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# Nutrient physiology of seaweeds: Application of concepts to aquaculture

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**Abstract:** Nutrients and light are key environmental factors that can determine biomass yield and productivity of an aquaculture system. Light is mainly controlled by the choice of the aquacultural site. Nutrients are controlled on the site and nutrient manipulations can control biomass yield, productivity, epiphytes and the amount of certain products formed. Carbon, nitrogen and phosphorus are the main three nutrient elements added to large scale aquaculture systems. This review focuses mainly on nitrogen and the nitrogen physiology of seaweeds. Important concepts such as nutrient uptake, assimilation, storage, response to nutrient additions, nutrient ratios, and critical tissue nitrogen levels are discussed in terms of their application to aquaculture.

For a particular species grown in an aquaculture system, it is important to know the C:N:P ratio of the seaweed under nutrient saturating conditions. This information will make the delivery of C, N, and P more economical. Nutrient uptake rates vary considerably with various physical, chemical and biological factors. In particular, light and temperature influence nutrient uptake rates, but we require further research to fully understand their effects. Nitrate uptake by some seaweeds is light dependent (i.e. diel periodicity), while ammonium uptake may be less dependent on light. Growth rates on different nitrogen sources ($NO_3^-$, $NH_4^+$, urea or combinations of these) should be tested. A wide variety of biological factors such as interplant variability, nutritional history, type of tissue, life history stages/age, surface area:volume ratio of the thallus and morphological changes such as the production of hairs may influence nutrient uptake rates.

Some seaweeds show three phases of uptake of the limiting nutrient (especially $NH_4^+$) when it is added to N-limited seaweeds. The "surge" uptake rate is greater than the growth rate and hence the seaweed can overcome its nitrogen deficiency. This "surge" uptake may be used to advantage in adding pulsed $NH_4^+$ additions at night to reduce competition with epiphytes. Epiphytes can also be controlled by starving the seaweed of N for several days until the tissue N (i.e. stored N) decreases to a critical level (below which the growth rate will decrease).

**Résumé :** Les sels nutritifs et la lumière sont les facteurs environnementaux clés qui déterminent les taux de biomasse et la productivité d'un système d'aquaculture. La disponibilité de la lumière est dépendante du choix du site d'aquaculture. Les sels nutritifs sont contrôlables sur un site et des manipulations de teneurs en sels nutritifs peuvent contrôler la production de biomasse, la productivité, les épiphytes et les quantités de certains produits du métabolisme. Le carbone, l'azote et le phosphore sont les éléments nutritifs majeurs qui sont utilisés à grande échelle en aquaculture. Cette revue est axée sur l'azote et la physiologie de l'azote chez les algues marines. Des concepts importants comme l'absorption des sels nutritifs, l'assimilation et les réponses à l'addition d'éléments nutritifs, les formes d'azote et les niveaux critiques d'azote dans les tissus sont présentés en relation avec leur application en aquaculture.

Pour des espèces particulières cultivées dans des sytèmes d'aquaculture, il est important de connaître le rapport C:N:P de l'algue en conditions de saturation en sels nutritifs. Cette information permet de rationaliser les apports de C, N, et P. Les taux d'absorption varient considérablement avec les facteurs physiques, chimiques et biologiques. En particulier, la lumière

et la température influent sur les taux d'absorption de sels nutritifs, mais des recherches supplémentaires sont nécessaires pour comprendre leurs effets. L'absorption de nitrates par certaines algues est dépendante de la lumière (périodicité circadienne) alors que l'absorption d'ammonium est moins dépendante de la lumière. Les taux de croissance à partir de différentes sources d'azote ($NO_3^-$, $NH_4^+$, urée ou combinaisons de ces formes) doivent être évalués. Une large variété de facteurs biologiques comme les variations individuelles, l'histoire de la nutrition, le type de tissu, les stades du cycle de vie et l'âge, le rapport surface:volume du thalle et les changements morphologiques comme la production de poils hyalins peuvent influer sur les taux d'absorption d'azote.

Certaines algues marines présentent trois phases d'absorption des éléments particulièrement limitant ($NH_4^+$) en situation de carence azotée. Le taux d'absorption "par à coup" est supérieur au taux de croissance et ainsi l'algue peut compenser son déficit en azote. Le taux d'absorption "par à coup" peut être utilisé pour réduire la compétition avec les épiphytes par additions pulsées de $NH_4^+$ la nuit. Les épiphytes peuvent aussi être contrôlés par privation des algues en azote pendant plusieurs jours, afin que la teneur en azote des tissus (N stocké) décroisse jusqu'à un niveau critique au dessous duquel le taux de croissance diminue.

*Keywords*: Seaweeds, physiology, nutrients, nitrogen, nutrient ratios, aquaculture, nutrient uptake

## Introduction

Seaweeds require a wide variety of nutrients for growth. Nitrogen and phosphorus are the two nutrients that limit seaweed growth and yields in most natural environments. When N and P are added in aquacultural practices, carbon then becomes limiting and therefore carbon is also added. Of these three important nutrient elements, this review will focus mostly on nitrogen.

In order to understand the nutrient physiology of seaweeds, it is necessary to understand basic concepts such as nutrient uptake rates, nutrient assimilation, nutrient storage, critical tissue nutrient concentrations and growth rates. Practical applications of these concepts include nutrient loading rates in relation to growth rates, nutrient pulsing to control epiphytes, and nutrient manipulation to enhance product formation (e.g. decreased phosphate concentration enhances carrageenan production).

