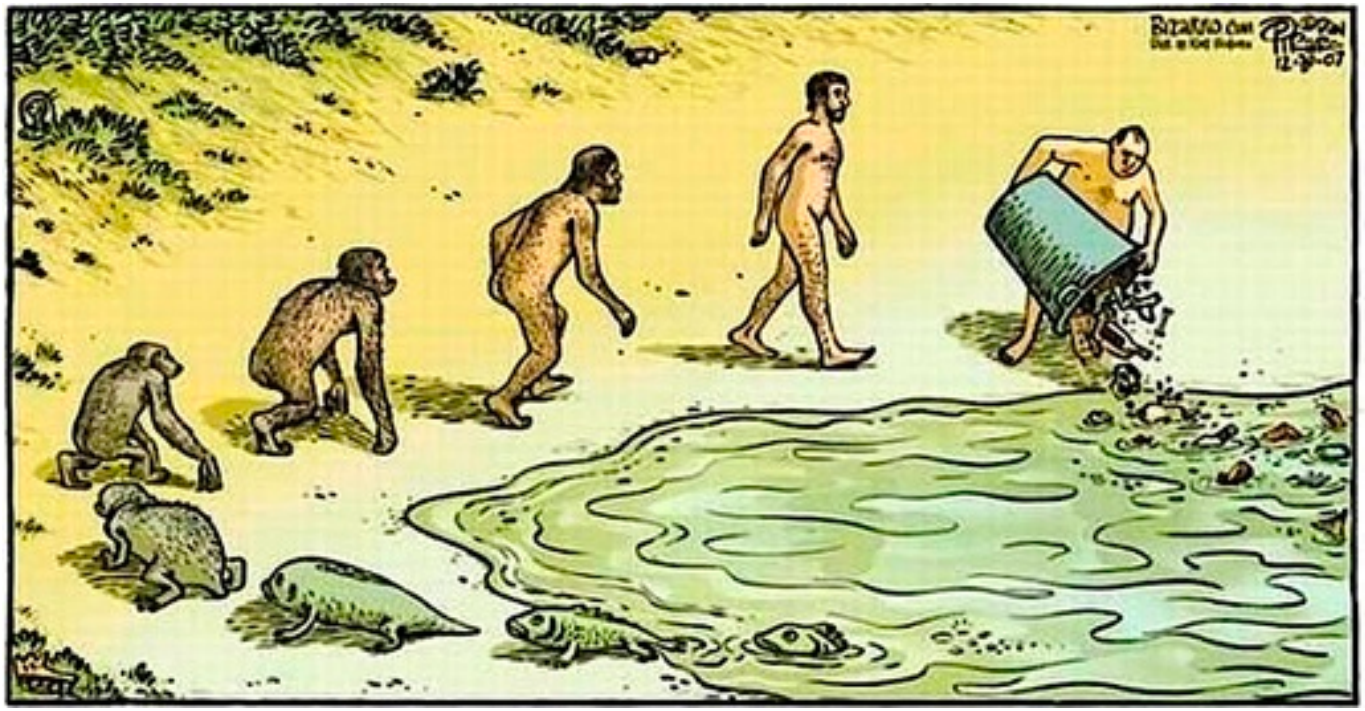
Nitrogen:
acquisition and supply



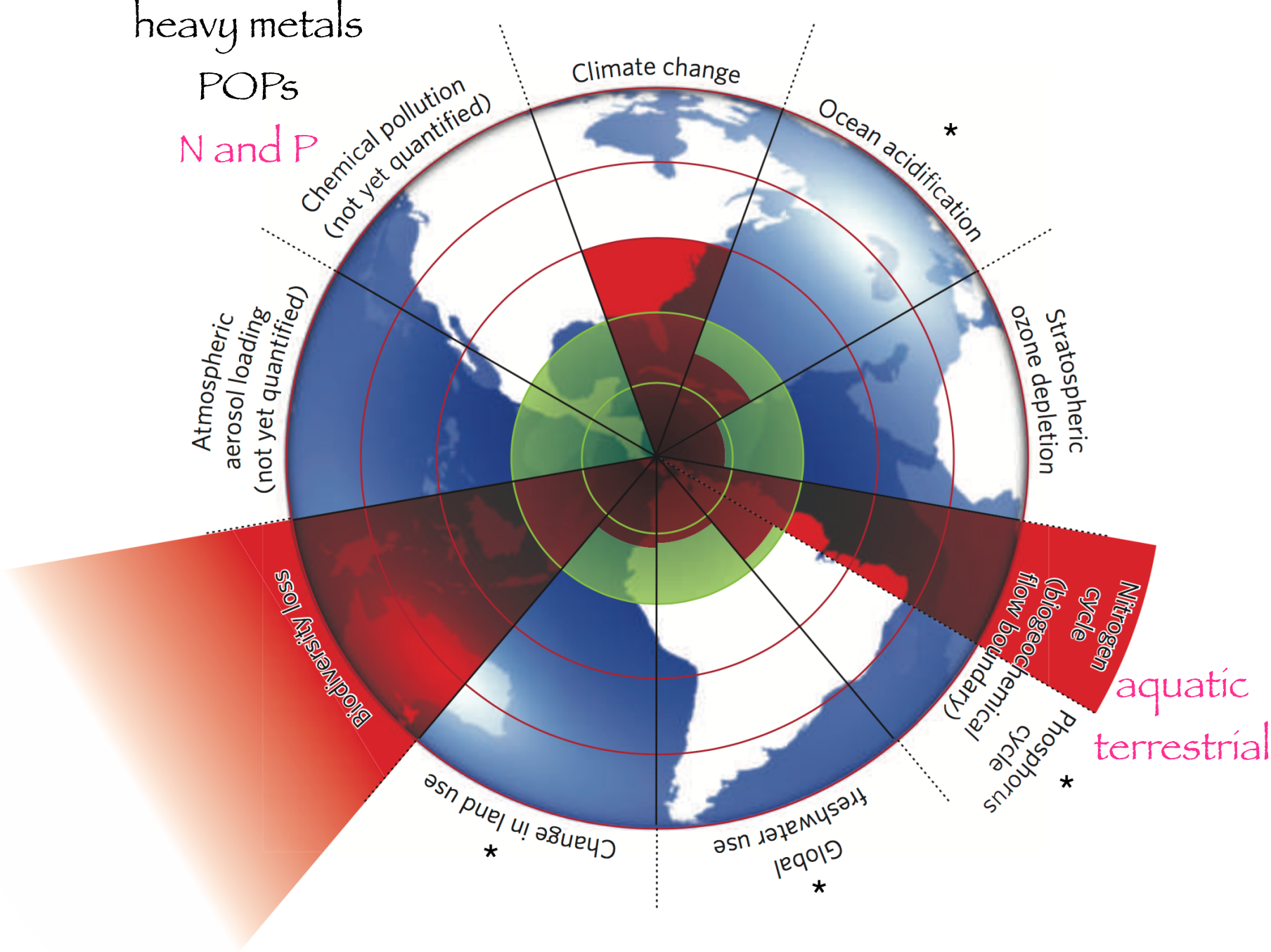
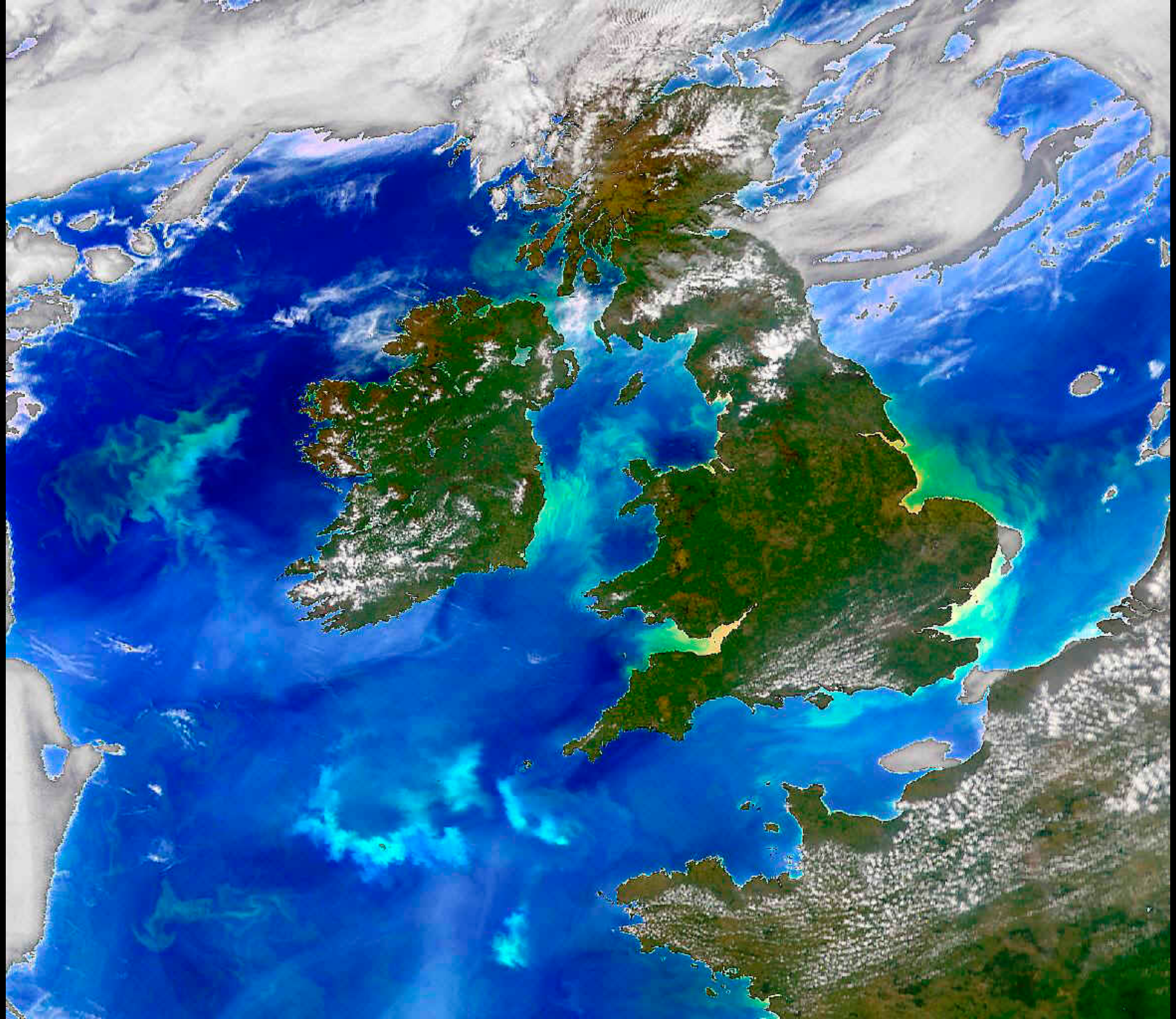
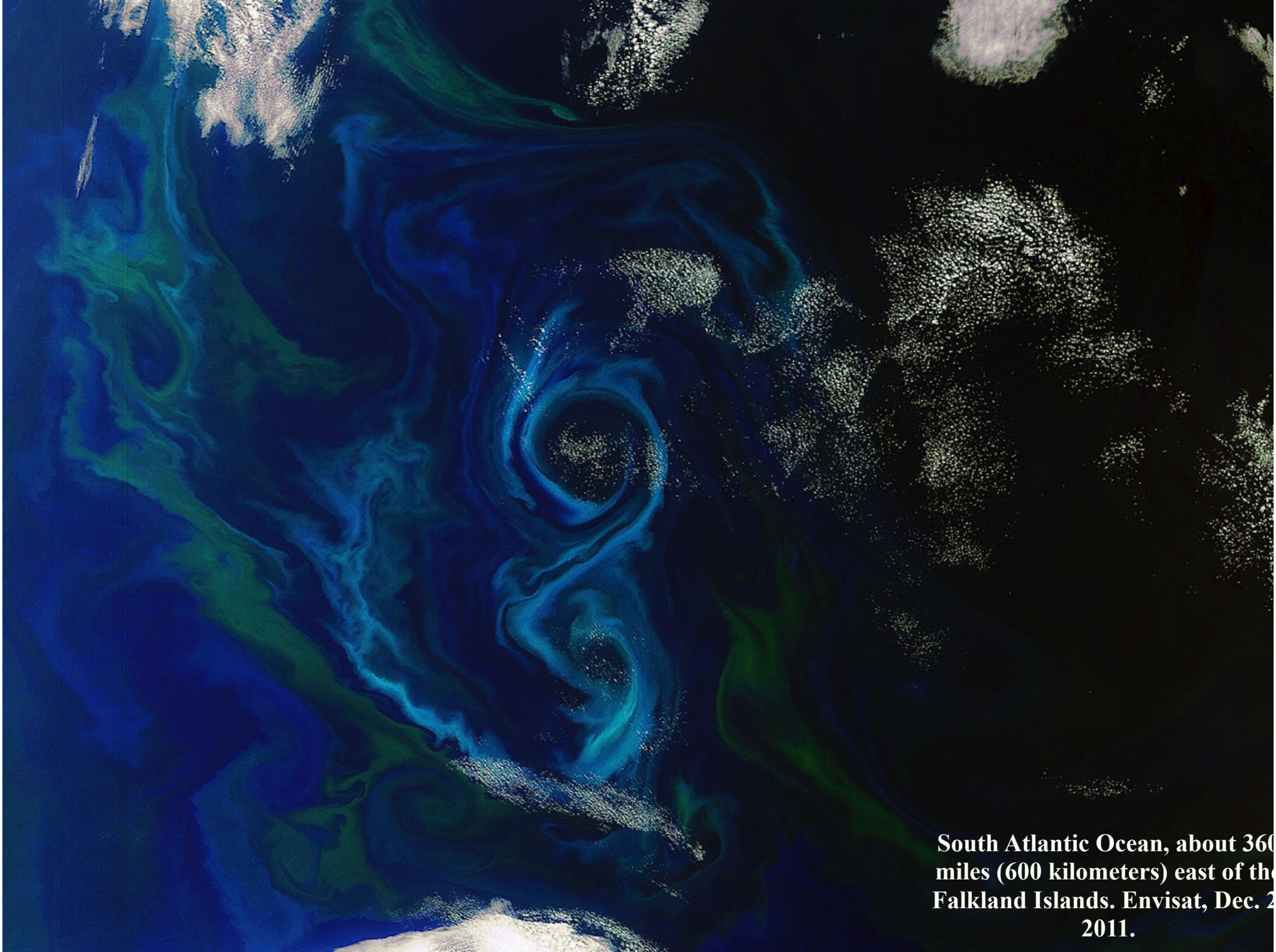


Figure 1 | Beyond the boundary. The inner green shading represents the proposed safe operating space for nine planetary systems. The red wedges represent an estimate of the current position for each variable. The boundaries in three systems (rate of biodiversity loss, climate change and human interference with the nitrogen cycle), have already been exceeded.





