Experimental assessment of the macroalgae *Ascophyllum nodosum* and *Fucus vesiculosus* for monitoring N sources at different time-scales using stable isotope composition

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Abstract

Stable isotope composition of brown macroalgae has been widely used to monitor N loading during the last decades but some of the required assumptions when using them to detect anthropogenic inputs remain untested. In this study several experiments were run with two key species, *A. nodosum* and *F. vesiculosus*, to determine internal nitrogen isotope dynamics. First, the equilibration of the isotopic values of the different parts of the thallus of these species was tested by growing them under different water sources. Then, nitrate uptake capacity and N transport along the frond were tested by ^{15}N enrichment experiments. The results indicate that although the growing tips had the highest uptake rates, older parts of the frond of both species have the capacity to incorporate N at low rates. No evidence of N transport along the thallus, from the tip to the basal segment of the frond or the converse was found. These results show that the growing tips of these macroalgae can be used to monitor N loadings at time scales from weeks (*F. vesiculosus*) to months (*A. nodosum*). The use of non-growing parts of the thallus to do retrospective studies cannot be recommended because of their measurable exchange of N with the surrounding water.

Keywords: stable isotopes, enrichment, growth rate, Phaeophyceae, DIN

1. Introduction

Concern with coastal eutrophication has increased in the last decades due to higher N loading associated with the growing human population in these areas. The ratio of the stable isotopes of N (^{15}N:^{14}N) in macroalgal tissues allows detecting the presence of anthropogenic N that is available for macroalgae in coastal waters, but also allows estimating the intensity of the effluents and detect disturbances before alteration in structure and function occur in the ecosystem (McClelland et al., 1997; McClelland and Valiela, 1998a, 1998b; Costanzo et al., 2001; Gartner et al., 2002; García-Sanz et al., 2010, 2011; Carballeira et al., 2013). The basis for the use of macroalgae and other biota for monitoring anthropogenic water sources is that different water sources may show characteristic isotopic signatures (Xue et al., 2009) due to different fractionation processes occurring through the N cycle (Montoya, 2007). All the different sources of N may also alter the baseline $\delta^{15}N$ of the macroalgae, as they use N as part of their metabolism, to synthetize structural components or to gain energy for growth (Gruber, 2008).

Among macroalgae, Fucaceae, as *Fucus vesiculosus* and *Ascophyllum nodosum*, have been widely used for monitoring loads of N and other substances (e.g. heavy metals) (Viana et al., 2010, 2011). As these species show apical growth, the tips have been traditionally used in monitoring studies. The growing tips can be feasibly related with previous weeks of growth (Viana et al., 2014, accepted) and hence with the environmental status at a particular time. First studies with Fucaceae were focused on detecting wastewater effluents (Hobbie et al., 1990; Savage and Elmgren, 2004) but later, they were reliable used to discern anthropogenic from natural sources (García-Sanz et al., 2010, 2011; Carballeira et al., 2013; Viana and Bode, 2013). Their high tolerance to broad salinity ranges have also enable to study the status of estuaries and

65 rias in both native populations (Bode et al., 2011, 2014; Viana et al., 2011; Raimonet et
66 al., 2013) and transplant studies in the field (Deutsch and Voss, 2006).

67 In any case, long-term monitoring is needed to track the ecological status of the
68 ecosystem or to feasibly interpret data obtained at a particular moment. Obtaining a
69 reliable and long-time monitoring series would require of a careful sampling plan
70 implemented during decades. Consequently there are only few examples of time series
71 using stable isotopes (Viana et al., 2011). That is the reason why some authors have
72 taken advantage of the long lifespan of the species considered, up to 15 yr in the case of
73 *A. nodosum* (Niell, 1979), and their apical growth to do retrospective studies. If growth
74 rates are known (Viana et al., 2014, accepted), different segments along the frond can be
75 related with past environmental or water conditions (Savage and Elmgren, 2004;
76 Raimonet et al., 2013; Carballeira et al., 2014). Moreover, *A. nodosum* fronds develop a
77 gas bladder in the tip that generally occurs once a year (David, 1943; Viana et al.,
78 2014). This annual bladder enables estimation of the minimum age of an individual and
79 definition of its annual growth (Niell, 1979; Viana et al., 2014). Retrospective analysis
80 would allow reducing the sampling effort in monitoring programs (Carballeira et al.,
81 2014).

82 The use of stable isotopes in the growing tips of these species for monitoring N
83 loadings requires some assumptions related to their physiology. For instance, net
84 fractionation processes (i.e. the preferential use of light against heavy isotopes) in
85 macroalgae are poorly understood. Fractionation during uptake in macroalgae is the best
86 studied. Experimental studies on different macroalgal species demonstrated that, at least
87 those macroalgae, did not exhibit concentration dependent N isotope fractionation
88 (Cohen and Fong, 2005; García-Sanz, 2009; Dudley et al., 2010). But there is no
89 information about fractionation processes during the subsequent processes within the

tissues, as absorption, accumulation or release of nitrogen. This is important as if fractionation factor is not known; the isotopic values in macroalgae can lead to misinterpretation of the contribution of anthropogenic sources (Bode et al., 2014).

The main assumption of retrospective studies is that only the growing tips of the thallus take up nitrogen and, therefore, the isotopic composition of a given section of the thallus would reflect the isotopic composition of the dissolved nitrogen in the surrounding water at the time of growth. To fully interpret the data obtained in these studies, some questions need to be answered. First, Fucaceae do not have specific transport tissues, but the pores of the sieve plates should enable a continuous system of cytoplasm for longitudinal translocation of materials (Moss, 1983). There is experimental evidence of such transport of organic ^{14}C, ^{86}Rb or ^{32}P (Penot and Penot, 1979; Diouris and Floc´h, 1984; Raven, 2003). If transport of nitrogen along the thallus also takes place, it would directly affect the retrospective identification of past nitrogen sources. Second, most studies assume that isotopic composition of tissues does not change for at least several months, given that these species generally show low variability in $\delta^{15}N$ values at monthly time scales (Gartner et al., 2002; Raimonet et al., 2013), but no data of N-specific uptake and turnover rate were available for this species.

To assess the feasibility of using *A. nodosum* and *F. vesiculosus* for isotopic differentiation of local N sources, two sets of experiments were made under laboratory conditions. The first experiment aimed to determine the equilibration of N isotopes in the growing tips and older parts of the fronds by growing them under water with different N origins. The second experiment aimed to detect nitrogen transport along their thalli and to test if all the parts of the frond have the capacity of taking up NO_3^- by using artificially ^{15}N-enriched water. The latter approach also allowed the estimation of N turnover rates in different sections of the thallus.

2. Material and Methods

2.1. Experiment 1: N isotope equilibration

Water samples –The first laboratory experiment was conducted with water from 3 different sites: water from an urbanized watershed, from a forested watershed, and from an oceanic influenced site which was considered the control. The first two sites are Childs River (CR) and Sage Lot Pond (SLP), which are part of the Waquoit Bay National Estuarine Research Reserve, Massachusetts (Fig. 1b). The Waquoit Bay estuarine system is a complex of sub-estuaries with different N inputs from their watersheds, and thus, with differing ambient N concentration and origin (Valiela et al., 1992; Valiela et al., 1997). The CR estuary (41º34' N, 70º32'W) is surrounded by the most urbanized watershed in the Waquoit Bay system. Nutrients (primarily nitrate) are delivered to the CR estuary from the watershed via groundwater flow (Valiela et al., 1992). In contrast, SLP (41º55'N, 70º50'W) has a forested watershed receiving a low N load, with NH_4^+ as the dominant dissolved inorganic nitrogen (DIN) form (Valiela et al., 1997), and the estuary is surrounded by salt marshes. The control site was at Nobska Beach (41º51'N, 70º65'W), which water is marine with no terrestrial or anthropogenic inputs draining in the area (Fig. 1a).