## Limiting Nutrients and Nutrient Ratios

The nutrient requirements of seaweeds are divided into three categories, macronutrients (e.g. N, P, C, etc.), micronutrients or trace elements (e.g. Fe, Zn, Cu, Mn, Mo, etc.) and vitamins (vitamin $B_{12}$, thiamine, and biotin). A more extensive list of these nutrients, their functions and examples of compounds in the seaweeds is given in Lobban & Harrison (1994; see Table 5.1). Most of these essential elements for growth are in relatively low concentrations in seawater relative to their concentration in the seaweed's tissue. For example, N and P are concentrated about 100,000 times by the seaweeds, while C is concentrated about 10,000 times over ambient seawater concentrations.

The definition of "limiting nutrient" dates back over 100 years to Liebig's Law of the Minimum. It states that the nutrient that is available in the smallest quantity with respect to the other nutrient requirements of the plant, will limit the rate of growth, assuming all other factors are optimal. It is important to remember that the seawater nutrient concentration is determined by the balance between the supply rate of the nutrient (water column mixing, nutrient regeneration, etc.) and the nutrient demand (uptake) by the seaweeds. Therefore a very low seawater nutrient concentration will not tell you whether the seaweed is slightly, moderately, or severely nutrient limited. Also, natural populations of seaweeds can obtain nutrients from sources other than the water column, for example, particulate material on their surface (Schaffelke, 1999) or animal excretion (e.g. Taylor & Rees, 1998). Tissue nutrient concentrations are thus required to determine the severity of nutrient limitation (see section later).

Nutrient concentrations are expressed as µM (equivalent to µmol $l^{-1}$ and µg-at $l^{-1}$ for all nitrogen compounds, except urea where 1 µM = 2 µg-at $l^{-1}$ since the urea molecule contains 2 atoms of N). Inorganic nutrient concentrations in surface waters vary with geographic location and in tropical regions concentrations can be low (at the limits of detection) year-round. For temperate regions, concentrations are typically maximal during the fall and winter and minimal between late spring and late summer. For example, in the NE Pacific winter concentrations of inorganic N ($NO_3^- + NO_2^- + NH_4^+$) are 30-40 µM, while inorganic P ($PO_4^{3-}$) is approximately 2 µM, yielding a N:P atomic ratio of approximately 16:1. The atomic ratio of C:N:P in phytoplankton is 106C:16N:1P (the Redfield ratio), while benthic plants (mean of 92 species) on average have a somewhat higher ratio of 550C:30N:1P (Atkinson & Smith, 1983). These ratios indicate that many seaweeds require less P and more N than phytoplankton and that many seaweeds are more prone to N limitation than phytoplankton.

The ratio of two nutrients that is required for maximal growth of a seaweed is called the optimum ratio. As stated above, the average N:P ratio for seaweeds is 30N:1P with a range from 10:1 to 80:1 (Atkinson & Smith, 1983). An

optimum atomic ratio of 30N:1P means that the seaweed will consume N and P in this ratio and therefore from an aquacultural point of view, it is most cost effective to add N and P in this ratio. If the N:P ratio in the seawater is > 30N:1P, the seaweed's growth will be limited by P, and surplus N may remain in the seawater medium or be stored in the seaweed's tissues. Similarly, when N:P <30:1 the seaweed's growth will be N-limited and surplus P may remain in the seawater or be stored in the seaweeds' tissues. Since each seaweed may have a different optimum N:P ratio, it is advisable to determine the optimum ratio for the species of interest, if it is not already known. The method has been described by Rhee (1978) for phytoplankton, and for seaweeds, it involves growing the seaweed in seawater with a wide range of N:P ratios and maintaining a steady state by using a flow through system and measuring the tissue N and P (plot of N or P $g_{dw}^{-1}$ vs N:P ratio in seawater).

## Nutrient Uptake Mechanisms and Rates

Nutrients may be taken up by several different mechanisms. Gases such as $CO_2$, $O_2$, and $NH_3$ and uncharged molecules may be taken up by passive transport (diffusion) if there is a favourable concentration gradient. Facilitated diffusion and ion channels have not been well studied for seaweeds. Active transport for inorganic nitrogen and phosphorus is implied if a plot of nutrient uptake rate (V) vs nutrient concentration (S) yields a rectangular hyperbola. The equation describing this curve is known as the Michaelis-Menten Equation, $V = V_{max}\ (S/K_s+S)$ where $V_{max}$ = maximal uptake rate and $K_s$ = half-saturation value, the nutrient concentration where $V = V_{max}/2$. $K_s$ is often used to compare a seaweed's ability to take up nutrients at low concentrations. However, because $K_s$ is dependent on $V_{max}$, seaweeds with the same $\alpha$ (initial slope of the V vs S rectangular hyperbola), but a different $V_{max}$ will have a different $K_s$. This difference in $K_s$ reflects their $V_{max}$ values and not their ability to take up nutrients at low concentrations. Therefore, the slope of the initial linear portion of the curve (at low S values), $\alpha$, is more useful than $K_s$ in comparing the uptake abilities of two species at low S (Harrison et al., 1989). A high $\alpha$ (steep linear slope) indicates a high affinity for nutrients at low seawater concentrations, while a high $V_{max}$ indicates an ability to rapidly take up nutrients when their concentration is high. Uptake rates are normalized to dry wt (µmol $g_{dw}^{-1}$ $h^{-1}$), surface area (µmol $cm^{-2}$ $h^{-1}$), or particulate/tissue nutrient which yields the specific uptake rate ($h^{-1}$).