South Atlantic Ocean, about 360
miles (600 kilometers) east of th
Falkland Islands. Envisat, Dec. 2
2011.

Nutrients

Introduction

Algae, like terrestrial plants, require nutrients for their persistence, growth and reproduction.

Algae are bathed in a nutrient medium (most of the time for intertidal species)…

No need for elaborate root systems - uptake is through the thallus surface**…** **(think: links with SA:V)**

Whereas many terrestrial plants have evolved symbiotic relationships with fungi or bacteria (i.e. mycorrhiza, root nodules) or specialised root systems, macroalgae have specialised nutrient uptake mechanisms to cope in low nutrient environments…

Nutrients

Much of the knowledge of algal nutrition we have today comes from research into algal culture media between the 1930s to 1970s.

These studies have identified a host of essential and beneficial nutrients.

Essential: According to E. Epstein (1972) it is essential if

(1) the plant can't complete a normal life cycle without it; or

(2) the element is part of some essential plant constituent or metabolite; e.g. Mg in chlorophyll-*a*, Cl required for oxidation of water during photosynthesis

Nutrients

An alternative view of when a nutrient is ‘essential’:

(i) The alga fails to grow or reproduce when the nutrient is limiting… same as in Epstein’s definition;

(ii) They cannot be replaced by another nutrient, i.e., no other nutrient has the same metabolic function as the limiting nutrient;

(ii) They have a direct effect rather than act in conjunction with another nutrient or through some interaction with another ‘factor’.

Nutrients

Essential nutrients classified into micro- and macro-quantities (hence micro- and macronutrients).

Reflects the relative concentrations in tissue or required in nutrient solutions (Table 5.2, Lobban and Harrison (1997); Table 4.4, Hopkins and Hüner, 2008); does not infer importance relative to the nutritional needs.

Macronutrients: mainly (but not always) structural, but some may be regulatory (e.g. Ca and Mg).

Micronutrients: catalytic and regulatory.

Nutrients

About 20 nutrients fulfil critical metabolic pathways in algae.

[see Table 5.1, Lobban and Harrison (1997) for essential nutrients and their functions]

Table 5.1. *Functions and compounds of the essential elements in seaweeds*

Element	Probable functions	Examples of compounds
Nitrogen	Major metabolic importance in compounds	Amino acids, purines, pyrimidines, amino sugars, amines
Phosphorus	Structural, energy transfer	ATP, GTP, etc., nucleic acids, phospholipids, coenzymes (including coenzyme A), phosphoenolpyruvate
Potassium	Osmotic regulation, pH control, protein conformation and stability	Probably occurs predominantly in the ionic form
Calcium	Structural, enzyme activation, cofactor in ion transport	Calcium alginate, calcium carbonate
Magnesium	Photosynthetic pigments, enzyme activation, cofactor in ion transport, ribosome stability	Chlorophyll
Sulfur	Active groups in enzymes and coenzymes, structural	Methionine, cystine, glutathione, agar, carrageenan, sulfolipids, coenzyme A
Iron	Active groups in porphyrin molecules and enzymes	Ferredoxin, cytochromes, nitrate reductase, nitrite reductase, catalase
Manganese	Electron transport in photosystem II, maintenance of chloroplast membrane structure	
Copper	Electron transport in photosynthesis, enzymes	Plastocyanin, amine oxidase
Zinc	Enzymes, ribosome structure(?)	Carbonic anhydrase
Molybdenum	Nitrate reduction, ion absorption	Nitrate reductase
Sodium	Enzyme activation, water balance	Nitrate reductase
Chlorine	Photosystem II, secondary metabolites	Violacene
Boron	Regulation of carbon utilization(?), ribosome structure(?)	
Cobalt	Component of vitamin B_{12}	B_{12}
Bromine[a]	Toxicity of antibiotic compounds(?)	Wide range of halogenated compounds, especially in Rhodophyceae
Iodine[a]		

[a]Possibly an essential element in some seaweeds.

Source: DeBoer (1981), with permission of Blackwell Scientific Publications.

Nutrients: `higher` plants

- 17 elements are essential for the growth of all higher plants

TABLE 4.4 The essential nutrient elements of higher plants and their concentrations considered adequate for normal growth.

Element	Chemical Symbol	Available Form	Concentration in Dry Matter (mmol/kg)
Macronutrients			
Hydrogen	H	H_2O	60,000
Carbon	C	CO_2	40,000
Oxygen	O	O_2,CO_2	30,000
Nitrogen	N	NO_3^-, NH_4^+	1,000
Potassium	K	K^+	250
Calcium	Ca	Ca^{2+}	125
Magnesium	Mg	Mg^{2+}	80
Phosphorous	P	HPO_4^-, HPO_4^{2-}	60
Sulfur	S	SO_4^{2-}	30
Micronutrients			
Chlorine	Cl	Cl^-	3.0
Boron	B	BO_3^{3-}	2.0
Iron	Fe	Fe^{2+}, Fe^{3+}	2.0
Manganese	Mn	Mn^{2+}	1.0
Zinc	Zn	Zn^{2+}	0.3
Copper	Cu	Cu^{2+}	0.1
Nickel	Ni	Ni^{2+}	0.05
Molybdenum	Mo	Mo_4^{2-}	0.001

Nutrients

Unlike macronutrients (C, H, O, N and P; these also make up the bulk of the algal dry matter), most other essential micronutrients (and vitamins) are present in much smaller amounts in nature and rarely limit algal growth to the extent that the macronutrients do.

Most nutrients required for algal growth are present at much lower concentrations in the external medium (seawater) compared to in the algal thallus or cell. For example, N and P are concentrated by about 100,000 times by the seaweed, while C is about 10,000 times more abundant with respect to the medium.

In many cases this indicates some sort of active uptake mechanism (the nutrient is moved against a concentration gradient, a process that requires metabolic energy expenditure). More on this later.

[Table 5.2, Lobban and Harrison (1997)]

Table 5.2 *Concentrations of some essential elements in seawater and in seaweeds*

Element	Mean concentration in seawater (mmol kg^{-1})	Mean concentration in seawater ($\mu g\ g^{-1}$)	Concentration in dry matter Mean ($\mu g\ g^{-1}$)	Concentration in dry matter Range ($\mu g\ g^{-1}$)	Ratio of concentration in seawater to concentration in tissue
Macronutrients					
H	105,000	10,500	49,500	22,000–72,000	2.1×10^{0}
Mg	53.2	1,293	7,300	1,900–66,000	1.8×10^{-1}
S	28.2	904	19,400	4,500–8,200	4.7×10^{-2}
K	10.2	399	41,100	30,000–82,000	1.0×10^{-2}
Ca	10.3	413	14,300	2,000–360,000	2.9×10^{-2}
C	2.3	27.6[a,b]	274,000	140,000–460,000	1.0×10^{-4}
B	0.42	4.50	184	15–910	2.4×10^{-2}
N	0.03	0.420[a,c]	23,000	500–65,000	2.1×10^{-5}
P	0.002	0.071	2,800	300–12,000	2.4×10^{-5}
Micronutrients					
Zn	6×10^{-6}	0.0004[a]	90	2–680	4.4×10^{-5}
Fe	1×10^{-6}	0.00006[a]	300	90–1,500	1.0×10^{-5}
Cu	4×10^{-6}	0.0002[a]	15	0.6–80	1.7×10^{-4}
Mn	0.5×10^{-6}	0.00003[a]	50	4–240	2.0×10^{-5}

[a]Considerable variation occurs in seawater (Bruland 1983).
[b]Dissolved inorganic carbon.
[c]Combined nitrogen (dissolved organic and inorganic).
Source: DeBoer (1981), including concentrations of elements in seawater from Bruland (1983), with permission of Blackwell Scientific Publications.

Nutrients

In addition to the micro- and macronutrients, vitamins are also required by many algae and other plants. The main vitamins are B12, thiamine and biotin which are often added to the many types of nutrients solutions used for experimental purposes to promote algal growth.

More recent studies have shown that algae are also capable of utilising dissolved organic compounds such as amino acids (source of N) and acetic acid (source of C) as additional sources of nutrients.

Nutrients

Early studies have identified N as the primary nutrient limiting algal growth in the marine environment (Dugdale, 1976).

The addition of N to seaweed aquaculture systems greatly enhances growth and production.

In some systems P is also limiting, and its addition can lead to similar growth enhancements as N additions.

Nutrients

Nutrient limitation

Microalgae and seaweeds differ in terms of the absolute requirements of C, N and P.

In microalgae, the C:N:P ratio is approximately 106:16:1, while in macroalgae it is 550:30:1.

Macroalgae therefore require about half the P needed by microalgae, and the large amount of C indicates that macroalgae have the capacity to store or use large amounts of C (either as storage polysaccharides or used for structural support).

The 106:16:1 ratio in microalgae is called the 'Redfield ratio' which has special significance to biological oceanographers.

Nutrients

N and P at a ratio of roughly 30:1 is required for optimal macroalgal growth, although in reality the range in not fixed but fairly variable:

10:1 is the optimal ratio to cultivate the commercially important red seaweed *Gracilaria gracilis*, but other studies have shown the optimal ratio to be higher at up to 80:1 for certain other seaweeds.

The optimal ratio can be determined experimentally, and this information is essential when it comes to working out fertilising strategies for seaweeds cultivated commercially in land-based tank systems. The knowledge is also used to determine a species' response to eutrophication in the natural environment as it can be used to predict the consequence of pollution.

Nutrients

Liebig's Law of the Minimum

Justus von Liebig (1803-1873; the 'Father of the Fertiliser Industry') published the Law of the Minimum which states that if one nutrient is deficient or lacking, algal growth will be poor even when all other elements are present at optimal or high concentrations.

In fact, the entire ecosystem productivity is limited by the nutrient that is depleted first.

Any deficiency of a nutrient, no matter how small an amount is lacking, will limit growth. If the deficient element is supplied, growth will increase up to the point where the supply of that element is no longer limiting.

Increasing the supply beyond this point is not helpful, as some other element would then be in a minimum supply and become the limiting factor.

Nutrients

The concentration of nutrients in the seawater is determined by the balance of nutrient requirement by the alga and the rate at which nutrients can be supplied to the alga.

The law of the minimum may also include factors (including some physical features of the environment and genetic characteristics intrinsic to the alga) that have been shown to influence algal growth.

Factors such as light intensity (affects the photosystems and the ability of the alga to take up and reduce nitrate), temperature (control the rates at which nutrients are taken up and assimilated), water motion (directly affects the ability of algae to acquire nutrients by affecting the boundary layer diffusion), algal density (enhances competition for nutrients and increases self shading, which leads to a host of follow-on effects), plant population and genetic capacities varieties and so forth may also impinge on the ability of algae to use nutrients.

Nutrients

Assume the optimal N:P ratio in the seaweed tissue is 30:1.

If N and P are present in seawater at exactly that ratio, the seaweed would grow optimally (at least for a given set of environmental conditions).

When the ratio in the external medium is >30:1?

e.g. 60:1…

growth will be limited by P

When the ratio is <30:1?

growth will be limited by N.

Luxury consumption

Any excess N or P will either remain in the culture medium or seawater, or be taken up by the alga and stored as unassimilated inorganic N or P in the vacuoles (in the short term), or assimilated into some organic compound (typically amino acids or some kind of protein in the case of N) but not used for growth.

When assimilated and stored in such a way, it may be kept internally (in the seaweed's tissue) until the limiting nutrient again becomes available when the stored nutrient is mobilised and reallocated to somatic or reproductive growth.

This principle where a nutrient is taken up and stored in excess of immediate requirements for growth is called 'luxury consumption', and is used as a survival strategy by many algae in unpredictable nutrient environments, or by macroalgal aquaculturists as a certain fertilising strategy.

Nutrients

Most of the discussion in the remainder of the lecture will use N as an example to demonstrate interesting and important points of algal nutrition.

This is because it is N that most often limits the growth of algae, but also because N pollution has negative environmental perturbations (eutrophication).

The theory can equally be applied to other nutrients, as the processes that govern uptake (and in many cases assimilation) are basically the same as for N.

Most concepts are equally applicable to macroalgae and microalgae.