Experimental design– Individual fronds of *A. nodosum* and *F. vesiculosus* were collected at Quissett Harbor and Nobska Beach respectively, in Woods Hole, Massachusetts (Fig. 1a); and were transported in coolers to the laboratory. Macroalgae were kept in tanks with continuous seawater flow (15.7±1.6 ºC) and low light intensities during the night (less than 12 hours) until the start of the experiment. *A. nodosum* fronds of 14.6±2.6 cm long and with 2 or 3 gas bladders, and *F. vesiculosus* fronds of

10.7±2 cm long were selected to run the experiment. Individuals with visible damage or epiphytes were avoided.

For each set of water treatments, macroalgae (n=4 for *A. nodosum*, n=3 for *F. vesiculosus*) were placed in three different 1 L Erlenmeyer flasks containing CR, SLP or Nobska unfiltered water. The study was run in triplicate with each replicate in a separate flask for each of the three treatments over a period of 22 days for *A. nodosum* and 12 days for *F. vesiculosus*. Samples were taken at the start of the experiment (t=0) and at subsequently exponential times, 4 times for *A. nodosum* and 3 times for *F. vesiculosus*. At each time, a macroalgal frond of each flask was sampled and frozen (-20 °C) before processing. The different time scales for each species were chosen based on the previous knowledge of growth rates. A control flask with no macroalgae was established for each water treatment and maintained under the same conditions as the experimental flasks.

For comparison with experimental individuals, native individuals of *F. vesiculosus* were collected along with water samples where present (i.e. CR and SLP) and analyzed for stable isotope composition. Local populations of *A. nodosum* were not found at the sites selected for water collection.

Experiments were carried out in a culture chamber with 18:6 light:dark cycle at light intensities varying between 390-450 µE m^{-2} s^{-1} under 18-20 ºC air temperature oscillation between night and day respectively. Water aeration was maintained with air pumps and diffusers and water temperature set at 24.08±0.06 ºC.

Water was replaced every 2 days to avoid nutrient depletion. Samples of water were collected before and after replacement to quantify the variation in DIN concentrations among times and sites and to check macroalgal consumption. Salinity and temperature

were measured with a portable conductivity meter (YSI Model 30) every time the water was changed.

The macroalgal samples used for $\delta^{15}N$ and N and C content were separated with a glass spatula. The growing tip (1 cm) was sampled at all sampling dates during the experiment for both species. Additionally to the tip, at the start of the experiment (t=0) and at the endpoint, all intervesicular segments were sampled in *A. nodosum* individuals, while for *F. vesiculosus* individuals only the basal segment of the frond was additionally sampled. All macroalgal samples were rinsed with Milli Q water and frozen (-20 °C) before processing. Later, samples were defrosted and dried (50 °C) until constant weight before grinding into a homogeneous powder prior to isotopic and elemental analysis.

Macroalgal growth–To measure macroalgal growth response to the different water samples, the wet biomass of each frond was recorded at the beginning of the experiment and at the time the frond was sampled. Individual growth rates (μ) were calculated as a percent increase in biomass per day (% d^{-1}):

$$\mu = \frac{100\left[\mathrm{Ln}\left(\frac{N_t}{N_0}\right)\right]}{t}$$

where N_t is the biomass on day t, N_0 is the initial biomass, and t is time in days of incubation (Lobban and Harrison, 1994).

Nutrient sampling and analysis–Changes in concentration of $NO_3^- + NO_2^-$, NH_4^+, and PO_4^{3-} were determined during the experiment to quantify differences in ambient nutrient concentrations among water samples. Water samples were frozen until analysis of nutrient concentrations. Nitrate and phosphate were determined using standard colorimetric assays in a Lachat Auto Analyzer (Cd reduction). Ammonium

concentrations were determined by spectrophotometry following the indophenol method. Detection limit was 0.25 µM for any of the three nitrogen species.

2.2. Experiment 2: ^{15}N enrichment experiment

An enrichment experiment was done to determine N-turnover rates in different sections of the thallus and to test: i) the occurrence of transport of N along the thallus, from the tip to the basal segment of the frond, ii) the occurrence of transport of N from the basal segment of the frond to the tip, and iii) to quantify the uptake rates of the growing tips and mature parts of the thallus.

As in the previous experiment, *A. nodosum* and *F. vesiculosus* were collected at Quissett Harbor and Nobska Beach respectively (Fig. 1a). Macroalgae were transported in coolers to the laboratory and maintained under the same pre-incubation conditions as previously described. For these experiments *A. nodosum* individuals were 23.2±0.9 cm long and had 4 gas bladders, and *F. vesiculosus* individuals were 12.7±1.1 cm. The selected individuals did not show apparent damage or epiphytes. Treatment water was created by adding a stock solution of 10 mM $K^{15}NO_3$ (99 atom % ^{15}N) to 2 L of a final volume of seawater (from Nobska). The final concentration was ~120 µM, with 98.8% atom % ^{15}N enrichment. Nitrate was selected as the tested nutrient, as it is a dominant inorganic nitrogen compound entering these estuaries.

To test i) and ii), experiments were divided in two periods: a first 4-h period under the stock solution, followed by a 24-h period under control seawater. During the first period, only the tips (i) or the basal segment of the frond (ii) of three different fronds of each species were submerged, while the non-submerged parts of the thallus were manually vaporized with control seawater at regular intervals (~20 min) to avoid desiccation. Macroalgae were maintained inside the culture chamber under the same

light and temperature conditions as in the previous experiment. After this first 4-hour period, individuals were gently washed with seawater and transferred individually to an Erlenmeyer flask with 1 L of control seawater. They were kept during 24 hours under the same conditions of temperature, light and aeration as in the previous experiment.

After both incubation periods, all individuals were immediately subsampled for stable isotope determinations. Each *A. nodosum* individual was divided into tip (1-1.5 cm fragment measured from the distal part) and intervesicular segments, and those of *F. vesiculosus* were divided into tip (1 cm fragment from the distal part) and regular length segments (~3 cm) from the tip to the base. The lateral vegetative or reproductive branches of *A. nodosum* or reproductive tips of *F. vesiculosus* were discarded.

To test iii) the uptake capacity of the tip and non-growing parts of the thallus, three fronds of each species were completely submerged in the treatment solution for 2 h. Macroalgae were maintained inside the culture chamber under the same light and temperature conditions as in the previous experiment. To exclude the possible transport of inorganic N along the thallus, macroalgae were subsampled immediately after the incubation period. Macroalgae were subsampled following the same procedure as previously described for i) and ii).

During the first period of the two first experiments and the second experiment, control individuals of *A. nodosum* (n=3) and *F. vesiculosus* (n=3) were maintained in the same conditions as the experimental individuals but in 1L Erlenmeyer flasks with control seawater.