Some seaweeds do not show a saturation of nutrient uptake rates at high environmental nutrient concentrations (as described by the Michaelis-Menten Equation). Their uptake rate increases linearly with high nutrient additions (e.g. 60-100 µM for $NO_3^-$ or $NH_4^+$; Harrison et al., 1986; Taylor et al., 1998) well beyond the normal environmental concentrations. In this case it is not possible to determine $V_{max}$ and it may be more appropriate to give the uptake rate at a high environmental concentration, or the uptake rate at the concentration of the nutrient addition for aquaculture conditions. Non-saturable uptake by seaweeds is commonly recorded for $NH_4^+$ and suggests passive uptake (e.g. DeBoer & Whoriskey, 1983, Taylor et al., 1998, Taylor & Rees, 1999, Campbell, 1999). Passive uptake of $NH_4^+$ can be explained by the conversion of $NH_4^+$ at the thallus surface to $NH_3$ (due to higher pHs at the cell surface caused by $CO_2$ uptake) which is passively taken up (see Hurd, 2000). Non-saturable uptake has also been recorded for $NO_3^-$ (Harrison et al., 1986) and urea (Phillips, unpublished) but the mechanisms behind these phenomena are unknown. For intertidal seaweeds from New Zealand, both saturated and non-saturated uptake of $NO_3^-$, $NH_4^+$ and urea can occur for different individuals of the same population (Phillips & Hurd, unpublished).

The three uptake kinetic parameters, $V_{max}$, $K_s$, and $\alpha$, for a particular seaweed, vary considerably due to various physical, chemical and biological factors, although only a small number of seaweeds have been studied. Physical factors such as light, temperature, desiccation and water motion influence uptake kinetics. For the kelp *Macrocystis*, $NO_3^-$ uptake has been shown to decrease with decreasing irradiance (Wheeler, 1982). However, there may be an interactive effect of tissue nitrogen status and the length of the incubation in light or darkness on nitrate uptake (Wheeler & Srivastava, 1984) although Kopczak (1994) found little influence of light or N-status on $NO_3^-$ uptake by *Macrocystis*. Light levels had no influence on $NH_4^+$ uptake by *Macrocystis* (Wheeler, 1982). For *Porphyra*, $NO_3^-$ is a better N-source than $NH_4^+$ for growth in high light (160 µmoles $m^{-2}$ $s^{-1}$) but in low light (50 µmoles $m^{-2}$ $s^{-1}$) growth rates are similar on either N-source (Hafting, 1999). Phosphate uptake rates of *Pelvetia canaliculata*, *Fucus spiralis* and *F. serratus* were similar in the light and dark, whereas for *Ulva* sp., rates decreased by 50% in the dark compared to light (Hurd, 1990). We know little of how temperature influences uptake rates, but in general a doubling of temperature doubles uptake rates (i.e. $Q_{10} = 2$). Seaweeds probably have an optimal range of temperatures over which uptake occurs and rates are likely to decrease above and below this range (Wheeler & Srivastava, 1984). When some mid to high intertidal seaweeds (e.g. *Fucus*) were desiccated during low tidal exposure, their N uptake rate was several times higher than normal during the first hour of submergence during flood tide (Thomas & Turpin, 1980), although this phenomenon was not observed for phosphate uptake (Hurd & Dring, 1991).

Water motion (stirring) is extremely important for seaweeds since it determines the thickness of velocity and diffusion boundary layers (DBL's) around the thallus and hence the movement of ions and gases to and from the thallus surface (Hurd, 2000). Laboratory studies in unidirectional flows indicate that for inorganic nitrogen, maximal uptake rates are achieved at a current speeds of 2-6 cm $s^{-1}$ (Wheeler, 1982; Gerard, 1982; Hurd et al., 1996; Hurd, 2000). Rates of photosynthesis are also reduced under slow flows (< 6 cm $s^{-1}$, e.g. Wheeler, 1980; Koch, 1993). For *Gracilaria conferta* it is the accumulation of $OH^-$ ions at the thallus surface that lowers photosynthetic rates in slow moving water (probably through its influence on the pH within the DBL), rather than the accumulation of $O_2$ or the reduced flux of dissolved inorganic carbon (DIC) (Gonen et al., 1995).

Chemical factors such as the nutrient concentration (already discussed) and the form of the limiting nutrient (e.g. $NO_3^-$ vs $NH_4^+$) may influence uptake rates. Some seaweeds (especially kelp) are able to take up $NO_3^-$ and $NH_4^+$ simultaneously and at the same rate (Bird, 1976; Harrison et al., 1986). Thus they are able to take up twice as much N per unit time compared to when only one N form is available for uptake. This observation suggests that the seaweed should be able to grow faster if both $NO_3^-$ and $NH_4^+$ are added to the seawater, although for *Gracilaria cornea* growth rates were similar when nitrogen was supplied as $NH_4^+$, $NO_3^-$ and $NO_3^-$ + $NH_4^+$ (and urea) (Navarro-Angula & Robledo, 1999). In contrast, many other seaweeds take up $NH_4^+$ preferentially over $NO_3^-$ and therefore $NH_4^+$ inhibits the uptake of $NO_3^-$ by up to 50% (DeBoer, 1981). The ability of some New Zealand seaweeds to take up $NH_4^+$ vs $NO_3^-$ varies seasonally, with $NO_3^-$ and $NH_4^+$ uptake rates being similar in summer, while $NO_3^-$ uptake is much lower than $NH_4^+$ uptake in winter (Phillips, unpublished). Urea is an excellent N source for some seaweeds (e.g. kelp), but other seaweeds show reduced growth on urea (DeBoer, 1981; Navarro-Angula & Robledo, 1999). For some intertidal seaweeds, urea uptake is negligible in winter but increases in summer to provide an important N source (Phillips, unpublished).

