

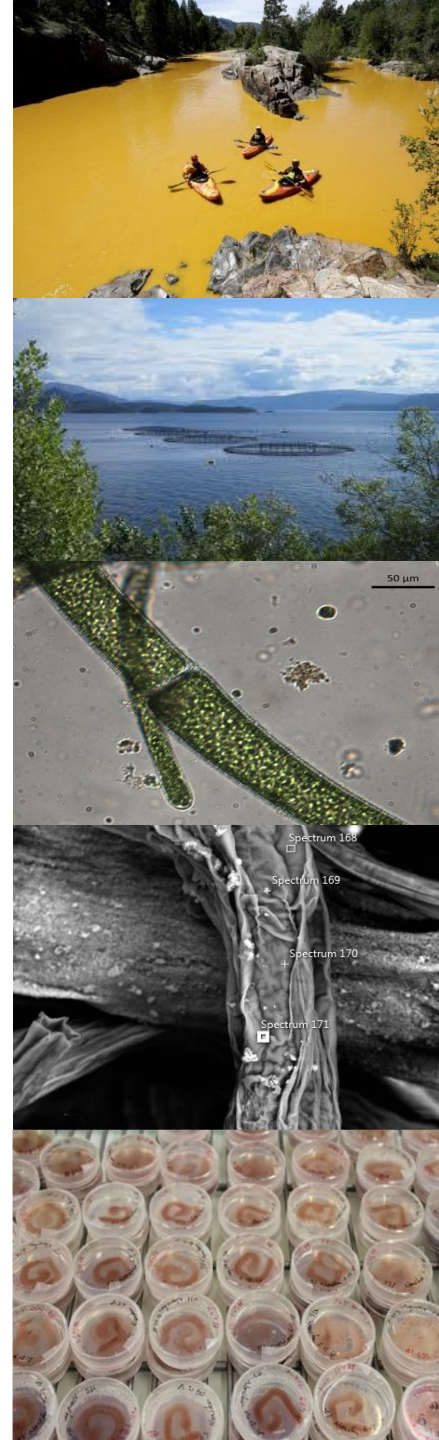
Algal biotechnology at SAMS: *An emphasis on bioremediation*

Michael Ross

Michael.ross@sams.ac.uk

Location

9th October, 2019



Contents

Past:

- *Wastewater treatment by filamentous macro-algae*
 - *Nutrient removal*
 - *Heavy metal bioremediation*

Present:

- Relevant research within SAMS
 - Selected algal biotech projects

Future:

- The future direction of research

Contents

Past:

- *Wastewater treatment by filamentous macro-algae*
 - *Nutrient removal*
 - *Heavy metal bioremediation*

Present:

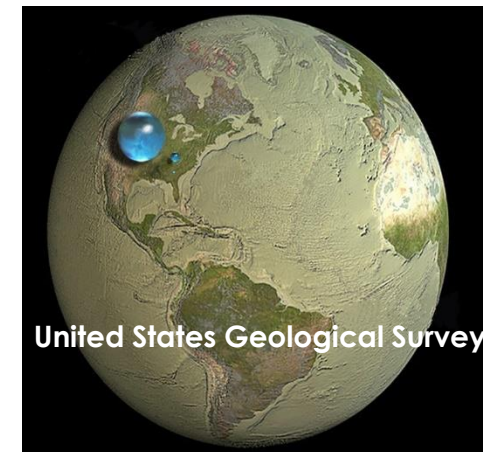
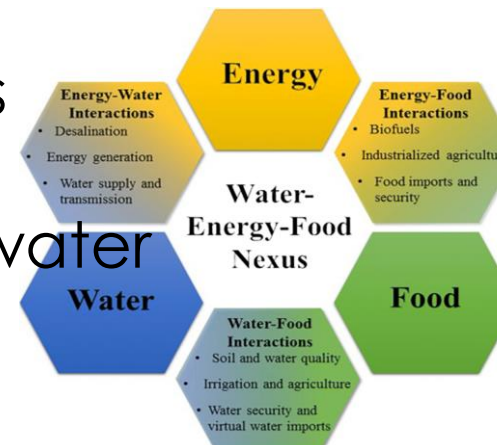
- Relevant research within SAMS
 - Selected algal biotech projects

Future:

- The future direction of research

Water as an asset...

- Clean water is critical for life on Earth
- As a resource it is essential for:
 - Agriculture
 - Aquaculture
 - Industry
 - Energy
 - Potable water
- However, water as a raw material, it is limited
 - 70% of Earth's surface covered in water
 - 3% of this is considered freshwater
 - 2.6% of this is inaccessible
 - 0.4% remains...albeit, with problems



...is under threat



Increasing awareness of the threats posed has led to policy change/legislation:

- WFD
- Paris Agreement
- Horizon 2020
- *UN Sustainable Development Goals*





THE UNIVERSITY
of EDINBURGH



Joint funding by NERC
and EPSRC for an E3P
DTP

EPSRC

Engineering and Physical Sciences
Research Council

NERC
SCIENCE OF THE
ENVIRONMENT

“Wastewater treatment by filamentous macroalgae”

Supervised by Dr Andrea J.C. Semião, Prof. John G. Day, Prof. Michele S. Stanley

1. Introduction
2. Materials & Methods
3. The rationale for implementing *Cladophora* sp. into WWT
4. Fresh weight determination of filamentous macroalgae
5. **Nitrogen uptake by *Cladophora*: influence on growth, nitrogen preference and biochemical composition**
6. **Biosorption of heavy metals from aqueous solutions by dried *Cladophora parriaudii***
7. Conclusions

Nitrogen uptake by *Cladophora*: influence on growth, nitrogen preference and biochemical composition

■ Eutrophication is characterised by:

- *Formation of an algal bloom*
- *Followed by its senescence, its death, and resultant decay*
- *Leading to anoxic conditions*
 - Dead zones characterised by $< 2 \text{ mL O}_2 \text{ L}^{-1}$



A) Eutrophication

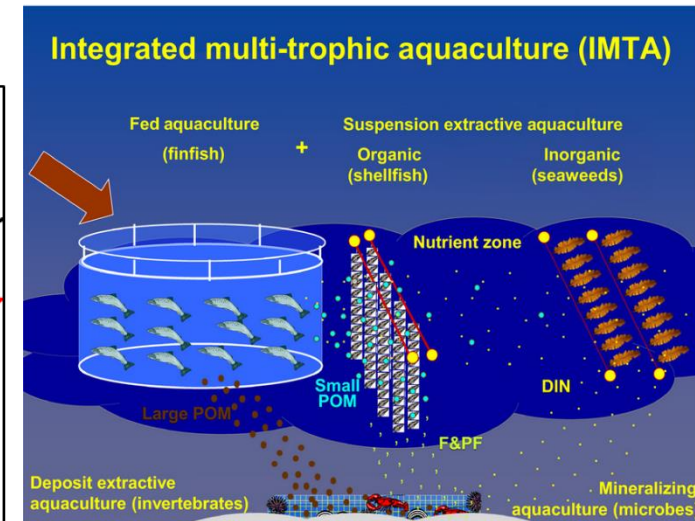
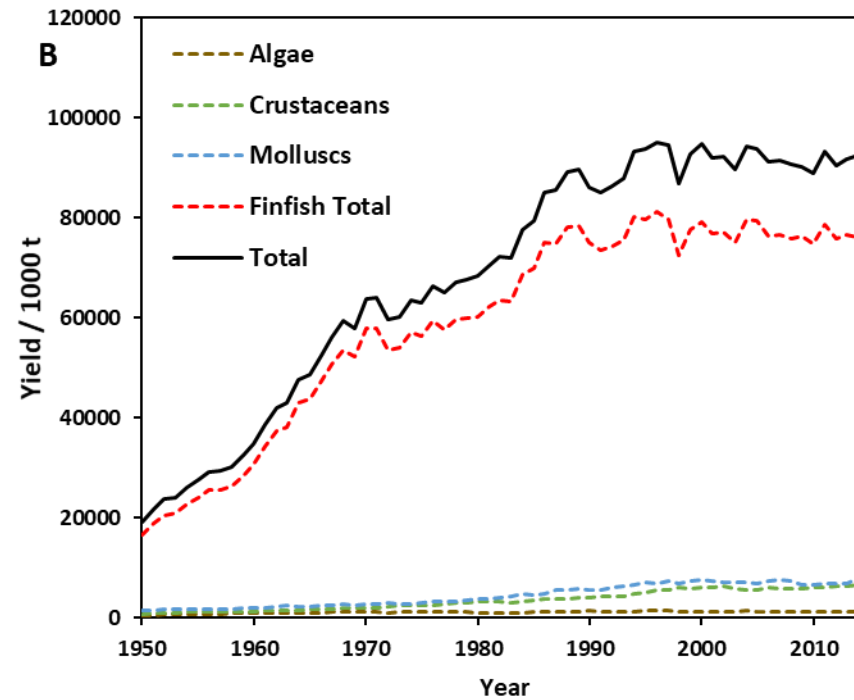
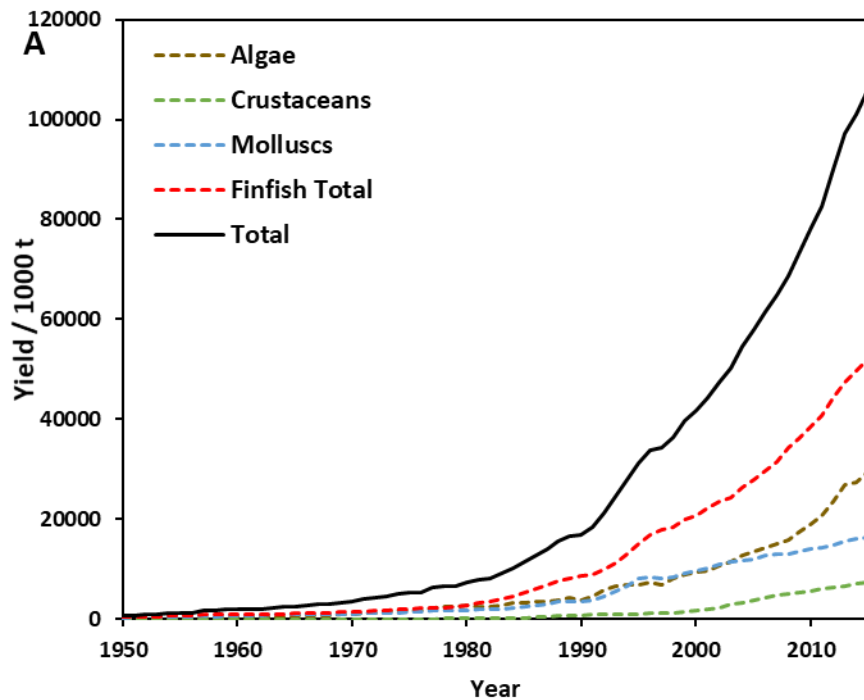