2.3. Internal nutrient content and $\delta^{15}N$ analysis

N stable isotope and elemental analyses for N and C content to estimate the tissue C:N were performed for all samples. Aliquots of ca. 2.5 mg of macroalgae samples

were used. Samples were placed in tin capsules and introduced into an isotope-ratio mass spectrometer (Thermo Finnigan Mat Delta Plus) via an element analyzer (Carlo Erba CHNSO 1108). Isotopic results are expressed in delta notation:

$$\delta^{15}N = [(^{15}N_{sample}:^{14}N_{sample}/^{15}N_{std}:^{14}N_{std})-1] \times 1000$$

where the standard (std) is atmospheric N_2. Precision (se of 5 replicates) was better than 0.05‰ for either IAEA-N-2, IAEA-N-1 or IAEA-NO-3 standards. The coefficient of variation of triplicate sample aliquots was always <2%.

2.4. Statistical analyses and calculations

Comparison of nutrient concentrations among water samples was done by analysis of variance (one-way ANOVA). Differences in the growth, $\delta^{15}N$ and C:N of the growing tips of macroalgae over the experiments were also tested using one-way ANOVA at each time separately using the site as fixed factor.

This test was also used to analyze differences among sites and macroalgal segments along the thallus at the end of the isotope equilibration experiment, and to study differences between macroalgal segments within individuals from the same site. In this case, when significant differences were detected, *a posteriori* Student-Neuman-Keuls (SNK) tests for multiple comparisons were used to detect differences among groups.

Experimental samples of the ^{15}N enrichment experiments were compared with the control samples to test the atom % ^{15}N enrichment using a paired-samples t-test, which compares two measurements of the same sample before and after the treatment. All tests were carried out with SPSS Statistical Software.

To estimate N uptake in the enrichment experiment we used the N specific uptake rate, which was calculated from appearance of the ^{15}N in the macroalgal tissue:

$$\text{N specific uptake} = \frac{\text{atom\%}^{15}\text{N}_f - \text{atom\%}^{15}\text{N}_i}{R \cdot t}$$

where atom % $^{15}N_f$ and atom % $^{15}N_i$ are the final and initial atom % ^{15}N enrichment of macroalgal thallus, *R* (%) is the calculated exponential average of the initial and final atom % enrichment of NO_3^-, and *t* is the time in hours.

The inverse of the N specific uptake-rate was used to estimate the turnover time (*tr*) in days that would take to renovate the total N of a particular macroalgal fragment.

3. Results

3.1. Experiment 1: N isotope equilibration rates

Concentrations of all inorganic nitrogen compounds during the experiment with *A. nodosum* in September were higher than those found during the *F. vesiculosus* experiment in August (Table 1). In the former case, water from CR had more nitrate and ammonium than water from the other sites but showed similar phosphate concentrations. In contrast, during the *F. vesiculosus* experiment, the oceanic-influenced site (Nobska) held larger nitrate and lower ammonium and phosphate concentrations than those at the other experimental sites, which had similar concentrations of all nutrients. In all cases, DIN:PO_4^{3-} values were low, indicating potential nitrogen limitation of algal growth.

The macroalgal growth differed between species, although the pattern was very similar among sites within the same species (Fig. 2). Overall growth of *A. nodosum* was higher than growth of *F. vesiculosus*. In all cases there was positive growth, but maximum growth was recorded after 6 d for *A. nodosum* and after 12 d for *F. vesiculosus* (Table 2). During the experiment with *F. vesiculosus*, no significant differences between sites were observed (Table 2). While during *A. nodosum*

277 experiment, significant differences were detected after 6 days of incubation, when
278 maximum growth was observed (Table 2).

279 The response of N isotope composition was different for each species (Table 2) but
280 similar for all water types assayed (Fig. 2). $\delta^{15}N$ values in the growing tips of both
281 species significantly differed during the experiment from initial values, especially in
282 *F. vesiculosus* (Table 2). Nevertheless, differences among fronds cultivated in different
283 water treatments were slight and remained close to the range of variation of the initial
284 values (6.7±0.1‰ in *A. nodosum* and 8.5±0.2‰ in *F. vesiculosus*, Fig. 2). These
285 changes were not large enough to reach the N isotopic values observed in native
286 individuals of *F. vesiculosus* in CR (6.9±0.1‰) or SLP (5.0±0.3‰).

287 As observed in the case of growth rates, tissue C:N of both species increased during
288 the experiment but there was no significant effect of culture water and only
289 *F. vesiculosus* maintained in SLP water had lower C:N values than those individuals
290 maintained in other water types (Fig. 2, Table 2). For all treatments, however, final C:N
291 values measured exceeded the range of values observed in the site of collection.

292 At the end of the experiment, differences between initial (t=0) and final values along
293 the thallus were especially noticeable in the tips, both for $\delta^{15}N$ and tissue C:N values
294 (Fig. 3, Table 3). In all parts of the frond, and for both species, the lowest isotopic
295 values were observed generally in individuals cultured in SLP water and the highest
296 values in those cultured in CR water (Fig. 3) thus approaching the isotopic values of
297 native macroalgae. The $\delta^{15}N$ values for growing tips of *A. nodosum* individuals
298 maintained in Nobska and SLP water were significantly different from other segments,
299 while no significant differences between segments from the same individual exposed to
300 CR water appeared (ANOVA, post hoc SNK test, $p \leq 0.01$). *F. vesiculosus* showed

301 significant differences between tip and the basal segment of the frond in individuals
302 under all culture regimes (ANOVA, post hoc SNK test, $p \leq 0.01$).

303 As *F. vesiculosus* was cultivated in its original water (Nobska), this can be used as
304 a control to find differences when macroalgae was cultivated in its original water and
305 two other water treatments (Sage Lot Pond and Childs River). N isotopic values of the
306 growing tips of macroalgae cultivated under water from Childs River were not
307 significantly different from the control at the endpoint of the experiment, while there
308 were statistical differences between the control and Sage Lot Pond. No significant
309 differences were found in C:N of the growing tips of macroalgae under the control and
310 the two other water treatments.

311 *3.2. Experiment 2: ^{15}N enrichment experiment*

312 The growing tip and the basal segment of the frond of both species when submerged
313 in ^{15}N enriched seawater significantly increased their ^{15}N content relative to non-
314 submerged parts of the frond and to control segments (Fig. 4a, b). Tips increased from
315 natural levels to average enrichments of 1.1% and 1.7% in *A. nodosum* and
316 *F. vesiculosus* respectively, while enrichment of the basal segment were only 0.4 and
317 0.8%, respectively. No evidence of enrichment was found in the emerged sections of the
318 thallus during this experiment.

319 The ^{15}N content in wholly-submerged fronds of both species significantly changed
320 after the treatment (Fig. 4c). As in the previous experiment, higher enrichment was
321 observed for *F. vesiculosus* than for *A. nodosum* individuals, and consequently N-
322 specific uptake rates were lowest in the latter (Table 4). Among *A. nodosum* individuals,
323 the basal segment showed the lowest enrichment, while in *F. vesiculosus* the segment

immediately under the growing tip showed the lowest enrichment together with the basal segment. The tips of both species were more enriched relative to other segments.

N uptake proceeded at low rates and N turnover times estimated from these rates were in general higher than the duration of the isotope equilibration experiments (Fig. 2). The average N turnover time of tip-submerged individuals was about 30 and 16 d for *A. nodosum* and *F. vesiculosus* respectively (Table 4). In contrast, when the basal segment was submerged, N turnover times averaged 7 months and 19 days for *A. nodosum* and *F. vesiculosus* respectively. Finally, when all the frond was submerged, turnover time of the tip for *A. nodosum* was longer (up to 6 months) than in the other treatments, although turnover at the basal segment of the fronds was maintained (Table 4). Turnover for intermediate segments was slightly faster (4-5 months) than at the tip or at the basal segment. In the case of *F. vesiculosus*, N turnover at the tip would need on average 11 d and only 21 d at the basal segment of the frond, while other algal segments showed intermediate turnover values.

