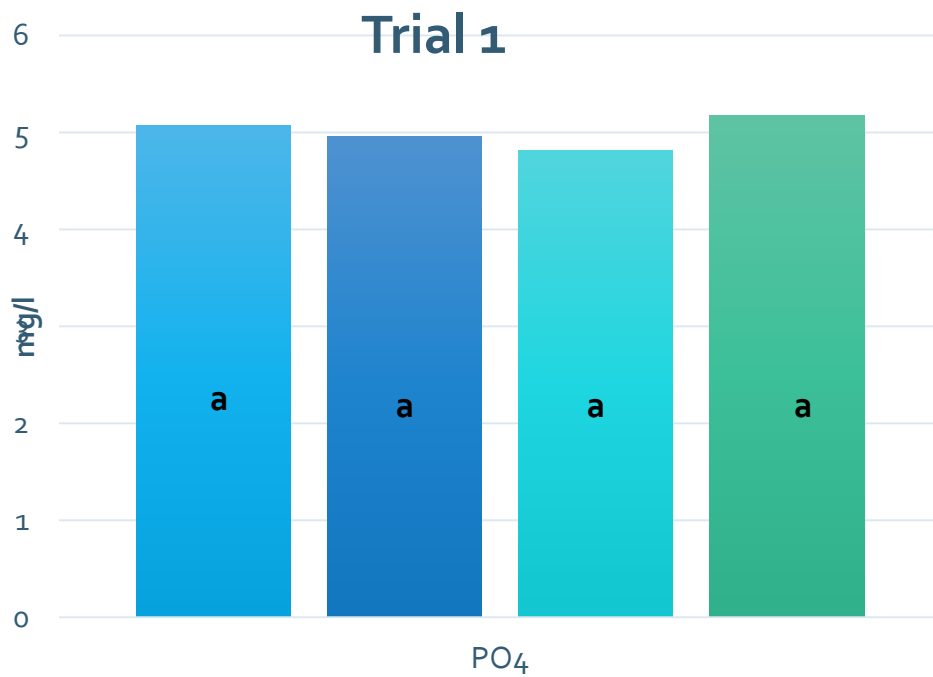
## Trial 2



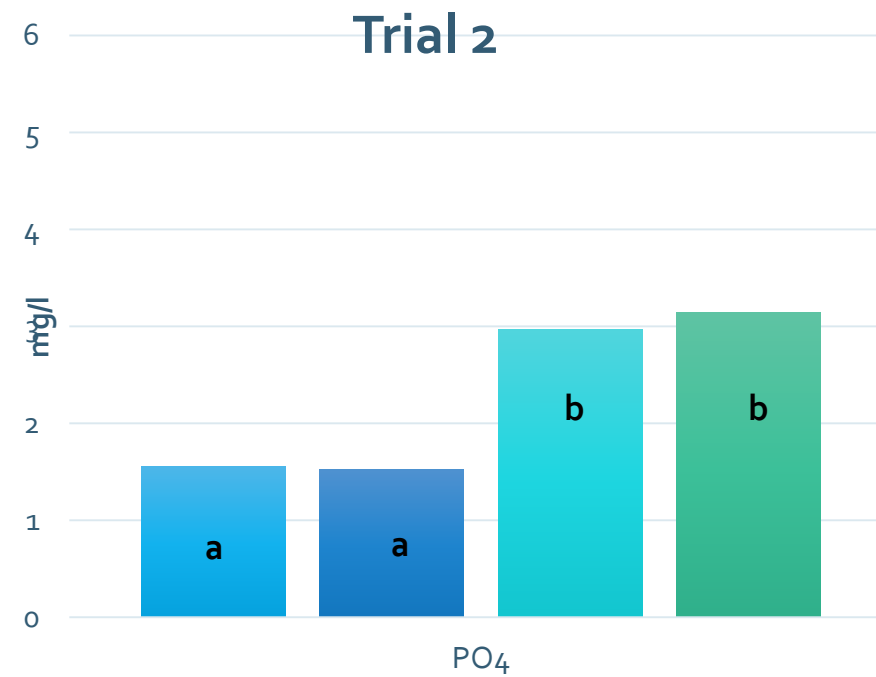
■ Bacteria 2 (gravel bed) ■ ULVA 2



# P- in water, orthophosphates



■ CW1 ■ CF1 ■ UW1 ■ UF1



■ CW2 ■ CF2 ■ UW2 ■ UF2





# Role of *Ulva* in sea bass growth

- Body Weight Increase (BWI), Specific Growth Rate (SGR), Feed Conversion Rate (FCR) and mortality did not show significant differences
- Fish in *Ulva*-RAS showed increased feed consumption when fed VO-based diets and decreased when fed FO-based diets compared to control fish.
- Fish in *Ulva*-RAS showed increased condition factor (K), regardless of feed consumption
- Protein content was significantly different between UF and CF in both trials and seems to be related to feed intake.
- Fat content was higher in UF in both trials
- In fish fed VO-diets, the % content of EPA and DHA in total lipids was lower in UF, however the total amount of these fatty acids in 100g of tissue was higher in UF as a result of higher fat content



# Role of *Ulva* in RAS water quality

- When TA-N levels were elevated (Trial 1), there was a removal of ~8% for ammonia from *Ulva* while the bacteria gravel bed retained ~53%.
- During the 2<sup>nd</sup> Trial where the bacteria gravel bed retained almost all ammonia (up to 93,89%), *Ulva* had no effect.
- *Ulva* seems to have no effect on nitrites reduction, since the bacteria gravel bed retains a high percentage of this nutrient.
- *Ulva* does not reduce significantly the levels of nitrates and phosphates, as it was expected, but on the contrary in some cases higher levels of PO<sub>4</sub> and NO<sub>3</sub> compared to controls, were observed.
- It is therefore possible, that *Ulva* demineralisation process has an additive effect on PO<sub>4</sub> and NO<sub>3</sub> concentrations.



# Conclusions / Future prospects



- *Ulva* could be used as an additional biofilter in RAS for improving water quality when  $\text{NH}_3$  is abundant.
- Indoor cultivated *Ulva* showed no biomass increase, probably due to nutrient rather than light limitation, therefore RAS could not be used as an *Ulva* production system.
- However, *Ulva* cultured in RAS shows improved quality, due to the decreased carbohydrates and the higher protein content.
- Fish in *Ulva*-RAS show stress tolerance (data not shown) and an increased condition factor.
- *Ulva* seems to contribute to fish welfare, which are probably recruiting dissolved or suspended matter related to *Ulva* presence.
- Therefore we can assume that the presence of *Ulva* has a positive effect on sea bass nutritional (EPA & DHA content) and physiological status, although not being a dietary component.
- Hence, the co-culture of sea bass and *Ulva* could result in a higher nutritional value of both species.
- Further investigation should be driven towards the effects of *Ulva* on fish health and welfare.



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# Merci de votre attention