

Can Activity of Enzymes Involved in Nutrient Assimilation be Useful as Indices of Nutrient Status in *Cladophora*?

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The recent resurgence of benthic algal growth in Lake Michigan near Milwaukee has caused public outrage and concern about ecosystem health. During the late 1970's - 1980's, blooms of the invasive *Cladophora* appeared in Great Lakes nearshore regions and research pinpointed increasing P inputs as the major causative factor.

Consequently, with the 1990's reoccurrence of *Cladophora* blooms, it was initially assumed that algal growth was being stimulated by increasing nutrient inputs to the lake from sewage, agricultural and industrial runoff. Freshwater habitats are usually considered to be P limited, and with the relationship between *Cladophora* growth and P found during the 1980's, the role of P was considered pivotal. However, P inputs to Lake Michigan have not increased significantly since the 1980's, rather with the implementation of P control measures, the P inputs have decreased over the last decade. Clearly a broader picture of nutrients and other factors need to be considered in understanding the current *Cladophora* problem in Lake Michigan near Milwaukee.

Our research addressed the issue of whether *Cladophora* growth is controlled by P availability? To do this, we targeted the following questions :

1. Does growth of *Cladophora* become seasonally limited by nutrients?
2. What is the water column availability of macronutrients P and N ?
3. Is there a seasonal variation in nutrient status in *Cladophora*? To assess this third question, we used three approaches -
 - Changes in nutrient content (stoichiometry)
 - Changes in photosynthetic 'capacity'
 - Variation in expression of enzymes of nutrient assimilation
 - Nitrate reductase – regulated by N availability
 - Alkaline phosphatase – only present when P is limiting

In addressing the question of what nutrients are available to *Cladophora*, we collected water samples over the summer growing period, from our 10 m site off Atwater beach, north of Linnwood intake facility (Fig. 1). We also assessed nutrient inputs to Lake Michigan via Milwaukee harbour, the largest point-source of nutrients in the region. Survey results are presented in Bootsma, Young and Berges (this volume).

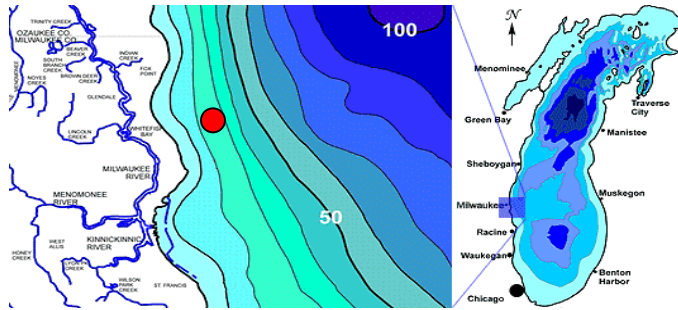


Figure 1. Atwater sampling site at 10 m depth off the coast N of Milwaukee harbor.

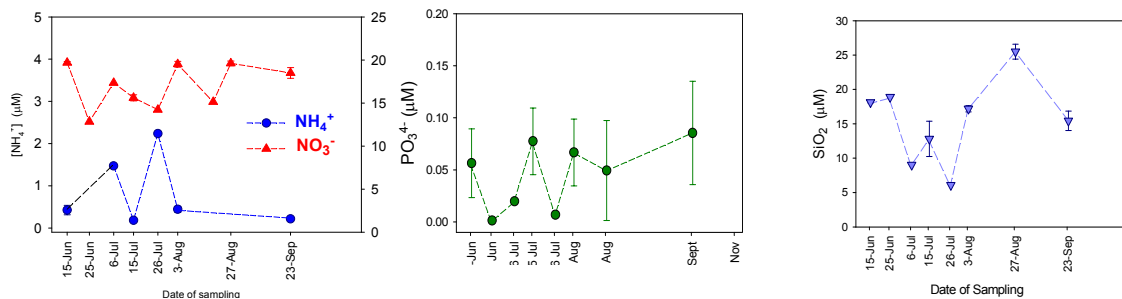


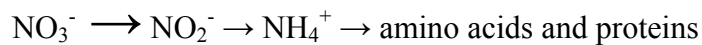
Figure 2. Seasonal variation in soluble nutrients in the water column at Atwater 10 m site. **A.** Inorganic nitrogen ($\text{NO}_3^- + \text{NO}_2^-$, NH_4^+) **B.** ortho-phosphate (PO_4^{3-}). **C.** Silicate (SiO_2).

The seasonal patterns of soluble nutrients in the water column at the Atwater sampling site are shown in Fig. 2. There were some oscillations in available inorganic N with levels of NO_3^- consistently high enough to support algal growth. The ammonium levels were lower, possibly as a result of preferential uptake by phytoplankton and benthic algae. Soluble phosphate availability was consistently very low over the growing season, suggesting possible limitation in P for algal growth. There was no evidence for draw-down of P from algal uptake over the growing season with slightly higher levels in Aug - Sept than at points earlier in the season. Silicate was consistently high enough to support growth of silicate-requiring algae (i.e. diatoms).