B) A “Dead Zone” in the Baltic Sea

84,000 km²

Target wastewater - Aquaculture

THE UNIVERSITY
of EDINBURGH



Aquaculture WW effluents
reported N concentrations in
the range of 50-124,900 μM N

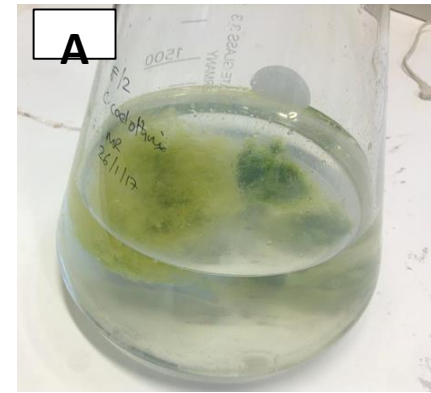
In DIN and DON forms

Global aquaculture yield versus capture fisheries (data taken from FAO)

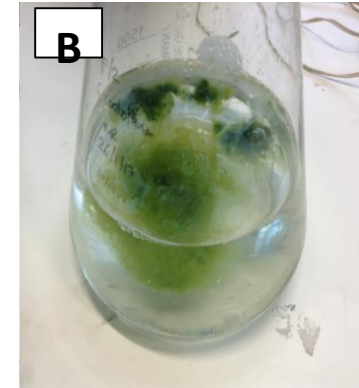
- Scottish aquaculture industry wants to double its revenue to £3.6 billion by 2030
 - *Increased environmental impact, disease, infrastructure and logistical challenges*
- Movement towards onshore recirculating aquaculture systems under trial – rolled out by 2021.

Cladophora

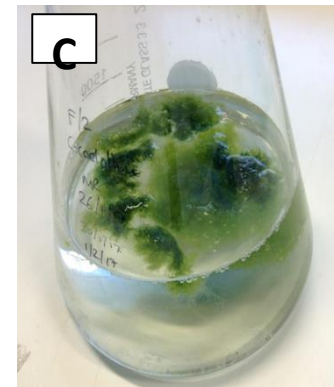
- Ubiquitous
- Often found in nutrient enriched conditions
- Nutrient and CO₂ removal
- High rates of growth
- Ease of cultivation/harvesting
- Resistance to grazers/herbivory
- Tolerant to a range of abiotic conditions
 - Incredibly robust



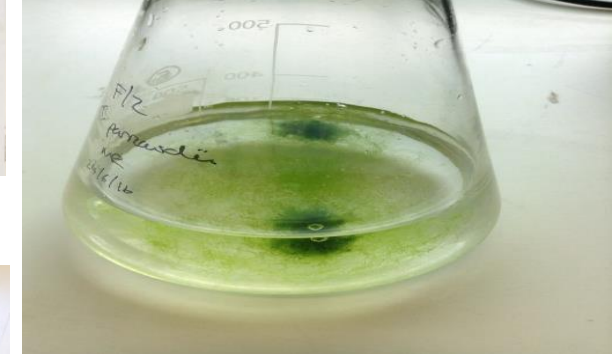
Long-term storage



4 days



6 days



Complexity of wastewaters

Given that wastewaters are highly dynamic environment; varying both temporally and spatially:

- How does Nitrogen affect the growth and composition of *Cladophora*?
- Are two closely related species able to uptake different N sources indiscriminately, across a range of concentrations?
- Does *Cladophora* have a preference for a certain N type?
- Is *Cladophora* capable of removing different nitrogen sources simultaneously?

Part I – Removal of single N sources

2 Species:

- *C. coelothrix* & *C. parriaudii*

Cultivation:

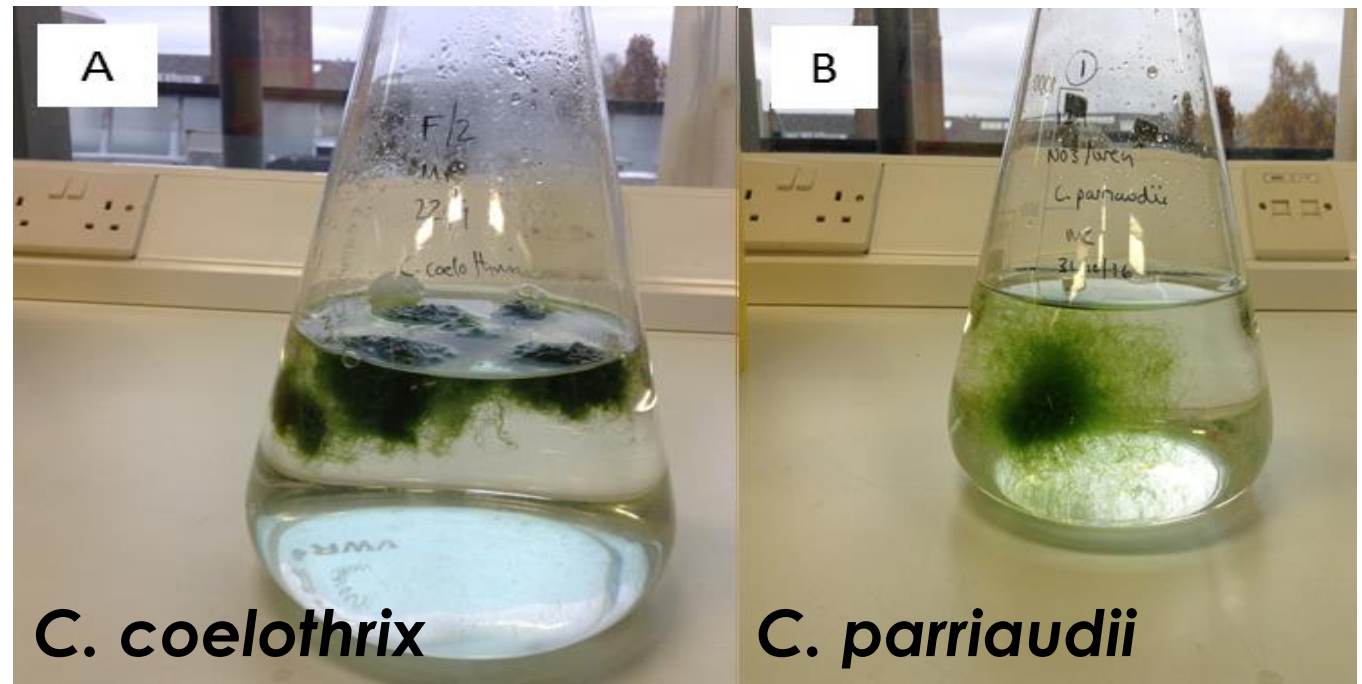
- 14 days, 24°C, 16/8 h L/D

4 Nitrogen Types:

- NH_4^+ , NO_2^- , NO_3^- , Urea

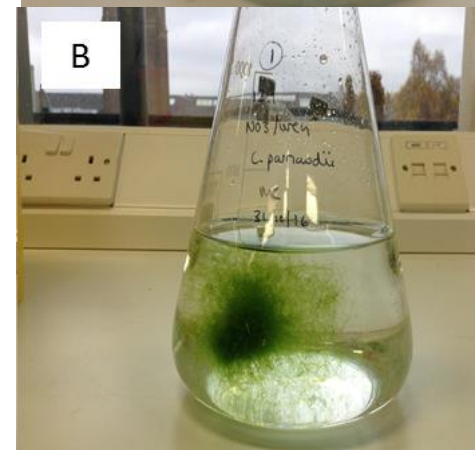
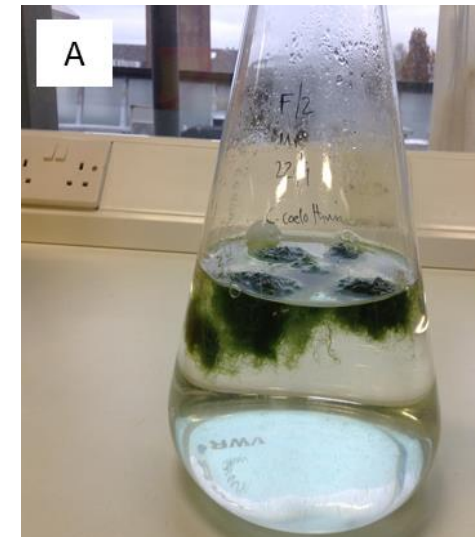
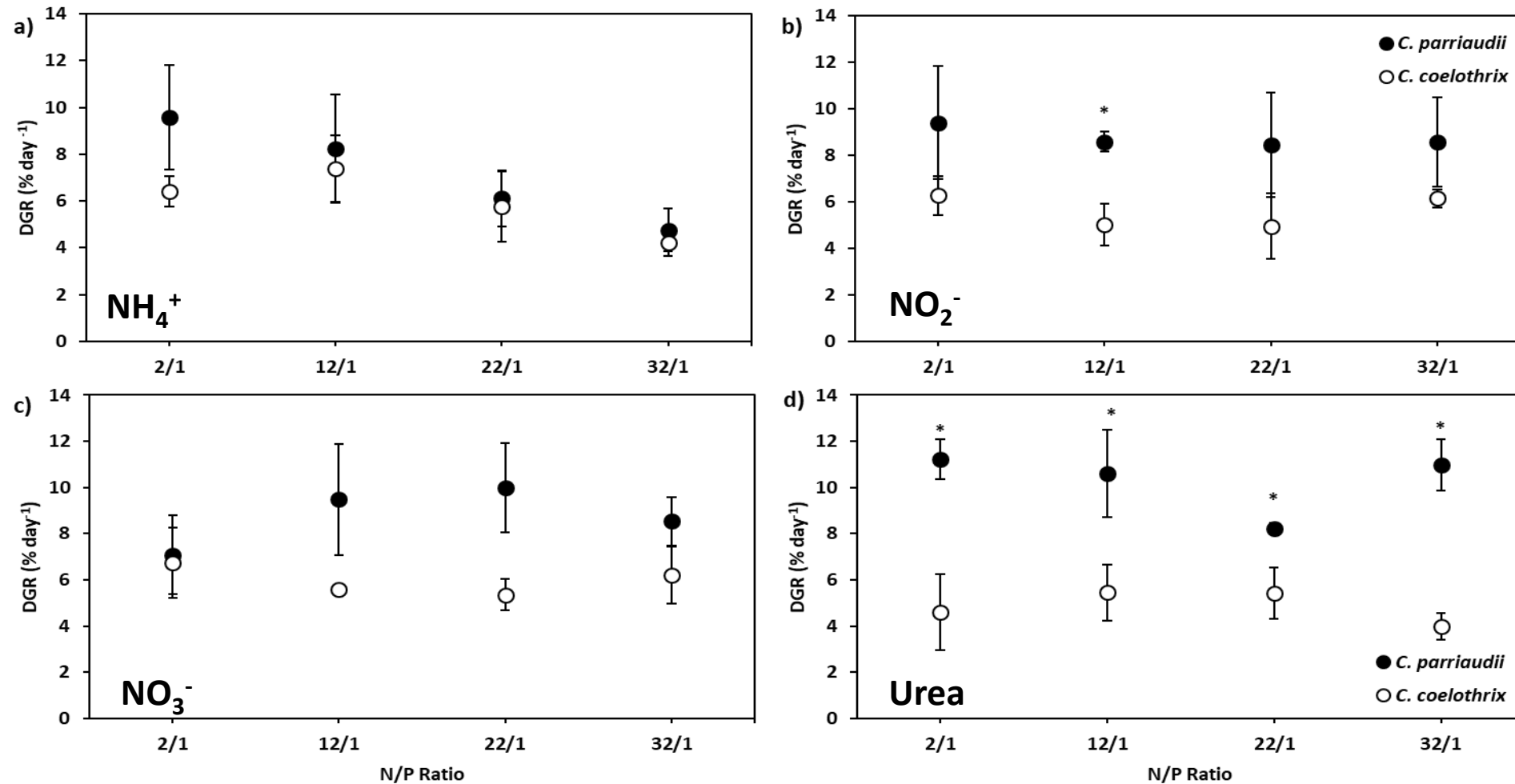
4 N/P ratios:

- 2/1, 12/1, 22/1, 32/1
- Equates to an initial [N] of 160, 960, 1760, 2560 μM N
 - In range of N concentrations found within aquaculture WW



Growth under single N sources

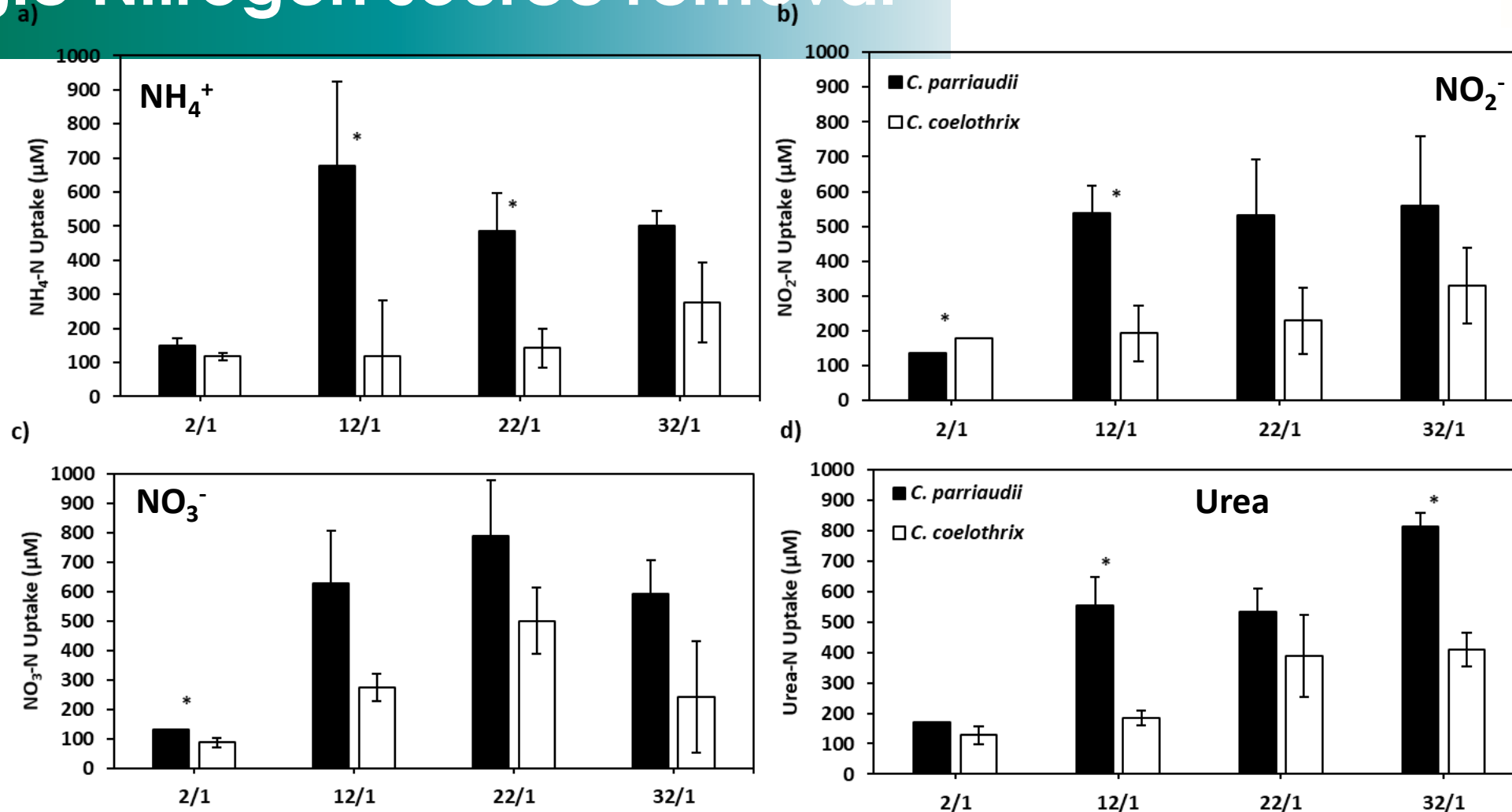
THE UNIVERSITY
of EDINBURGH



Daily growth rate of *C. coelothrix* (white) and *C. parriaudii* (black) cultivated under different Nitrogen

