Marine Biology 85, 269–278 (1985)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rhodophyta)

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Abstract

Porphyra perforata J. Ag. was collected from a rocky land-fill site near Kitsilano Beach, Vancouver, British Columbia, Canada and was grown for 4 d in media with one of the following forms of inorganic nitrogen: NO_3^-, NH_4^+ and NO_3^- plus NH_4^+ and for 10 d in nitrogen-free media. Internal nitrogen accumulation (nitrate, ammonium, amino acids and soluble protein), nitrate and ammonium uptake rates, and nitrate reductase activity were measured daily. Short initial periods (10 to 20 min) of rapid ammonium uptake were common in nitrogen-deficient plants. In the case of nitrate uptake, initial uptake rates were low, increasing after 10 to 20 min. Ammonium inhibited nitrate uptake for only the first 10 to 20 min and then nitrate uptake rates were independent of ammonium concentration. Nitrogen starvation for 8 d overcame this initial suppression of nitrate uptake by ammonium. Nitrogen starvation also resulted in a decrease in soluble internal nitrate content and a transient increase in nitrate reductase activity. Little or no decrease was observed in internal ammonium, total amino acids and soluble protein. The cultures grown on nitrate only, maintained high ammonium uptake rates also. The rate of nitrate reduction may have limited the supply of nitrogen available for further assimilation. Internal nitrate concentrations were inversely correlated with nitrate uptake rates. Except for ammonium-grown cultures, internal total amino acids and soluble protein showed no correlation with uptake rates. Both internal pool concentrations and enzyme activities are required to interpret changes in uptake rate during growth.

Introduction

Seasonal fluctuations in nitrogen supply in coastal environments impose nutrient-dependent growth patterns on most marine macrophytes (Chapman and Craigie, 1977; Gagné *et al.*, 1982; Rosenberg and Ramus, 1982). The study of the relationship between growth rate and nitrogen supply has been complicated by the fact that macroalgae store intracellular nitrogen, which can be utilized during times of nitrogen deficiency (Buggeln, 1974; Chapman and Craigie, 1977; Gerard, 1982; Rosenberg and Ramus, 1982). It has also been shown that nitrogen uptake rates (D'Elia and DeBoer, 1978) and nitrogen reserves (Bird *et al.*, 1982) in marine macrophytes can be influenced by past nitrogen supply (D'Elia and DeBoer, 1978; Probyn and Chapman, 1982).

This study is the first comprehensive evaluation of the effect of various forms of inorganic nitrogen and nitrogen starvation on more than one stage of nitrogen utilization (uptake, storage and assimilation). The examination of several phases of nitrogen utilization gives a more complete understanding of the control of nitrogen procurement. Regulation of nitrogen procurement and metabolism in response to a fluctuating nitrogen supply could be an adaptive advantage in an environment where the growth of macroalgae is nitrogen-limited.

Materials and methods

Species and culture conditions

Young non-reproductive *Porphyra perforata* J. Ag. (Rhodophyta) whole thalli were collected in early March 1982 from a rocky land-fill site on English Bay, Kitsilano, Vancouver, British Columbia, Canada. They were immediately transported to the laboratory and rinsed with filtered (0.45 μm) seawater.

Forty grams wet weight of plant material (whole thalli) were added to 10 litres of filtered (0.45 μm) enriched (all

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nutrients at *f*/20 minus nitrogen: Guillard and Ryther, 1962) nitrogen-deficient natural seawater in 12-litre flat-bottomed boiling flasks. This seawater had been previously stripped of nitrogen ($< 1.0\,\mu M$ nitrate and $< 0.2\,\mu M$ ammonium) by incubating 1 g wet weight of *Ulva* sp. per litre for 12 to 24 h at 10 °C at $150\,\mu E\,m^{-2}\,s^{-1}$. Eight cultures were incubated in a 10 °C cold room under an irradiance of $150\,\mu E\,m^{-2}\,s^{-1}$ provided by fluorescent lighting (Vita-lite) on a 12 h:12 h light:dark cycle. The cultures were stirred continuously with 5 cm magnetic stirring bars at 120 rpm. One culture was enriched with $50\,\mu M$ nitrate, another with $50\,\mu M$ ammonium and a third with $50\,\mu M$ ammonium plus $50\,\mu M$ nitrate. No nitrogen was added to the fourth culture. These nitrogen concentrations were chosen because they were high enough to allow maximum growth, but still fell within the range of field concentrations. Lower concentrations would also have supported maximum growth rates, but the nutrient would have been depleted too quickly in cultures with a high biomass. Duplicate cultures were maintained in order to produce sufficient biomass for subsequent tests. The first three cultures (nitrogen-sufficient) were maintained for 4 d and the starved culture was maintained for 10 d, with the media changed after 5 d.

The nitrate and ammonium concentrations in the flasks were monitored during the culture period and periodic additions were made to maintain concentrations at $50\,\mu M$. Five μmol $NaHCO_3$ was added for every μmol of nitrogen in the medium to ensure that the cultures did not approach carbon limitation.

Internal soluble-nitrogen content, uptake rates, and nitrate reductase activity, were measured each day at 11.00 hrs, 3 h after the beginning of the light period as described below.

