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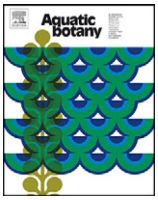

# Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies

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## ABSTRACT

An important life history trait of macroalgae species is the physiological ability to cope with nutrient limiting conditions, which seasonally occur in temperate coasts while other environmental factors are adequate (*e.g.*, sufficient light). Nitrogen (N) and Phosphorus (P) uptake kinetics and field growth limitation were investigated in the perennial *Bifucaria bifurcata*, the opportunistic *Ulva intestinalis*, and the summer-annual *Nemalion helminthoides* from Asturian coasts (N Spain). We performed 4 nutrient uptake experiments (ammonium, nitrate, nitrate + ammonium, and phosphate) and monitored the growth and N content of field individuals in the presence/absence of artificial nutrient supply to assess potential growth limitations. *B. bifurcata* was not actively growing during summer thus low nutrient demands probably relied on stored pools and/or the low background nutrient levels in seawater, as generally observed for perennials. Corresponding N content and uptake rates in this species were the lowest. The opportunistic *U. intestinalis* showed kinetics suitable for assimilating N quickly at high external concentrations in order to fulfill the high nutrient demands that support its fast-growing strategy. This response is well adapted to seasons and sites of high nutrient loading but signs of nutrient starvation during summer (decreasing growth and N content) were found in the pristine studied area. *N. helminthoides* showed an intermediate response in terms of thallus N content and uptake affinity, together with an inducible activation of nitrate uptake. This response assured the uptake of transient nutrient pulses without the nutrient storage response of perennials, or the costly enzymatic machinery of opportunistics. This allows *N. helminthoides* to effectively exploit low background nutrient conditions interrupted by transient peaks during spring–summer, when most ephemerals found difficulties to survive and perennials suspend their active growth. P uptake did not differ greatly between species suggesting its secondary importance compared to N in the tested algae.

## 1. Introduction

Low nutrient availability is one of the major environmental factors limiting macroalgal growth in temperate areas (Hanisak, 1979; Conolly and Drew, 1985) playing a *bottom-up* control of species composition (*e.g.*, Lotze et al., 2000; Worm and Lotze, 2006). Particularly during summer, macroalgae experience periods of very low ambient N and P concentrations. The way a species overcomes the fluctuating nature of nutrient supply depends on its ability to take up and assimilate nutrients, store these in nutrient-rich compounds, and on the capacity to sustain maximal growth with low nutrient demands (Fujita, 1985; Pedersen and Borum, 1996, 1997; Pedersen et al., 2010). This is a crucial part of the ecological strategy adopted by a seaweed (but see Lotze and Schramm, 2000), in turn closely related to other algal traits, for example physiological responses to optimize photosynthesis or defences against grazers, that involve the synthesis of N-rich metabolites (*e.g.*, Toth et al., 2007; Figueroa et al., 2010). In terms of nutrition, we found different strategies of slow-growing perennials, which develop large nutrient stored pools, whereas fast-growing opportunistics show remarkably nutrient uptake potential to profit from conditions of high nutrient supply. Strategies of other annuals, particularly those that develop during late spring to summer in the absence of significant nutrient supplies, are however poorly understood (reviewed in Sears and Wilce, 1975).

Slow-growing perennials rely on the N and P stored from autumn and winter (when nutrient availability in seawater is high) for growing during spring as daylength and temperature increase. Then they show reduced growth rates during summer, lowering their nutrients demands when nutrient conditions become

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*Abbreviations:* DW, dry weight; FW, fresh weight; SE, standard error.

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unfavourable, and eventually profiting from high light by developing Carbon (C) reserves (*e.g.*, Martínez and Rico, 2002). This strategy allows perennials to be relatively tolerant to the nutrient summer limitation reported for temperate areas taking advantage of other benign conditions, particularly light availability (Fujita, 1985; Wheeler and Björnsäter, 1992; Lüning, 1993; McGlathery et al., 1996; Martínez and Rico, 2002). As a result, perennials are thought to gain dominance in pristine temperate rocky shores (Pedersen and Borum, 1997; Pedersen et al., 2010).

On the other hand, opportunistic ephemeral macroalgae have high nutrient demands due to their fast growth and thus tend to allocate more energy into high-affinity uptake systems not developing large stored nutrient pools (Pedersen and Borum, 1997; Lotze and Schramm, 2000; Smit, 2002; Phillips and Hurd, 2003; Pedersen et al., 2010). The uptake and assimilation of nutrients in these species are coupled in order to sustain high growth and reproductive maturation, enhancing the synthesis of aminoacids and proteins, and other key nutrient-rich organic molecules (see McGlathery et al., 1996; and references therein). This ecological strategy is well suited to seasons of high nutrient and light supply and adequate temperature, such as late winter to spring in temperate areas. And also to sites of high nutrient loading, normally in the vicinity of waste-water discharges and in eutrophic-polluted areas, eventually resulting in macroalgal blooms in such impacted locations at variable moments over the year (Morand and Briand, 1996; Moran and Merceron, 2005; Worm and Lotze, 2006). Nevertheless, in temperate pristine coasts their presence is normally limited by the low nutrient background levels in seawater during summer, insufficient to fulfill opportunistics' high nutrient demands (Fujita, 1985; Pedersen and Borum, 1996, 1997; Pedersen et al., 2010).