Enzyme Analyses:

The rationale for examining enzyme activity in *Cladophora* is that enzyme activities in algae are regulated in response to available nutrients and are thus a physiological index of nutrient status (Beardall et al. 2001).

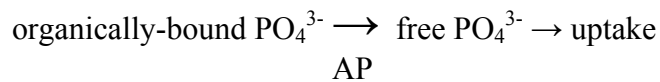
Inorganic N assimilation by algae follows this pathway :



NR

Nitrate reductase (NR) catalyses the initial reduction of NO_3^- to NO_2^- , which is believed to be the rate-limiting step in uptake and assimilation of NO_3^- into amino acids and proteins. The activity of NR is regulated in response to available NO_3^- , NO_2^- and NH_4^+ ; NR expression requires the presence of NO_3^- and light and is suppressed by high ambient concentrations of NH_4^+ , in most algae (Berges et al. 1995; Young et al. 2005).

Phosphate acquisition similarly involves expression of enzymes. Much of the P available in the aquatic environment is not available for uptake by algae because it is bound to organic chelators. A widely distributed enzyme which helps cleave orthophosphate from the organic chelator is alkaline phosphatase (AP). It has been shown in algae that the expression of AP activity is greatly elevated under conditions of low P availability (Dyhrman and Palenik 1997). Thus AP activity can be used as an index of P limitation in algae.

**Enzyme assays - how they work**

Nitrate Reductase : *Cladophora* is frozen in liquid nitrogen at the sampling site and stored until assaying. Tissue samples are ground in liquid nitrogen and extracted into a buffer containing constituents to preserve protein function (Young et al. 2005). Small volumes of the extract are incubated in replicate tubes with the substrates NO_3^- and NADH supplied in excess. The reaction is stopped at various times. The concentration of NO_2^- is measured spectrophotometrically in clarified extracts. The increase in NO_2^- concentration is plotted and linear rate is proportional to the activity of NR enzyme.

Alkaline Phosphatase: Freshly collected *Cladophora* tissue is stored in Lake Michigan water. Intact pieces of *Cladophora* are incubated with artificial fluorometric substrate, methyl umbelliferone- PO_4^{3-} (MUP). The change in fluorescence over time is measured and is indicative of cleavage of the PO_4^{3-} from MUP. AP enzyme activity is proportional to rate of fluorescence change, compared with a standard curve.

Seasonal patterns in NR and AP activity were measured in *Cladophora* from the Atwater site over the summer growing period (Figs. 3, 4). There was some correlation between NO_3^- available in the water column, and NR activity in *Cladophora* (Fig. 3).

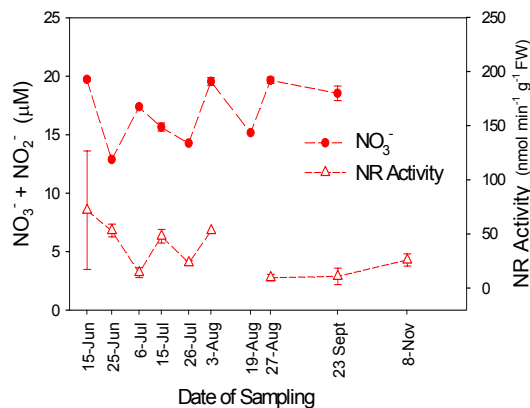


Figure 3. Seasonal variation in nitrate reductase (NR) activity in *Cladophora* and water column NO_3^- at Atwater 10 m site. Points are mean \pm standard deviation, $n \geq 2$ (NO_3^-), $n = 8$ (APA).

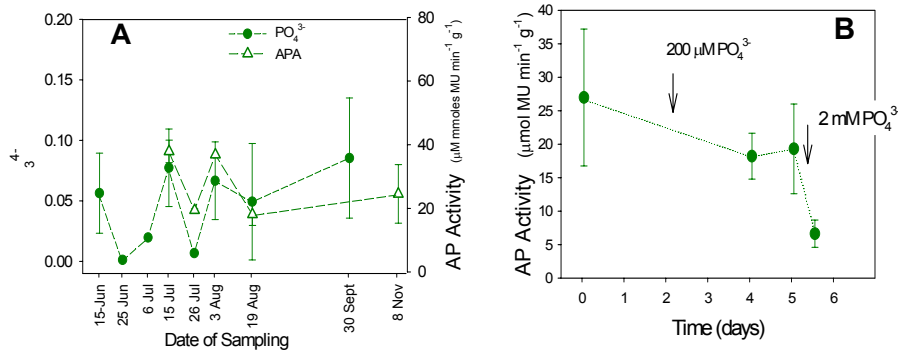


Figure 4. **A.** Seasonal variation in alkaline phosphatase (AP) activity in *Cladophora* and water column soluble PO_4^{3-} from Atwater 10 m site. Points are mean \pm std dev, $n \leq 2$ (PO_4^{3-}), $n = 8$ (APA). **B.** Suppression of *Cladophora* alkaline phosphatase activity by PO_4^{3-} -enrichment in laboratory culture. Points are means \pm std dev, $n = 8$.