A wide variety of biological factors such as inter-seaweed variability, nutritional history, type of tissue, life history stages/age, surface area:volume ratio of the thallus, and morphological changes such as the blade morphology or the production of hairs may influence nutrient uptake rates. The difference in uptake rates among different seaweeds of the same species can be substantial (>2x), and therefore a wide variety of individuals of a particular species must be sampled (Harrison et al., 1986). Young tissue has much higher uptake rates than older tissue and therefore one must be careful when uptake rates are determined only on portions of the thallus (e.g. the whole thallus may be too large for most containers) (e.g. Wallentinus, 1984; Hurd & Dring, 1990). Early life history stages usually have higher uptake rates than mature thalli of the same species (Thomas et al., 1985). For example, the kelp *Laminaria groenlandica* is a perennial and first year plants have higher uptake rates than the third year plants (Harrison et al., 1986).

Seaweeds with a high surface to volume ratio generally have a higher nutrient uptake rate (more membrane surface for uptake) (Wallentinus, 1984; Hein et al., 1995; Taylor et al., 1998). Plants growing in different environmental conditions undergo changes in blade morphology which could influence uptake rates, although for *Macrocystis* there was no difference in the $NO_3^-$ or $NH_4^+$ uptake rates of morphologically distinct blades (Hurd et al., 1996). Some seaweeds produce hyaline hairs under low N or P conditions (Whitton, 1988). The structure and development of hairs varies between species, but they are typically 2-6 mm long, have thin walls, a large central vacuole (DeBoer & Whoriskey, 1983; Hurd et al., 1993; Oates & Coale, 1994) and some exhibit cytoplasmic streaming (DeBoer & Whoriskey, 1983; Whitton, 1988). Seaweeds with hairs often have higher nutrient uptake rates, however, the increased uptake rates could also be due to the low tissue N or P since these seaweeds were grown in low nutrients to stimulate hair production.

The last but possibly the most important biological factor to strongly influence nutrient uptake rates is the nutritional history of the seaweed. Several decades ago, Conway et al. (1976) found that when N-limited phytoplankton were given $NH_4^+$, three phases of uptake (surge, internally controlled and externally controlled uptake) occurred over several hours and Pedersen (1994) observed three identical phases of $NH_4^+$ uptake by N-limited *Ulva lactuca* (Fig. 1). When the uptake rate was several times higher than the growth rate, they termed this phenomenon 'surge' uptake. This enhanced uptake rate allows the cell to overcome its previous nutrient deficiency, since it is able to 'catch up' by taking up the limiting nutrient much faster than it is required for growth. Later, Parslow et al. (1984) expanded this previous finding for $NH_4^+$ to include $PO_4^{3-}$ which showed 'surge' uptake. In contrast, there is typically a lag in $NO_3^-$ uptake for the first hour after a $NO_3^-$ addition, presumably due to a shutdown of the enzymes required for nitrate assimilation.

Some nutrient-limited seaweeds also show a decrease in the uptake rate of the limiting nutrient with time after it has been added to seawater. For this reason it is important to include the time period over which measurements of nutrient uptake rates are made for nutrient-limited seaweeds. Hence, one should designate the uptake rate period as a superscript (e.g. $V^{0\text{-}5\ min}$). These methods are described in detail for phytoplankton by Harrison et al. (1989) and further applications to seaweeds are described in

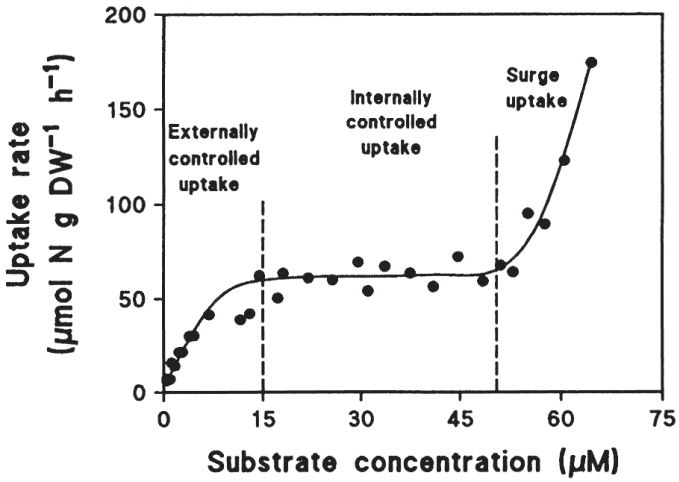


**Figure 1**. Uptake rate vs substrate ($NH_4^+$) concentration showing three distinct phases of uptake for *Ulva lactuca*. The experiment began on the right side of the x-axis (at T = 0) and proceeded to the left until the substrate ($NH_4^+$) concentration reached 0 (from Pedersen, 1994).

**Figure 1**. Taux d'absorption en fonction de la concentration en substrat ($NH_4^+$) montrant les trois phases distinctes de l'absorption chez *Ulva lactuca*. L'expérience a débuté sur le côté droit de l'axe des x (à T = 0) et s'est poursuivie jusqu'à ce que la concentration en substrat ($NH_4^+$) atteigne 0 (d'après Pedersen, 1994).

