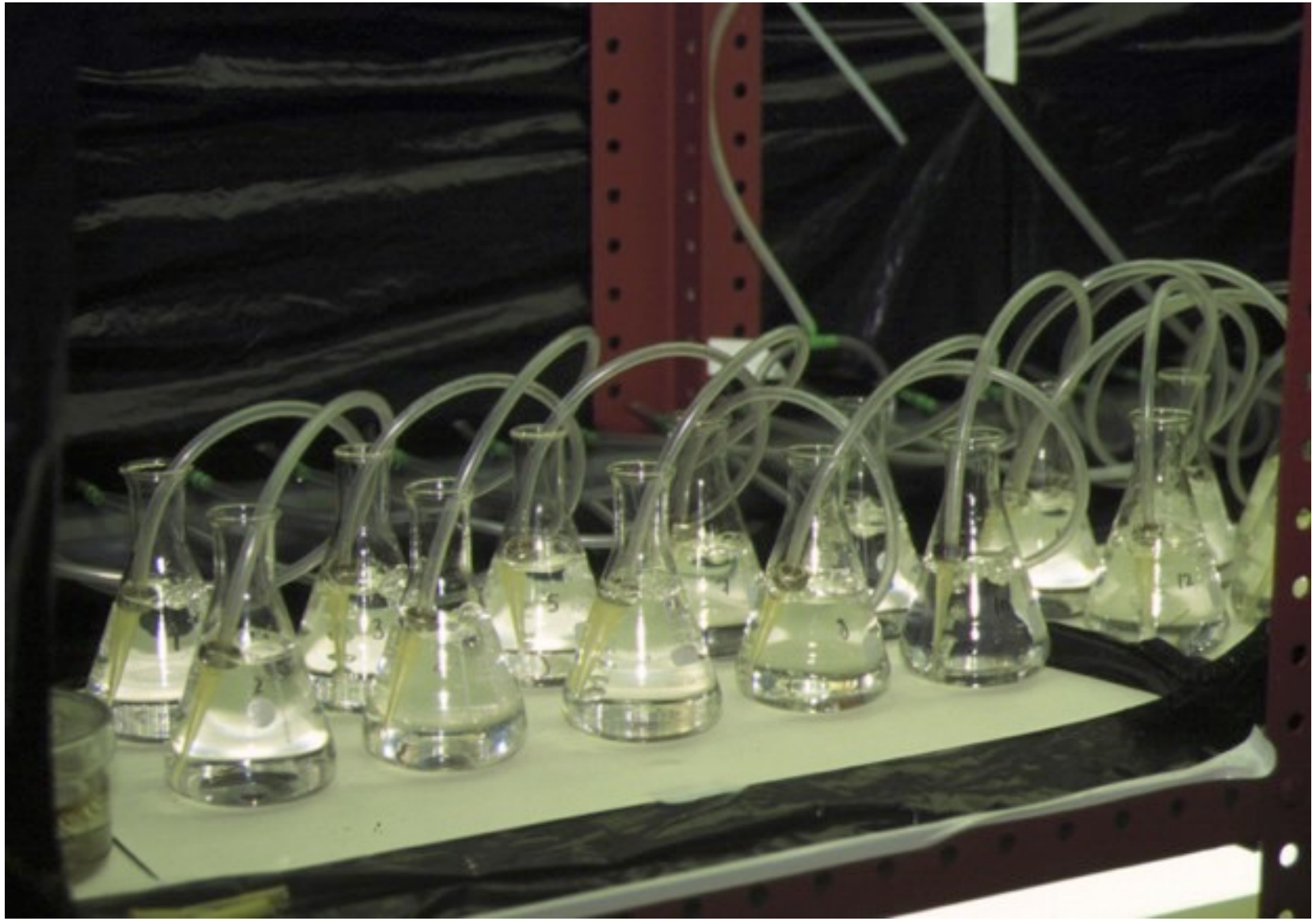
Uptake kinetics experiments

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

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

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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.

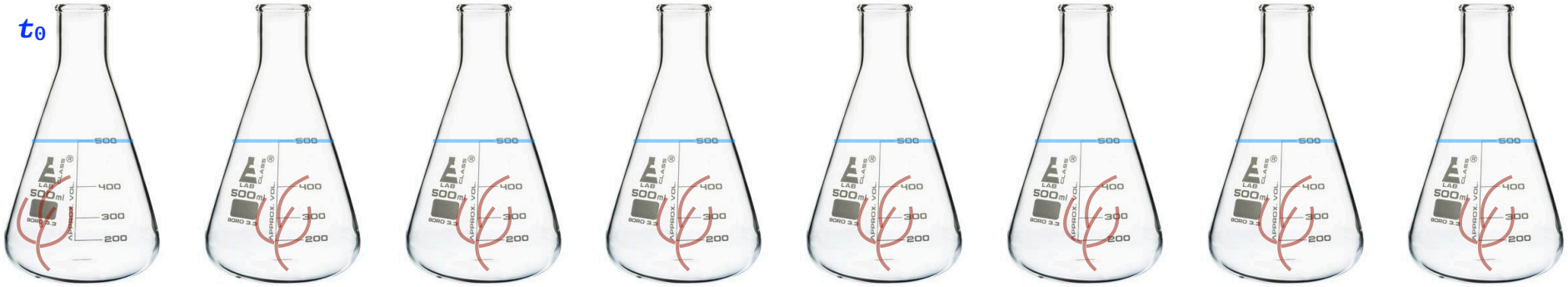
The growth rate and productivity of algae is, in part, controlled by the concentration of dissolved inorganic nitrogen (DIN) in the aqueous medium surrounding the thallus (Dugdale 1967, Chapman and Craigie 1977, Rosenberg and Ramus 1982, Lavery and McComb 1991). The ability of an alga to utilise N for 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.