Single Nitrogen source removal

THE UNIVERSITY
of EDINBURGH



Total nitrogen removal by *C. coelothrix* (white) and *C. parriaudii* (black)

Part II – Simultaneous removal of multiple N sources

■ *C. parriaudii*

Cultivation:

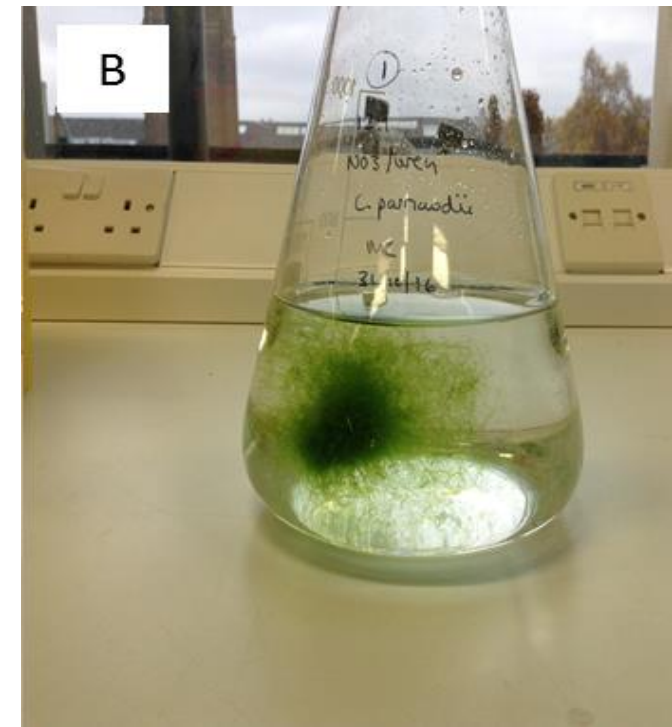
- 14 days, 24°C, 16/8 h L/D

2 N/P ratios:

- 2/1, 12/1, 22/1, 32/1
- Equates to an initial [N] of 160, 960 μM N

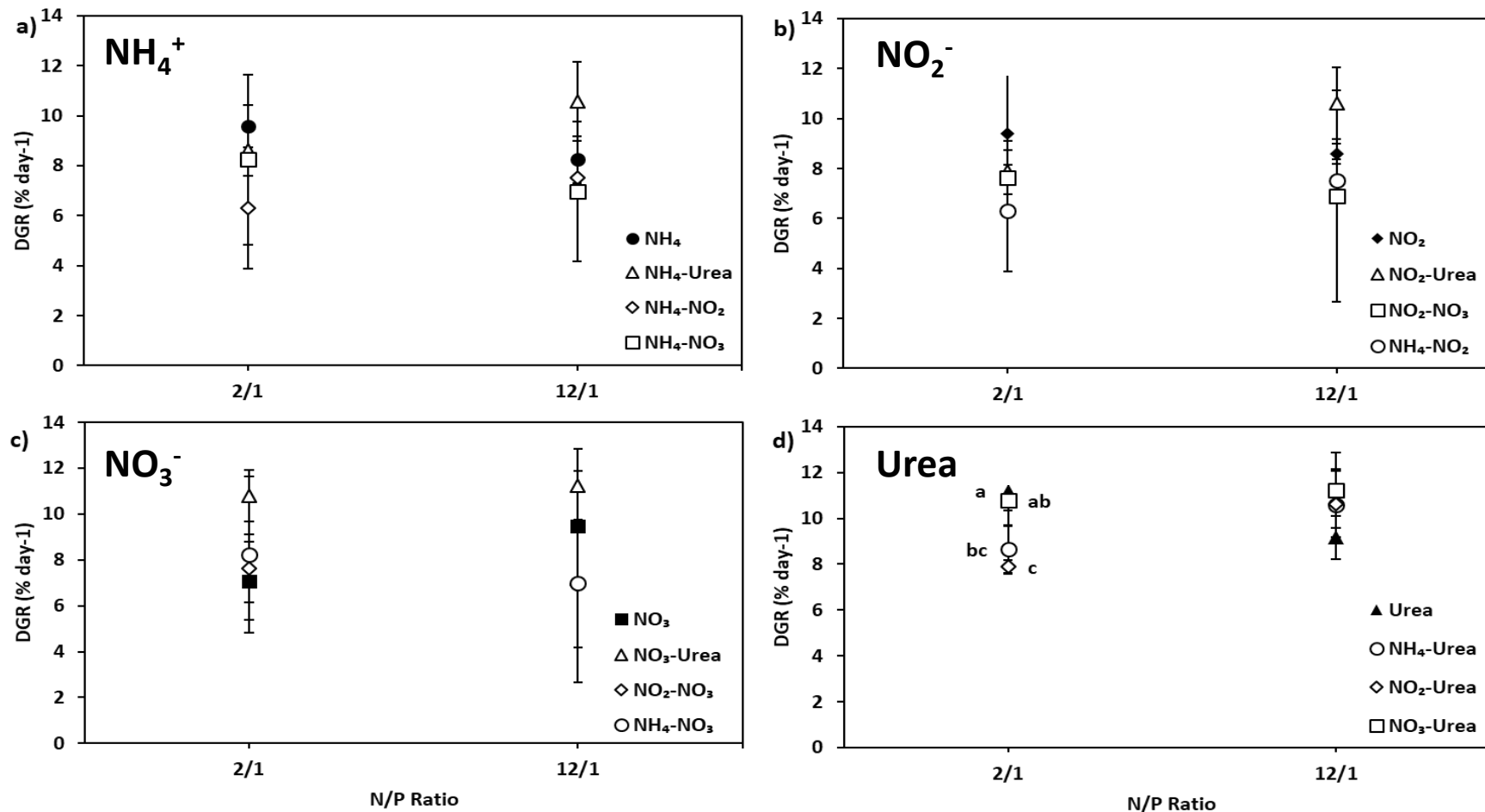
10 Nitrogen Types:

- Individual N sources and N-combinations
 - *E.g. $\text{NH}_4\text{-NO}_2$, $\text{NO}_2\text{-Urea}$*