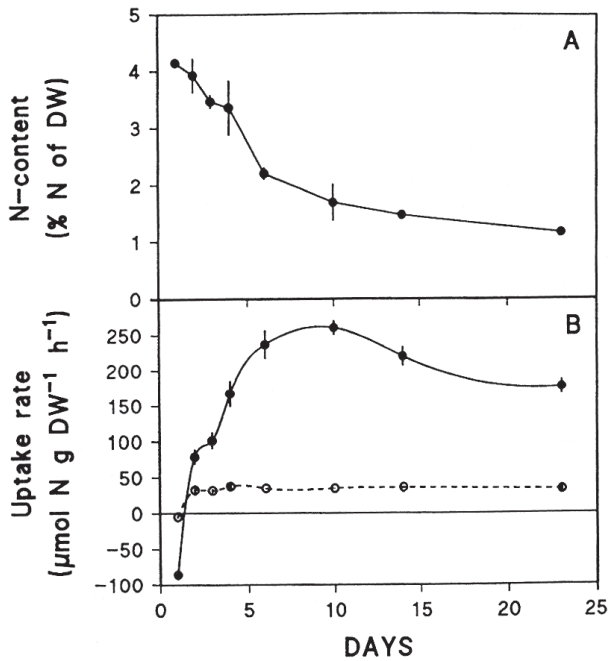


**Figure 2.** Time course changes in tissue N content and uptake rates during 24 days of N starvation of *Ulva lactuca*. **A** Changes in the tissue N during the starvation experiment (mean ± 95% CL). **B** Changes in surge uptake rate (•) and maximum internally controlled uptake rate or assimilation rate (o) during 24 days of N starvation (mean ± 95% CL) (from Pedersen, 1994).

**Figure 2.** Cinétique de l'évolution de l'azote tissulaire et des taux d'absorption durant 24 jours de privation d'azote chez *Ulva lactuca*. **A** Evolution de l'azote tissulaire durant l'expérience de privation (moyenne ± 95% CL). **B** Evolution des taux d'absorption "par à coup" (•) et taux maximum d'absorption interne contrôlée ou taux d'assimilation (o) durant 24 jours de privation d'azote (moyenne ± 95% CL) (d'après Pedersen, 1994).

Thomas & Harrison (1987), Lobban & Harrison (1994), and Pedersen (1994). In *Ulva lactuca*, Pedersen (1994) found that the surge $NH_4^+$ uptake over the first 15 min increased five times as tissue N decreased from 4 to 2%, but a further decrease in tissue N to 1% resulted in a decrease in surge uptake (Fig. 2). These observations suggest that surge uptake reaches its maximum at an "optimal" N stress; in the case of *Ulva*, it was after ten days of N starvation. However, the assimilation rate (the internally controlled uptake rate where $NO_3^-$ is converted into ammonium and amino acids) was not affected by the decline in tissue N during the 24 days of N starvation. Fast-growing seaweeds such as *Ulva* exhibit a high surge uptake (5 x > growth), while slower-growing seaweeds like *Fucus* and *Codium* show little surge uptake when they are N-limited and resupplied with $NH_4^+$ (Pedersen & Borum, 1997). It is interesting to note that during this brief period of surge $NH_4^+$ uptake, the uptake rate of non-limiting nutrients such as $PO_4^{3-}$ or $CO_2$ are often considerably reduced in phytoplankton (Conway et al., 1976; Turpin, 1983); similar measurements have not been conducted for seaweeds.

The other two phases of uptake of the limiting nutrient occur after 'surge' uptake (Fig. 1). They are internally and externally controlled uptake. It has been suggested that the internally controlled uptake rate is regulated by the assimilation of $NO_3^-$ to $NH_4^+$ where the rate limiting step is the conversion of $NO_3^-$ to $NO_2^-$ by the enzyme nitrate reductase or in the case of ammonium, the conversion of $NH_4^+$ to amino acids.

A summary of values for the nutrient uptake kinetic parameters is given by Lobban & Harrison (1994; see Table 5.4). There is no apparent difference in the values for the three algal classes (Phaeophyta, Rhodophyta and Chlorophyta), and $K_s$ values for $NO_3^-$ and $NH_4^+$ range from 2-20 µM and $V_{max}$ ranges from 6 to > 100 µmol $g_{dw}^{-1}$ $h^{-1}$. Part of this variability in uptake values may be due to the variety of methods used to determine uptake rates (Harrison & Druehl, 1982; Harrison et al., 1989; Pedersen, 1994). Wallentinus (1984) grouped the seven species of seaweed that she studied into two categories: (1) high $NH_4^+$ uptake species such as *Cladophora* which are short-lived, opportunistic, have a high surface area:volume ratio (it is filamentous and has numerous hairs) and a high $V_{max}/K_s$ (high $\alpha$) and (2) low $NH_4^+$ uptake species (e.g. *Fucus*) that are late successional, long-lived, have a low surface area:

volume ratio (thick thallus) and a low $V_{max}/K_s$ (low $\alpha$). Even though seaweeds have highly variable nutrient uptake parameters within and among species, Hein et al., (1995) showed that most seaweeds have a significantly lower $V_{max}$, higher $K_s$, a lower $\alpha$, and a lower SA:V ratio than microalgae. Therefore, based on these uptake rate parameters, most seaweeds cannot compete with microalgae when nutrients are limiting (Hein et al., 1995). However, many seaweeds have a substantial capacity to store nutrients when they are plentiful and these stored nutrients are utilized to maintain their growth rate during periods of nutrient limitation (Chapman & Craigie, 1977; Fujita, 1985).

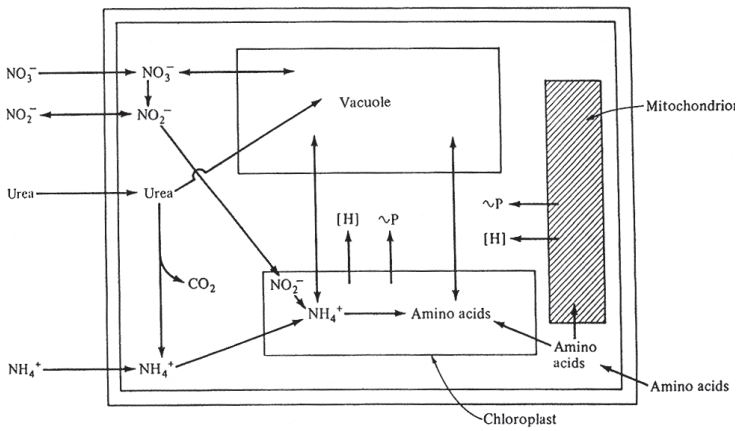


**Figure 3**. Main features of nitrogen uptake and assimilation in an eukaryotic algal cell (from Syrett, 1981).

**Figure 3**. Principales caractéristiques de l'absorption et de l'assimilation de l'azote dans une cellule algale (d'après Syrett, 1981).