Internal soluble nitrogen content

One gram wet weight of plant material was ground in a mortar with 25 ml hot water and 0.5 g sand (washed and ignited). The slurry was centrifuged at $2\,000 \times g$ for 6 min. Part of this extract was used to analyse for internal nitrate using a Technicon AutoAnalyzer. All extractions were done in triplicate. The efficiency of this extraction procedure was determined by re-extracting the material four times (Thomas, 1983).

The remaining portion of the above extract was analysed for soluble protein (Leggett-Bailey, 1967), ammonium (Davis *et al.*, 1973) and total amino acids (Lee and Takahashi, 1966). Due to the possibility of incomplete extraction and interference from other compounds during the colorimetric analysis, these results are not quantitative, but increasing or decreasing trends can be distinguished.

Nitrate and ammonium uptake experiments

Two grams wet weight of plant material (whole thalli) were placed in a 500 ml Erlenmeyer flask with 400 ml filtered enriched seawater (see "Species and culture conditions") with either $25\,\mu M$ nitrate, $25\,\mu M$ ammonium, or $25\,\mu M$ nitrate plus $25\,\mu M$ ammonium. The irradiance, provided by fluorescent lights (Vita-lite), was $300\,\mu E\,m^{-2}\,s^{-1}$. The medium was continuously stirred with a magnetic stirring bar at 120 rpm, producing an average current velocity of approximately 10 cm s^{-1}. The uptake medium was kept at 12 °C with a water-jacketed cooling system. The nitrate and ammonium concentrations in the uptake medium were monitored continuously for 30 min using a Technicon AutoAnalyzer (Davis *et al.*, 1973). An "initial" uptake rate determined over the first 10 to 20 min of exposure to the incubation medium (designated hereafter as V_{0-15}) was often followed by an increased uptake rate (an "induced" rate, designated hereafter as V_{15-30}). At the end of the incubation period, the thalli were removed and dried to a constant weight (24 to 48 h) at 60 °C in order to normalize the uptake rate to dry weight of the thalli.

Nitrate reductase activity

An *in vitro* nitrate reductase assay with the optimum assay conditions for *Porphyra perforata* was used (Thomas, 1983; Thomas and Harrison, in preparation). One gram wet weight of plant material was used per assay. These extracts were stored at −10 °C prior to analysis for soluble protein (Leggett-Bailey, 1967).

All analyses were done in triplicate. The complexity of methods and the absolute requirement for performing all the analyses at the same time of day made additional sampling impossible. Since patterns were complex, all curves were fitted by eye in order to show general trends.