Invasive nature

- Beijing olympics: Qingdao green tide

Source of N

Sources of nitrogen

Nitrogen is brought into the oceans via the atmosphere (as gas) and rivers (mostly nitrates and some ammonium); it may also be lost from the ocean as nitrous oxides (NO_x) and gaseous nitrogen (N_2)

Recycling of nitrogen in the oceans is of major importance

N_2 is only used by cyanobacteria, but nitrate (and nitrite) and ammonium is used by most photoautotrophs in the oceans

Nutrient	Relative
gaseous N_2	95%
NO_3^-	~5 %
NO_2^-	~0 %
NH_4^+	~0.1 %

DIN:
$NO_3^- + NO_2^- + NH_4^+$

Cycling

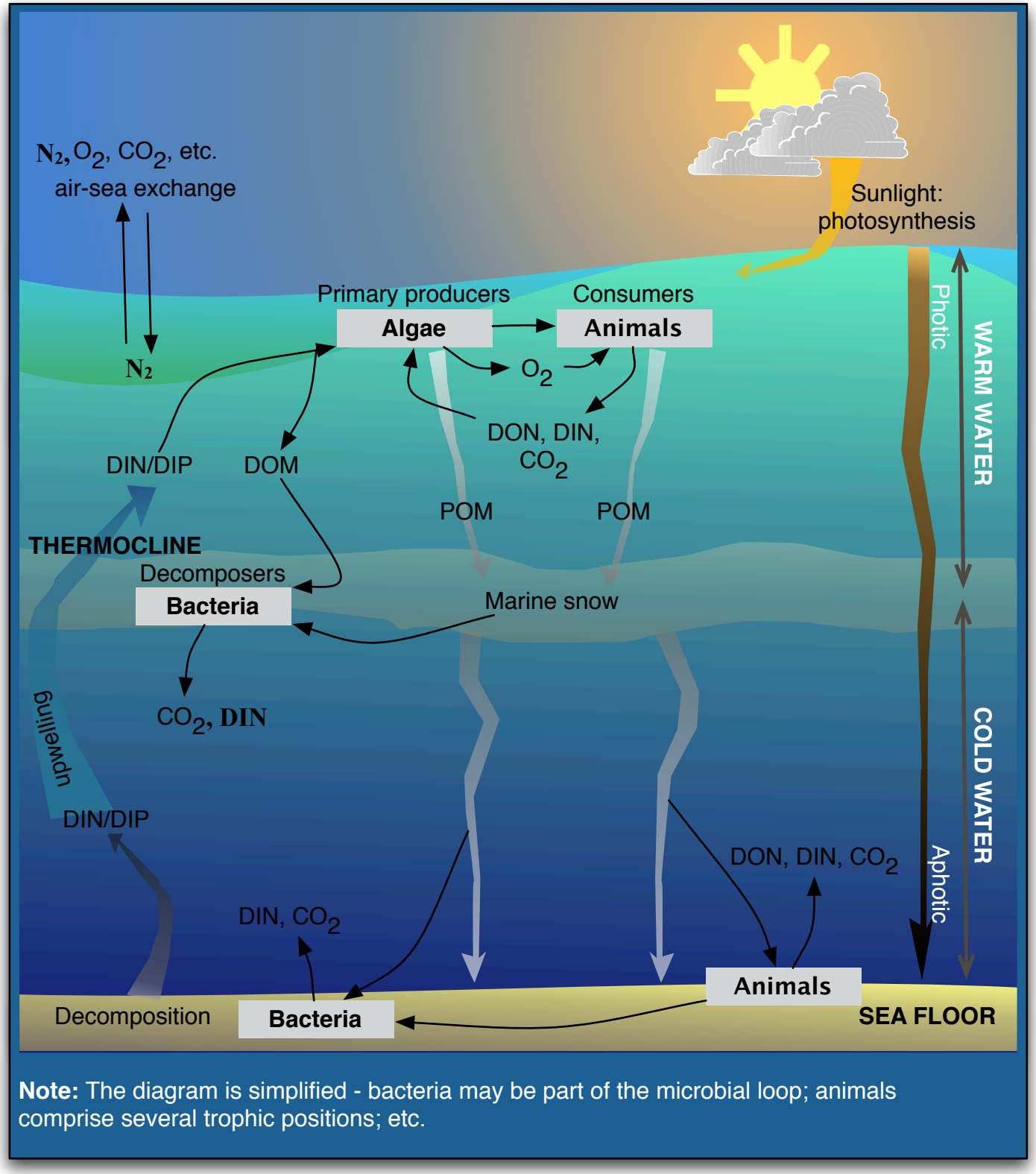
P, N, Si in the oceans

Nutrient uptake by algae (phytoplankton) occurs in the euphotic zone where photosynthesis occurs. Nutrients are removed from the euphotic zone and transferred to the deeper ocean as dead organisms (detritus) sink to the ocean floor. Here, organic matter is remineralised, i.e. brought back into solution as nutrients. This process requires oxygen.

The ocean cannot support highly productive ecosystems except where nutrients are returned to the euphotic zone from below (upwelling).

Nutrient concentrations usually increase with depth, while oxygen concentration decreases. Departures from this trend are caused by advection of different water masses.

Note:
Cyanobacteria take up N_2 and convert it into bio-available N (DIN), prior to it being used by algae.



Note: The diagram is simplified - bacteria may be part of the microbial loop; animals comprise several trophic positions; etc.

Cycling

In the previous slide, the basic components of the N and P cycles are:

- DIN: dissolved inorganic N
- DIP: dissolved inorganic P
- DON: dissolved organic N
- DOP: dissolved organic P
- POM: particulate organic matter

Nitrogen

Units of measurement

Nutrient concentrations are usually expressed in µM units (µmol l^{-1}), but µg-at l^{-1} may also be used.

NH_4^+ & NO_3^- (µmol l^{-1})	NH_4^+ & NO_3^- (µg-at l^{-1})
2	**?**
3	**?**
12	**?**
21	**?**
30	**?**

$$MM = g.mol^{-1}$$

$$MM = \frac{g}{mol}$$

Molecular Mass N = 14.0067 $g.mol^{-1}$

Have: 2 $\mu mol.l^{-1}$ NH_4^+

How many µg N?

$$14.0067 = \frac{\mu g}{2\ \mu mol}$$

28.0134 µg

Note that these calculations (*i.e.* those involving macronutrients N and P) are based only of the atom of interest, *e.g.* N in this example, within the overall molecule.

The Prefixes Used with SI Units

Prefix	Symbol	Meaning	Scientific Notation
exa-	E	1,000,000,000,000,000,000	10^{18}
peta-	P	1,000,000,000,000,000	10^{15}
tera-	T	1,000,000,000,000	10^{12}
giga-	G	1,000,000,000	10^{9}
mega-	M	1,000,000	10^{6}
kilo-	k	1,000	10^{3}
hecto-	h	100	10^{2}
deka-	da	10	10^{1}
—	—	1	10^{0}
deci-	d	0.1	10^{-1}
centi-	c	0.01	10^{-2}
milli-	m	0.001	10^{-3}
micro-	μ	0.000 001	10^{-6}
nano-	n	0.000 000 001	10^{-9}
pico-	p	0.000 000 000 001	10^{-12}
femto-	f	0.000 000 000 000 001	10^{-15}
atto-	a	0.000 000 000 000 000 001	10^{-18}

Nitrogen

Environmental concentrations…

In tropical areas and some temperate seas and oceans such as the temperate coastal areas of Australia, concentrations can be almost immeasurable all year round.

In coastal upwelling areas along the west coasts of all major continents (the California Current along western shores of North America; the Benguela Current along the west coast of southern Africa; the Canary Current along the west coast of north Africa; and the Humboldt Current which runs along South America) nutrient concentrations vary seasonally, with maximal nutrient concentrations (total inorganic N = 20 – 40 μM; inorganic P = ~2 μM) during the major upwelling season and lowest concentration the rest of the time (generally <4 μM total inorganic N; <0.2 μM inorganic P).

In upwelling systems, nutrients may become available in pulses every week or two, each one lasting one to several days, depending on local oceanographic and climatic conditions.

Nitrogen

Most natural marine ecosystems fall within the two categories mentioned above – systems with year round low nutrient status are called oligotrophic, while the upwelling systems are called mesotrophic).

Eutrophic systems have unnaturally high amounts of nutrients.

Let's look at the history of eutrophic systems, and why they exist.

Nitrogen

Usually N limitation is not the problem... (although in aquaculture...)

A problem of global proportions

The Haber-Bosch process has successfully short-circuited the nitrogen cycle so that the distribution of nitrogen globally was significantly modified. Today more nitrogen is fixed annually by anthropogenic processes than by natural nitrogen fixation. Modifications to the nitrogen cycle originally manifested itself in increased agricultural production — this is the desired effect and is seen as beneficial — but gradually negative effects were witnessed in aquatic and coastal marine environments.

Eutrophication has been described as the oldest problem of water quality caused by human activities in lakes and coastal ecosystems and has today become a problem of global proportions.

Nitrogen

The Nitrogen Bomb

By learning to draw fertilizer from a clear blue sky, chemists have fed the multitudes. they've also unleashed a fury as threatening as atomic energy

By David E. and Marshall Jon Fisher
Photographs by James Worrell
DISCOVER Vol. 22 No. 04 | April 2001

See 'The Nitrogen Bomb (2001).pdf' on iKamva.

Also see:
The Scientist Who Killed Millions and Saved Billions
https://www.youtube.com/watch?v=EvknN89JoWo

Eutrophication

One consequence of eutrophication in natural systems is the development of blooms of unwanted ('nuisance') opportunistic macroalgae such as *Ulva*, *Enteromorpha* and *Cladophora* spp. (and phytoplankton in certain systems).

Blooms are unsightly, but there is a host of other effects such as shifts in community composition (*i.e.* species diversity, abundance, and biomass), and in severe cases anoxia and dystrophic events due to the decomposition of algal biomass after blooms.

A system's response to eutrophication varies greatly, and depends on the scale of the nutrient inputs. The term 'bloom' used describes the situation where a system becomes dominated by a high biomass with an incredibly low diversity of species (note the similarity here with upwelling and mariculture systems).

Blooms

Bloom development is as much influenced by increased nutrient pools over and above that which is available naturally, as by the morphology (and hence physiology) of the algal thallus.

Refer to the Functional Form model (Littler & Littler, 1980); basically, it states that macroalgae with a large SA:V such as membranous *Ulva* spp., or finely branched species such as *Cladophora* spp. have faster rates of nutrient uptake than species with a coarser or thicker thallus construction (Wallentinus, 1984).

The higher rate of nutrient uptake of opportunistic species (with high SA:V) imparts a competitive advantage over other groups of macroalgae under eutrophic conditions (Rosenberg & Ramus, 1984).

An ecophysiological understanding

We will focus on the physiological properties of nuisance macroalgae that give them a competitive advantage in eutrophic systems. In order to find out why only certain species respond to nutrient additions we will also examine the physiology of those species that do not bloom, but which persist or even thrive under oligotrophic or mesotrophic conditions.

There are also other points that may be of interest:

What is the source of nutrients to primary producers in very oligotrophic systems?

How is the physiology of seaweed nutrient uptake (uptake kinetics) studied?

In order to understand the nutrient physiology of seaweeds and the mechanisms seaweeds employ to cope with transient or more consistent nutrient environments, it is important that we first look at the basic concepts of seaweed nutrition.

Entry of DIN into cells

water column/soil water → across boundary layer → across cell wall → across cell membrane (plasmalemma) → into cytoplasm

Uptake kinetics

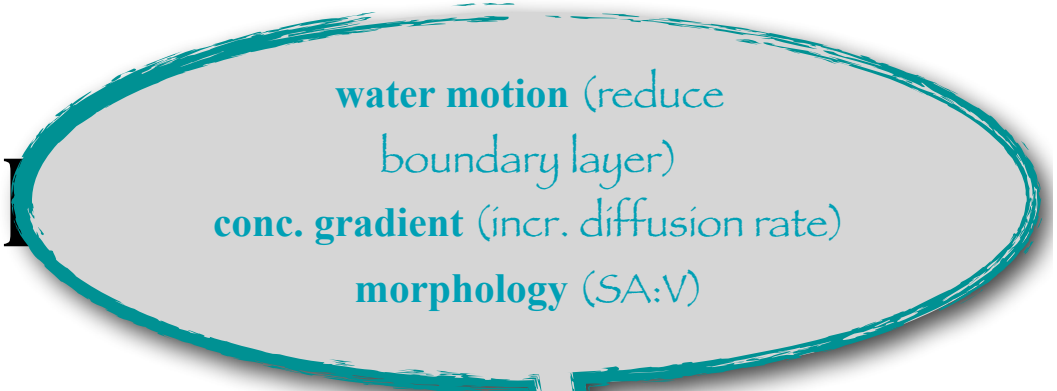
The transport of solutes across solid-fluid boundaries plays an integral role in nutrient cycling in marine and aquatic systems.

The movement of nutrients from the aqueous medium into seaweeds is described by a two-stage process:

(i) the uptake rate is influenced by the diffusion across the boundary layer, the thin layer of water adjacent to the algal surface.

(ii) reaction kinetics inside the thallus, mediated by the seaweed itself, makes up the final stage of uptake. *When fast diffusion rates can be sustained, nutrient acquisition is kinetically controlled (limited); conversely, uptake of N is controlled (limited) by diffusion rates when kinetic reactions are faster than what can be supplied across the boundary layer*.

take



The transport of so tes across solid-fluid boundaries play n integral role in nutrient cycli in marine and aquatic systems.

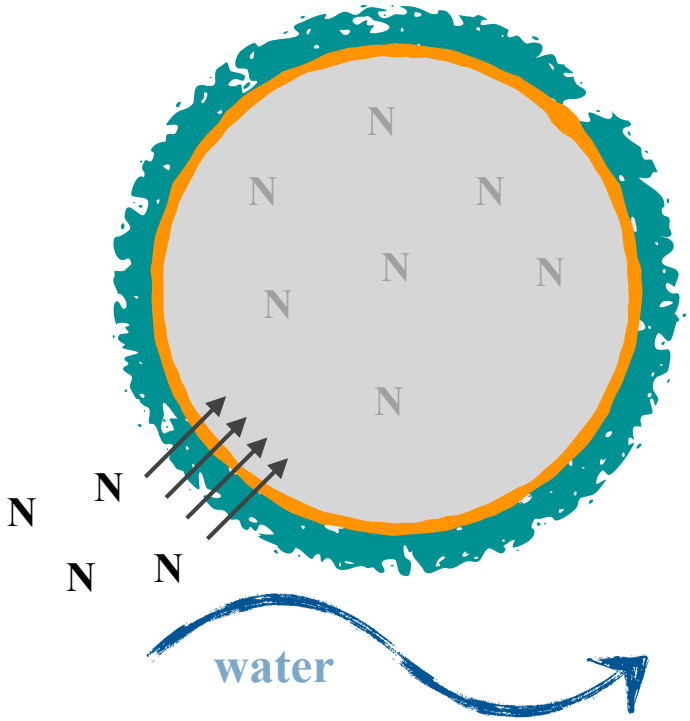
The movement of nutrient rom the aqueous medium into s aweeds is described by a two-stage pro ss:

(i) the uptake rate is influenced by the diffusion across the oundary layer, the thin layer of water adjac nt to the algal surface.