## Nutrient Assimilation and Storage

When nitrate is taken up by seaweeds it can be stored intracellularly in the vacuole and cytoplasm or reduced to nitrite via the enzyme nitrate reductase (Fig. 2). Nitrite is transported from the cytoplasm to the chloroplasts where it is reduced to ammonium via the enzyme nitrite reductase. Similarly, urea is taken up and stored in the vacuole or cytoplasm and reduced to ammonium via the enzyme urease. Ammonium is taken up or formed from $NO_3^-$ or urea and it is converted into amino acids via glutamine synthetase in the chloroplasts (Fig. 3). Nitrate storage occurs when the uptake of nitrate is greater than the conversion rate of $NO_3^-$ to $NO_2^-$ due to factors such as low nitrate reductase activity. Intracellular pools of $NO_3^-$ and $NH_4^+$ and amino acids can be measured by using various solvents, including boiling water, which ruptures cells to release the inorganic and organic nitrogen compounds (Fujita et al., 1988). Intracellular $NO_3^-$ pools can make up to 5-10% of the total tissue N, while intracellular $NH_4^+$ pools make up < 1%. When seaweeds are N starved, intracellular $NO_3^-$ pools decrease to undetectable levels in a few days, while $NH_4^+$ and amino acid pools decrease to 50% of the original level (Thomas & Harrison, 1985). Seaweeds produce a variety of amino acids, but alanine is the most abundant amino acid in *Macrocystis pyrifera* and *Gracilaria tikvahiae* (Bird et al., 1982), while citrulline and arginine are the most abundant in *Gracilaria secundata*, (Lignell & Pedersen, 1987). Large amounts of citrulline and the dipeptide citrullinylarginine are important N storage compounds in *Chondrus crispus* (Laycock et al., 1981), *Gracilaria*, and other red seaweeds (Laycock & Craigie, 1977). Additions of N commonly result in an increase in N-containing photosynthetic pigments such as chlorophyll and phycobilins (Dawes, 1995; Vergarra et al., 1995). These pigments can act as N storage compounds and they are readily degraded during N limitation (Lapointe & Duke, 1984; Rico & Fernández, 1996). However, the role of N-containing pigments as N-stores will vary with species, and for *Chaetomorpha linum* and *Undaria pinnatifida* they are not considered important N-stores (McGlathery et al., 1996; Dean, 1998). Because many seaweeds can store enough N to allow them to grow at maximal rates for several days without nitrogen (Fujita, 1985), this characteristic has been used to advantage in controlling epiphyte growth in aquaculture systems. We know comparatively little about phosphorus storage in macroalgae, but one study revealed that storage occurs as polyphosphate granules in *Chondrus crispus* (Chopin et al., 1997).

## Growth Kinetics

The principles of growth kinetics in relation to substrate concentration were derived from bacteria growing on organic substrates (Monod, 1942). The relationship between growth rate ($\mu$) and substrate concentration (S) is described by a rectangular hyperbola whose equation is $\mu = \mu_{max}$ ($S/K_s + S$) where $\mu_{max}$ = maximal growth rate ($h^{-1}$) and $K_s$ = the substrate concentration at which $\mu = \mu_{max}/2$. DeBoer et al. (1978) applied this relationship between the limiting nutrient concentration and growth kinetics to the red seaweed *Agardhiella subulata* and found that the $K_s$ value was < 0.5 µM for $NO_3^-$, $NH_4^+$ and urea. For phytoplankton, growth rate is also related to the cell quota (Q) for the limiting nutrient (the amount of the limiting nutrient per cell). This relationship between growth rate ($\mu$) and cell quota (Q) has become known as the Droop Equation (Droop, 1968) which is $\mu = \mu_{max}$ ($1 - Q_{min}/Q$). The application of the Droop Equation derived for

phytoplankton yielded a similar equation for seaweeds but with the introduction of a new parameter, the critical tissue N content (Hanisak, 1979; 1990; Pedersen & Borum, 1997). This relationship between tissue N and growth rate indicates that when the tissue N falls below the critical tissue N, the growth rate begins to decrease (Fig. 4). The critical tissue N value is important to know for aquacultural systems because a seaweed can decrease its tissue N during N limitation/starvation to the critical tissue N without an accompanying decrease in growth rate. This concept is useful in the control of epiphytes which generally do not have such a large storage capacity as the host seaweeds or microalgae (e.g. benthic diatoms). The critical tissue N varies from 0.7 to 3.2% dry wt and is about 50 to 80% of the maximum tissue N (Lobban & Harrison, 1994; see Table 5.9).