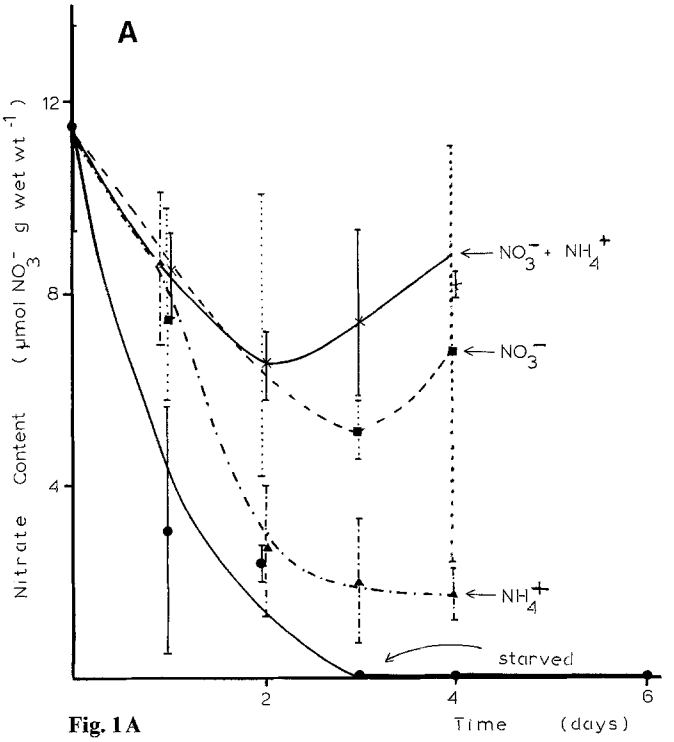

Fig. 1A

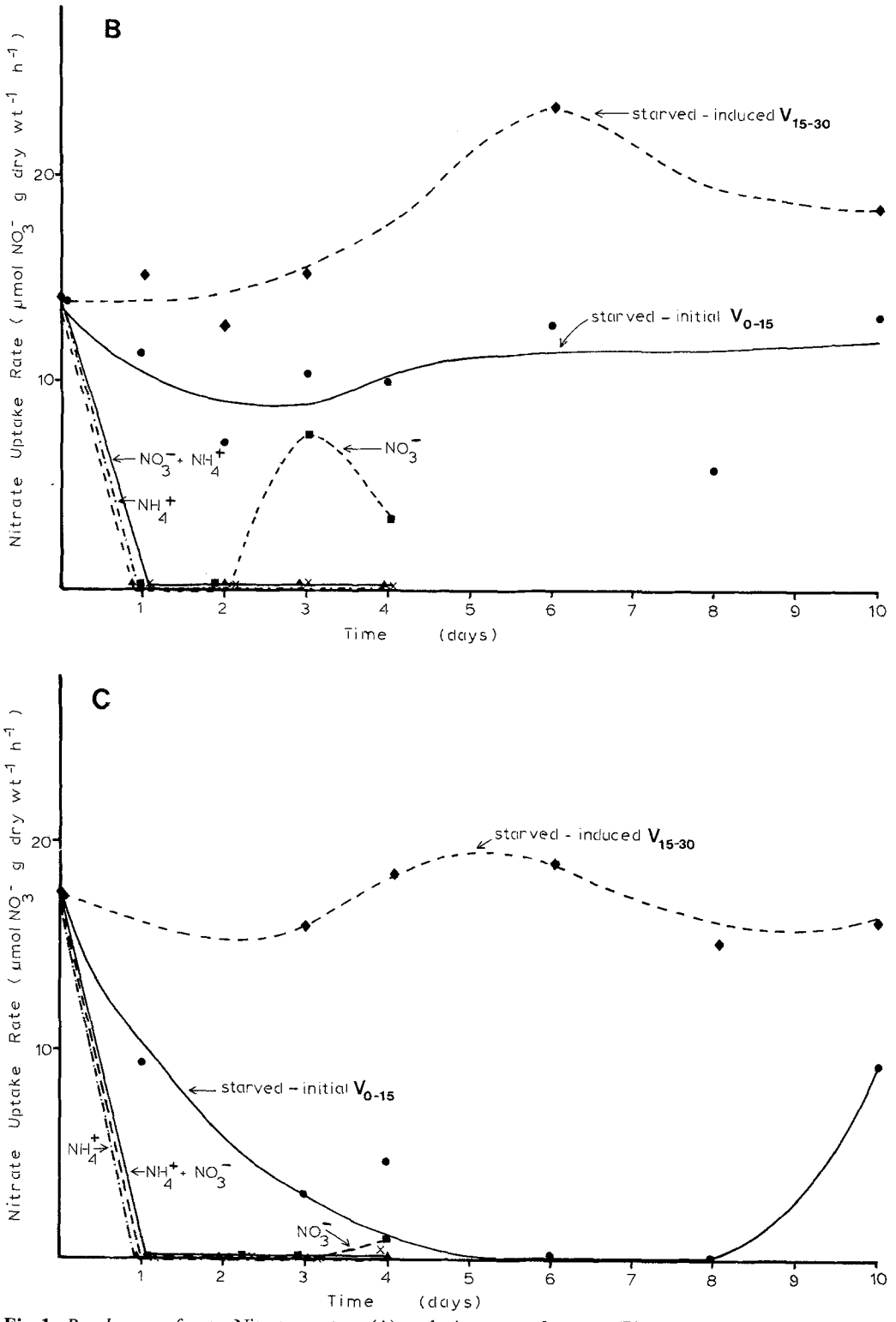

Fig. 1. *Porphyra perforata.* Nitrate content (A) and nitrate uptake rates (B) (C). Uptake rates were measured in the presence of 25 μM nitrate (B) or 25 μM nitrate plus 25 μM ammonium (C) in the uptake medium. Thalli were preconditioned for 0 to 10 d on 50 μM nitrate, 50 μM ammonium, 50 μM nitrate plus 50 μM ammonium, or nitrogen-starved. Starved-initial uptake rates (V_{0-15}) (–●–) refer to uptake rates over the first 15 min of the experiment, while starved-induced uptake rates (V_{15-30}) (- - ◆ - -) refer to rates after 15 min. Error bars represent ± one standard deviation