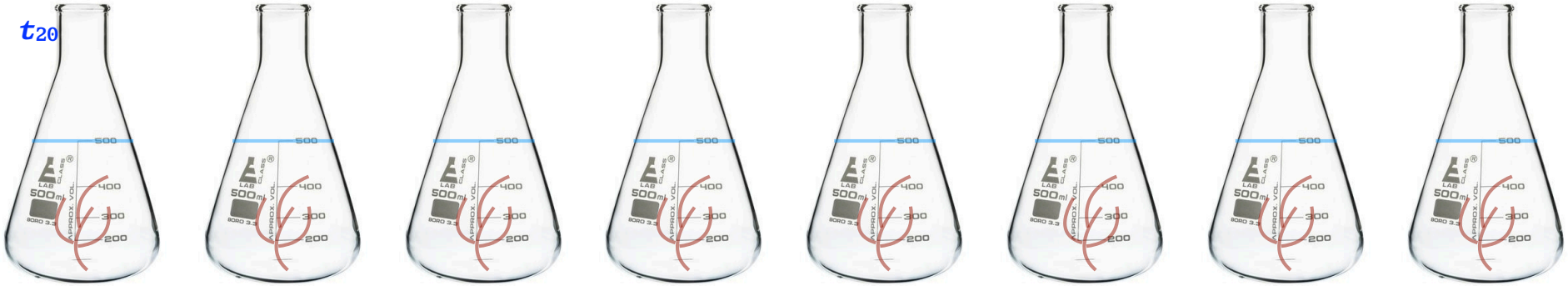
Flask 1 Flask 8

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?

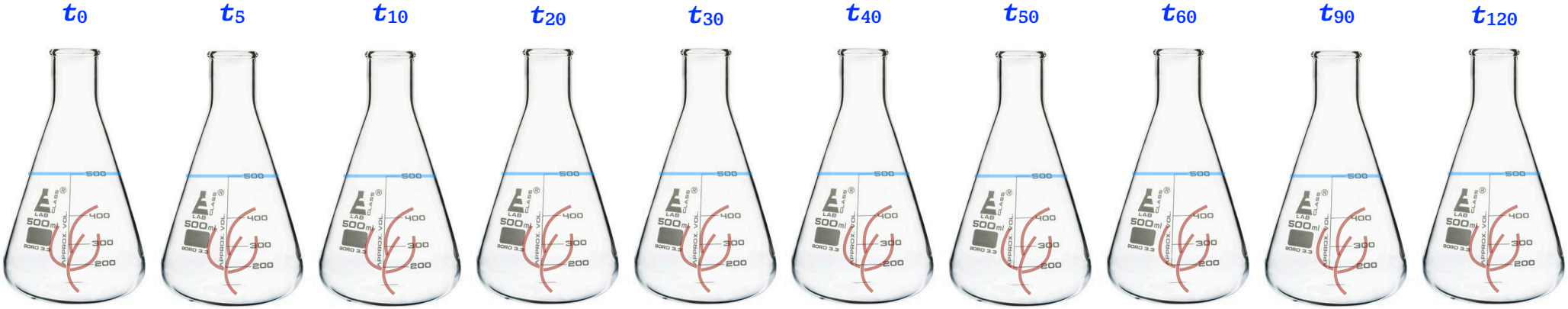
`> 23.5µg N.g-1 × 3 = 70.50µg N.g-1.hr-1`

Perturbation experiment

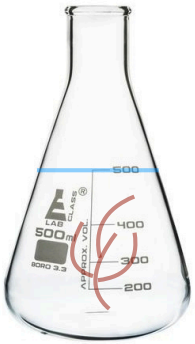
Set-up:
Nutritional history: 4µM NO_3^- for four weeks
Algal mass: (*e.g.*) ~4.5 g
Culture volume: 500 ml
Temperature: 20°C
Nutrient tested: NO_3^-
Incubation time: variable
Etc.

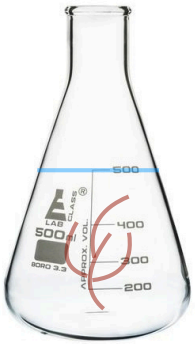
Flask 1 (one of several replicates, each measured repeatedly — at intervals — over 120 minutes)

Flask 1



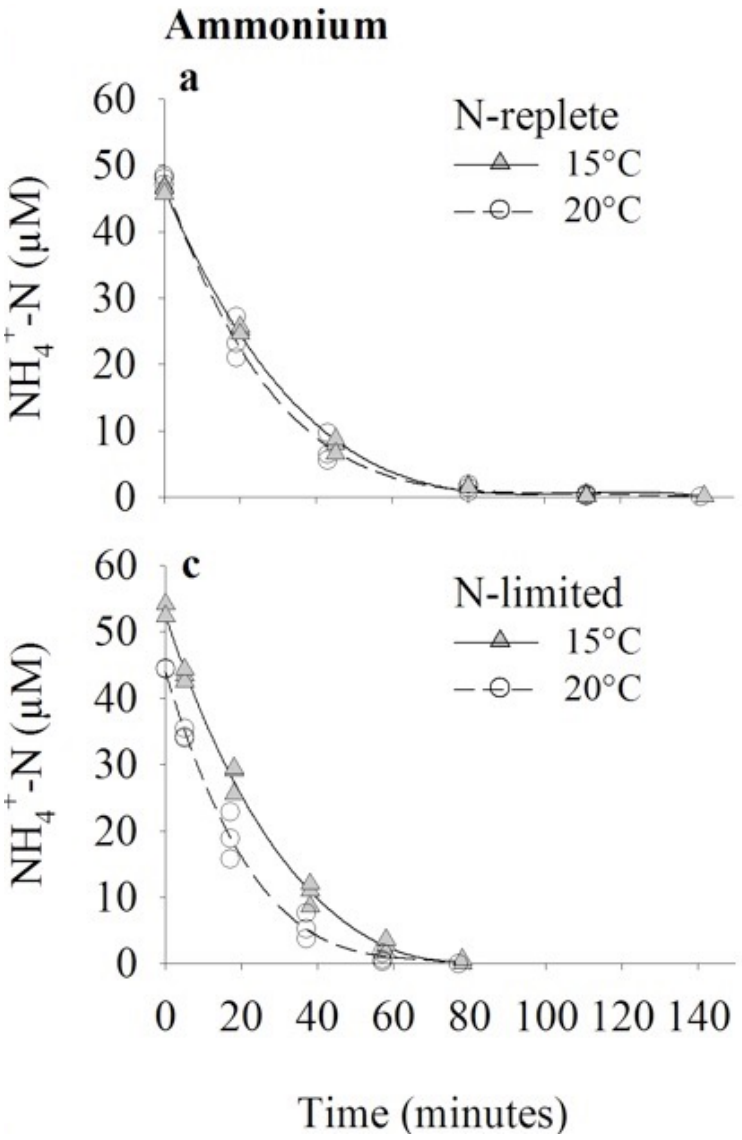
Control flask 1 (treated in similar way as the experimental flasks)





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?