4. Discussion

4.1. Variation of $\delta^{15}N$ in macroalgal growing tips

As both macroalgae show apical growth, isotope composition of the tips was expected to change according to the isotope composition of the surrounding water at faster rates than other parts of the thallus. These changes would ideally lead to a complete isotope equilibration between the algal tissue and the water in absence of isotope fractionation. The results of the experiments in this study revealed that the tips of both *A. nodosum* and *F. vesiculosus* required a long time to converge with the $\delta^{15}N$ values typical of native plants when exposed to water with different isotopic composition. The time required largely exceeded the duration of the experiments (up to

22 d), as N turnover rates varied between 11 d (*F. vesiculosus*) and 6 months (*A. nodosum*). Similar delays in the equilibration of $\delta^{15}N$ values in apical tissues of *F. vesiculosus* when changing the surrounding water were reported in *in situ* transplant studies with *F. vesiculosus* (Deutsch and Voss, 2006) while much faster equilibration was observed for other brown (García-Sanz, 2009), red or green macroalgal species (Naldi and Wheeler, 2002; Teichberg et al., 2008). Such delays can be due to low growth and N uptake rates, strong isotope fractionation, low ambient N or to the initial nitrogen content, and isotope composition of the individuals assayed.

Both macroalgae evidence logistic growth, with highest rates during their first year of life. *F. vesiculosus* can grow in length up to 2 cm $month^{-1}$ at the season of maximum growth but more often rates are as low as 0.6 cm $month^{-1}$ (Viana et al., accepted). The growth for *A. nodosum* is much slower, but individuals of this species can live for more than 10 yr (Viana et al., 2014). Low growth rates also imply lower N requirements and uptake than fast growing species (Pedersen and Borum, 1997). Such low requirements would explain N-specific uptake rates <0.1 d^{-1} even at high ambient N concentrations as those employed in the enrichment experiment in this study (Table 4), and consequently long N turnover times in these macroalgae.

Strong isotope fractionation is not likely to occur. Previous studies with Fucaceae (García-Sanz, 2009) and other macroalgae (Cohen and Fong, 2005) did not find significant N isotope fractionation related to nutrient concentrations, in contrast with diatoms (Wada and Hattori, 1978; Pennock et al., 1996). The rates of change in $\delta^{15}N$ in our experiments would have been faster than observed if fractionation were a significant factor, as the light isotopes would have been preferred. For instance, the assayed *F. vesiculosus* with mean initial $\delta^{15}N$ = 8.5‰ would have converged to values typical of individuals native of the water origin locations (5.0 to 6.9‰) but they did not.

373 The concentration of ambient N may have also affected changes in macroalgal $\delta^{15}N$.
374 The water employed in the experiments had nutrient concentrations typical of summer
375 in the study area, when uptake by primary producers depletes nutrients (Tomasky et al.,
376 1999). N sources, rather than total N concentration determines $\delta^{15}N$ in the water and
377 ultimately in primary and secondary producers (McClelland and Valiela, 1998b; Viana
378 and Bode, 2013). Experiments with other species showed that macroalgal $\delta^{15}N$ did not
379 change with water N concentrations as long as the $\delta^{15}N$ of dissolved N was constant
380 (Cohen and Fong, 2005; García-Sanz, 2009). Furthermore, nutrient uptake in
381 *F. vesiculosus* is less dependent on substrate concentration than in green or red algae
382 (Pedersen and Borum, 1997). In our experiment with water of different origins, the low
383 concentrations of dissolved N did not prevent the individuals of both species from
384 growing in weight and maintaining C:N values characteristic of non N-limited algae
385 (Niell, 1976), thus suggesting that the slight changes in $\delta^{15}N$ were not a direct
386 consequence of water N concentration.

387 The relatively high nitrogen content (1.2±0.3% for *A. nodosum*, 1.4±0.1% for
388 *F. vesiculosus*) and the enriched $\delta^{15}N$ values of macroalgae at the starting point could
389 have also influenced isotopic equilibration. Slow-growing brown macroalgae usually
390 rely on their internal N pools during periods of low nutrient supply, as in summer
391 seasons in temperate areas (Lehvo et al., 2001; Villares et al., 2013). During these
392 periods growth rates and external nutrient demand are lowered while the macroalgae,
393 eventually profiting from high light levels, develop carbon reserves, thus increasing
394 tissue C:N, as observed in our experiments (Fig. 2). Naldi and Wheeler (2002) also
395 observed that high total N content of thalli influenced nitrate uptake rates in green and
396 red macroalgal species. Low external N demand along with large differences in $\delta^{15}N$
397 values between the macroalgal tissue and the surrounding water (as suggested by the

398 $\delta^{15}N$ values of native macroalgae), may be the main determinants of the rate of isotopic
399 equilibration in our incubations with *F. vesiculosus*. Other experiments with
400 transplanted individuals of this species in the field also found small or no changes in
401 their tissue $\delta^{15}N$ after days of incubation (Deutsch and Voss, 2006).

402 *4.2. N uptake and turnover along the thallus*

403 The results of the enrichment experiments showed that both species do not transport
404 recently absorbed N along their thallus, at least during 24 h after uptake (Fig. 4).
405 Despite their internal structure (i.e. symplastic pathway) suited for transport (Raven,
406 2003), only carbon photosynthetic assimilates were reported to translocate along the
407 thallus of some Fucaceae (Diouris and Floc´h, 1984). Inorganic nitrogen transport,
408 however, was reported for other brown macroalgae, such as Laminariales (Mizuta et al.,
409 1996; Hepburn et al., 2012). These algae have nutrient requirements different from
410 those of Fucales as they show basal meristematic growth, which means that they grow
411 where the blade and the stipe meet (Lobban and Harrison, 1994). In contrast, Fucales
412 show mostly apical growth and therefore concentrate N demands in the tips of the
413 thallus (Topinka, 1978), although as demonstrated by our enrichment experiment
414 (Fig. 4c), all sections of the thallus are able to take up inorganic N from the water. As N
415 transport have relatively high energy and oxygen requirements (Raven, 2003), this
416 process can be avoided if both assimilation and uptake occur in the same part of the
417 thallus. In Laminariales, N uptake and assimilation occur at different rates in the
418 different parts of the thallus, resulting in gradients along the frond (Mizuta et al., 1996).

419 Despite their apical growth, variation in $\delta^{15}N$ values along the thallus has been
420 reported for *Fucus* species (Savage and Elmgren, 2004; Raimonet et al., 2013) and in
421 the present study (Fig. 3). If transport is excluded, such intra-individual variation might

422 be due to differential uptake and growth, or to isotope fractionation in the different
423 sections of the thallus.