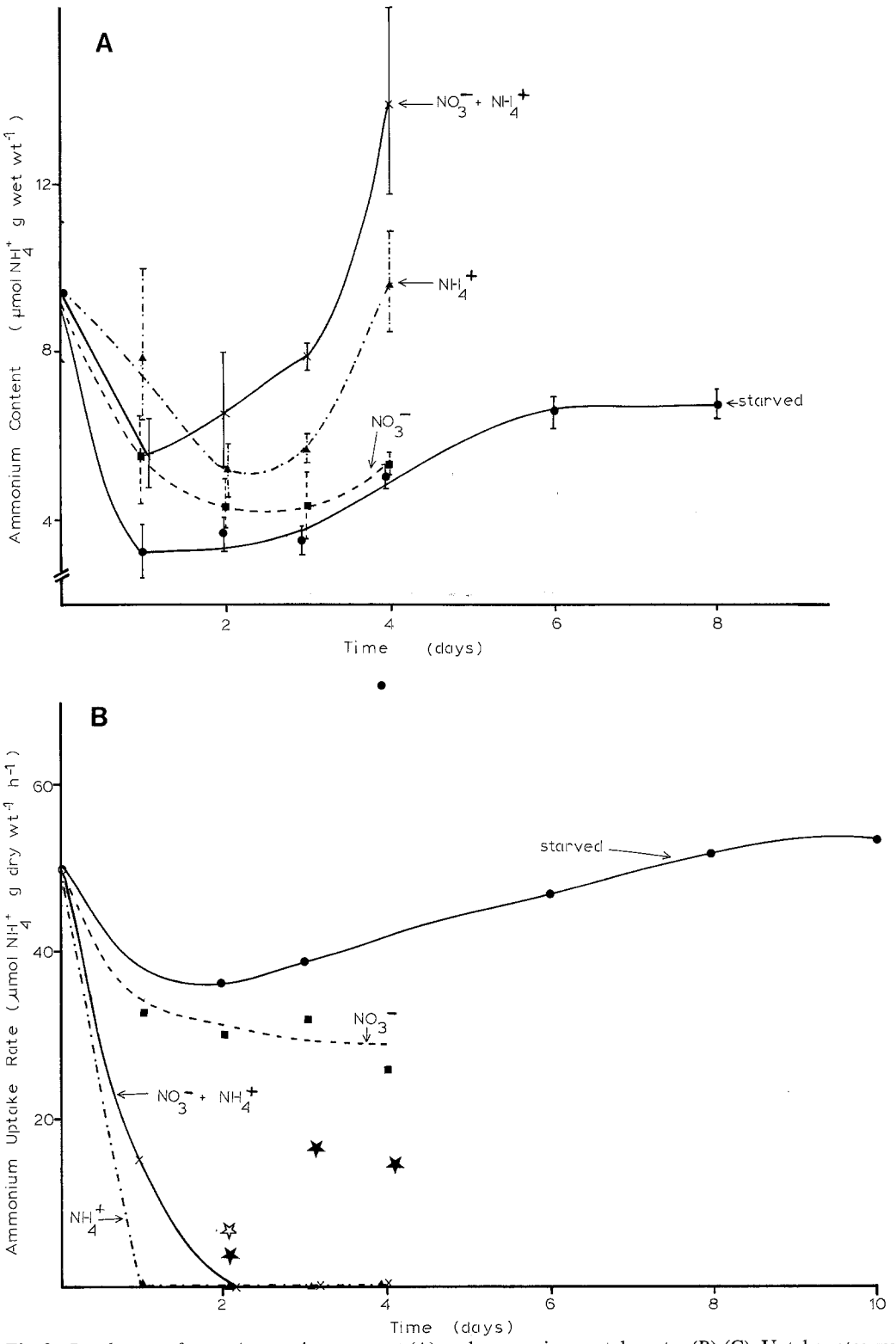

Fig. 2. *Porphyra perforata.* Ammonium content (A) and ammonium uptake rates (B) (C). Uptake rates were measured in the presence of 25 μM ammonium (B), or 25 μM ammonium plus 25 μM nitrate (C) in the uptake medium. Thalli were preconditioned for 0 to 10 d on 50 μM nitrate, 50 μM ammonium, 50 μM nitrate plus 50 μM ammonium, or nitrogen-starved. The ammonium and ammonium plus nitrate-grown thalli occasionally showed transient (< 5 min) unsustained uptake rates at the start of the uptake experiment. These rates are indicated by ★ and ☆, respectively. Error bars represent ± one standard deviation