Growth under multiple N sources

THE UNIVERSITY
of EDINBURGH

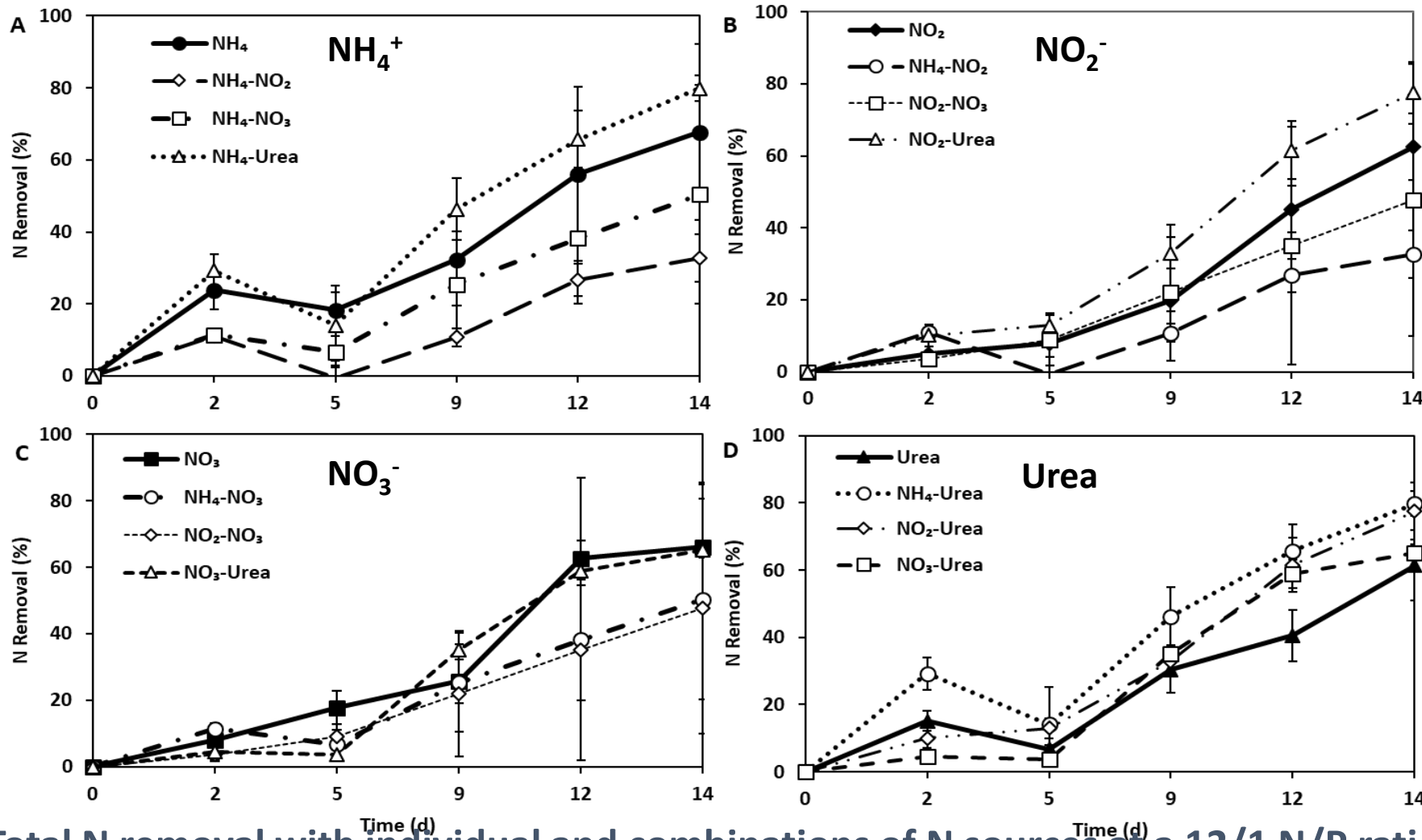


- Growth generally better with individual N sources (solid black symbol)
- Except Urea, enhanced growth with additional N forms
- Also, enhanced growth of the other N types

Nitrogen type influences growth rate

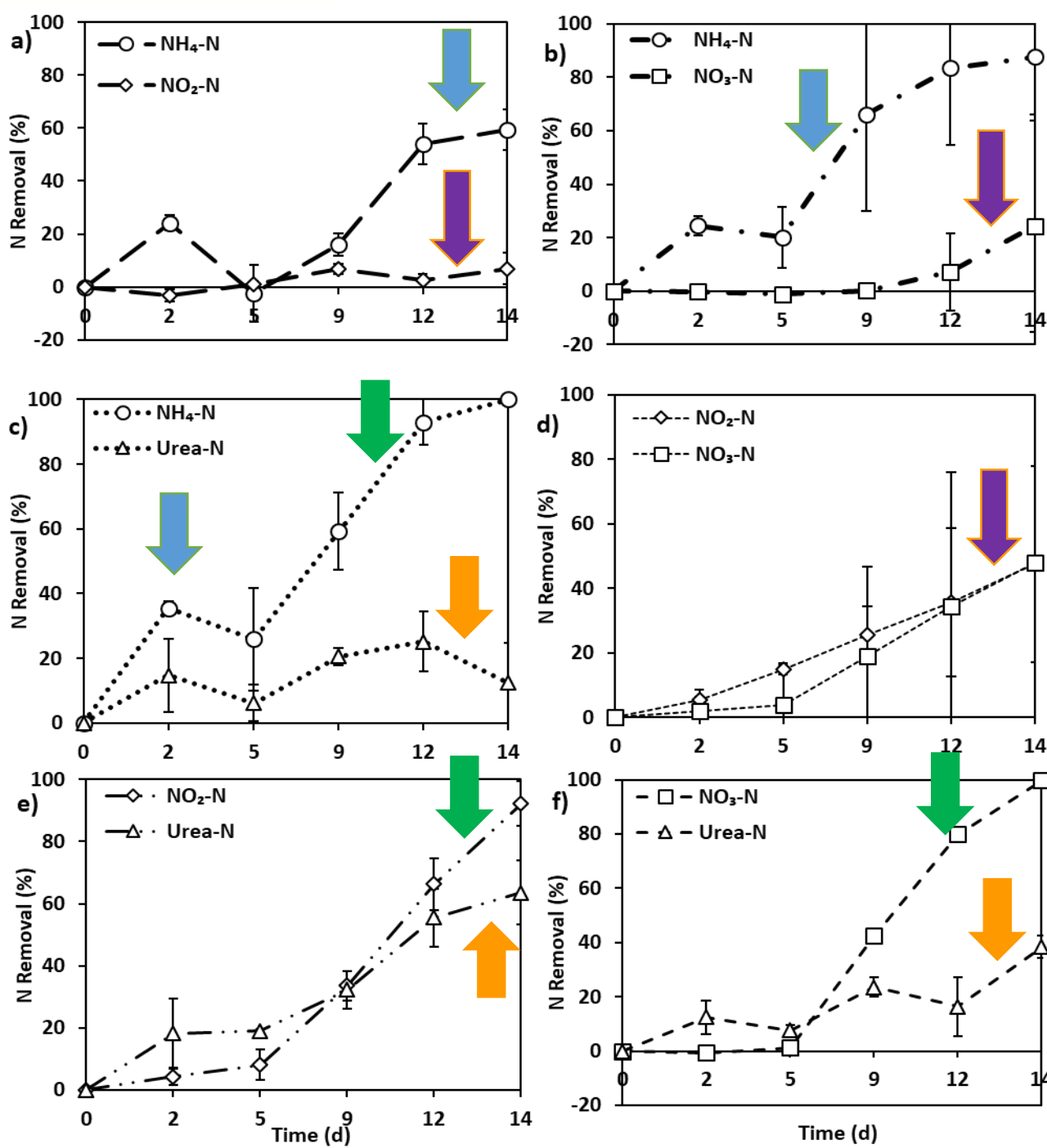
DGR of *C. parriaudii* cultivated with individual and combinations of N sources at a 2/1 and 12/1 N/P ratio

Total Nitrogen removal – Multiple N



- Again, individual N sources (solid black lines) had greater removal
- With the exception of urea, which performed better in the presence of an alternative N source
- However, the presence of urea enhances the Total N removal
- Uncertainty over Nitrogen preference ...???

Total N removal with individual and combinations of N sources at a 12/1 N/P ratio



Individual N type removal by *C. parriaudii*
when cultivated with Nitrogen combinations
of N sources at a 12/1 N/P ratio

- NH₄⁺ is the preferred N source
- NO₂⁻ and NO₃⁻ have almost identical uptake patterns
 - Suppressed when cultivated with NH₄⁺
- Urea removed secondarily
- Presence of urea enhances uptake of other N forms present

Biochemical composition

Biochemical composition of *C. parriaudii* when after cultivation under different
nitrogen regimes

Nitrogen Source	Protein (% DW)		Carbohydrate (% DW)	
	2/1	12/1	2/1	12/1
NH ₄ ⁺	6.2 (± 1.8)*	13 (± 0.7)*	51.1 (± 2)*	31.1 (± 1.3)*
NO ₂ ⁻	5.4 (± 1.3)*	10.9 (± 1.2)*	41.7 (± 6.7)	38.5 (± 3.1)
NO ₃ ⁻	6.8 (± 1.2)	9.7 (± 0.5)	41.1 (± 2)*	32 (± 1.9)*
Urea	6.5 (± 0.6)	8 (± 0.9)	43.8 (± 11.9)	37.6 (± 3.6)
NH ₄ -NO ₂	10.8 (± 3.3)	15 (± 0.4)	45.6 (± 6.2)	39.2 (± 1.5)
NH ₄ -NO ₃	8.3 (± 2.9)	12.1 (± 1.9)	47.2 (± 5.9)	36.1 (± 6.5)
NH ₄ -Urea	5.1 (± 0.2)*	11.4 (± 1.8)*	51.5 (± 0.9)	40.6 (± 4.5)
NO ₂ -NO ₃	8.4 (± 3)	11.9 (± 1.1)	38.8 (± 5.6)	38.2 (± 2.2)
NO ₂ -Urea	6.5 (± 1.1)*	12 (± 0.9)*	54 (± 8.1)	42.7 (± 2.2)
NO ₃ -Urea	6.1 (± 0.2)*	10.7 (± 0.4)	48.4 (± 1.3)	42.8 (± 3.1)