424 In the enrichment experiment we showed that both species were able to incorporate
425 dissolved nitrogen when submerged (Fig. 4). The process of nitrogen uptake and
426 assimilation in macroalgae involves transport from the water column and assimilation
427 into organic compounds, followed by incorporation into proteins and macromolecules
428 for growth (McGlathery et al., 1996). Growth is the most important N sink in
429 macroalgae. In mature segments, N demand for structural pools is not as important as in
430 growing tips, this would explain why N uptake at the non-growing segments was only
431 half the uptake rate measured at the tips of *F. vesiculosus*, when all the frond was
432 submerged (Table 4). For *A. nodosum* there was also a marked difference in the uptake
433 rates of the tip and those of the mature segments, at least when only one of the sections
434 was submerged. These results agree with studies reporting higher N uptake in apical
435 fronds and whole young plants or germlings and lowest in slower-growing older fronds
436 and stipes of *F. spiralis* (Topinka, 1978; Rosenberg et al., 1984) and differential ^{15}N
437 enrichment along thalli regions of *F. vesiculosus* (Döhler et al., 1995).

438 Non-apical segments of *A. nodosum* and *F. vesiculosus* individuals can store N to
439 use in metabolic processes other than growth. For instance, N can be accumulated as
440 inorganic (NO_3^- and NH_4^+) and organic compounds (as phycobiliproteins) and can be
441 found in algal pigments (Hanisak, 1983) although NH_4^+ storage capacity is limited due
442 to toxicity (Haines and Wheeler, 1978; Lotze and Schramm, 2000).

443 The net short-term N uptake recorded along the thallus implies that $\delta^{15}N$ values of
444 different sections would change with the isotopic composition of the surrounding water
445 at rates depending on their initial $\delta^{15}N$ value, and on the processes affecting isotope

fractionation within each section. Nitrogen release, both in organic and inorganic forms, has been observed for some green and red macroalgae (Naldi and Wheeler, 2002; Tyler and McGlathery, 2006) and was interpreted as the result of isotopic equilibration of internal and external pools (Fujita et al., 1988) or to stress due to sudden changes in the proportion of different N sources (Naldi and Wheeler, 2002). Fractionation during uptake in brown macroalgae is not likely to occur (García-Sanz, 2009), although in other primary producers it was observed to result in lower nitrogen isotopic values in the tissues than in the water (Pennock et al., 1996). On the other hand, the release of preferentially light N isotopes may explain the higher enrichment of the tip sections compared to other parts of the thallus, as found in our experiments (Figs. 3 and 4) and in other studies (Raimonet et al., 2013). As far as we know, there are no reports of N release in the species considered in our study, but it can be expected that this process is restricted to the most metabolically active tissues.

4.3. Implications for the use of A. nodosum *and* F. vesiculosus *to monitor land-derived nitrogen sources*

The results of the present study are of application when using *A. nodosum* and *F. vesiculosus* to study the impact of anthropogenic N sources on littoral ecosystems both analyzing native populations and in incubation experiments, the latter applicable when these species are not naturally present in the impacted area. Taking advantage of the apical growth and long life span of both species, Savage and Elmgren (2004) interpreted $\delta^{15}N$ values in different sections of the thallus of *F. vesiculosus* in a retrospective study to monitor changing N loadings. The underlying assumptions were that annual growth occurred only at the tips and, by knowing the rate of growth, each section of the thallus could be dated and associated to a particular period of exposure to the ambient N. Thus, $\delta^{15}N$ of the sections would reflect past N sources if mature

segments do not equilibrate N contents with the surrounding water and if there is no transport of N along the thallus. Other studies, however, questioned this application for retrospective studies as they found contrasting patterns of change along the thallus that could not be related to ambient N (Raimonet et al., 2013).

The enrichment experiment in this study demonstrated that all sections of the thallus of both species take up N from the ambient water when submerged. Even when there was no transport of the N along the thallus and the rates of uptake at the mature parts of the frond were lower that at sections located at or near the tip this uptake would affect the $\delta^{15}N$ of the sections. These results explain why previous studies found contrasting patterns of change of $\delta^{15}N$ along the thallus of *F. vesiculosus* as the $\delta^{15}N$ of each section changes with the isotopic composition of the water at different rates. Therefore, it is not possible to obtain unbiased estimates of past N sources from the $\delta^{15}N$ of different sections of the thallus of these macroalgae. Furthermore, determinations of $\delta^{15}N$ from pooled samples of different sections would produce $\delta^{15}N$ values resulting from a mixture of past and present N sources, depending on the amount of matter from sections with different turnover rates. Pooled samples of the whole individual can be also be misinterpreted if individuals of different lengths (i.e. ages) are used. $\delta^{15}N$ of the tips can, however, be used as monitors of N sources in ambient water averaged over scales of 15 days (*F. vesiculosus*) and up to 6 months (*A. nodosum*). This range of integration times is particularly appropriate to differentiate chronic pollution from point discharges that may have little impact on the macroalgae.

Besides the use of natural populations, these macroalgae can be used in transplantation or laboratory experimental incubations with different water types to determine potential impacts of different N sources (Deutsch and Voss, 2006). In this case, the turnover and equilibration times of the tips, as determined in the present study,

need to be taken into account when determining the duration of the incubations. Otherwise the results will not reflect the actual impact of the ambient N sources.

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510 **References**

511 Bode, A., Varela, M., Prego, R., 2011. Continental and marine sources of organic matter
512 and nitrogen for rías of northern Galicia (Spain). Mar. Ecol. Prog. Ser. 437, 13-26.

513 Bode, A., Fernández, C., Mompeán, C., Parra, S., Rozada, F., Valencia-Vila, J., Viana,
514 I.G., 2014. Differential processing of anthropogenic carbon and nitrogen in benthic food
515 webs of A Coruña (NW Spain) traced by stable isotopes. Deep-Sea Res. Pt. II 106, 198-
516 206.

517 Carballeira, C., Rey-Asensio, A., Carballeira, A., 2014. Interannual changes in $\delta^{15}N$
518 values in *Fucus vesiculosus* L. Mar. Pollut. Bull. 85, 141-145.

519 Carballeira, C., Viana, I.G., Carballeira, A., 2013. $\delta^{15}N$ values of macroalgae as an
520 indicator of the potential presence of waste disposal from land-based marine fish farms.
521 J. Appl. Phycol. 25, 97-107.

522 Cohen, R.A., Fong, P., 2005. Experimental evidence supports the use of $\delta^{15}N$ content of
523 the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to
524 determine nitrogen sources to estuaries. J. Phycol. 41, 287-293.

525 Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M.,
526 2001. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 42,
527 149-156.

528 David, H.M., 1943. Studies in the autecology of *Ascophyllum nodosum* Le Jol. J. Ecol.
529 31, 178-198.

530 Deutsch, B., Voss, M., 2006. Anthropogenic nitrogen input traced by means of $\delta^{15}N$
531 values in macroalgae: Results from in-situ incubation experiments. Sci. Total Environ.
532 366, 799-808.

533 Diouris, M., Floc´h, J.Y., 1984. Long-distance transport of ^{14}C-labelled assimilates in
534 the Fucales: directionality, pathway and velocity. Mar. Biol. 78, 199-204.

535 Döhler, G., Hagmeier, E., David, Ch., 1995. Effects of solar and artificial UV
536 irradiation on pigments and assimilation of ^{15}N ammonium and ^{15}N nitrate by
537 macroalgae. J. Photoch. Photobiol. B 30, 179-187.

538 Dudley, B.D., Barr, N.G., Shima, J.S., 2010. Influence of light intensity and nutrient
539 source on δ^{13}C and δ^{15}N signatures in *Ulva pertusa*. Aquat. Biol. 9, 85-93.