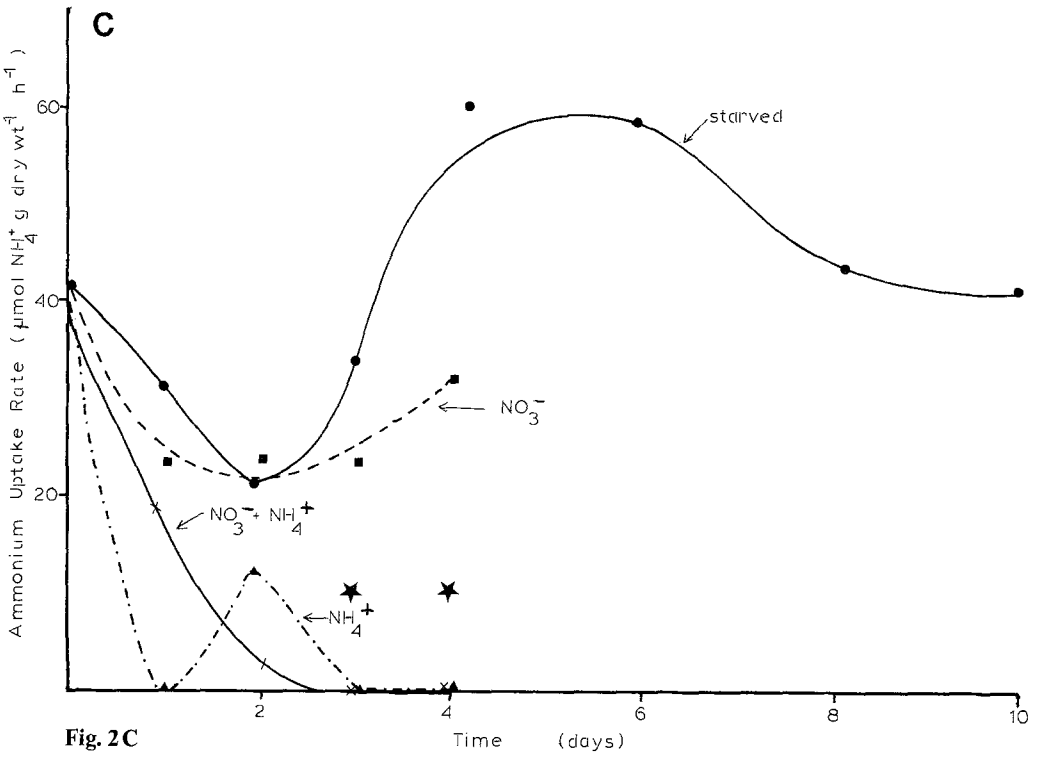

Fig. 2C

Results and discussion

Internal soluble nitrogen content

The internal nitrate content of the ammonium-grown and the starved *Porphyra perforata* decreased substantially within 48 h, and the internal nitrate content of the starved plants reached zero after 3 d (Fig 1A). The nitrate-grown plants and the nitrate plus ammonium-grown plants maintained mean internal nitrate levels not significantly different from initial values. The mean ammonium content of all extracts decreased during the first 2 d of culturing but only the content of starved plants was significantly below initial values (Fig. 2A). After 4 d, the ammonium content of the plants exposed to ammonium (the ammonium or nitrate plus ammonium cultures) was 2 to 3 times greater than that of the other plants. There were non-significant increases in the ninhydrin-positive material (assumed to be primarily amino acids) in all tissue extracts except for nitrogen-starved plants (Fig. 3A). The soluble protein contents of the plants from the various cultures were similar (Fig. 3B).

Nitrogen starvation caused a significant decrease in internal nitrate content in *Porphyra perforata*. Little or no decrease was observed in internal ammonium, total amino acids and soluble protein. Large and utilizable reserves of nitrate have been found in other seaweeds, especially the kelps (Chapman and Craigie, 1977; Wheeler and Srivastava, 1984), but are certainly not always present. Bird *et al.* (1982) and Rosenberg and Ramus (1982) found that amino acids and proteins were a more important rapidly utilizable nitrogen pool than nitrate in *Gracilaria foliifera*. On the other hand, Best (1980) showed that amino acid content of *Ceratophyllum demersum* was not affected by a growth-stimulating increase in either ammonium or nitrate in ambient seawater.

The internal nitrate content of the ammonium-grown plants did not reach zero, as it did in the starved plants. It is possible that in the ammonium-incubated plants ammonium was taken up and assimilated rather than the plant utilizing its pre-existing internal nitrate reserves. The nitrate reductase activity of the starved culture was still high even when the internal nitrate was reduced to undetectable levels (Fig. 3C). The marked decrease in internal nitrate content was most likely due to the increase in reductase activity which was stimulated by 3 d of nitrogen starvation.

Nitrate reductase activity

The initial nitrate reductase activity of the plants from the field was 72 μmol NO_2^- g^{-1} protein h^{-1} (Fig. 3C). Nitrogen-starved plants exhibited an increase in activity up to Day 3, after which activity decreased rapidly. A similar significant increase occurred among the nitrate-grown plants on the third day. Nitrogen starvation also caused a transient increase in nitrate reductase activity in phytoplankton (Morris and Syrett, 1965). The subsequent decrease in activity following more severe nitrogen starvation results in inactivation of the enzyme system which is metabolically costly for the cell to maintain during a time of nitrogen stress. Yet, even under 10 d of nitrogen starvation, *Porphyra perforata* maintained a low level of nitrate reductase activity. A transient increase in nitrate reductase activity in nitrate-grown plants has been observed previously in *Giffordia mitchellae* (Weidner and Kiefer, 1981).