Other annuals sustain active growth during late spring to summer, attaining their maximal size and reproductive development during the most unfavourable seasons in terms of nutrition. These *summer-annual* species take advantage of benign light conditions but cope with nutrient limiting levels that prevent the active growth of perennials, or even the survival of many opportunistic ephemeral macroalgae (Sears and Wilce, 1975; Kapraun and Zechman, 1982; Lewis, 1983; Lindstrom et al., 1999). Summer-annuals achieve this response without significant nutrient stored pools since they perennate as microscopic phases during the rest of the year (*e.g.*, Sears and Wilce, 1975). They are not bloom-forming species, nor associated with pollution incidents, suggesting a different ecological strategy from opportunistic algae. Previous research suggests an endogenous control of their life cycle regulated by photoperiod aimed to optimize favourable high light conditions (Pato et al., 2011). However their nutritional strategy and potential nutrient limitation while developing in the field, their responses to available nutrient sources, or their short-term nutrient storage ability remain largely unknown (but see Guo and Mathieson, 1992; Kiirikki and Blomster, 1996; Pato et al., 2011).

In this study, the N and P uptake kinetics and field growth limitation of three intertidal seaweeds showing the above mentioned ecological strategies were comparatively investigated. The target species are common in the temperate North Atlantic (Lüning, 1990). *Bifurcaria bifurcata* R. Ross (Cystoseiraceae, Phaeophyta) represents the perennial slow-growing strategy, and is frequently found forming almost monospecific stands in rocky sites in northern Spain. It is thus considered a foundation species of the intertidal community. *Ulva intestinalis* Linnaeus (Ulvaceae, Chlorophyta) is an opportunistic bloom-forming ephemeral macroalgae (Fong et al., 1998; Lotze and Schramm, 2000), fast-growing at early spring in the studied area before the decline of nutrients levels from May onwards (Rico and Fernández, 1996; Martínez and Rico, 2002). It remains however in polluted areas thorough the summer in locations close to the studied site (authors pers. obs.). Finally, *Nemalion helminthoides* (Velley) Batters (Liagoraceae, Rhodophyta) shows a summer-annual life cycle (*e.g.*, Dixon and Irvine, 1977), with a macroscopic phase growing fast and reproducing from mid May to early August in northern Spain, thus facing very low nutrient conditions (Pato et al., 2011). We performed 4 separate nutrient uptake experiments (ammonium, nitrate, nitrate + ammonium, and phosphate) and also assessed the effect of overcoming nutrient limitation for growth and nutrient thallus content under natural conditions by *in situ* adding fertilizer pellets.

**Table 1**
Range of initial nutrient concentrations, and date of execution of the 4 uptake experiments. Each species (*Bifurcaria bifurcata*, *Ulva intestinalis* and *Nemalion helminthoides*) was subjected to 12 different concentrations of the assayed nutrient.

| Nutrient source | Initial nutrient concentrations assayed | Date of uptake experiment |
|---|---|---|
| Ammonium | 0.70 (0.02) to 59.15 (0.90) | 07-June-05 |
| Nitrate | 2.02 (0.02) to 68.41 (0.08) | 11-June-05 |
| Nitrate + ammonium | | |
| Nitrate | 0.59 (0.01) to 63.90 (0.13) | 15-June-05 |
| Ammonium | 2.81[a] (0.04) | |
| Phosphate | 0.05 (0.002) to 30.11 (0.51) | 19-June-05 |

Values in brackets represent SE. $n = 3$ except otherwise stated.

[a] $n = 42$, significant ammonium levels were measured after 120 or 210 min depending on the species.

We hypothesized a distinct strategy of the three species to profit from available nutrients, in particular from pulses associated with upwelling events and fresh water runoff that occurs in the studied area (*e.g.*, Botas et al., 1990; Martínez and Rico, 2008). We expect the perennial *B. bifurcata* to benefit only little from such pulses in contrast to the opportunistic *U. intestinalis.* We expect however the former to be growth-limited during summer in response to its high nutrient demand for growth. Finally, our previous research suggested that the survival of *N. helminthoides* in the studied area is based on its ability to profit from such pulses (Pato et al., 2011).

## 2. Materials and methods

### 2.1. Uptake experiments

### 2.1.1. Collection of samples and preincubation

Vegetative fronds of the three species were collected the day before assaying their uptake kinetics (4 dates in June 2005), during the morning low tide, from the rocky intertidal site of *La Concha de Artedo* (Asturias, northern Spain, 43°34′N, 6°11′W). The material was transported to the laboratory (4 °C, in darkness) after collection, and then preincubated in 8 L glass flasks filled with aged (i.e. low nutrients), filtered (AcroPak 1000 capsule W/supor® membrane 0.8/0.2 μm) seawater supplemented with half-strength Von Stosch's medium (Guiry and Cunningham, 1984) but the specific nutrient to be assayed (N for ammonium and nitrate and P for the phosphate uptake experiments respectively). Flasks were left in a walk-in cold room (15 °C, in darkness) before the experiment, i.e. one day, to let algae to acclimate to the experimental conditions and avoid limitation by the not-assayed nutrients. Adequate mixing was assured with air pumps.

### 2.1.2. Experimental procedure

To evaluate nutrient uptake kinetics, we measured the decrease of nutrient concentration at different time intervals in Erlenmeyer flasks filled with 200 mL of medium set at 12 different initial substrate concentrations (Table 1). Four experiments were performed following the protocol in Martínez and Rico (2004): ammonium, nitrate and phosphate *alone* experiments, and nitrate in the presence of a fixed concentration of ammonium representing mean field conditions (Table 1). Several fronds of each species (together about 1 g FW) were put into the