540 Fujita, R.M., Wheeler, P.A., Edwards, R.L., 1988. Metabolic regulation of ammonium
541 uptake by *Ulva rigida* (Chlorophyta): a compartmental analysis of the rate-limiting step
542 for uptake. J. Phycol. 24, 560-566.

543 García-Sanz, M., 2009. Estudio y desarrollo de indicadores biológicos para evaluar el
544 alcance espacial de vertidos procedentes de granjas marinas. Universidad de Barcelona,
545 Spain, PhD dissertation, 205 pp.

546 García-Sanz, T., Ruiz, J.M., Pérez, M., Ruiz, M., 2011. Assessment of dissolved
547 nutrients dispersal derived from offshore fish-farm using nitrogen stable isotope ratios
548 (δ^{15}N) in macroalgal bioassays. Estuar. Coast. Shelf Sci. 91, 361-370.

549 García-Sanz, T., Ruiz-Fernández, J.M., Ruiz, M., García, R., González, M.N., Pérez,
550 M., 2010. An evaluation of a macroalgal bioassay tool for assessing the spatial extent of
551 nutrient release from offshore fish farms. Mar. Environ. Res. 70, 189-200.

552 Gartner, A., Lavery, P., Smit, A.J., 2002. Use of δ^{15}N signatures of different functional
553 forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage
554 dispersal. Mar. Ecol. Prog. Ser. 235, 63-73.

555 Gruber, N., 2008. The marine nitrogen cycle: Overview and challenges, in: Capone,
556 D.G., Bronk, D.A., Mulholland, M.R., Carpenter, E.J. (Eds.), Nitrogen in the marine
557 environment. Elsevier Inc, pp. 1-50.

558 Haines, K.C., Wheeler, P.A., 1978. Ammonium and nitrate uptake by the marine
559 macrophytes *Hypnea musciformis* (Rhodophyta) and *Macrocystis pyrifera*
560 (Phaeophyta). J. Phycol. 14, 319-324.

561 Hanisak, M.D., 1983. The nitrogen relationships of marine macroalgae, in: Capone,
562 D.G., Bronk, D.A., Mulholland, M.R., Carpenter, E.J. (Eds.), Nitrogen in the marine
563 environment. Elsevier Inc, pp. 699-730.

564 Hepburn, C.D., Frew, R.D., Hurd, C.L., 2012. Uptake and transport of nitrogen derived
565 from sessile epifauna in the giant kelp *Macrocystis pyrifera*. Aquat. Biol. 14, 121-128.

566 Hobbie, J.E., Fry, B., Larsson, U., Elmgren, R., 1990. Sewage derived ^{15}N in the Baltic
567 traced in *Fucus*. Eos 71, 190.

568 Lehvo, A., Bäck, S., Kiirikki, M., 2001. Growth of *Fucus vesiculosus* L. (Phaeophyta)
569 in the Northern Baltic proper: energy and nitrogen storage in seasonal environment.
570 Bot. Mar. 44, 345-350.

571 Lobban, C.S., Harrison, P.J., 1994. Seaweeds ecology and physiology. Cambridge
572 University Press.

573 Lotze, H.K., Schramm, W., 2000. Ecophysiological traits explain species dominance
574 patterns in macroalgal blooms. J. Phycol. 36, 287-295.

575 McClelland, J.W., Valiela, I., 1998a. Linking nitrogen in estuarine producers to land-
576 derived sources. Limnol. Oceanogr. 43, 577-585.

McClelland, J.W., Valiela, I., 1998b. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Mar. Ecol. Prog. Ser. 168, 259-271.

McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnol. Oceanogr. 42, 930-937.

McGlathery, K.J., Pedersen, M.F., Borum, J., 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). J. Phycol. 32, 393-401.

Mizuta, H., Maita, Y., Yanada, M., Hashimoto, S., 1996. Functional transport of nitrogen compounds in the sporophyte of *Laminaria japonica*. Fisheries Sci. 62, 161-167.

Montoya, J.P., 2007. Natural abundance of ^{15}N in marine planktonic ecosystems, in: Michener, R., Lajtha, K. (Eds.), Stable isotopes in ecology and environmental science. Blackwell Publishing Ltd, pp. 176-201.

Moss. B., 1983. Sieve elements in the Fucales. New Phytol. 93, 433-437.

Naldi, M., Wheeler, P.A., 2002. ^{15}N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): Comparison of net nutrient disappearance, release of ammonium and nitrate, and ^{15}N accumulation in algal tissue. J. Phycol. 38, 135-144.

Niell, F.X., 1976. C:N ratio in some marine macrophytes and its possible ecological significance. Bot. Mar. 19, 347-350.

599 Niell, F.X., 1979. Sobre la biología de *Ascophyllum nodosum* (L.) Le Jol. en Galicia. III.
600 Biometría, crecimiento y producción. Inv. Pesq. 43, 501-518.

601 Pedersen, M.F., Borum, J., 1997. Nutrient control of estuarine macroalgae: growth
602 strategy and the balance between nitrogen requirements and uptake. Mar. Ecol. Prog.
603 Ser. 161, 155-163.

604 Pennock, J.R., Velinsky, D.J., Ludlam, J.M., Sharp, J.H., Fogel, M.L., 1996. Isotopic
605 fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*:
606 implications for $\delta^{15}N$ dynamics under bloom conditions. Limnol. Oceanogr. 41, 451-
607 459.

608 Penot, M., Penot, M., 1979. High speed translocation of ions in seaweeds. Z.
609 Pflanzenphysiol. 95, 265-273.

610 Raimonet, M., Guillou, G., Mornet, F., Richard, P., 2013. Macroalgae $\delta^{15}N$ values in
611 well-mixed estuaries: Indicator of anthropogenic nitrogen input or macroalgae
612 metabolism? Estuar. Coast. Shelf Sci. 119, 126-138.

613 Raven, J.A., 2003. Long-distance transport in non-vascular plants. Plant Cell Environ.
614 26, 73-85.

615 Rosenberg, G., Probyn, T.A., Mann, K.H., 1984. Nutrient uptake and growth kinetics in
616 brown seaweeds: Response to continuous and single additions of ammonium. J. Exp.
617 Mar. Biol. Ecol. 80, 125-146.

618 Savage, C., Elmgren, R., 2004. Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace
619 decrease in sewage influence. Ecol. Appl. 14, 517-26.

620 Teichberg, M., Fox, S.E., Aguila, C., Olsen, Y.S., Valiela, I., 2008. Macroalgal
621 responses to experimental nutrient enrichment in shallow coastal waters: growth,
622 internal nutrient pools, and isotopic signatures. Mar. Ecol. Prog. Ser. 368, 117-126.

623 Tomasky, G., Barak, J., Valiela, I., Behr, P., Soucy, L., Foreman, K., 1999. Nutrient
624 limitation of phytoplankton growth in Waquoit Bay, Massachusetts, USA: a nutrient
625 enrichment study. Aquat. Ecol. 33, 147-155.

626 Topinka, J.A., 1978. Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). J. Phycol. 14,
627 241-247.

628 Tyler, A.C., McGlathery, K.J., 2006. Uptake and release of nitrogen by the macroalgae
629 *Gracilaria vermiculophylla* (Rhodophyta). J. Phycol. 42, 515-525.

630 Valiela, I., Collins, G., Kremer, J., Lajtha, K., Geist, M., Seely, B., Brawley, J., Sham,
631 C.-H., 1997. Nitrogen loading from coastal watersheds to receiving estuaries: new
632 method and application. Ecol. Appl. 7, 358-380.