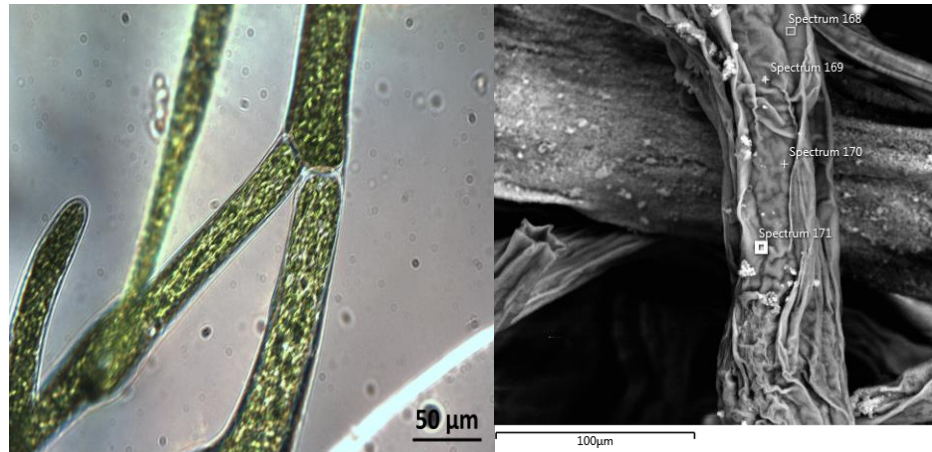
- Trade-off between carbohydrate & protein content with N/P ratio
- Cultivation with urea yields carbohydrate rich biomass

Conclusions from Nitrogen Removal

- Species-specific differences in growth, N uptake, and ability to utilise certain N forms:
- *C. parriaudii* is capable of complimentary N uptake:
- A preference is shown towards NH_4^+
- Urea was typically removed secondarily
- The presence of urea in the media enhanced uptake of any other N form present
- Final biochemical composition is strongly influenced by nutrient regime
 - Findings from this study could lead to a paradigm shift in algal cultivation & WWT practices
 - Species selection to species capable of complimentary N and C uptake
 - Cultivation using alternative, cheaper, and more easily assimilable N sources
 - Tailoring nutrient regimes to produce biomass of varying composition for specific applications/commercially exploitable attributes

Heavy Metal Biosorption

Putting the 'bio' into biosorption:
heavy metal removal from aqueous solutions by
dried *Cladophora parriaudii*





pollution –

Mercury in canned tuna still a concern

‘Jaw-dropping’ levels of heavy metals found in whales
Contamination threatens human food supply



Scientists discovered high levels of aluminum, chromium, lead, mercury, and other metals in sperm (Chris Bangs/Associated Press/File 2001)



Pollutants cause huge rise in brain diseases

Scientists alarmed as number of cases triples in 20 years
The numbers of sufferers of brain diseases, including Alzheimer's, Parkinson's and motor neurone disease, have soared across the West in less than 20 years, scientists have discovered.



Marine Biological Association
Incorporated by Royal Charter

[About](#)
[Research](#)
[Membership](#)
[Education](#)
[Facilities](#)
[Services](#)

Historical impact: Sex changes caused by toxic paint led to international bans

MBA research demonstrating how toxic anti-fouling paints used on boats resulted in population crashes of marine snails led to worldwide bans on their use.

Mining

Deep-sea mining possibly as damaging as land mining, lawyers say

Environmental and legal groups warn of potential huge effects on Indigenous people and the environment

● Sign up to receive the top stories in Australia every day at noon

Ben Doherty

@bendoherthycorro

Wed 18 Apr 2018
04.02 BST





< 2620

68



▲ Deep-sea mining off Papua New Guinea's coast. Legal and environmental and Indigenous groups who live nearby. Photograph



Fracking in America leading to possible mass heavy metal contamination

Tuesday, November 01, 2016 by: JD Heyes
Tags: frac sand, Heavy metals, Wisconsin



quences



(mg L ⁻¹)	Lead (mg L ⁻¹)
.712	0.0003 – 0.4
	15.8 – 32.2
	0.01

atic environment and the ecosystem

Industrial Effluent Drinking Quality

The pre poses a

Conventional metal removal technologies...



...have their downsides...

- High CAPEX/OPEX
- Generation of toxic by-products/sludge
- Regeneration of exhausted materials
- Use of hazardous chemicals
- Cost/source of raw materials
- Reduced efficiency when treating large volumes of WW containing low metal concentrations

So, there is a need to improve existing methods or develop alternative techniques for the treatment of metals, sustainably.



Contents

Past:

- *Wastewater treatment by filamentous macro-algae*
 - *Nutrient removal*
 - *Heavy metal bioremediation*

Present:

- Relevant research within SAMS
 - Selected algal biotech projects

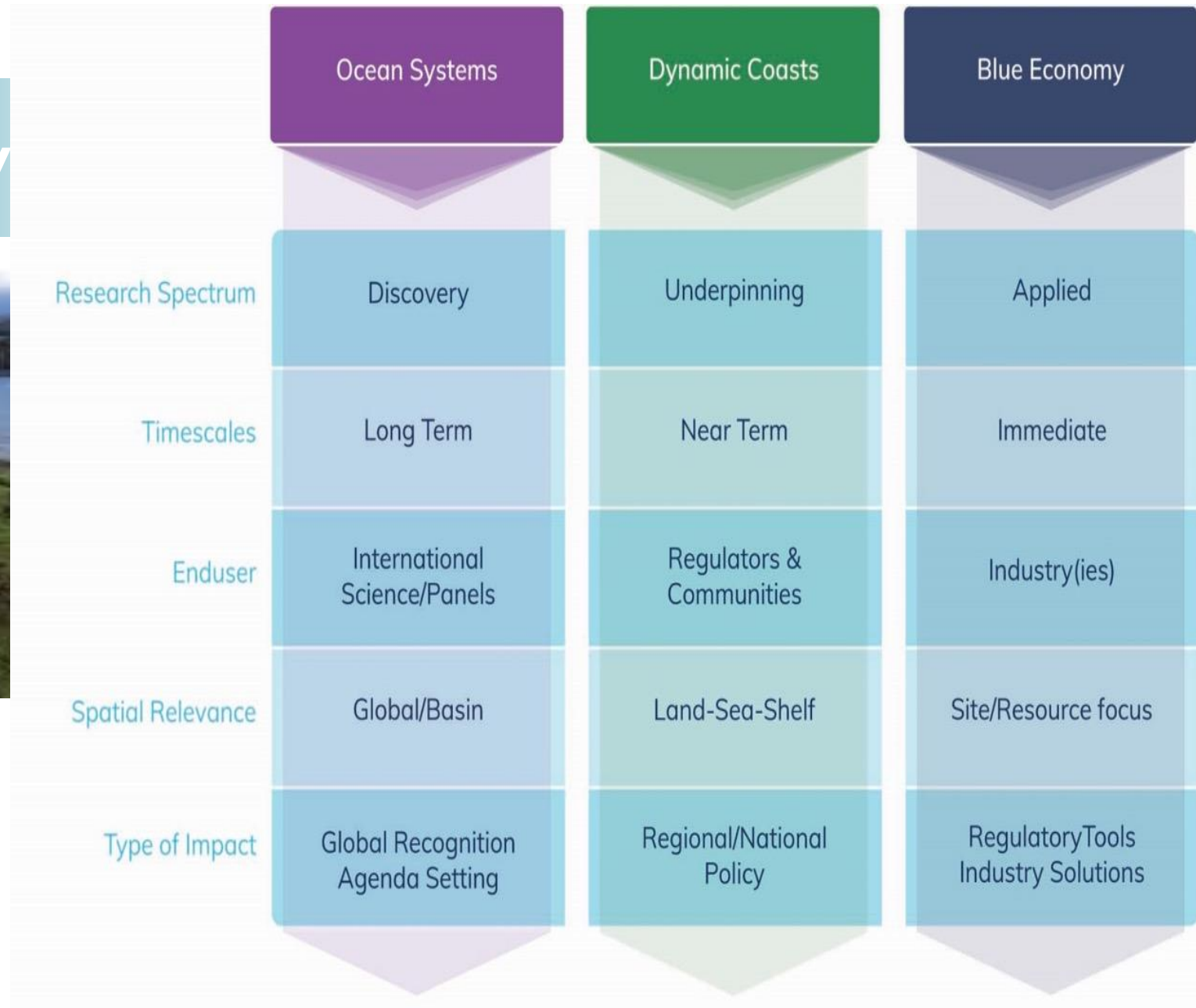
Future:

- The future direction of research

New research strategy



The Blue Economy has a focus on marine biotechnology, aquaculture, renewable energy, and oil and gas decommissioning...