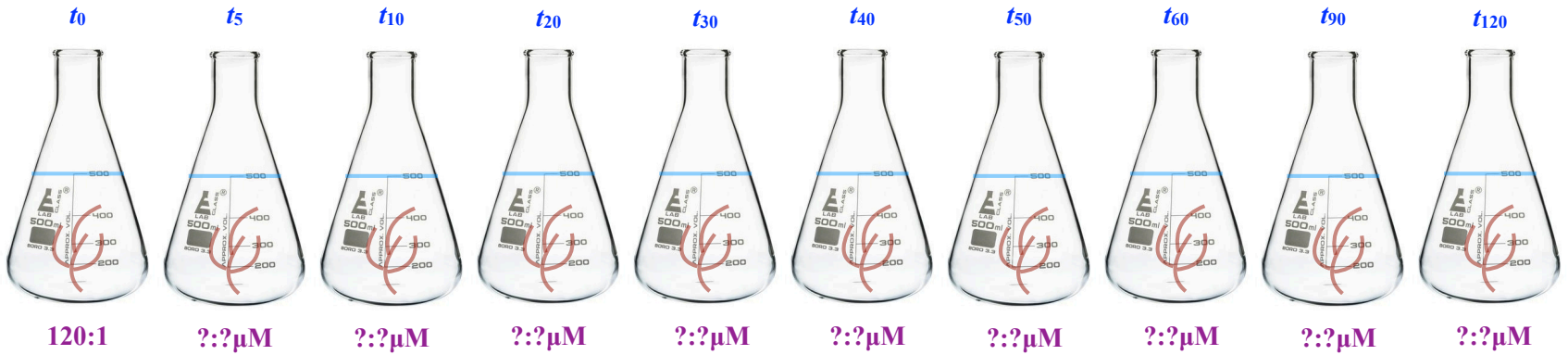
`> 175.09µg N - 149.17µg N = 25.92µg N`

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

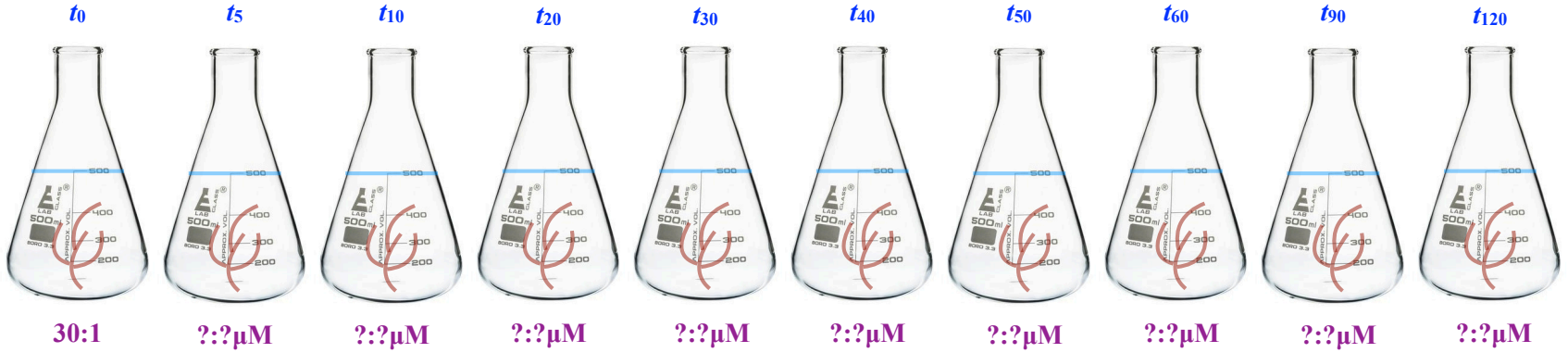
`> 25.92µg N / 4.5g = 6.48µg N.g-1`

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

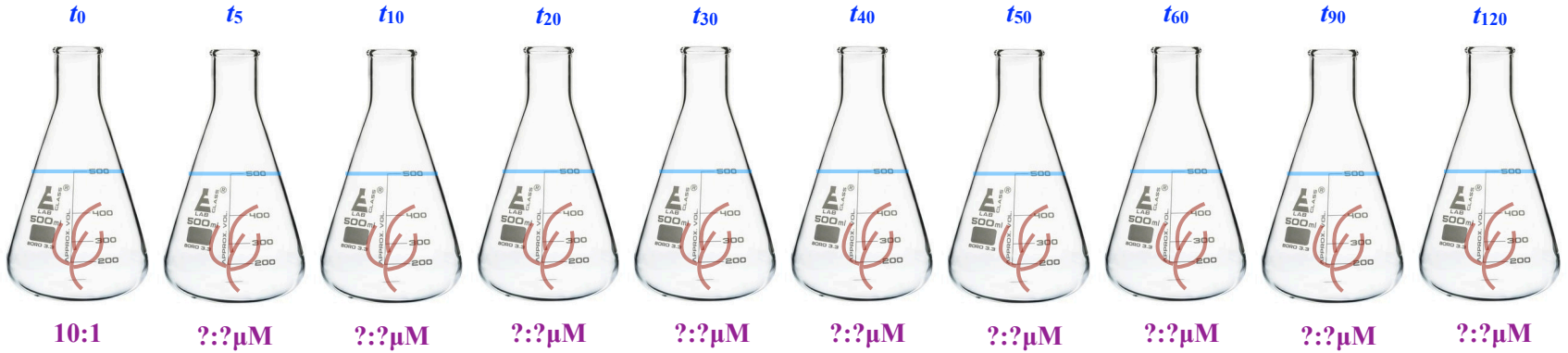
`> 6.48µg N.g-1 × 12 = 77.76µg N.g-1.hr-1`