633 Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., DeMeo-
634 Anderson, B., D'Avanzo, C., Babione, M., Sham, C.-H., Brawley, J., Lajtha, K., 1992.
635 Coupling watersheds and coastal waters: Sources and consequences of nutrient
636 enrichment in Waquoit Bay, Massachusetts. Estuaries 15, 443-457.

637 Viana, I.G., Bode, A., 2013. Stable nitrogen isotopes in coastal macroalgae: Geographic
638 and anthropogenic variability. Sci. Total Environ. 443, 887-895.

639 Viana, I.G., Fernández, C., Bode, A., 2014. Growth and production of new recruits and
640 adult individuals of *Ascophyllum nodosum* in a non-harvested population at its southern
641 limit (Galicia, NW Spain). Mar. Biol. 161, 2885-2895.

642 Viana, I.G., Fernández, C., Bode, A., accepted. Ecology of *Fucus vesiculosus*
643 (Phaeophyceae) at its southern distributional limit: growth and production of early
644 developmental stages. Eur. J. Phycol.

645 Viana, I.G., Aboal, J.R., Fernández, J.A., Real, C., Villares, R., Carballeira, A., 2010.
646 Use of macroalgae stored in an Environmental Specimen Bank for application of some
647 European Framework Directives. Water Res. 44, 1713-1724.

648 Viana, I.G., Fernández, J.A., Aboal, J.R., Carballeira, A., 2011. Measurement of $\delta^{15}N$ in
649 macroalgae stored in an environmental specimen bank for regional scale monitoring of
650 eutrophication in coastal areas. Ecol. Indic. 11, 888-895.

651 Villares, R., Fernandez-Lema, E., Lopez-Mosquera, E., 2013. Seasonal variations in
652 concentrations of macro- and micronutrients in three species of brown seaweed. Bot.
653 Mar. 56, 49-61.

654 Wada, E., Hattori, A., 1978. Nitrogen isotope effects in the assimilation of inorganic
655 nitrogenous compounds by marine diatoms. Geomicrobiol. J. 1, 85-101.

656 Xue, D., Botte, J.,De Baets, B., Accoe, F., Nestler, A., Taylor, P.,Van Cleemput, O.,
657 Berglund, M.,Boeckx, P., 2009. Present limitations and future prospects of stable
658 isotope methods for nitrate source identification in surface- and groundwater. Water
659 Res. 43, 1159-1170.
660

Table 1. Sampling dates, and mean (±se) values of salinity, nutrient concentrations (µM) and DIN:PO_4^{3-} during the N isotope equilibration experiments with *A. nodosum* and *F. vesiculosus* exposed to water from Childs River, Sage Lot Pond and Nobska (Fig. 1). Significant differences among nutrient concentrations in the different sites are shown (***: p≤0.001, **: p≤0.01, *: p≤0.05, one-way ANOVA).

	A. nodosum			*F. vesiculosus*		
	Childs River	Sage Lot Pond	Nobska	Childs River	Sage Lot Pond	Nobska
Dates	29 August- 20 September 2013			2 August- 14 August 2013		
Salinity	24.57±0.89	27.04±0.45	31.04±0.05	25.85±0.40	26.33±1.28	31.10±0.32
Nutrient concentrations (µM)						
$NO_3^- + NO_2^-$	5.98±2.58	2.08±0.29	1.85±0.14*	1.07±0.13	1.28±0.15	2.03±0.18**
NH_4^+	5.12±1.46	3.12±0.65	1.15±0.09**	2.19±0.01	0.85±0.13	0.57±0.04***
PO_4^{3-}	1.70±0.51	1.06±0.12	1.25±0.12	1.55±0.24	0.75±0.15	1.23±0.09**
DIN:PO_4^{3-}	7.02±2.38	4.99±0.79	2.20±0.3	1.11±0.33	2.29±0.78	2.39±0.55*

Table 2. Results of one-way ANOVA analysis at each sampling time (2, 6, 12 and 22 days from the start of the experiment) to analyze the variation in growth (% d^{-1}), $\delta^{15}N$ (‰) or C:N in the tips of *A. nodosum* and *F. vesiculosus*. The variability in the tips of both species is compared when grouped by sites (Childs River, Sage Lot Pond or Nobska) and the initial values (t0) as fixed factors. Significant differences at different times are shown (***: p≤0.001,**: p≤0.01 *: p≤0.05).

	2 days				6 days				12 days				22 days			
	SS	df	MS	F	SS	df	MS	F	SS	df	MS	F	SS	df	MS	F
A. nodosum																
Growth	8.27	8	0.00	-	55.04	8	0.15	178.72***	5.21	8	0.58	1.49	29.23	8	3.66	0.99
$\delta^{15}N$	3.2	11	0.18	3.4	1.42	11	0.11	1.7	2.19	11	0.13	3.15	3.57	11	0.11	8.31**
C:N	744.59	11	26.86	6.58**	1323.73	11	37.81	9.00**	1307.43	11	106.14	1.44	673.71	11	49.79	1.84
F. vesiculosus																
Growth	0.02	8	0.00	-	0.00	8	0.00	-	1.26	8	0.12	2.45	-	-	-	-
$\delta^{15}N$	1.57	12	0.047	8.01**	1.71	12	0.07	5.62*	2.17	12	0.08	6.02**	-	-	-	-
C:N	164.87	12	10.31	2.33	430.98	12	18.56	4.74*	1146.96	12	39.64	6.65**	-	-	-	-

Table 3. Results of analysis of variance (one-way ANOVA) and SNK post-hoc comparison tests of $\delta^{15}N$ (‰) and C:N in different segments of *A. nodosum* and *F. vesiculosus* fronds (n=3) at the endpoint of the study (Fig. 3). Site (CR, Childs River; SLP, Sage Lot Pond and N, Nobska) and initial values (t0) were set as fixed factors. The tip and BS segments correspond to the growing apical segment and the basal segment of the frond respectively. S1 and S2 segments for *A. nodosum* correspond to the intervesicular segments numbered from the tip to the base. P values are significant when ≤0.05. n.s.: non significant. The tip and BS segments correspond to the growing apical segment and the basal segment of the frond respectively. S1 and S2 segments for *A. nodosum* correspond to the intervesicular segments numbered from the tip to the base.

		$\delta^{15}N$				C:N			
Species	Macroalgal segment	df	F	p value	post-hoc	df	F	p value	post-hoc
A. nodosum									
	Tip	11	8.308	0.008	t0<SLP<CR=N	11	1.8	0.218	n.s.
	S1	11	13.7	0.002	t0<SLP<CR=N	11	1.0	0.428	n.s.
	S2	11	8.7	0.007	CR>t0=SLP=N	11	1.3	0.34	n.s.
	BS	11	15.6	0.001	CR>SLP=N>t0	11	8.7	0.007	t0<CR=SLP=N
F. vesiculosus									
	Tip	12	6.0	0.016	t0=SLP<CR=N	12	6.6	0.012	t0=SLP<CR=N
	BS	11	0.6	0.625	n.s.	11	1.7	0.238	n.s.

Table 4. Variation of N specific uptake (mean±se, $days^{-1}$) and turnover time (days) in the different macroalgal segments of *A. nodosum* and *F. vesiculosus* when, i) the tip was submerged in an enriched seawater solution (Fig. 4a, b), ii) the basal segment of the frond (BS) was submerged in an enriched seawater solution (Fig. 4c, d), and iii) the entire frond was submerged in an enriched seawater solution (Fig. 4e, f). The tip in both species corresponds to the growing apical segment. S1, S2 and S3 segments correspond to intervesicular and 3-cm segments in order from the tip to lower down the frond in *A. nodosum* and *F. vesiculosus* respectively.