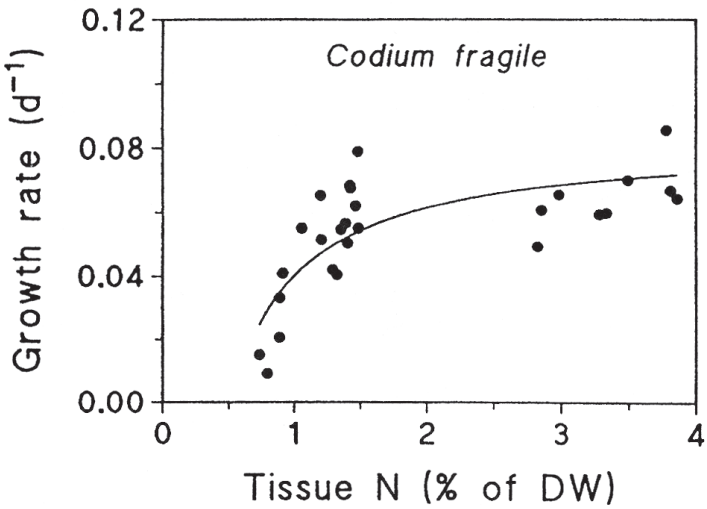


**Figure 4**. Relationship between growth rate and tissue N concentrations for *Codium fragile* (from Pedersen & Borum, 1997). Critical tissue N is about 1.5%.

**Figure 4**. Relations entre les taux de croissance et les concentrations tissulaires en azote pour *Codium fragile* (d'après Pedersen & Borum, 1997). Le niveau critique en N est d'environ 1,5 %.

In the field it is possible to determine the period of N limitation for a particular seaweed by determining the critical tissue N in the laboratory and measuring the seasonal change in tissue N. Pedersen & Borum (1996) showed that faster growing seaweeds such as *Ulva lactuca* had a high critical tissue N (4% of DW) and field values for tissue N were below 4% DW for 4-5 months, indicating that growth rate was reduced for this period due to N limitation. In contrast, slower growing seaweeds such as *Fucus vesiculosus* had a tissue N of about 1.5% DW and the field values for its tissue N were below 1.5% for only a brief (one month) period in late summer. In the classical field study of blade growth of *Laminaria*, Chapman & Craigie (1977) showed that even though $NO_3^-$ in the seawater reached undetectable levels by late March and early April, blades continued to grow at high rates using the $NO_3^-$ and other compounds stored in its tissues, indicating that this kelp had considerable N storage capacity. For seaweeds that have limited storage capacity, such as *Gracilaria gracilis*, nitrogen pulsing twice a week produced twice as much yield, and seaweeds with higher pigment and protein contents, than seaweeds pulsed once a week (Smit et al., 1997).

The nutrient management strategy will vary depending on whether the goal is biomass yield or product formation. When N or P are limiting, carbon may be allocated to commercially valuable cell wall materials such as agar, carrageenan and alginate (DeBoer, 1979; Kraemer & Chapman, 1991; Chopin et al., 1995). For example, the carrageenan content is the highest when the tissue P content is the lowest (Chopin et al., 1995; Chopin & Wagey, 1999). Therefore P starvation can be used to advantage to increase carrageenan content and yield. The properties of gels can also be manipulated by changing the alga's nutrient status. Agar strength, for example, increases with increasing N or P content, and melting and gelling properties are also influenced by algal nutrient status (Craigie et al., 1984; Martinez & Buschmann, 1996; Sousa-Pinto et al., 1996). As light levels influence growth rates and hence seaweed nutrient requirements, both light and nutrient supply can be manipulated to optimize agar and carrageenan yield and/or product properties (Hemmingson & Furneaux 2000).

## Applications of Nutrient Physiology to Seaweed Aquaculture

### *Offshore cultivation*

Aquaculturalists growing seaweeds on lines or nets offshore (e.g. Laminariales, *Porphyra*) have relatively little control over the environmental factors experienced by their seaweeds. However, some environmental variables can be manipulated and a thorough knowledge of a seaweed's nutrient ecophysiology can allow optimal manipulation to enhance growth rates and/or product. For example, periods of N-limited growth may be overcome by the addition of fertilizer by attaching porous pots containing slow-release fertilizer to the lines (see Neushul et al., 1992). By tracking the seasonal changes in tissue N and P to determine periods of nutrient limitation, N and P can then be added in an appropriate ratio. Also, the form of N ($NO_3$, $NH_4^+$, or $NO_3$+ $NH_4^+$) that promotes the highest growth rates can be applied. The kinetic parameters $V_{max}$ and the

initial slope ($\alpha$) will vary seasonally for some seaweeds, e.g. *Undaria pinnatifida* (Dean, 1998) and the concentration and frequency of fertilizer additions can be varied appropriately. For seaweeds with a high $\alpha$ or low $V_{max}$, it may be more efficient to add low concentrations of nutrient over sustained periods, while periodic pulses of high nutrient concentrations may be better for seaweeds with a low $\alpha$ and high $V_{max}$. Another method of enhancing nutrient supply is to co-cultivate seaweeds with commercially valuable marine animals, such as mussels or salmon, which provide an additional supply of N and P (Petrell et al., 1993; Martinez & Buschmann, 1996; Troell et al., 1997). Co-cultivation is beneficial as it can lead to increased yield and decrease the risk of eutrophication by farm wastes. For desiccation-tolerant intertidal seaweeds such as *Porphyra*, epiphyte control is achieved by exposing the lines to the air, which kills desiccation-intolerant epiphytes and may enhance nutrient uptake by *Porphyra* upon re-submersion.

Water motion can also be manipulated to some degree for offshore seaweed farms. To avoid damage by waves and strong currents, long-line and net style marine farms have to be located in relatively calm sea conditions, such as wave-sheltered bays. Most natural seaweed beds probably encounter turbulent flow and it is unlikely that seawater velocities experienced are low enough to cause mass transfer-limited growth (Hurd, 2000). However, for a densely planted seaweed farm located in relatively slow flows, the reduction in seawater velocity caused by the seaweeds and lines/nets could lead to the restricted transport of nutrients to the seaweed surface. This can be alleviated by increasing the tension of the lines (to increase the relative velocity at the thallus surface) of long-line style farms, or by fertilization which increases the nutrient concentration, thereby increasing transport across the diffusion boundary layer (Neushul et al., 1992).