Flask 1 Flask 1



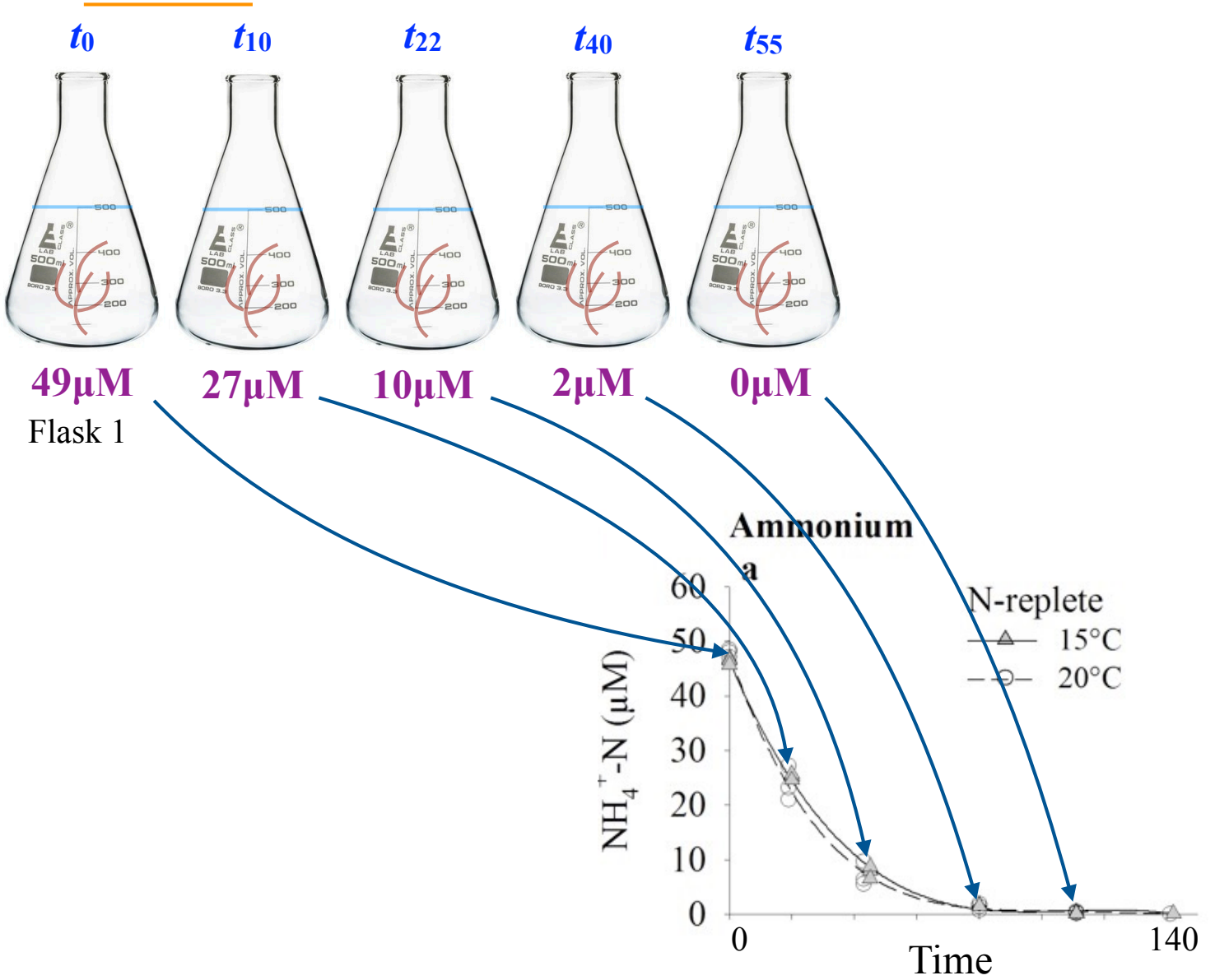
Flask 3 Flask 3



Flask 5 Flask 5

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**

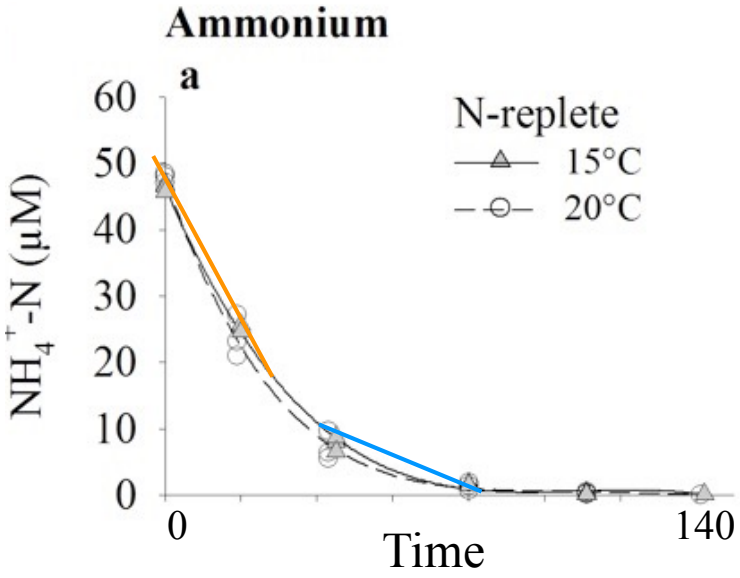
Δy *Δy* *Δy*

(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.

Uptake kinetics: V *vs.* S

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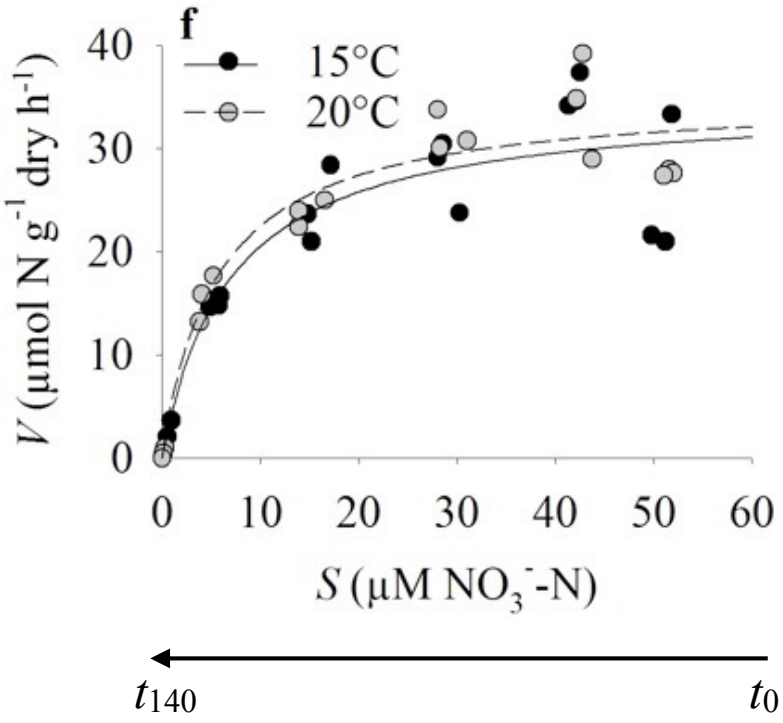
The Michaelis-Menten equation assumes that uptake is unidirectional so that no losses occur after uptake.