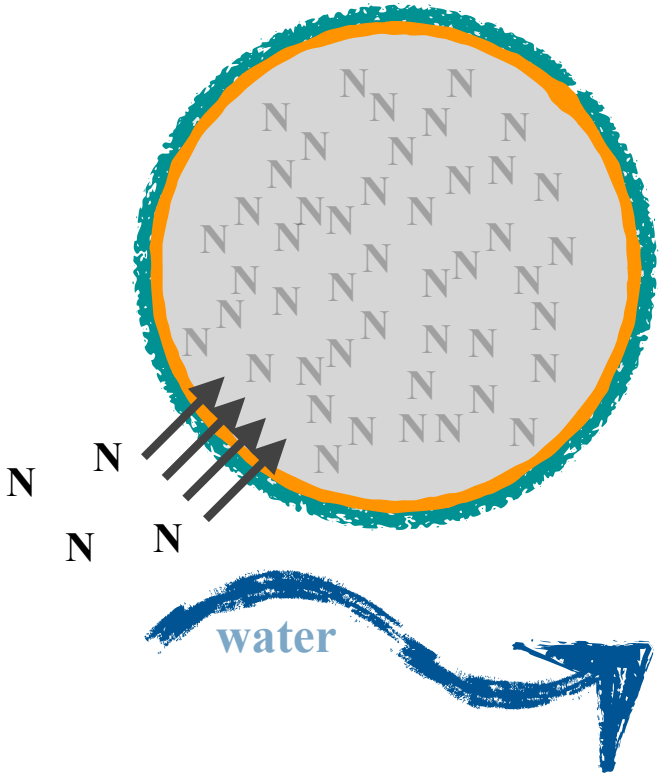
(ii) reaction kinetics inside the thallus, ediated by the seav eed itself, makes up the final stage of uptake. *When fast diffusion rates can be sustained, nutrient acquisition is kinetically controlled (limited); conversely, uptake of N is controlled (limited) by diffusion rates when kinetic reactions are faster than what can be supplied across the boundary layer*.

Boundary layer thickness & diffusion rate *vs.* water movement

cytoplasm
cell membrane
boundary layer

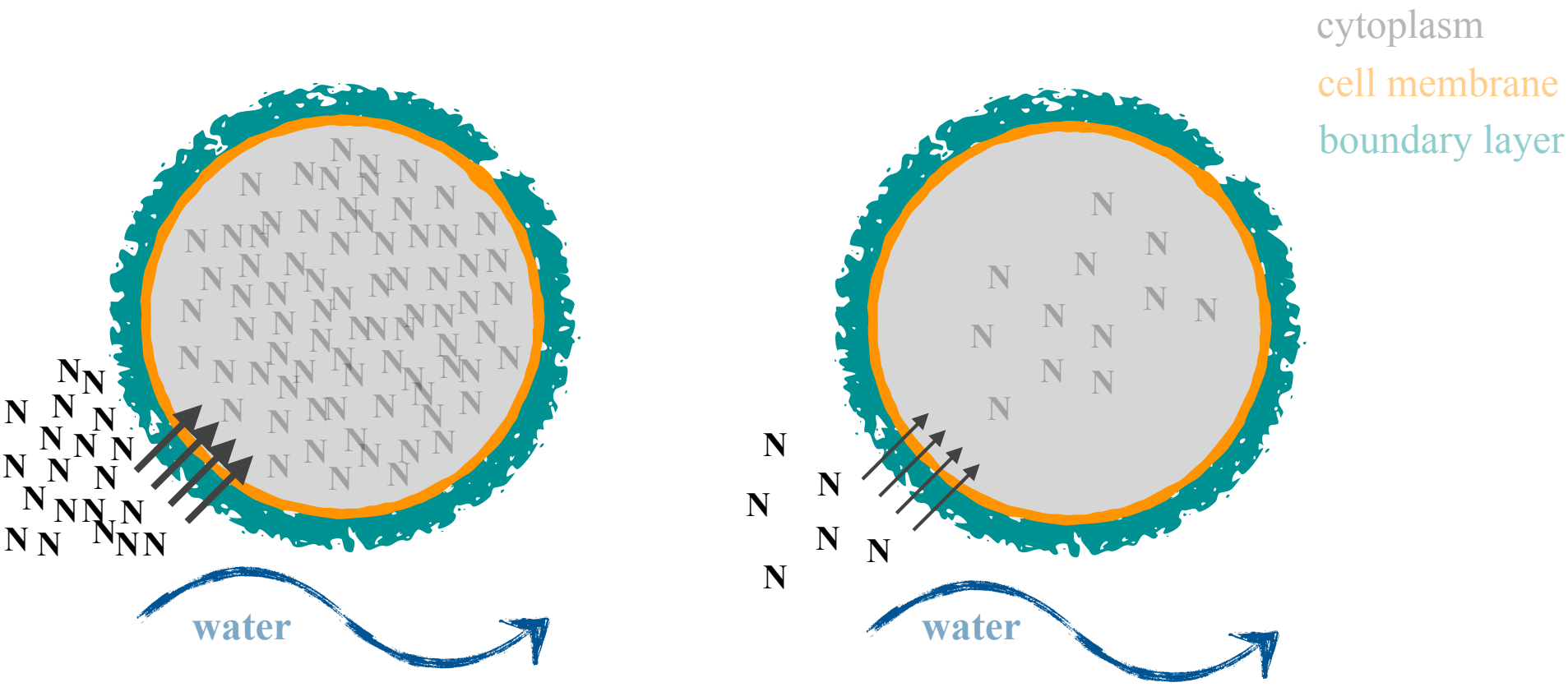


slow flow
thick boundary layer
high resistance to diffusion
slow diffusion
lower transport across cell membrane



fast flow
thin boundary layer
low resistance to diffusion
fast diffusion
greater transport across cell membrane

Concentration gradient *vs.* diffusion rate



high [DIN] in water
large concentration gradient
fast rate of diffusion

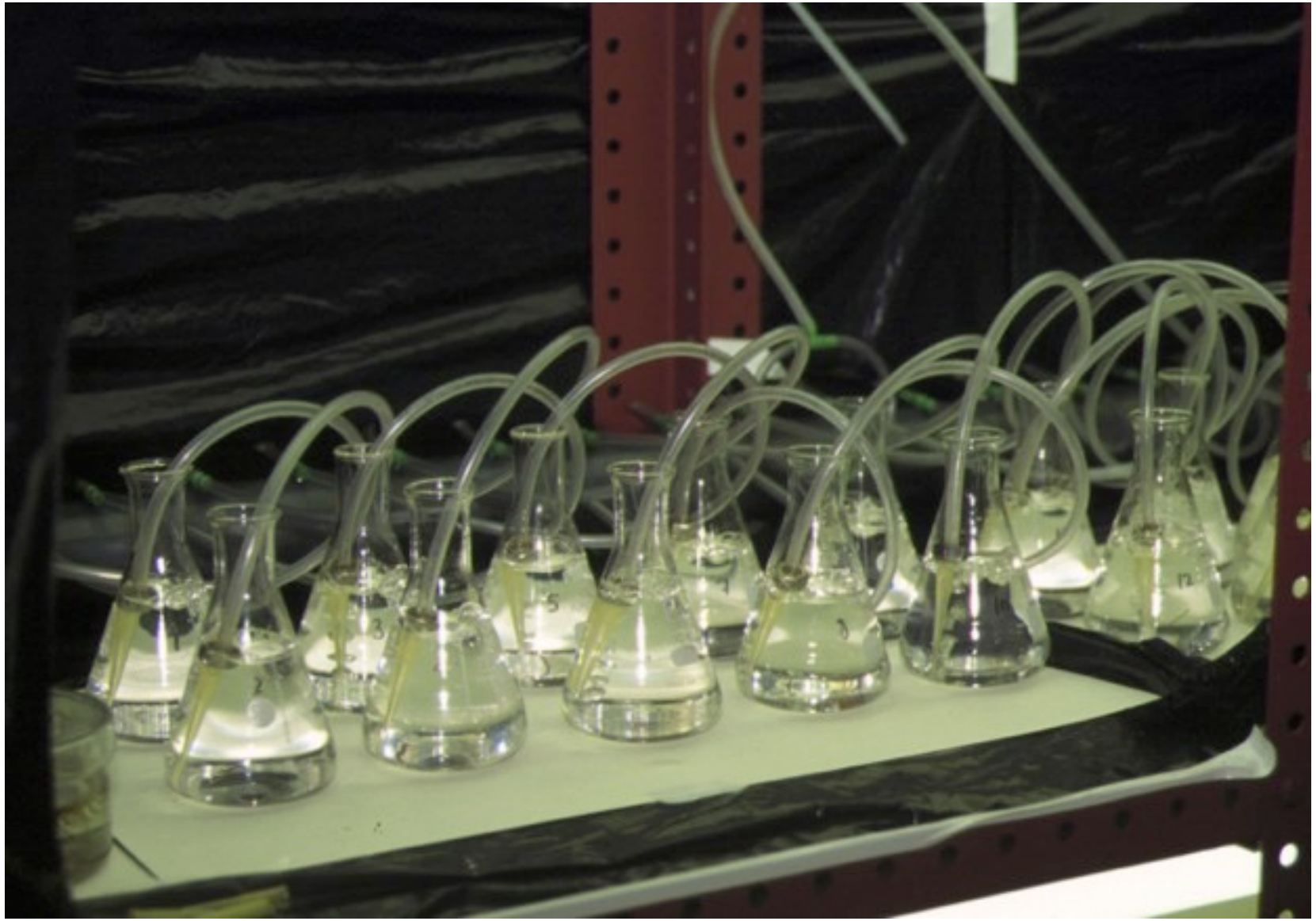
low [DIN] in water
small concentration gradient
slow rate of diffusion

Uptake kinetics experiments

The whole uptake process of nitrogen by algae can be measured *in situ* or under controlled conditions in the laboratory.

Nutrient uptake is often determined by measuring the disappearance of a nutrient from the culture medium over a time interval after the addition of the alga (using either the 'batch-mode' or 'perturbation' approach').

Such experiments allow the calculation of depletion curves, and from the depletion curve the uptake kinetics can be determined.



Depletion curves

Depletion curves display the decrease in concentration over a period of time from when an algal sample is placed into a nutrient solution until the end of the experiment when all/most nutrients had been taken up.

Depletion curves allow us to asks the question, "How much N does a unit of seaweed take up in a unit of time?"

- A convenient 'unit' of seaweed is a gram (but it is your choice… *select something sensible*).
- A convenient 'unit' of time is an hour (but it is your choice… *pick something sensible*).
- "How much N" generally implies "how many µg of N," but you may also work in µmol units…
  - Note, the unit 'µM' is not appropriate when asking "how much N?"

Required reading

Botanica Marina Vol. 45, 2002, pp. 196–209

Nitrogen Uptake by *Gracilaria gracilis* (Rhodophyta): Adaptations to a Temporally Variable Nitrogen Environment

A. J. Smit

Botany Department, University of Cape Town, PO Box Rondebosch, 7700, Cape Town, South Africa, ajsmit@botzoo.uct.ac.za

The physiology of nitrogen acquisition was determined for *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham in a series of perturbation experiments with the aim of examining uptake kinetics in response to transiently variable N. Experiments were designed to determine how variables such as history of exposure to nutrients, NO_3^--N and NH_4^+-N concentrations and interactions, temperature and water motion affect parameters of linear and Michaelis-Menten models. A third 'Michaelis-Menten parameter' (α) is introduced here and used to extract additional ecological relevant information from the model. Ammonium-nitrogen uptake was best described by a linear, rate-unsaturated response, with the slope increasing with N-limitation, indicating that *Gracilaria* is more efficient at acquiring nutrients when internally stored N pools were impoverished. Temperature also affected the slope of the linear regression in the case of N-replete material. Nitrate-nitrogen uptake was suppressed by approximately 38 % in the presence of NH_4^+-N at concentrations above 5 µM, and the seaweed displayed a higher affinity for NH_4^+-N than for NO_3^--N at low temperatures. Nitrate-nitrogen uptake followed a rate-saturating mechanism best described by the Michaelis-Menten model. Increased temperature enhanced the affinity for NO_3^--N only in N-limited thalli, while nutrient limitation enhanced affinity irrespective of temperature. The maximal velocity of uptake (V_{max}) and the half saturation constant (K_s) appeared to vary with experimental conditions, but these differences were not statistically significant. Water motion was shown to reduce 'diffusion transport limitation' experienced by the alga under conditions of low external dissolved inorganic nitrogen (DIN) concentrations, so that the rate of N uptake responds with a 4.5-fold increase under conditions of enhanced water motion. All results suggest that *Gracilaria gracilis* is well suited to remain productive in an upwelling environment dominated by the transient availability of DIN through the use of a high affinity system for NO_3^--N and non-saturable uptake of NH_4^+-N. Water motion interacts strongly with nutrient concentration, and may alleviate N limitation by reducing boundary-layer resistance to diffusion. Practical application of the results of this study is discussed in terms of significance to mariculture.

Introduction

Gracilaria gracilis (Stackhouse) Steentoft, Irvine *et* Farnham occurs as free-living, largely monospecific beds in a limited number of sheltered coastal water bodies on the west coast of southern Africa. Studies on the ecology, ecophysiology and cultivation of *Gracilaria gracilis* in Namibia and South Africa have been reported by Anderson *et al*. (1989), Rotmann (1990), Molloy (1992), Anderson *et al*. (1993), Dawes (1995), Anderson *et al*. (1996a, 1996b), Smit *et al*. (1997), Smit (1998), Anderson *et al*. (1998, 1999) and Smit and Bolton (1999). According to one study (Anderson *et al*. 1996a), low environmental nutrient concentrations appear to be responsible for the low growth rates of *Gracilaria* at certain times of the year. In other systems, similar seasonal changes in growth rates and production of seaweeds have also been ascribed to nutrient limitation (e. g. Rosenberg and Ramus 1982, Lapointe and Duke 1984, Fujita *et al*. 1989, Borum and Sand-Jensen 1996). It is well known that the addition of N can greatly enhance the growth rate and production of seaweeds under certain conditions (Lapointe and Ryther 1979, Smit *et al*. 1997). Anderson *et al*. (1996a) also suggest that site-related differences in growth rate of *G. gracilis* may be caused by differences in water movement at these sites.