### *Tank culture*

In tank culture, it is possible to control all environmental factors and thus knowledge of nutrient eco-physiology is very important for maximising growth rates and/or the product required. Bidwell & McLachlan (1985) demonstrated the value of conducting ecophysiological laboratory studies before the scale-up of large outdoor culturing facilities. They studied carbon uptake by *Chondrus crispus* and then applied these laboratory results to the tank design and cultivation of *C. crispus* in large outdoor raceways (Bidwell et al., 1985).

In order to calculate the nutrient supply rate to a tank, one must know the basic light, temperature and nutrient requirements of your species. Knowledge of the optimum nutrient ratio is necessary to determine the most economical addition of N and P. Knowing the critical tissue N, the growth rate, and the biomass in the tank, one can calculate the nutrient supply rate ($\mu$mol N $l^{-1}$ $d^{-1}$) to the tank. Species with a high growth rate, a high critical tissue N level and large intracellular N pools (e.g. *Ulva*) will require a very high N supply rate (up to 30 x slow growing species) in order to prevent N limitation.

As previously discussed for off-shore seaweed cultivation, water motion is essential for on-shore tank cultivation. Two basic methods can be used to obtain water motion: (1) the seaweeds are attached in a tank or race-way and water is pumped over the algae, or (2) the seaweeds are free-floating within a tank and water movement is achieved by bubbling air or $CO_2$ through the tank, and the seaweeds circulate within the tank (Bidwell et al., 1985). Advantages of the second method are that: (1) water circulation can be generated using $CO_2$ which will alleviate carbon limitation, and (2) for some seaweeds the dynamic light regime that they experience as they are circulated from the tank surface to depth can enhance growth rates (Greene & Gerard, 1990, but see Kübler & Raven, 1996). However, a potential disadvantage of seaweeds moving with the current is that the relative seawater velocities at the thallus surface will be lower than for seaweeds which are anchored and have water flowing over them (Gonen et al., 1993; 1994). For *Gracilaria conferta* cultured unattached in tanks, velocity reduction was greatest within large, densely branched specimens (Gonen et al., 1993) and such velocity dampening could reduce the supply of nutrients to the middle of the seaweed thallus. As described above, mass transport limitation can be overcome by increasing the nutrient concentration and/or the relative water velocity.

Several decades ago, studies were conducted on marine phytoplankton to determine if nutrient supply rate (flux rate) (Harrison & Davis, 1979) or nutrient pulsing (size and frequency of the addition of the limiting nutrient; Turpin & Harrison, 1979) was more important in determining species succession in outdoor chemostats containing natural phytoplankton assemblages. They found that the nutrient flux rate was more important than nutrient pulsing (Harrison & Davis, 1979). A high nutrient flux was selected for the fast growing diatoms, while a low flux rate was selected for slower growing flagellates or large slow growing diatoms (Turpin & Harrison, 1979, 1980). Variation in the size or frequency of the nutrient addition at a constant nutrient supply rate tended to select for certain species within the group of species selected by the nutrient supply rate (Harrison & Davis, 1979). Pickering et al. (1993) applied these concepts of nutrient flux and nutrient pulsing to the problem of epiphyte control in *Gracilaria*. They found that the total nutrient flux was the most important factor that determined growth rate and epiphyte abundance. Epiphytes were the most abundant at the highest nutrient flux. They found that adding N at 7-10 day intervals for *Gracilaria*

(but this will depend on the doubling time of the species) produced epiphyte starvation between pulses, while the growth of *Gracilaria* was affected little. Similarly, weekly pulses of 0.5 mM $NH_4^+$ controlled epiphyte growth in *Gracilaria conferta* (Friedlander et al., 1991). However, as Hanisak (1990) cautions, the nutrient demand (uptake rate) of the seaweed can change quickly as environmental factors such as light and temperature change and therefore the nutrient flux should be changed accordingly.

The species' N storage capacity, or importantly, how long it takes the tissue N to decline to the critical N level (a few days to several weeks), is critical information for epiphyte control. The longer the period between N pulses (without reducing the growth rate of the cultivated species), the better the epiphyte control. It is ideal if the seaweed exhibits surge $NH_4^+$ uptake when it reaches its critical N level since the pulse will be taken up faster and therefore there will be less time for the epiphytes to take up the $NH_4^+$ pulse. A night-time $NH_4^+$ pulse would reduce the uptake of $NH_4^+$ by the epiphytes because their storage capacity is limited and further $NH_4^+$ uptake via growth (cell division) is reduced in the dark. Ammonium may be toxic to some species in the 1 mM range and ammonium pulse concentrations should be less than 1 mM, unless your species has been studied for $NH_4^+$ toxicity. Ammonium pulsing has several advantages over $NO_3^-$ pulses. The assimilation of $NH_4^+$ reduces the pH through the release of protons (Goldman et al., 1982) and offsets the pH increase due to $CO_2$ uptake while the uptake of $NO_3^-$ increases the pH. Ammonium is usually taken up faster than $NO_3^-$ because of the possibility of surge uptake that is usually not present for $NO_3^-$. In addition, $NH_4^+$ uptake is usually light independent, in contrast to light dependent $NO_3^-$ uptake. Finally, from an energetic perspective, it takes eight electrons to reduce $NO_3^-$ to ammonium and it is therefore theoretically more efficient to supply ammonium rather than nitrate. This energetic efficiency may only be important in aquaculture situations where light limits the growth of the seaweed.

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