Mathematically, it is equivalent to the Monod equation, but where the Michaelis-Menten relationship describes nutrient uptake rate as a function of substrate concentration, the Monod model relates growth rate to substrate availability [by assuming that cells are always in equilibrium with their surroundings and that growth is exponential, which it is not always, especially for the more complex algae such as Gracilaria and Ecklonia that are capable of luxury consumption].

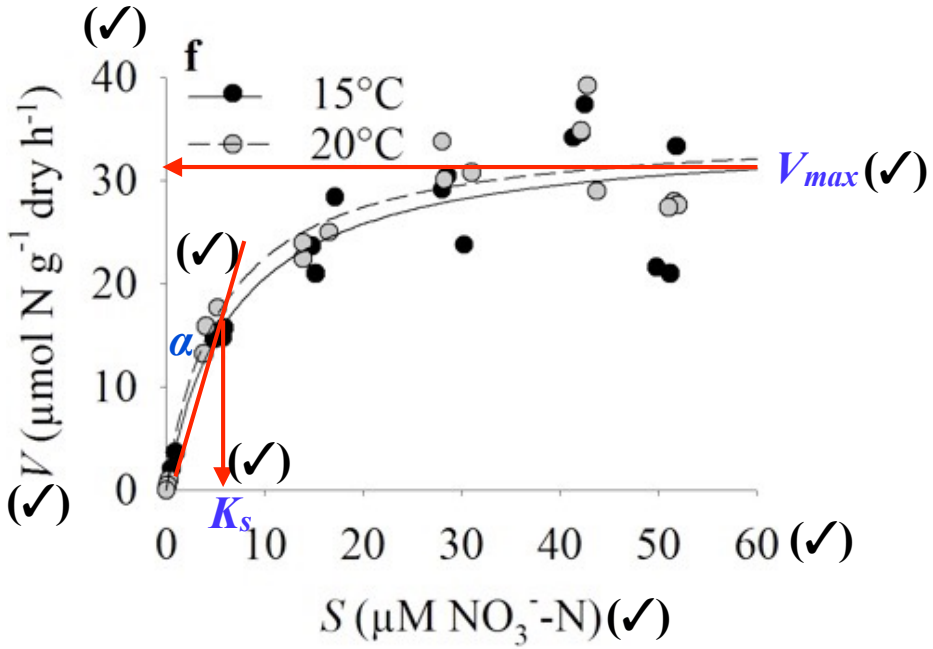
Michaelis-Menten

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



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

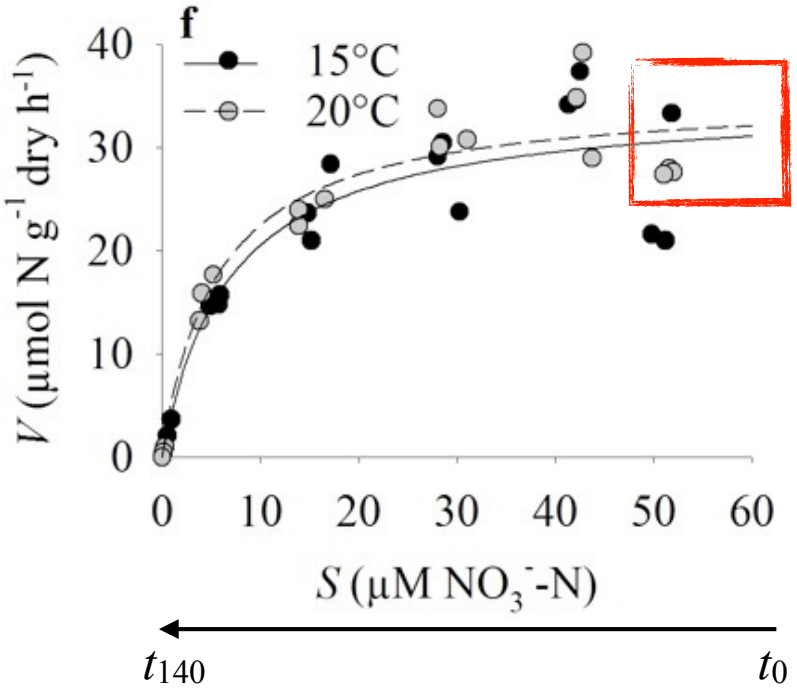
(ii) it may be the case that some other upper limit is imposed on the rate at which nutrients can be incorporated into thallus tissue.

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.