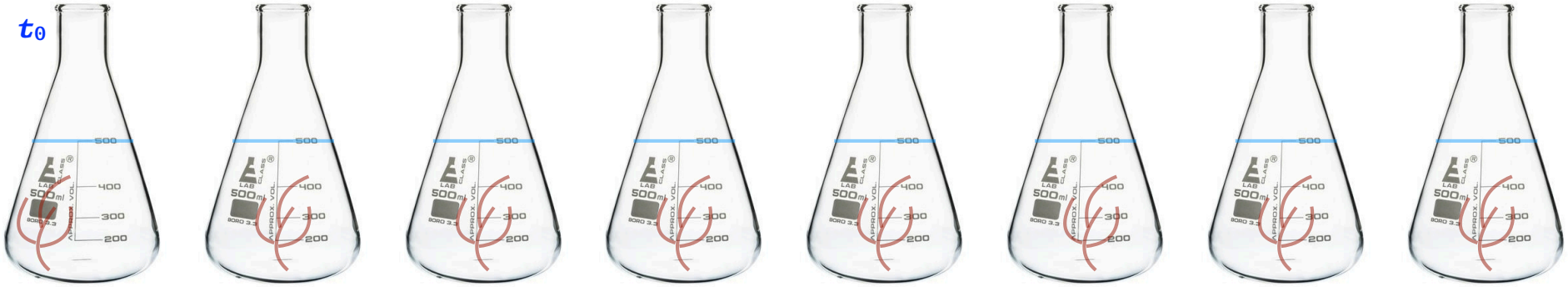
The growth rate and productivity of algae is, in part, controlled by the concentration of dissolved inorganic nitrogen (DIN) in the aqueous medium surrounding the thallus (Dugdale 1967, Chapman and Craigie 1977, Rosenberg and Ramus 1982, Lavery and McComb 1991). The ability of an alga to utilise N for biomass production is determined by the rate at which DIN can traverse the boundary layer adjacent to the outer cell layer of the thallus, and the rate at which this N takes part in biochemical processes (Wheeler 1980, Koch 1994, Sanford and Crawford 2000). Consequently, external physical processes acting on the boundary layer govern the rate of diffusion, whereas enzymatic processes (i. e. within the algal cells) determine the second stage of uptake. Understanding N nutrition of seaweeds thus requires the integration of at least three controlling factors, i. e. N concentration in the growth

Multiple flask (a.k.a. batch-mode)

Set-up:
Nutritional history: 4μM NO_3^- for four weeks
Algal mass: (*e.g.*) ~4.5 g
Culture volume: 500 ml
Temperature: 20°C
Nutrient tested: NO_3^-
Incubation time: 20 minutes in each flask
Etc.

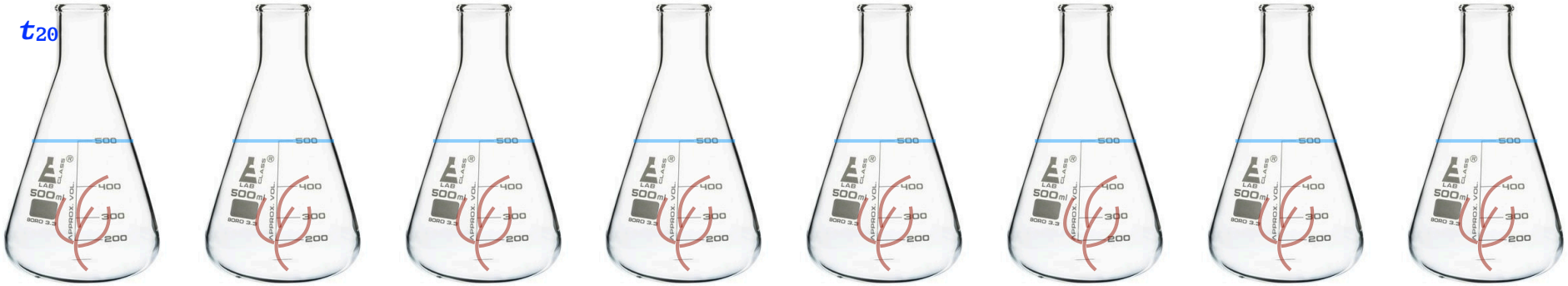
Flask 1 Flask 8

time: t_0



25μM 20μM 15μM 10μM 7.5μM 5μM 2.5μM 0μM

time: t_{20}



?μM ?μM ?μM ?μM ?μM ?μM ?μM ?μM

The multiple flask experiment does not result in depletion curves, and we must calculate V directly for each flask... this can then be used to make a Michaelis-Menten model (see later)

Calculations (*e.g.* flask 1)
t_0: 25µM NO_3^--N
t_{20}: 9.9µM NO_3^--N
Algal mass: (*e.g.*) 4.5 g
Culture volume: 500 ml

Repeat for every other flask

Calculating *V* from the multiple flask data

Step 1: How much N taken up in 20 minutes?

`> 25µM - 9.9µM = 15.1µM` … *this is the reduction in the N concentration but it says nothing about how much (*i.e. *the mass) N was removed …*

So, let's work with the mass of N instead.

Step 2: Convert concentrations to mass N present per flask at the start and end.

Knowing that µM = µmol.L^{-1}, how many µg N is 25µmol.L^{-1}, and how many µg N is 9.9µmol.L^{-1}?

MM = µg.µmol^{-1}, and the MM of N is 14.0067g.mol^{-1}, therefore…

`> 14.0067 = xµg / 25µmol = 350.17µg N`

and

`> 14.0067 = xµg / 9.9µmol = 138.67µg N`

This is the mass of N in 1 L at the start and end … *but we have only 500 mL in the culture flask*!

So, what mass of N in 500mL?

So, initially we had …

`> 350.17µg N / 2 = 175.09µg N`

… and after 20 minutes we had …

`> 138.67µg N / 2 = 69.34µg N`

Step 3: How much N does the 4.5g alga take up in 20 minutes?

`> 175.09µg N - 69.34µg N = 105.75µg N`

Step 4: How much N does 1 g alga take up in 20 minutes?

`> 105.75µg N / 4.5g = 23.5µg N.g-1`

Step 5: If 23.5µg N.g^{-1} is taken up in 20 minutes, how much in 1 hr?

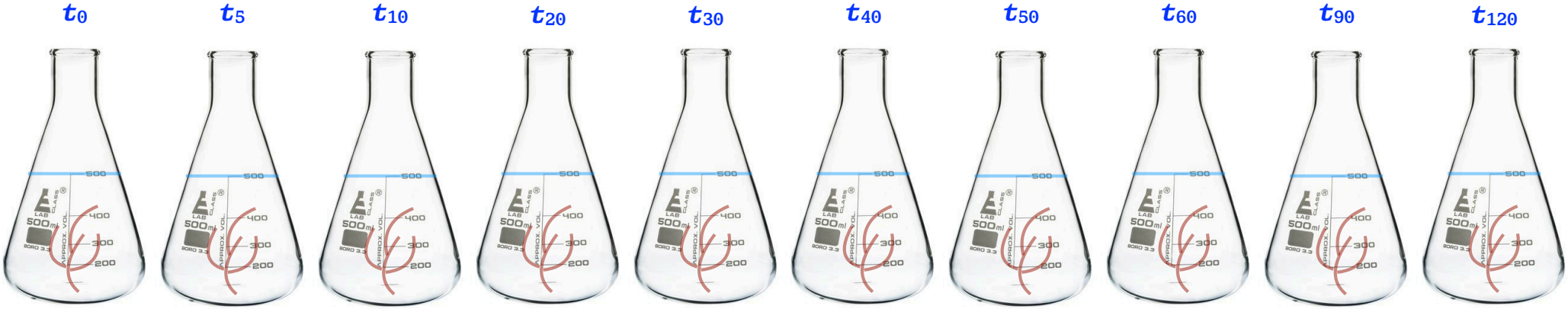
`> 23.5µg N.g-1 × 3 = 70.50µg N.g-1.hr-1`

Perturbation experiment

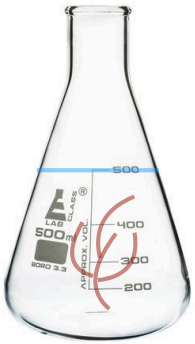
Set-up:
Nutritional history: 4µM NO_3^- for four weeks
Algal mass: (*e.g.*) ~4.5 g
Culture volume: 500 ml
Temperature: 20°C
Nutrient tested: NO_3^-
Incubation time: variable
Etc.

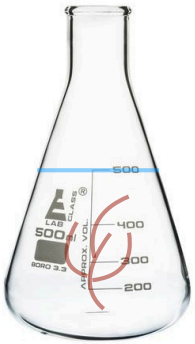
Flask 1 (one of several replicates, each measured repeatedly — at intervals — over 120 minutes)

Flask 1



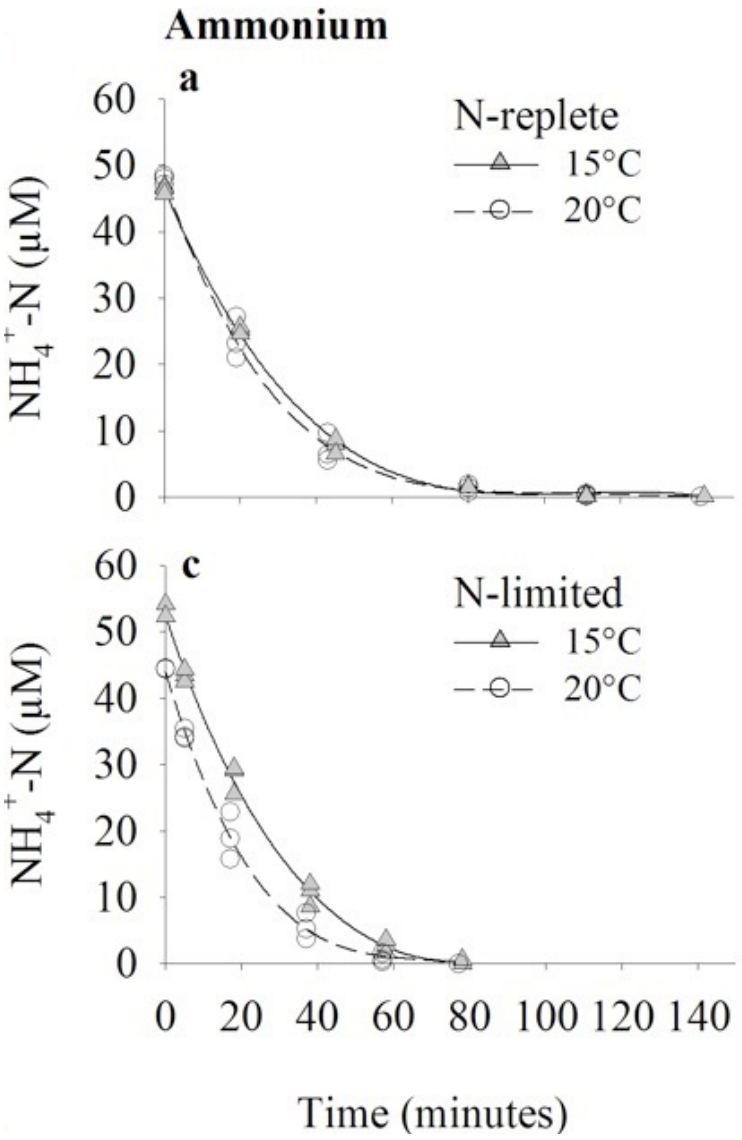
Control flask 1 (treated in similar way as the experimental flasks)





By making a curve of the concentration, *S* (here in µM), *vs.* time, *t*,
one gets the depletion curve seen in the next slide...
The depletion curve is then used to make the Michaelis-Menten model (see later).

Depletion curves obtained from a perturbation experiment



Calculations (*e.g.* first time interval, $t_5 - t_0$:)
t_0: 25µM NO_3^--N
t_5: 21.3µM NO_3^--N
Algal mass: (*e.g.*) 4.5 g
Culture volume: 500 ml

Repeat for every consecutive time interval, *i.e.*
$t_{10} - t_5$
then
$t_{20} - t_{10}$
etc.

Calculating *V* from the depletion curves

Step 1: How much N taken up in 5 minutes?

`> 25µM - 21.3µM = 3.7µM` … *this is the reduction in the N concentration but it says nothing about how much (i.e. the mass) N was removed …*

So, let's work with the mass of N instead.

Step 2: Convert concentrations to mass N present per flask at the start and end.

Knowing that µM = µmol.L^{-1}, how many µg N is 25µmol.L^{-1}, and how many µg N is 21.3µmol.L^{-1}?

MM = µg.µmol^{-1}, and the MM of N is 14.0067g.mol^{-1}, therefore…

`> 14.0067 = xµg / 25µmol = 350.17µg N`

and

`> 14.0067 = xµg / 21.3µmol = 298.34µg N`

This is the mass of N in 1 L at the start and end … *but we have only 500 mL in the culture flask!*

So, what mass of N in 500mL?

So, initially we had …

`> 350.17µg N / 2 = 175.09µg N`

… and after 20 minutes we had …

`> 298.34µg N / 2 = 149.17µg N`

Step 3: How much N does the 4.5g alga take up in 5 minutes?

`> 175.09µg N - 149.17µg N = 25.92µg N`

Step 4: How much N does 1 g alga take up in 5 minutes?