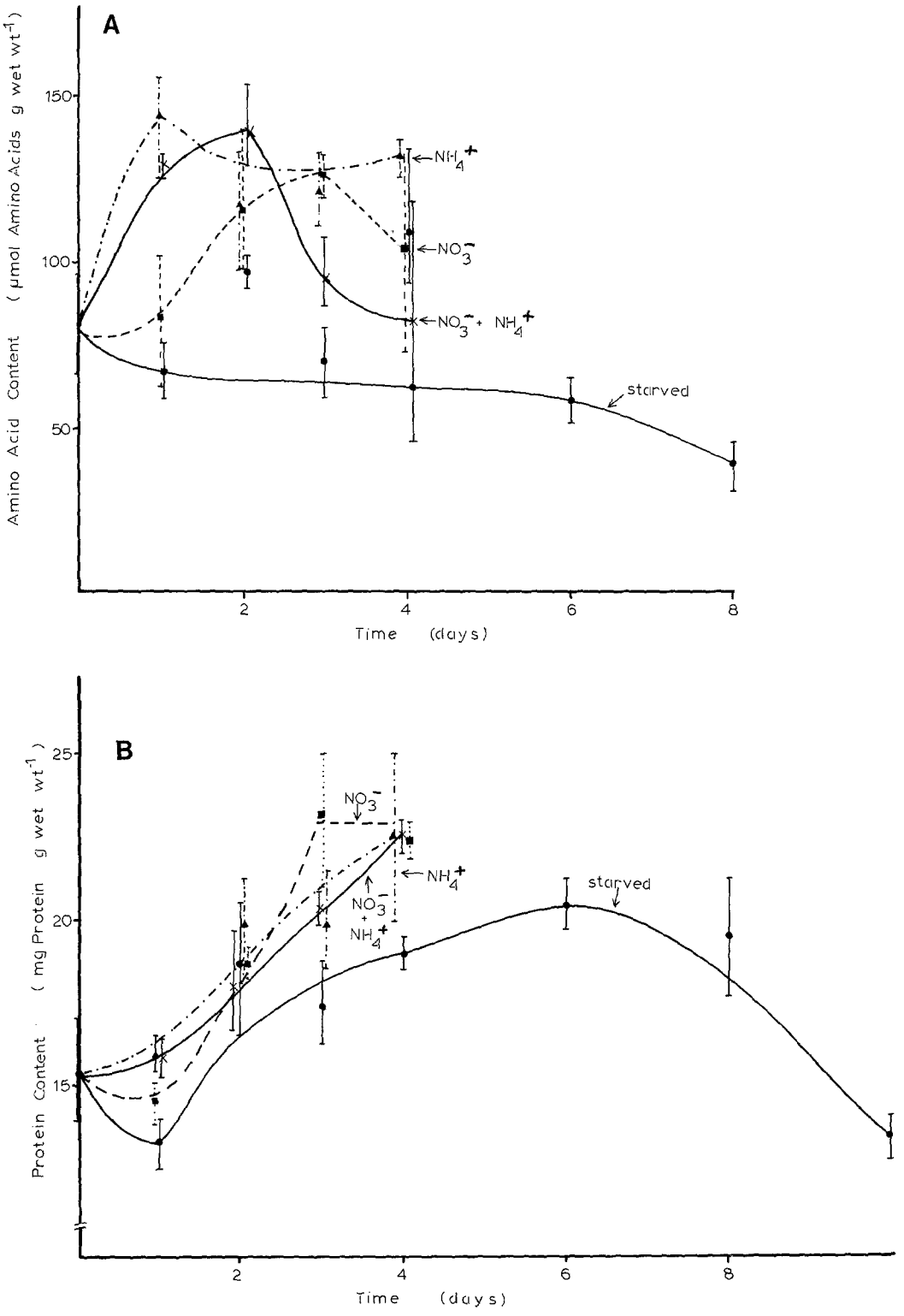

Fig. 3. *Porphyra perforata.* Soluble amino-acid content (A), soluble protein content (B), and nitrate reductase activity (C). Thalli preconditioned for 0 to 10 d on 50 μM nitrate (- - ■ - -), 50 μM ammonium (- · ▲ · -), 50 μM nitrate plus 50 μM ammonium (– × –), or nitrogen-starved (–●–). Error bars represent ± one standard deviation, $n = 3$

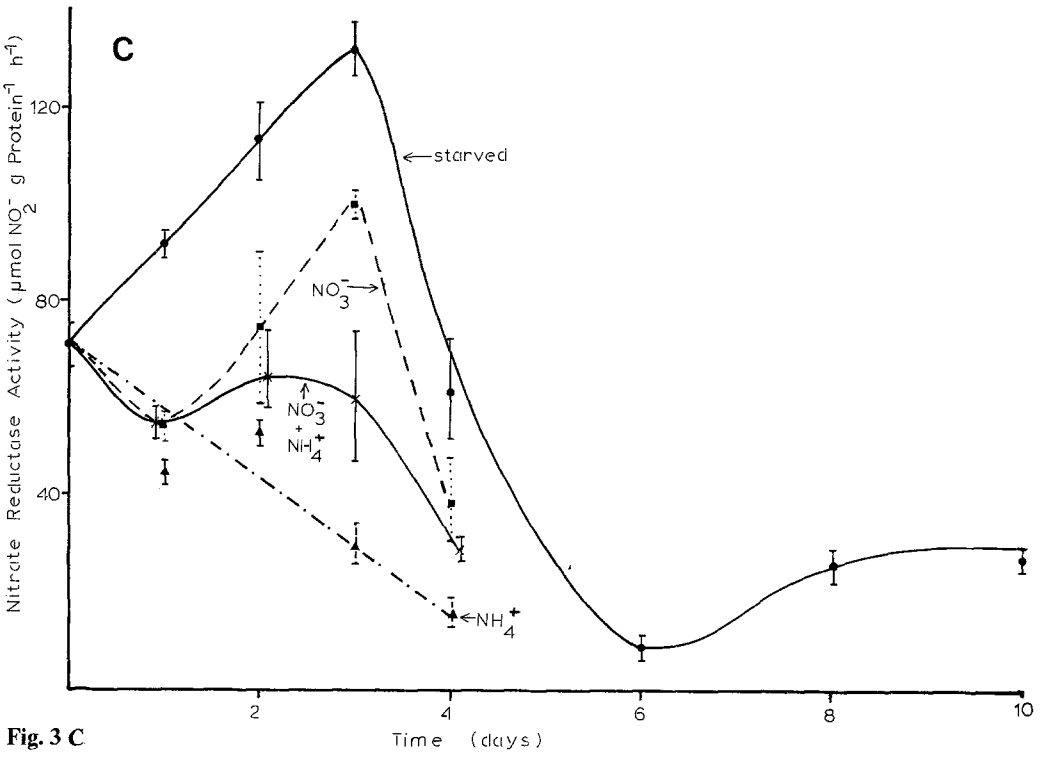

Fig. 3 C

Growth on ammonium resulted in a rapid decrease in nitrate reductase activity (Fig. 3 C). The plants grown on nitrate plus ammonium maintained a nitrate reductase activity of approximately 60 μmol NO_2^- g^{-1} protein h^{-1} for the first 3 d, which was similar to that of the field plants.