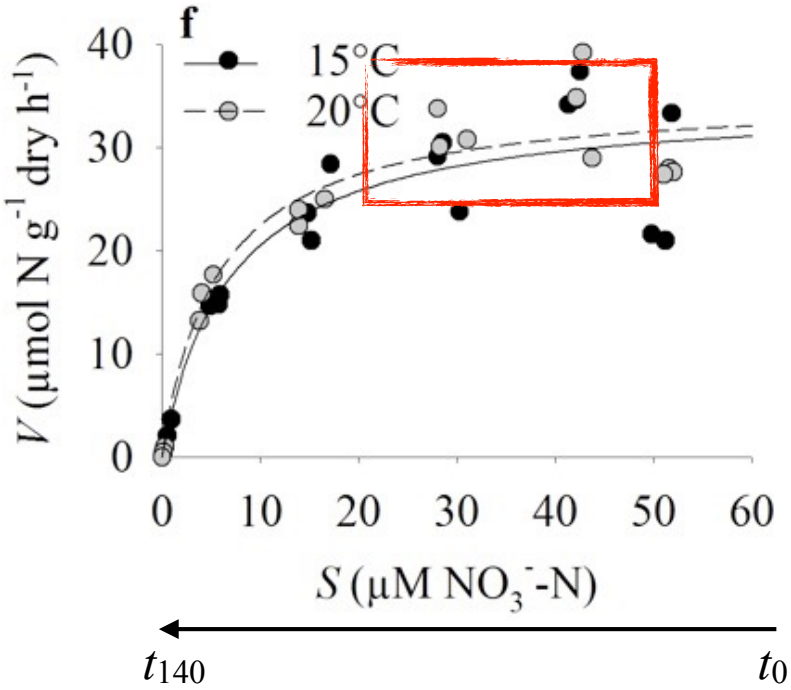
Phases of active uptake

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

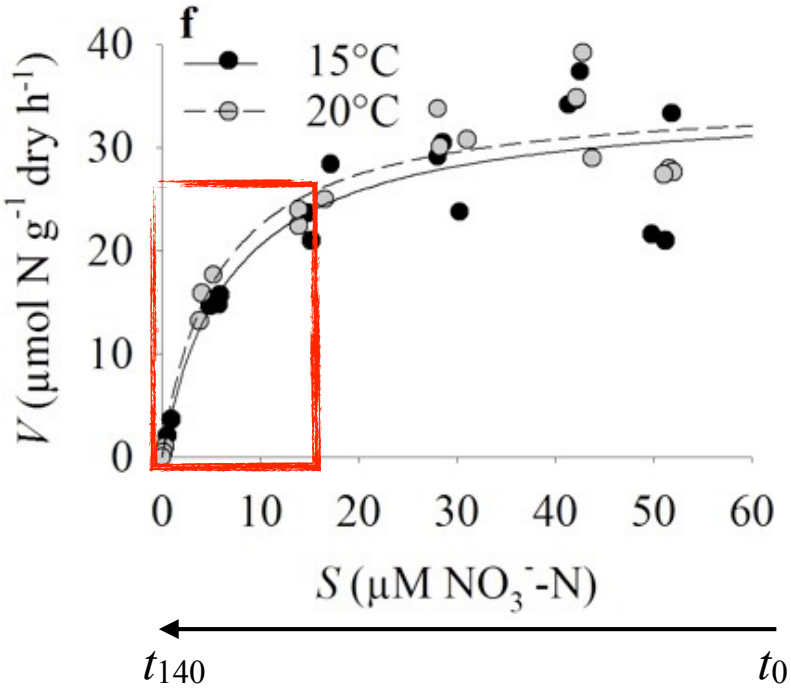
Surge uptake results from a concentration gradient between the alga and the external medium when it is first exposed to higher nutrient regimes. This surge in nutrient uptake abruptly ceases several minutes into the experiment as a result of feedback inhibition from pools of inorganic nitrogen and amino 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

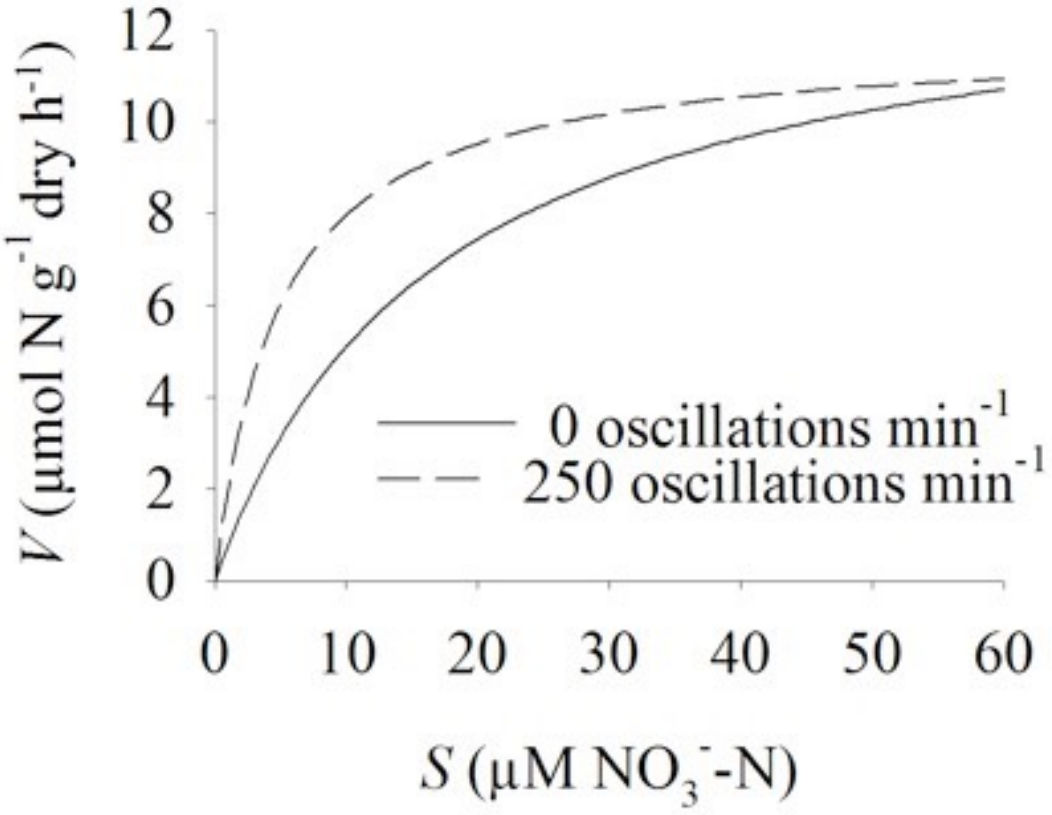
Usually, kinetic studies manipulate S (independent variable). However, water motion also affects V (but not V_{max}) via its effect on the boundary layer.

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).