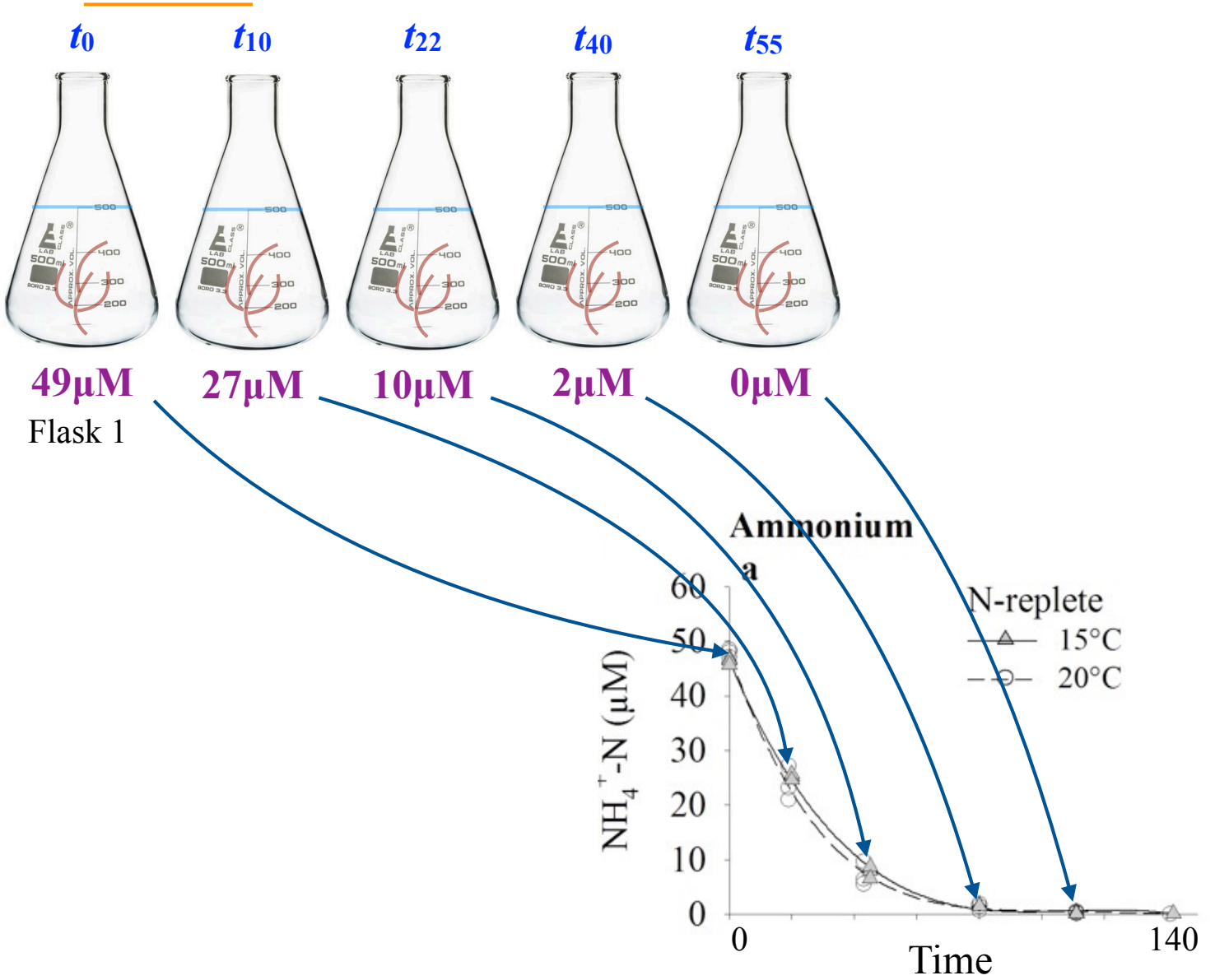
`> 25.92µg N / 4.5g = 6.48µg N.g-1`

Step 5: If 5.76µg N.g^{-1} is taken up in 5 minutes, how much in 1 hr?

`> 6.48µg N.g-1 × 12 = 77.76µg N.g-1.hr-1`

Perturbation experiment

Set-up
Nutritional history: 4µM NO_3^- for four weeks
Algal mass: (*e.g.*) ~4.5 g
Culture volume: 500 ml
Temperature: 20°C
Nutrient tested: NH_4^+
Etc.



Perturbation experiment

Set-up
Nutritional history: 4µM NO_3^- for four weeks
Algal mass: (*e.g.*) ~4.5 g
Culture volume: 500 ml
Temperature: 20°C
Nutrient tested: NH_4^+
Etc.

(i.e. Δt)

Δx *Δx* *Δx*

Time (*t*) **t_0** **t_{10}** **t_{22}** **t_{40}** **t_{55}**

Substrate conc. (*S*) **49µM** **27µM** **10µM** **2µM** **0µM**

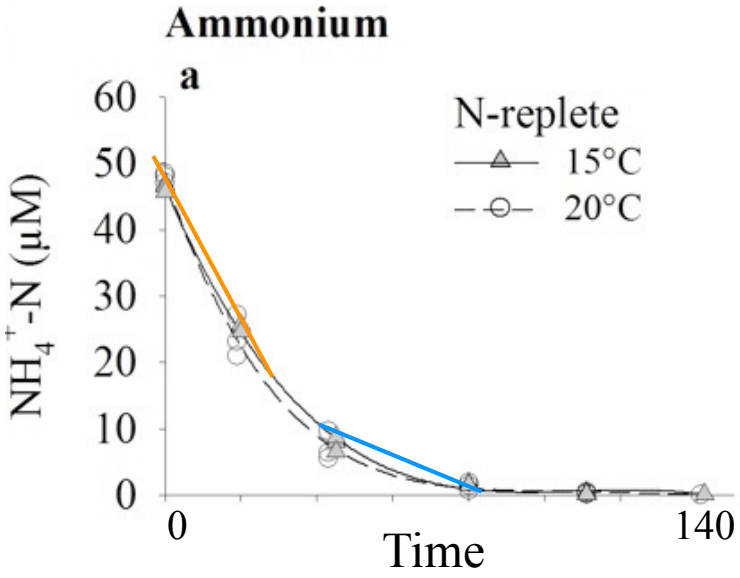
Δy *Δy* *Δy*

(i.e. ΔS)

slope = $(S_0 - S_{10}) / (t_0 - t_{10})$

$V = \Delta S / \Delta t$

where *V* is uptake rate
S is substrate concentration, but *S* needs to be converted to µmol or µg N within the 500 ml culture volume first



Q&A

Q: “my question is on the uptake rate *V*. The calculation for *V* is equal to the calculation for slope, however in the assignment we are asked to correct *V* for the seaweed mass. My question is whether we are suppose to divide the slope of the time interval, by the dry mass in order to correct *V* for mass to arrive at an answer for *V*”

A: “The calculation for *V* is equal to the calculation for slope” ... what you mean is, on a graph of concentration of nutrient remaining in solution (*S*) *vs.* time, the calculation of the slope over a certain time interval is equal to the uptake rate (*V*) over that time interval.

That uptake rate was affected by the mass of the seaweed in the culture vessel (*i.e.* ~4.5 g as stated on the slides). So, *V* will be the uptake rate obtained by ~4.5 g of seaweed. But we need to express uptake rate per unit of seaweed mass, and for convenience we use 1 g... This is seen in the unit for uptake rate, *viz*. *V* (μmol $N.g^{-1}.h^{-1}$), or in words, micromoles of N taken up per gram of seaweed per hour. So, to get to the ‘$.g^{-1}$’ bit, you divide *V* by the mass of the seaweed in the experimental flask, which is 4.5 g.

Uptake mechanisms

Uptake may be of one of three types: (1) active; (2) passive transport and (3) facilitated diffusion transport.

Uptake kinetics: *V vs. S*

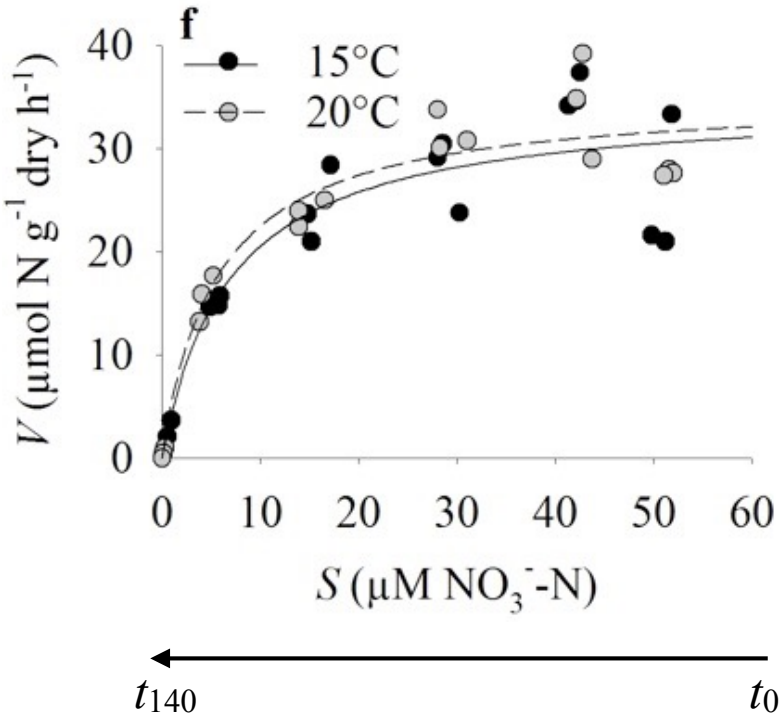
(*i.e.* uptake rate as a function of substrate concentration)

Uptake rate (*V*) for the active mechanism is often described as a hyperbolic function of substrate concentration (*S*), by analogy to the Michaelis-Menten expression used to model enzyme catalysed reactions.

The Michaelis-Menten equation assumes that uptake is unidirectional so that no losses occur after uptake.

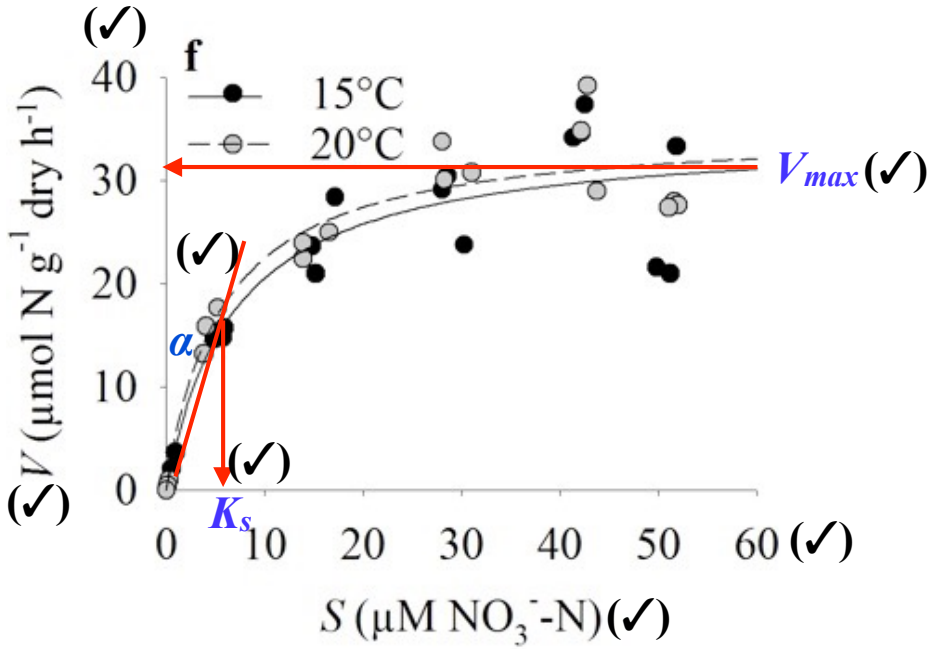
Michaelis-Menten

$$V = V_{\max} \cdot \frac{S}{K_s + S}$$



Michaelis-Menten

V_{max}, which is the [extrapolated or theoretical] maximal rate of uptake of the nutrient of interest under the experimental conditions, and K_S, which the half saturation constant and is numerically equivalent to the value of S, the substrate concentration, where $V = ½\ V_{max}$.



Active uptake

K_s describes the affinity of the carrier site to a particular nutrient: the lower K_s, the higher the affinity (high affinity: better cope under low nutrient concentrations).

The parameter α is the initial slope of the Michaelis-Menten model calculated at concentrations of less than K_s. It also it indicates the affinity for a particular nutrient and the ease at which the alga is able to respond with a faster nutrient uptake rate upon increases of the nutrient in the external medium.

α is independent of V_{max} (while K_s is not independent of V_{max}) which makes it more useful than K_s in indicating affinity for a nutrient; it is useful for comparing the competitive abilities of different algae.

The ecological meaning attached to V_{max}, on the other hand, is that it describes the maximal rate at which nutrients can be taken up when it is available in abundance.

Active uptake

The transfer of ions or molecules across a membrane against an electrochemical-potential gradient:

External concentrations: usually in the micromolar range;

Internal concentrations: usually in the millimolar range;

i.e. passive diffusion unlikely

Active uptake

It is energy-dependent: adding a metabolic inhibitor or changing the temperature will affect uptake rate because they affect energy production.

ATP is the most likely energy source. The primary transport reaction in which ATP is consumed is the transport of H^+ across cell membranes by H^+-pumping ATPases. This results in an H^+ electrochemical-potential and pH gradient, which drives secondary ion transport.

Such coupled transport may arise from the transport of different ions at different sites, either i) in opposite directions (antiport or counter-transport) or ii) in the same direction (symport or co-transport).

In algae: H^+-linked co-transport of sugars and thiourea; Na^+-driven co-transport may also take place in microalgae (seawater is high in Na^+ and low in H^+).

Active uptake

Other characteristics: selectivity of ions; saturation of the carrier system (*e.g.* Michaelis-Menten, see later)—these are also characteristic of facilitated diffusion.

Active transport is seen in most algae (but...)