There was a correlation between AP activity and the soluble PO_4^{3-} available in the water column (Fig. 4A). AP activity in *Cladophora* was demonstrated to be responsive to water column available P. In laboratory tests, when 2 mM PO_4^{3-} was added, APA was significantly reduced from 20 to 6 $\mu\text{mol MU min}^{-1}\text{g}^{-1}$ FW within 5 hours (Fig. 4B).

Conclusions about enzymes

- Nitrate reductase activity oscillates with available NO_3^- - no evidence for inorganic N limitation of *Cladophora* growth
- Alkaline phosphatase activity (APA) in *Cladophora* is responsive to available P, with rapid suppression of APA following PO_4^{3-} enrichment of growth medium.
- Elevated alkaline phosphatase activity in *Cladophora* indicates P limitation of *Cladophora* growth mid - late summer
- In conjunction with other indices of nutrient availability and internal nutrient concentrations, enzyme activity can be a useful additional parameter for examining nutrient status of *Cladophora* (and other algae).

Estimating Photosynthesis using Chlorophyll *a* Fluorescence

The relationship between photosynthesis and available light was measured using a rapid *in situ* measurement of *Cladophora* using a pulse amplitude modulated fluorometer (PAM; Walz GmbH, Germany). Photosynthesis vs Irradiance curves were modelled to derive maximum photosynthesis rate, the initial slope of the P v I curve (alpha) and the irradiance at which the onset of light limitation occurs (I_k) (Fig. 5).

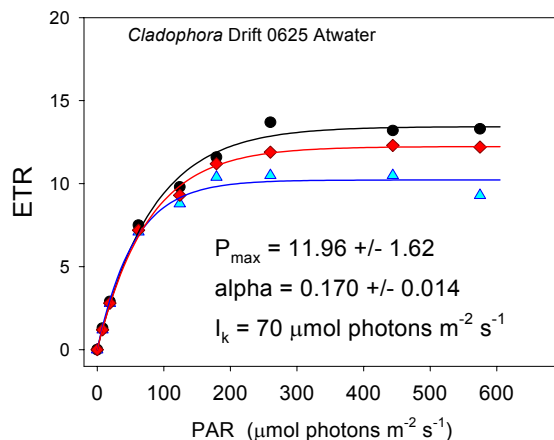


Figure 5. Three replicate measurements of P v I curves of *Cladophora* at the 10 m Atwater site on 25 June, 2004. The lines represent models of P v I relationship from Webb et al. (1974). Values of P_{max} , alpha and I_k are modelled means (std dev) of the three replicate curves.

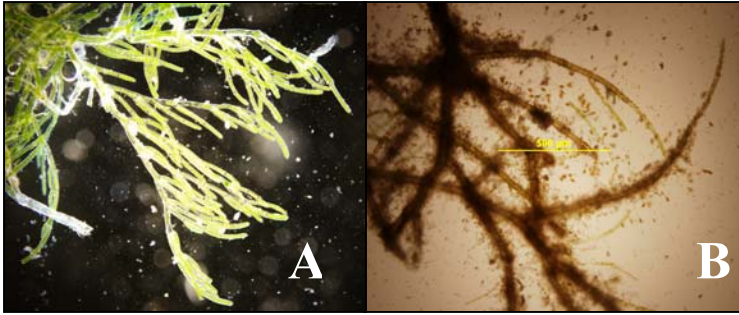


Figure 6. A. Healthy *Cladophora* collected from Bradford Beach Nov 2004. B. *Cladophora* collected from Atwater 10 m site Aug 2004, encrusted with epiphytic diatoms.

When we collected *Cladophora* from Lake Michigan at the 10 m Atwater site, macroscopically it appeared to be brown, rather than green. When observed under a microscope, the filaments of *Cladophora* were shown to be heavily epiphytised with, predominantly, diatoms (Fig. 6). This should be considered when thinking about what the *Cladophora* represents - a community of organisms rather than just the macroalga. The heavy epiphyte load may represent a stress to *Cladophora* as the epiphytes will shade light, and may strip the water of nutrients and inorganic carbon, reducing availability of those resources for *Cladophora*. This may be a stress factor contributing to the detachment of *Cladophora* from the hard substratum during the summer.

Overall Conclusions

- P limitation is evident in *Cladophora* based on
 - Consistently low soluble phosphate
 - AP activity
 - Nutrient stoichiometry (see Bootsma et al. chapter)
- If there are excess P inputs to nearshore Lake Michigan
 - it is being taken up immediately by benthic algae or phytoplankton
 - it is still insufficient to support unlimited *Cladophora* growth
 - P cycling within the benthos may be more important
- Light is probably limiting to *Cladophora* photosynthesis for the majority of the growing period
- Heavy epiphyte loads will further increase light limitation for *Cladophora* and may exacerbate nutrient limitation.

Literature Cited

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