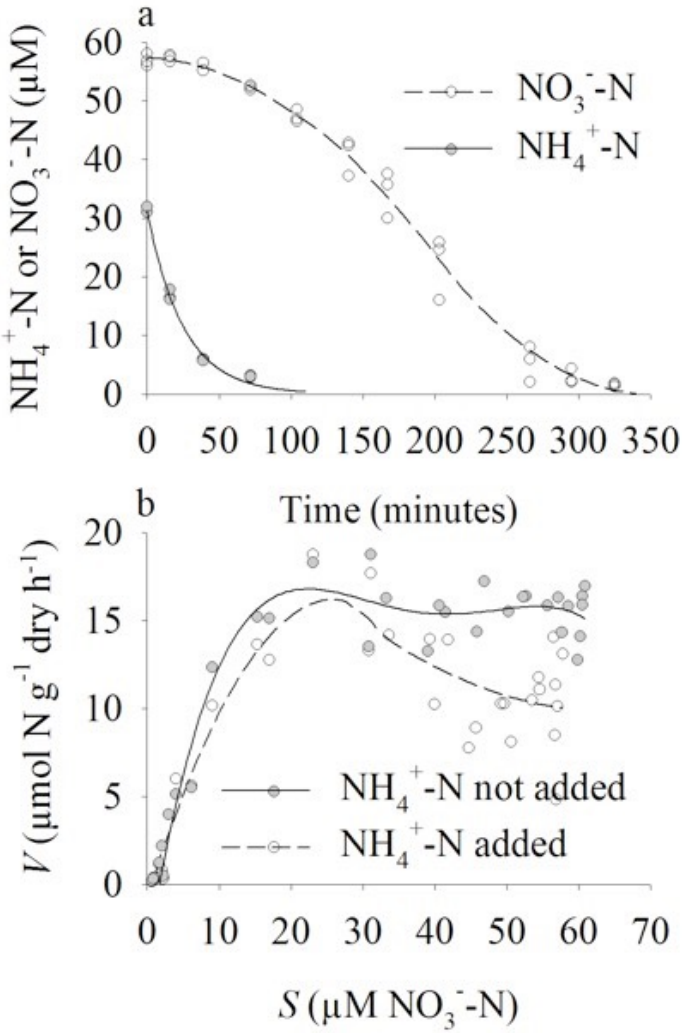
The magnitude of V_{max} on the other hand, should be independent of any external process, since it represents the maximum rate at which the alga is able to metabolise DIN that is already inside the cells subsequent to physical transport across the BL and cell membrane.

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.

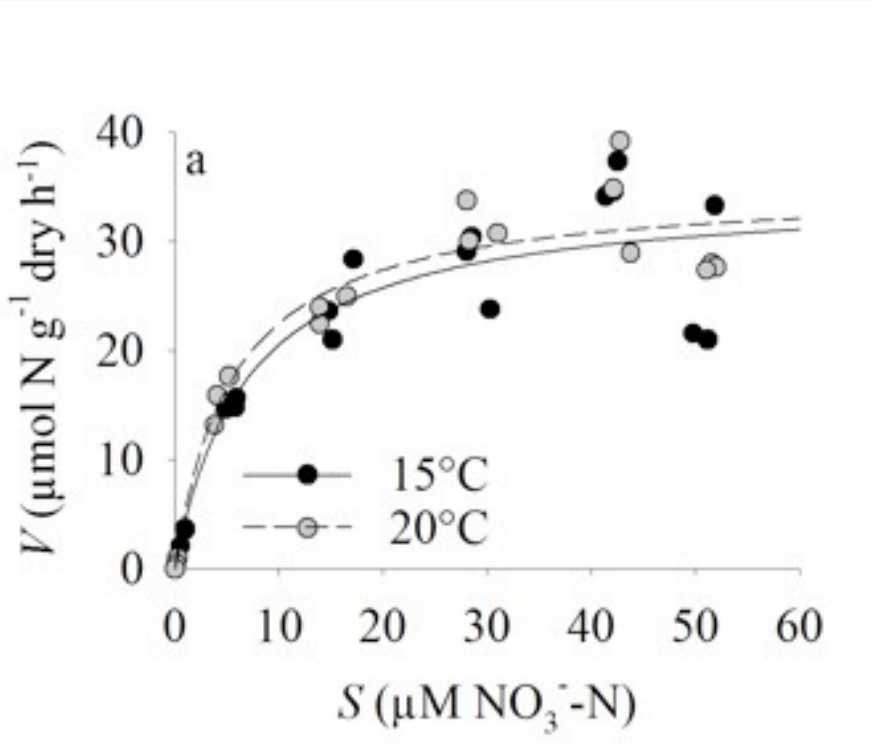
Nitrogen


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)