Active uptake

The hyperbolic geometry of the *V* vs. *S* curve suggests that in most cases uptake is not simply a passive process relying on diffusion alone, but that it is active or facilitated, *i.e.*

(i) that it is controlled to some extent by factors intrinsic to the alga itself; or

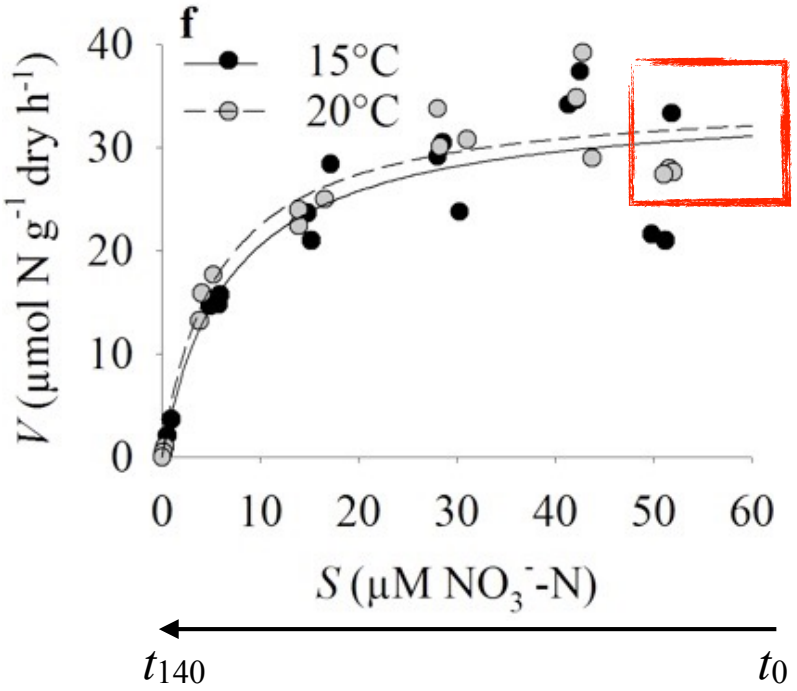
(ii) it may be the case that some other upper limit is imposed on the rate at which nutrients can be incorporated into thallus tissue.

Active uptake

The parameters V_{max} and K_s have an ecological meaning since they describe the nutrient uptake ability of a species under specific environmental conditions and allows for comparisons of nutrient uptake kinetics among species and studies.

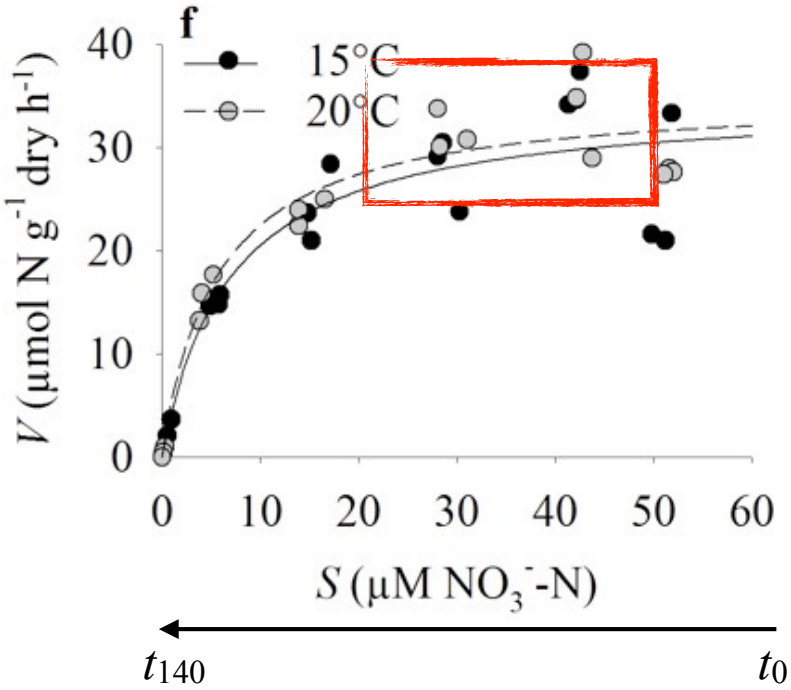
Phases of active uptake

The hyperbolic model can also be discussed in terms of phases of nutrient uptake called the (i) surge phase, the (ii) internally controlled phase, and the (iii) externally controlled phase (Pedersen, 1994).



(i) The surge phase

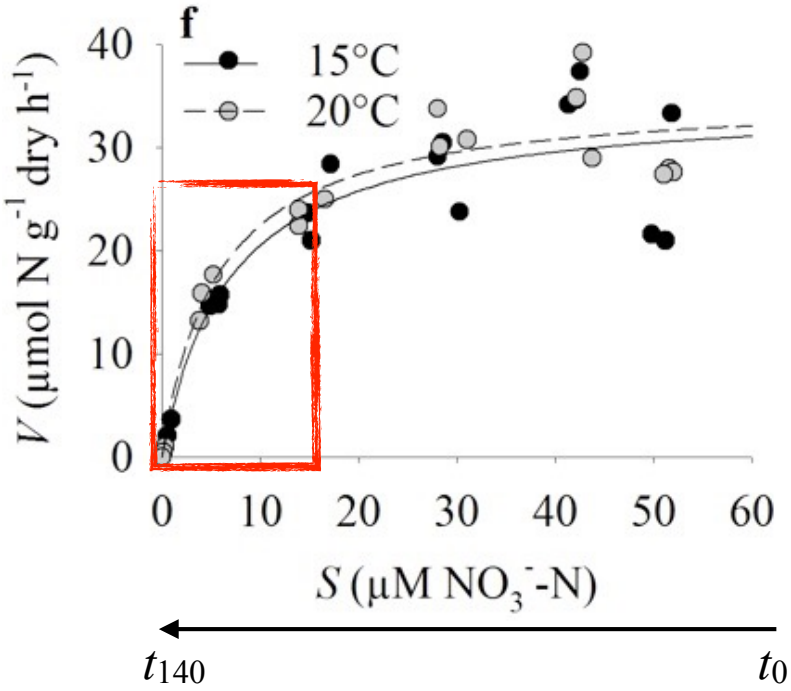
Surge uptake results from a concentration gradient between the alga and the external medium when it is first exposed to higher nutrient regimes. This surge in nutrient uptake abruptly ceases several minutes into the experiment as a result of feedback inhibition from pools of inorganic nitrogen and amino acids (as the internal pools fill, the concentration gradient is decreased down to a point where the mass influx of nutrient can no longer proceed).



(ii) The internally-controlled (kinetically-) phase

The rate-limiting step is the rate at which N (etc.) is catalysed to amino acids, macromolecules, *etc.*, and eventually biomass (this is V_{max}).

Any metabolic process that would require an increased utilisation of amino acids (*etc.*) will maximise the internally-controlled phase.



(iii) The externally-controlled phase

A.k.a. the physically controlled phase.

The limit is placed on uptake by the rate by diffusion of N (*etc.*) across the boundary layer, or…

…if rate of diffusion is greater than the mass transfer of N (*etc.*) to the outside of the BL, it is limited by the mass flow of N (*etc.*).

The limitation of uptake by externally controlled physical factors is called diffusion transport limitation, or mass transport limitation.

…

The rate of transport of a nutrient from moving water to the algal surface takes place through the boundary layer surrounding the seaweed.

The rate of diffusion is directly related to the concentration gradient across the boundary layer, but inversely proportional to the thickness of the layer.

Diffusion transport limitation can therefore be alleviated by increasing the concentration of the nutrient in the external medium, or by decreasing the thickness of the boundary layer through increasing the water movement past the thallus.

Coupling: growth-nutrient

When the addition of nutrient to the culture medium leads to an 'immediate' growth response, growth and nutrient uptake are coupled, as is the case with the opportunistic bloom-forming species.

In the case where the seaweed is capable of luxury consumption there will generally be a lag between nutrient uptake (or supply) and growth, and in this case growth is uncoupled from nutrient uptake.

Nitrogen

Factors affecting uptake rates and kinetics:

- Water movement (boundary layer effects)
- The type of DIN
- Nutritional history
- Light intensity
- Temperature
- SA:V (or functional form; incl. inter-species differences, effects of morphological plasticity, *etc.*)
- The combination of passive (diffusion) uptake with active uptake—results in a bi-phasic response
- Others...

Q&A

Q: “Does the uptake rate of one nutrient affect the uptake rate of another?”

A: Plants will take up the form of a nutrient for which it has the highest affinity. For example, when nitrate and ammonia/ammonium are both available in the same culture medium, many algae would take up ammonia/ammonium preferentially to nitrate because it requires less energy to take it up. Ammonia/ammonium is taken up passively through diffusion alone, whilst nitrate requires the input of energy to drive active uptake. But this depends on all sorts of other things, such as the concentration gradient (a function of both the internal and external nutrient concentrations and the forms in which they are available), water movement, the light intensity, temperature, pH, etc.

Water movement

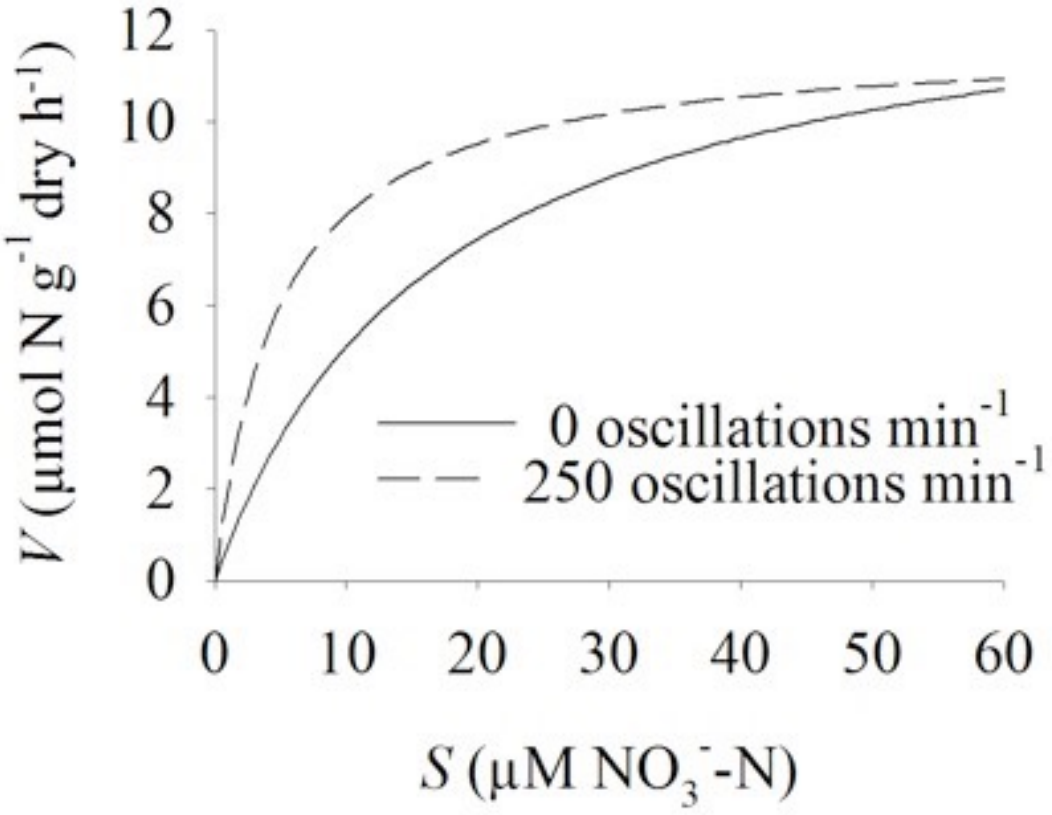
Usually, kinetic studies manipulate S (independent variable). However, water motion also affects V (but not V_{max}) via its effect on the boundary layer.

Water motion alters V by acting on α and K_s, the model parameter that describes the phase of uptake that is under physical (external) control (D'Elia & DeBoer, 1978).

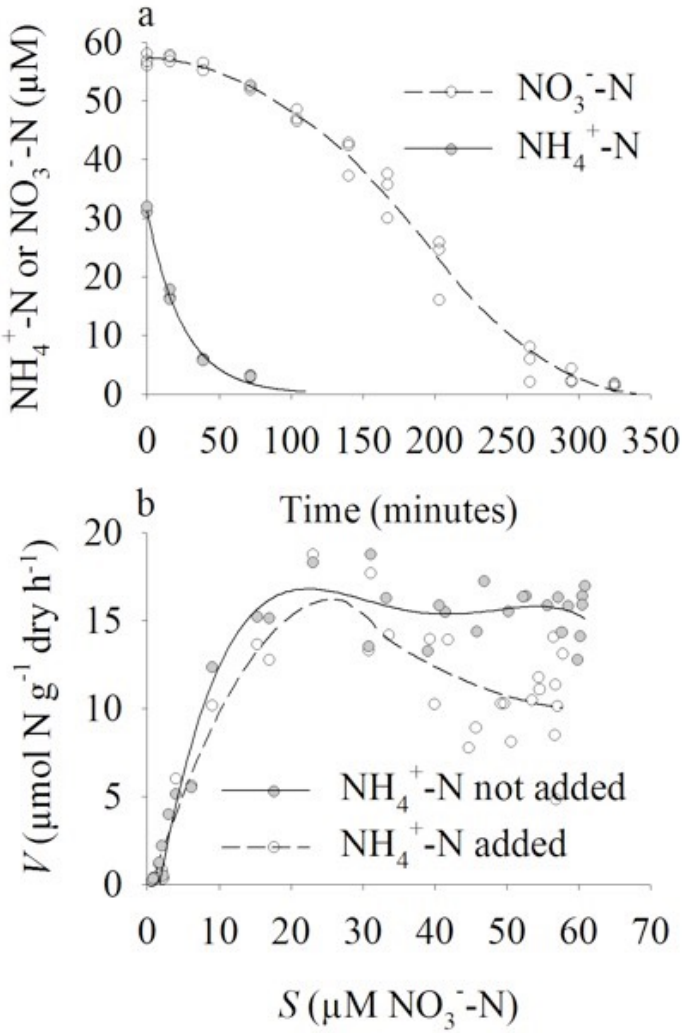
The magnitude of V_{max} on the other hand, should be independent of any external process, since it represents the maximum rate at which the alga is able to metabolise DIN that is already inside the cells subsequent to physical transport across the BL and cell membrane.

The velocity of nutrient uptake at a given S approaches V_{max} only when the rate of water motion is sufficient to suppress transport limitation; alternatively, an adequately high concentration gradient can overcome transport limitation.