Nitrate reduction was not "turned off" as rapidly as nitrate uptake by the presence of ammonium (cf. Figs. 1 C and 3 C). A similar delay has been shown in phytoplankton (Dortch *et al.*, 1979). Eppley *et al.* (1969) found that ammonium inhibited nitrate reductase activity in phytoplankton. This inhibition has also been observed in higher plants (MacKown *et al.*, 1982) such as corn, and in the marine macrophyte *Giffordia mitchellae* (Weidner and Kiefer, 1981).

Nitrate uptake rates

Nitrate uptake in the starved plants was not constant over the 30 min period when nitrate uptake was being continuously monitored. For the first 15 min, nitrate uptake rates (V_{0-15}) were low, but then nitrate uptake rates increased (V_{15-30}), presumably due to an induction phenomenon (Fig. 1 B, C). It appears that the uptake system for nitrate does not remain fully active in a nitrogen-starved thallus, but that it can be rapidly reactivated by a pulse of nitrate. This has been observed in phytoplankton (Dortch *et al.*, 1982), and it is assumed to be due to a reallocation of metabolic energy during starvation. It is certainly an adaptive advantage for a nitrogen-starved plant to have nitrate uptake readily inducible. This induction of nitrate uptake rate was not seen in nitrogen-replete plants, although the field plants showed no nitrate uptake for the first 1 to 2 min after nitrate was added to the uptake medium.

The nitrate uptake rate of *Porphyra perforata* plants taken directly from the field was 13 to 18 μmol g^{-1} dry wt h^{-1} (Fig. 1 B, C). When plants were grown on saturating concentrations of nitrate for 4 d, nitrate uptake increased during Days 3 and 4 if ammonium was not present in the uptake medium (Fig. 1 B). This delay in the induction of nitrate uptake in nitrate-grown cultures might be associated with the increased nitrate reductase activity occurring on the third day (Fig. 3 C). Prior to this increase in nitrate reductase activity, if nitrate were taken up it would only be slowly assimilated, resulting in the filling of nitrate pools and a subsequent reduction in the rate of nitrate uptake.

The presence of ammonium in the uptake medium inhibited the initial nitrate uptake rate (V_{0-15}) for thalli starved for 8 d (Fig. 1 C). After 10 d of nitrogen starvation, this inhibition appeared to be overcome. Preferential uptake of ammonium may reduce the energy required by the plant because it is not necessary to reduce nitrate to ammonium (Syrett, 1962). Partial inhibition of nitrate uptake by ammonium is common in marine macrophytes (D'Elia and DeBoer, 1978; Haines and Wheeler, 1978; Hanisak and Harlin, 1978; Gordon *et al.*, 1981) and phytoplankton (Conway, 1977; McCarthy *et al.*, 1977; Maestrini *et al.*, 1982). The results of the present study suggest that this no longer occurs after a certain period of nitrogen starvation. The presence of ammonium inhibited initial nitrate uptake (V_{0-15} but not induced nitrate uptake

(V_{15-30}). This suggests that initial and induced nitrate uptake do not involve the same uptake mechanism. Harrison *et al.* (in preparation) observed a similar response in *Laminaria groenlandica.*

At the end of the 30 min incubations in the nitrate uptake experiments, ammonium concentrations in the uptake flasks ranged from 10 to 15 μM. Concentrations below this may not have had an inhibitory effect on initial nitrate uptake (V_{0-15}). D'Elia and DeBoer (1978) found a similar response in *Gracilaria tikvahiae* incubated in 5 μM ammonium and Harrison *et al.* (in preparation) made the same observation in the kelp *Laminaria groenlandica.* Much lower ammonium concentrations (1 μM) can cause inhibition of nitrate uptake in phytoplankton (Conway, 1977; McCarthy *et al.*, 1977).

Ammonium uptake rates

The initial ammonium uptake rate (V_{0-15}) in the starved culture was high, but this rate was often short-lived and decreased after 10 to 15 min in the ammonium uptake medium. This was the opposite pattern to nitrate uptake in the nitrate uptake medium (Fig. 4). This transient increase in ammonium uptake has been shown for nitrogen-starved *Gracilaria tikvahiae* (D'Elia and DeBoer, 1978) and phytoplankton (Conway *et al.*, 1976). Enhanced initial ammonium uptake also occurred in both the ammonium-grown and the ammonium plus nitrate-grown plants. The reason for the enhanced initial uptake rate in these latter plants is unknown. It has been suggested that ammonium uptake may be controlled by a small internal pool which fills or empties rapidly (Conway *et al.*, 1976; Dortch, 1980). The depletion of this pool might have occurred during the time after the thalli were removed from the ammonium culture medium and before the start of the uptake experiment. However, this period was usually only of a few minutes, a time which is considered insufficient to deplete nitrogen reserves. Such a surge uptake of ammonium in phytoplankton (Conway *et al.*, 1976; McCarthy and Goldman, 1979; Turpin and Harrison, 1979) and other macrophytes (Thomas, 1983) usually takes several hours to develop after ammonium is depleted from the medium.