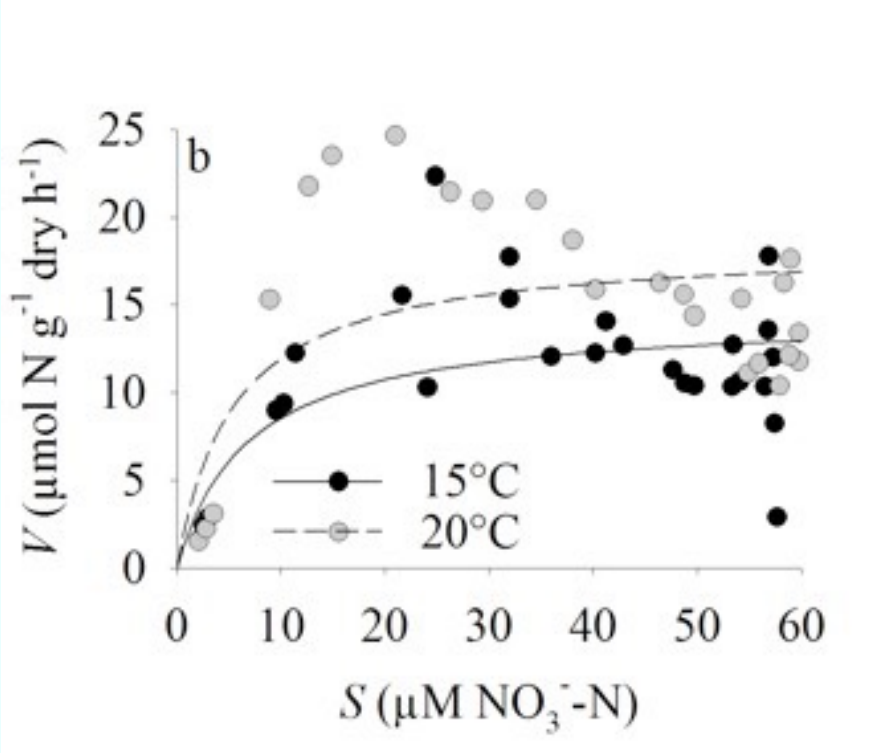
NH_4^+ *vs.* NO_3^-


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



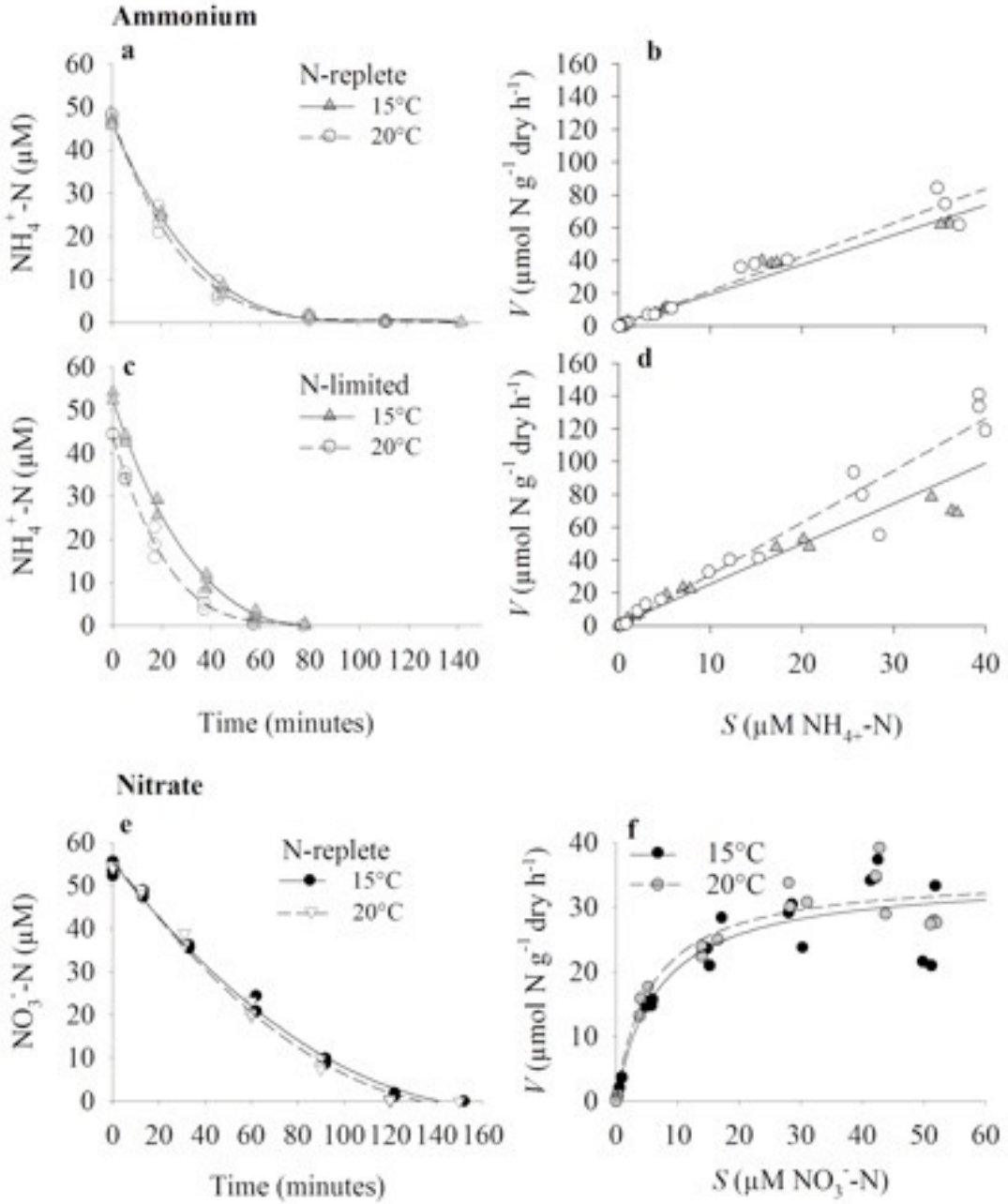
N-limiting

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

Some other ions may also enter cells via passive transport (these have a lower permeability through membranes which are electrically polarised; they also have a sphere of hydration which makes them somewhat larger than uncharged molecules).

These ions (usually cations) are usually driven by electro-potential or chemical-potential differences (combined called the electrochemical potential gradient) that exist between the in- and outside of cells (brought about by metabolic activity) - they are driven down the gradients. Such gradients can bring ions into cells against concentration gradients.

Passive uptake

So, some algae ammonia/ammonium may accumulate in the cells by a factor of 10^3—how is this possible? In seems to go against a concentration gradient...

Passive transport

Lipid bilayer: hydrophillic outside, hydrophobic inside - the inside is a barrier for charged ions.

Non-electrolytes diffuse through the cell membrane at a rate proportional to their solubility in lipid, and inversely proportional to their size. Ammonia (NH_3) can rapidly cross the membrane by diffusion across the first water-lipid interface (the one facing towards the outside), then dissolving in the lipid portion of the membrane, diffusion across the lipid-water interface in the cell's inside, and lastly dissolving in the aqueous cytoplasm.

The p*Ka* of ammonia/ammonium is 9.4; the pH of seawater is ~ 8.2 - i.e. only 5 - 10% of the total ammonium + ammonia is present as ammonia.

Passive uptake

At a higher pH, such as is sometimes found in dense cultures or tide pools the pH may increase to ~ 9.4; now > 50% of the total ammonium is present as ammonia. Under these conditions ammonia can rapidly diffuse into the cells.

The pH of the cytoplasm/vacuoles is ~ 7 - 7.5, and ammonia is rapidly protonated to ammonium; since ammonium is charged it cannot diffuse back to the outside. The process of converting ammonia to ammonium (acid trapping) maintains a low ammonia concentration inside the cell, and a high influx of ammonia is thus maintained (seemingly against a concentration gradient).

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).

Other factors affecting uptake

Much more detail is provided in Lobban & Harrison's book. Whether or not these factors affect nutrient uptake is highly species dependent and so much variability exists that it would be pointless to try and summarise everything here.

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.

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