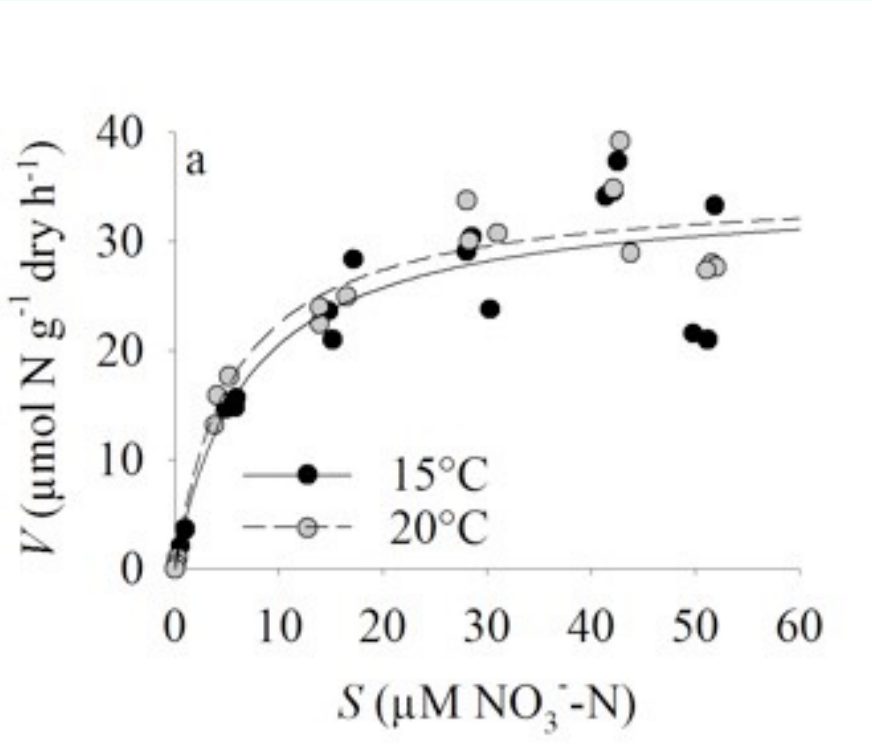
Nitrogen


12
10
8
6
4
2
0
V (µmol N g-1 dry h-1)
0 oscillations min-1
250 oscillations min-1
0
10
20
30
40
50
60
S (µM NO3--N)

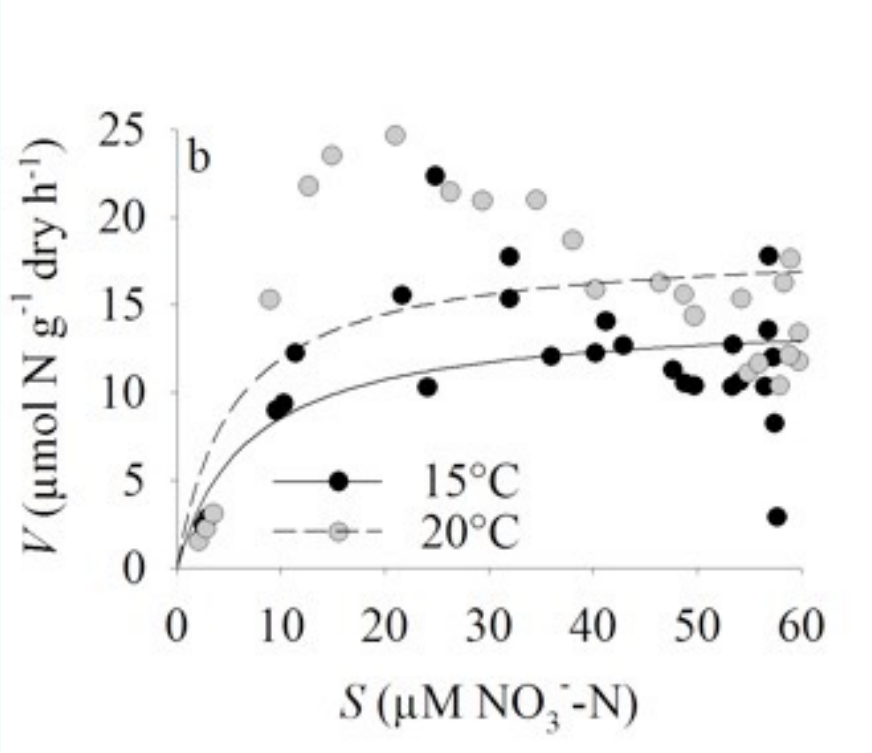
NH_4^+ *vs.* NO_3^-


a
60
50
40
30
20
10
0
NH_4^+-N or NO_3^--N (μM)
NO_3^--N
NH_4^+-N
0 50 100 150 200 250 300 350
Time (minutes)
b
20
15
10
5
0
V (μmol N g^{-1} dry h^{-1})
NH_4^+-N not added
NH_4^+-N added
0 10 20 30 40 50 60 70
S (μM NO_3^--N)

Nutritional history



N-replete



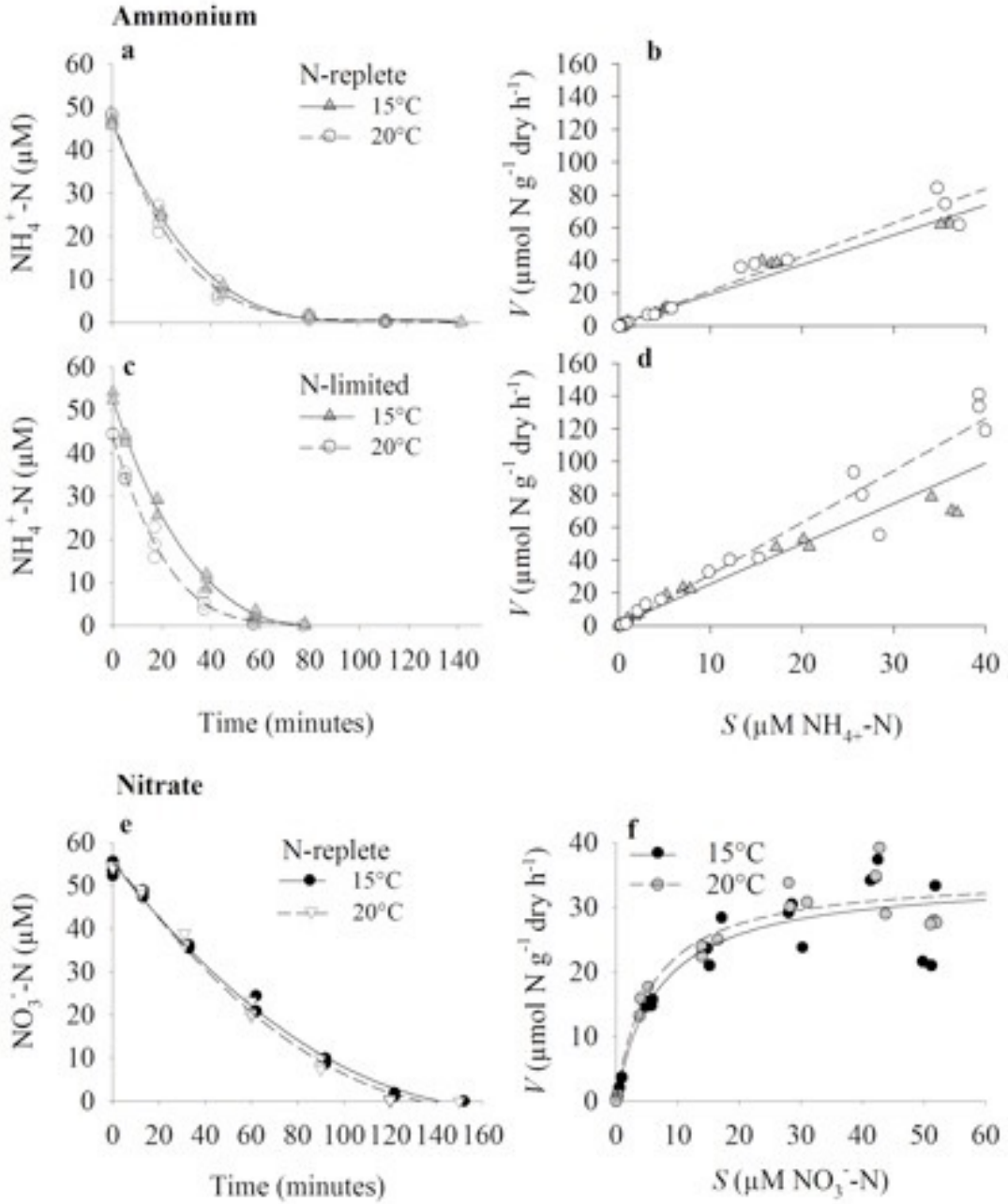
N-limiting

Different types of DIN

In some instances the uptake of nutrients (*e.g.* ammonium) does not appear to be saturated even under high experimental concentrations irrespective of past nutritional history and then other models must be sought to describe the relationship (Smit, 2002).

The transport (uptake) rate is directly proportional to the electrochemical-potential gradient (as determined by the difference is conc. between the exterior and interior of the cells).

This is usually the second type of nutrient uptake, *i.e.* passive uptake (unlike the Michaelis Menten type, which is active).


Ammonium
a
N-replete
15°C
20°C
b
c
N-limited
d
Time (minutes)
S (µM NH4+-N)
V (µmol N g-1 dry h-1)
Nitrate
e
f
S (µM NO3--N)

Passive uptake

Passive uptake occurs without the expenditure of metabolic energy.

Usually indicative of the uptake of gasses such as CO_2, O_2 and NH_3 (ammonia), or other uncharged molecules.

Such uptake processes are usually not affected by temperature.

Passive uptake

Note that the nature of the linear relationship describing the passive uptake process prevents us from calculating the K_s or V_{max} parameters (it is essentially possible to calculate and plot the Michaelis-Menten model for linear data, but parameter estimates obtained in such a way is not meaningful in ecological terms because the high concentrations at which the estimated values would fall do not occur in nature).

α (the slope of the linear regression) still exists and is equivalent (both in its calculation and in the theoretical sense) to α in the non-linear case.

Rate-unsaturated uptake has also been demonstrated for some seaweeds for nitrate and urea, but the mechanism here remains unknown.

Nutritional history

The nutritional history of the seaweed can complicate matters (selection of models, parameter estimation, and interpretation) since it often has a marked effect on the shape of the V vs. S relationship:

(i) Nitrogen limitation may change the typical hyperbolic response to a biphasic (D'Elia & DeBoer, 1978) or linear type (Fujita, 1985). This process is often called rate-unsaturated uptake, and points to an underlying passive uptake (diffusion) mechanism.

(ii) Nitrogen limitation may increase V_{max}.

Light environment

Light intensity, through its effect on photosynthesis, may influence nutrient uptake rates in active uptake processes:

(i) it is responsible for the production of ATP (energy) that is required for active transport against a concentration gradient.

(ii) it provides the C framework into which the inorganic N is eventually assimilated (proteins, amino acids, DNA, pigments, etc.).

(iii) increased growth rate occurs with increasing light intensity (up to a point).

(iv) due to effects on the nitrate reductase enzyme: the stimulatory effect of light intensity has been demonstrated for nitrate; its absence in ammonium uptake is probably because it is taken up chiefly *via* a passive process.

Photoperiod

Photoperiod affects nitrate uptake, possibly because nitrate reductase activity displays a diel periodicity in terms of activity and synthesis.

Temperature

Temperature mostly affects active rather than passive processes.

An increase in temperature increases metabolic rate: active uptake and general cell metabolism has an approx. Q_{10} of 2 (*i.e.* a 10°C increase in temperature results in a doubling of the rate.)

For passive (*i.e.* driven by physical factors) transport, Q_{10} is much less, typically 1.0 to 1.2.

Temperature effects are ion-specific and depends on the species.

Other factors affecting uptake

SA:V can affect nutrient uptake, and is often responsible for much of the variability observed between species (see the functional form model) or even within a species or within one individual (the functional form approach can be extended to different parts of the macroalgal thallus, or to different morphological strains of a species).

Different species have diff. uptake responses, e.g. *Ulva*: linear (passive); *Gracilaria*: Michaelis-Menten (active).

Other factors responsible for influencing uptake rates include desiccation, the type and concentration of the nutrient (some of this has been discussed), interactions with other nutrients (*e.g.* nitrate uptake is inhibited in the presence of ammonium), biological interactions (competition, density), intrinsic biological factors (production of hyaline hairs, reproductive state, age of the thallus, changes in morphology, genetic influences).

Facilitated uptake

Resembles passive transport in that it takes place down an electrochemical gradient.

Carrier proteins or ion channels in the membrane assist in bringing the ions into the cells.

Similar to active transport in that

(i) it can be saturated and data are described by an Michaelis-Menten-like equation.

(ii) only specific ions are transported.

(iii) it is susceptible to competitive and non-competitive inhibition.

References

D'Elia & DeBoer (1978) Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. J. Phycol. 14: 266-272.

Dugdale (1967) Nutrient limitation in the sea: Dynamics, identification, and significance. Limnology and Oceanography 12: 685-695.

Fujita (1985) The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J Exp. Mar. Biol. Ecol. 92: 283-301.

Droop (1974) Some thoughts on nutrient limitation in algae. J. Phycol 9: 264-272.

Droop (1977) An approach to quantitative nutrition of phytoplankton. J. Protozool. 24: 528-532.

Littler & Littler (1980) The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. Am. Nat. 116: 25-44.

Pedersen (1994) Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): Nature, regulation, and the consequences for choice of measuring technique. J. Phycol. 30:980-986.

Rosenberg & Ramus (1984) Uptake of inorganic nitrogen and seaweed surface area:volume ratios. Aquatic Bot. 19: 65-72.

Schaffelke (1999) Particulate organic matter as a novel nutrient source for tropical macroalgae. J. Phycol. 35: 1150-1157.

Smit (2002) Nitrogen uptake by *Gracilaria gracilis* (Rhodophyta): adaptations to a temporally variable nitrogen environment. Bot. Mar. 45: 196-209.

Taylor & Rees (1998) Excretory products of mobile epifauna as a nitrogen source for seaweeds. Limnol. Oceanogr. 43: 600-606.

Wallentinus (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar. Biol. 80: 215-225.