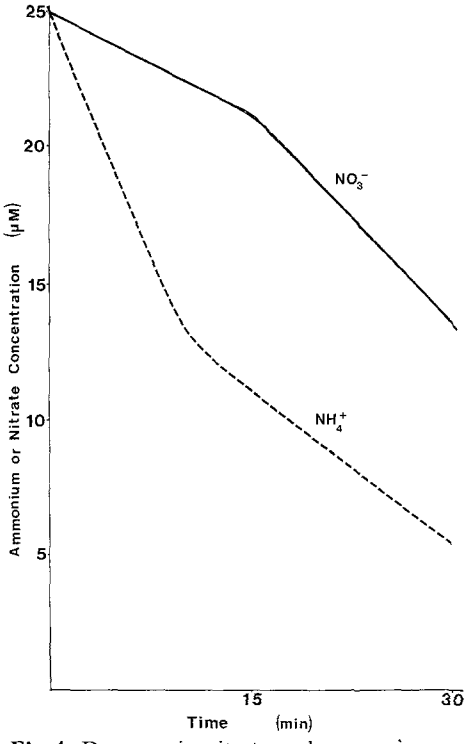

Fig. 4. Decrease in nitrate and ammonium concentrations in uptake medium due to nitrate and ammonium uptake of *Porphyra perforata* thalli after 6 d of nitrogen starvation Nitrate and ammonium concentrations were monitored continuously by a Technicon AutoAnalyzer

The ammonium uptake rates of the cultures grown on ammonium or ammonium plus nitrate, decreased dramatically within 1 and 2 d, respectively (Fig. 2 B, C). After 2 to 4 d of preconditioning, both cultures showed a brief (< 5 min) initial period of ammonium uptake (approximately 10 μmol g^{-1} dry wt h^{-1}) at the start of the ammonium uptake experiments. These brief periods of uptake and the normal levels of nitrate reductase activity indicate that these cultures were still healthy, but probably nitrogen-saturated and therefore maintaining very low (undetectable over 30 min) nitrogen uptake rates.

The ammonium uptake rates for the plants taken directly from the field ranged from 40 to 50 μmol g^{-1} dry wt h^{-1} (Fig. 2 B, C). The field plants had higher uptake rates than the nitrogen-replete cultures in the laboratory. The reason for this is unknown. It is unlikely that the field plants were partially nitrogen-limited, considering the relatively high nitrate and ammonium concentrations (17 and 4 μM, respectively) in the surface water at the time of collection.

The cultures grown on nitrate alone maintained an ammonium uptake rate similar to that of the field plants. This suggests that the mechanism to maintain high ammonium uptake rates that is normally associated with nitrogen-deficient plants, was also present in plants grown only on nitrate. This has also been observed for marine phytoplankton (Syrett, 1962). It is possible that nitrate reduction is the rate-limiting process (Dortch *et al.*, 1982). This suggests that the rate of supply of ammonium via nitrate uptake and reduction is much less than the rate of supply of ammonium by direct uptake. Generally, ammonium uptake rates were higher than nitrate uptake rates. One of the later steps in nitrogen metabolism which is not activated when nitrate reduction is slow, may be the factor maintaining high ammonium uptake rates in nitrate-grown plants. It has been postulated that ammonium uptake may be controlled by the level of certain amino acids (Dortch, 1980). Neither total amino acid content nor soluble protein content appeared to be the critical controlling factor, although the concentration of specific amino acids would have to be measured to test Dortch's suggestion. Ammonium is apparently a better nitrogen source for growth of marine macrophytes than nitrate (Yamada, 1961; DeBoer *et al.*, 1978), since nitrate must be

converted to ammonium before it is assimilated and this reduction requires energy (Nicholas, 1959). If nitrate reduction is the rate-limiting step, then nitrate pools should fill and nitrate uptake should be reduced to match the rate of nitrate reduction, assuming that the maximum level of internal nitrate is fixed. The observation that nitrate uptake was dramatically reduced after 24 h supports this suggestion.

This study illustrates not only the effect of nitrogen supply on several stages of nitrogen utilization, but also the interactions between these stages (uptake, accumulation, assimilation). Uptake rates were influenced by the form and amount of nitrogen supplied. Nitrate uptake was inversely correlated with internal nitrate. In the case of nitrogen-replete plants, nitrate uptake was correlated with nitrate reductase activity which should be influenced by the presence of ammonium. Further study on specific amino acid pools and enzyme activities will aid the interpretation of changes in uptake rates. Identification of such interactions are necessary to understanding the control of nitrogen procurement.

Acknowledgements. This research was supported by the Natural Science and Engineering Research Council of Canada (NSERC). An NSERC Postgraduate fellowship supported one of us (TET) during this research period. We acknowledge the use of facilities at Bamfield Marine Station, British Columbia, for some experiments and thank P. A. Thompson for his assistance with the AutoAnalyzer.

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Date of final manuscript acceptance: December 27, 1984.
Communicated by N. D. Holland, La Jolla