SAMS Research projects

[Home](#) » [Science](#) » [Projects](#)

SAMS science projects



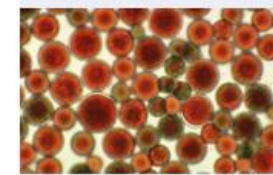
Arctic ABC

... designing innovative autonomous drifting observatories for ice covered Arctic waters



3D-Marg

... predicting biomass and volume of marine growth using 3D-photogrammetry



ABACUS

...producing high-value products from microalgae



ACIDCOAST

... understanding how coastal zone governance and management can effectively respond to ocean acidification



ALFF

...training 15 PhD students in omic-empowered research on the micro- and macroalgal microbiomes



ALLIANCE

... matching users to services, facilities and experts to take a blue biotech product to market



AlterEco

... a novel monitoring framework for shelf sea ecosystem drivers



AquaSpace

... identifying key constraints to the growth of the aquaculture industry in Europe



Arctic PRIZE

... investigating how change in sea ice and ocean properties will affect Arctic ecosystem structures



ARISE

Can we detect changes in the Arctic ecosystem?



ASSEMBLE Plus

... improving the long-term sustainability of marine stations



ATLAS

... assessing the Atlantic's deep-sea ecosystems to plan for sustainable blue growth

TomAlg

A Phyconet Business Interaction
Voucher funded project between
SAMS & BioPower Technologies Ltd.



PHYCONET

high value products from microalgae

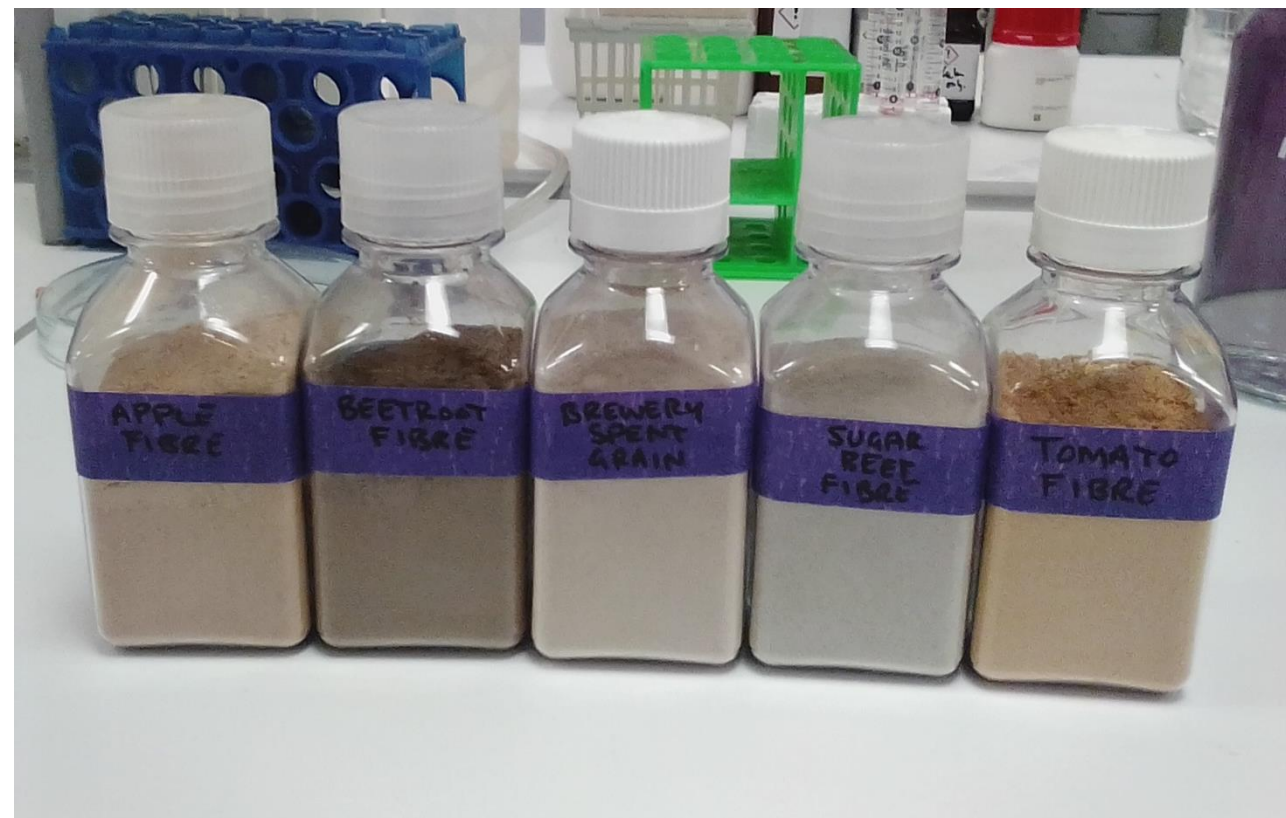


BBSRC Networks in Industrial Biotechnology
and Bioenergy (BBSRC NIBB)

The use of micronization powders from by-products of the food and drinks industry to supplement algal growth

The experimental aims were to:

- Determine the **solubility** of the powders in both fresh- and sea-water
- Screen the impact that the powders had on growth of 4 micro-algal species



TomAlg

Species selected were:

- ***Cylindrotheca fusiformis* (CCAP 1017/2)**
 - Pennate diatom, potential mixotroph
- ***Nannochloropsis oceanica* (CCAP 849/10)**
 - Eustigmatophyte, use in aquaculture and biofuel production
- ***Chlorella vulgaris* (CCAP 211/21A)**
 - Halotolerant, previous experience of mixotrophy
- ***Monodopsis subterranea* (CCAP 848/1)**
 - Freshwater species, capacity to accumulate nitrogen





ALGAE for a BIOMASS applied to the PRODUCTION of added value COMPOUNDS

3-year project aiming at a business-oriented and technology-driven development of a new algal biorefinery for high-value products.

Started in May 2017, brings together 2 large industries, 3 algal SMEs and 4 RTOs



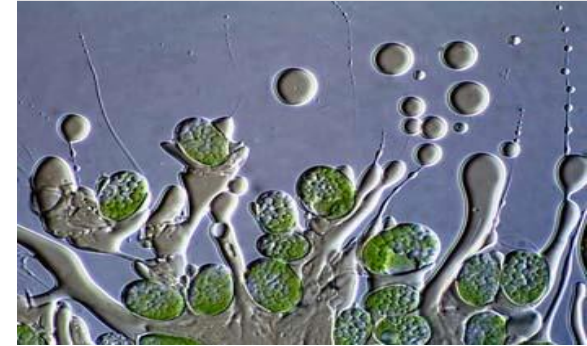
Algal Bioenergy 1



BioMara was a joint UK and Irish project that aimed to investigate the viability of production 3rd generation **biofuels** from marine biomass.

- Microalgae for biodiesel
- Macroalgae for biogas and alcohols

S.P. Slocombe, Q. Zhang, M.E. Ross, A. Anderson, N.J. Thomas, A. Lapresa, A. Rad-Menendez, C.N. Campbell, K.D. Black, M.S. Stanley, Unlocking nature's treasure-chest: screening for oleaginous algae, Scientific Reports. 5 (2015)



EnAlgae was a large INTERREG programme, bringing together 19 partners across Europe.

It aimed to use algal resources to mitigate CO₂ emissions, produce biomass, and bioenergy

Algal Bioenergy 2



Macrofuels aims to develop the next generation of macro-algal based **biofuels for transportation** via advanced **biorefinery** processes

SeaGas is a project assessing the financial viability of farming **sugar kelp** for bioenergy production via **anaerobic digestion**.



Together these projects aim to:

- Improve seaweed **cultivation** practices
- Investigate **seaweed storage** to ensure access to a **year-round** supply of a biomass feedstock
- Environmental Impact assessments (**EIA**)
- Develop efficient **anaerobic digestion** operational procedures for processing seaweed residues



IMPAQT or the Intelligent Management System for IMTA.

Aims to pave the way towards a more environmentally friendly and higher yielding European aquaculture industry.

- Design and implementation of **sensors** for **monitoring** and **management** of IMTA systems.



INTEGRATE

... fostering green growth, eco-innovation and environmental efficiency
by developing IMTA practice

INTEGRATE: an eco-innovative solution to foster sustainability in the Atlantic area.

The project aims to:

- Develop tools to increase competitiveness while removing barriers to the **green growth** of the aquaculture sector
- Improve the quality and **public image** of aquatic products

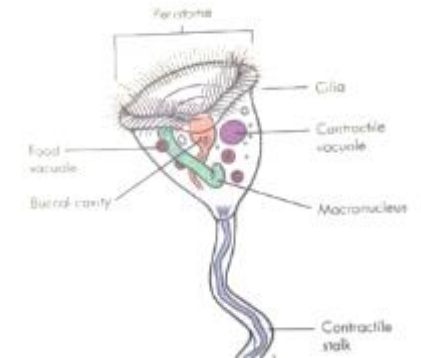


Polycultures (the more species, the merrier)

Naturally evolving community:

- ❖ Higher stability
- ❖ Higher resource efficiency
- ❖ Higher resilience
- ❖ No GMO, no extremophiles, no exotic species
- ❖ Precedent from terrestrial crops
- ❖ Low experiment repeatability
- ❖ Low predictability
- ❖ Lower “target compound” production

“**Paradox of the Plankton** describes the situation in which a limited range of resources supports an unexpectedly wide range of plankton species”



Culture crashes are more likely in monocultures

GENIALG – GENetic diversity exploitation for Innovative macro-ALGal biorefinery.

To improve production and exploitation of *Saccharina latissima* and *Ulva rigida*

- Selection and breeding
- Germplasm, pheno- and geno-typing for biobanking purposes
- Socio-economic benefits of seaweed farming



GENIALG

... increasing the sustainable production
of sugar kelp and sea lettuce



GlobalSeaweedSTAR

... growing the research and innovation
capability of developing countries
engaged in seaweed farming

GlobalSeaweedSTAR – To safeguard the future of the seaweed industry in developing countries, aiming to:

- Improve research capabilities, knowledge, and build capacity in developing countries
- Develop best cultivation practices, management and disease mapping and prevention
- Foster socio-economic growth, enablement, outreach and equality

Global Challenges Research Funding available via this project.

Enablement



ALFF is a recently completed platform which trained 15 PhD students in omics and the relationship between algae and the microbial community. These research topics broadly covered:

- Identification, classification and utilisation of algal symbionts
- Investigation of inter- and intra-species signalling in the natural environment
- Use of state-of-the-art genomics, molecular and biochemical techniques to understand these interactions



ALFF

...training 15 PhD students in omic-empowered research on the micro- and macroalgal microbiomes



ASSEMBLE Plus

... improving the long-term sustainability of marine stations

ASSEMBLEPlus: Association of European Marine Biological Labs Expanded

Allows scientists from academia, industry, and policy to access marine biological faculties, facilities, and resources.

It is a partnership of 32 marine stations

- Starter cultures
- Media
- DNA
- Sequencing/Taxonomy
- ABS Advice (Nagoya Protocol)
- Patent Depository
- Confidential Deposits
- Culture optimisation
- Training Courses
- Knowledgebase – one-stop shop



Currently, researchers outside the UK can access marine cultures for free. Apply for ASSEMBLEPlus Remote Access

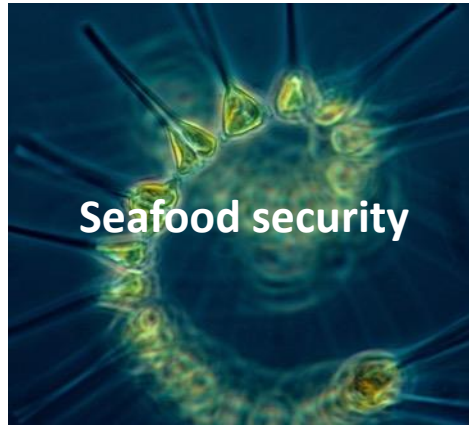
SRSL

Enterprise

**SAMS Research Services Ltd
Specialist marine consultancy and
survey services**

Enabling Productive Oceans...

SAMS Research Services Ltd (SRSL) provides specialist marine consultancy and survey services, underpinned by cutting-edge science. We enable our clients to understand and mitigate the risks involved in industry interaction with the marine environment; to maximise productivity and sustainability.

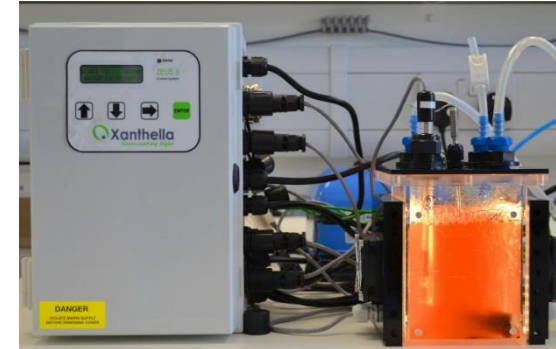


Xanthella

- Design and manufacturing company supplying Photobioreactors for R&D, scale-up and industrial biomass production.

ENBIO project:

- Deployed 16 Pandora PBR's in Ardnamurchan
 - Use of excess energy from Combined Heat & Power biomass burning
 - CO₂ from distillery waste fermentation
- Production of 1.5 t/y Spirulina



Contents

Past:

- *Wastewater treatment by filamentous macro-algae*
 - *Nutrient removal*
 - *Heavy metal bioremediation*

Present:

- Relevant research within SAMS
 - Selected algal biotech projects

Future:

- The future direction of research

Water Quality Innovation Group

WQIG is an Knowledge Exchange partnership with an aim to increase UHI's collaborative research with other academic, industrial, governmental, and third party sectors. Key research themes:

- **Water Analysis**
- **Monitoring and Assessment**
- **Water treatment and remediation**
- **Integrated catchment management**



WQIG Take Home Messages

- Water is a precious resource requiring:
 - Stewardship
 - Common quality and accreditation measures
- A holistic change in how we consider and treat water/wastewater. With an emphasis on:
 - Circular economy
- A need for innovative and improved technologies to treat current waste streams
- Pharmaceuticals are a high priority:
 - Many human pharma's detected in natural waters
 - Inefficient removal from conventional WWTP
 - Fully emerged...not emergent



ALLIANCE FOR
WATER STEWARDSHIP



DIAGEO



Scottish
Water

Always serving Scotland

CREW

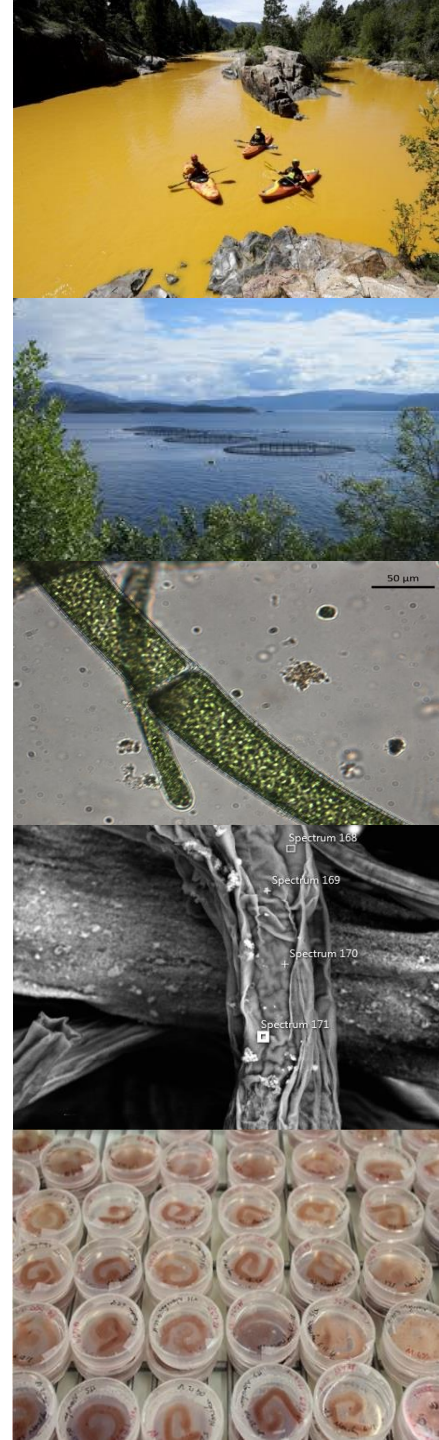
CENTRE OF
EXPERTISE
FOR WATERS

Conclusions

SAMS and staff have a vast experience in delivering algal biotech related projects, and has a strong interest in the bio-economy.

- Wastewater treatment
 - Nutrient
 - Heavy metals
 - Emerging microcontaminants
- Biorefineries
- Bioenergy
- Aquaculture

We would greatly welcome collaborations and contributions to help drive future research.



Thank you

Michael.ross@sams.ac.uk

Prof. Michele Stanley
Prof. John Day
Dr. Andrea Semião
Dr. Lucie Novoveska
Dr. Jean-François Sassi

Daniela Catania
Eilidh MacLeod
Naomi Thomas
Katharine Davis
Rory McColl