Species	Experiment	Macroalgal segment	N specific uptake ($days^{-1}$)	Turnover time (days)
A. nodosum				
	i	Tip	0.0409±0.0104	29.27±9.62
	ii	BS	0.0048±0.0004	209.17±14.66
	iii	Tip	0.0053±0.0001	188.15±5.33
		S1	0.0085±0.0002	118.04±2.44
		S2	0.0087±0.0007	115.97±10.27
		S3	0.0062±0.0004	162.43±10.8
		BS	0.0044±0.0001	227.67±5.57
F. vesiculosus				
	i	Tip	0.0665±0.0100	15.74±2.35
	ii	BS	0.0525±0.0100	19.06±2.35
	iii	Tip	0.0949±0.0047	10.59±0.5
		S1	0.0522±0.0017	19.2±0.62
		S2	0.0722±0.0050	13.98±0.9
		S3	0.0721±0.0036	13.95±0.73
		BS	0.0476±0.0021	21.1±0.96

Figure legends

Fig. 1. Location of the study sites at Cape Cod, Massachusetts, USA. Enlarged panel **a** shows Quissett Harbor and Nobska Beach, where *A. nodosum* and *F. vesiculosus* were sampled respectively. Open symbols in panels **a** and **b** indicate the sites where the water samples were taken (Basemap: USGS).

Fig. 2. Changes in mean±se (n=3) growth in wet biomass (% d^{-1}), $\delta^{15}N$ (‰) and tissue C:N in *A. nodosum* (a, c, e) and *F. vesiculosus* (b, d, f) during 22 and 12 d of incubation respectively using water of three different locations. Square symbols are the mean values at time 0 and the dashed lines the range of variation. Analysis of variance results shown in Table 2.

Fig. 3. Variation between initial (time 0) and endpoint $\delta^{15}N$ values ($\Delta\delta^{15}N$, mean±se, ‰) and tissue C:N (ΔC:N, mean±se) for different sections of the thallus of *A. nodosum* (a, c) and *F. vesiculosus* (b, d) individuals (n=3) growing under water of three different locations (Childs River, Sage Lot Pond and Nobska). Tip and intervesicular segments numbered from the tip to the base (BS) are shown for *A. nodosum* and tip and basal segment (BS) for *F. vesiculosus*.

Fig. 4. Mean (±se) variation of atom % ^{15}N enrichment along the fronds (n=3) of *A. nodosum* (a, c, e) and *F. vesiculosus* (b, d, f) when either: the tip was submerged in an enriched seawater solution (a, b), the basal segment of the frond (BS) was submerged in an enriched seawater solution (c, d), or the entire frond was submerged in an enriched seawater solution (e, f). The tip in both species corresponds to the growing apical segment. S1, S2 and S3 segments correspond to the intervesicular segments and to 3-cm segments in order to the closeness to the tip in *A. nodosum* and *F. vesiculosus* respectively. Significant differences between the experimental and the control frond

values are indicated by asterisks (*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, paired-samples t-test).

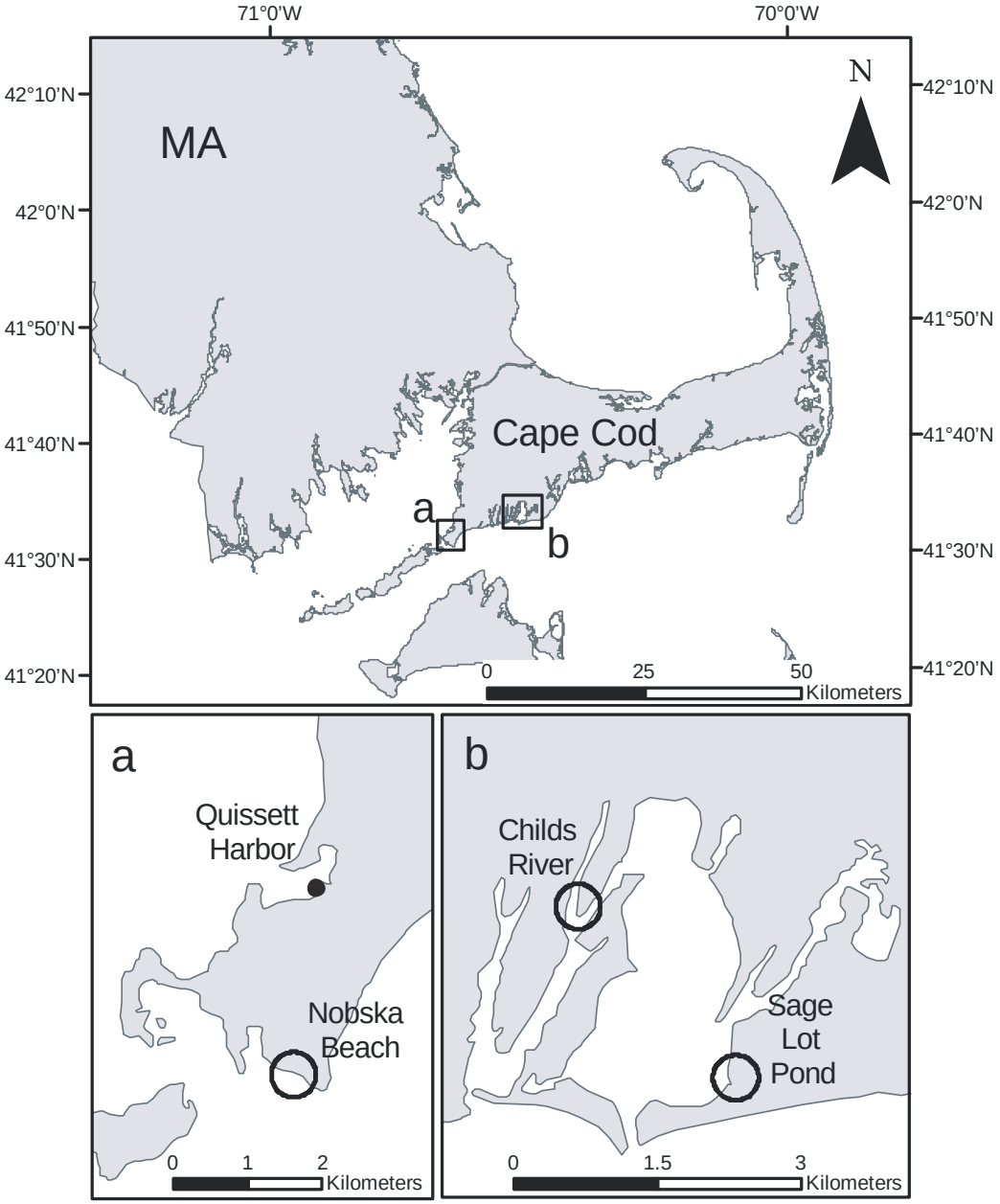
71°0'W
70°0'W
42°10'N
42°0'N
41°50'N
41°40'N
41°30'N
41°20'N
N
MA
Cape Cod
a
b
0
25
50
Kilometers
a
Quissett
Harbor
Nobska
Beach
0
1
2
Kilometers
b
Childs
River
Sage
Lot
Pond
0
1.5
3
Kilometers

Fig. 1.

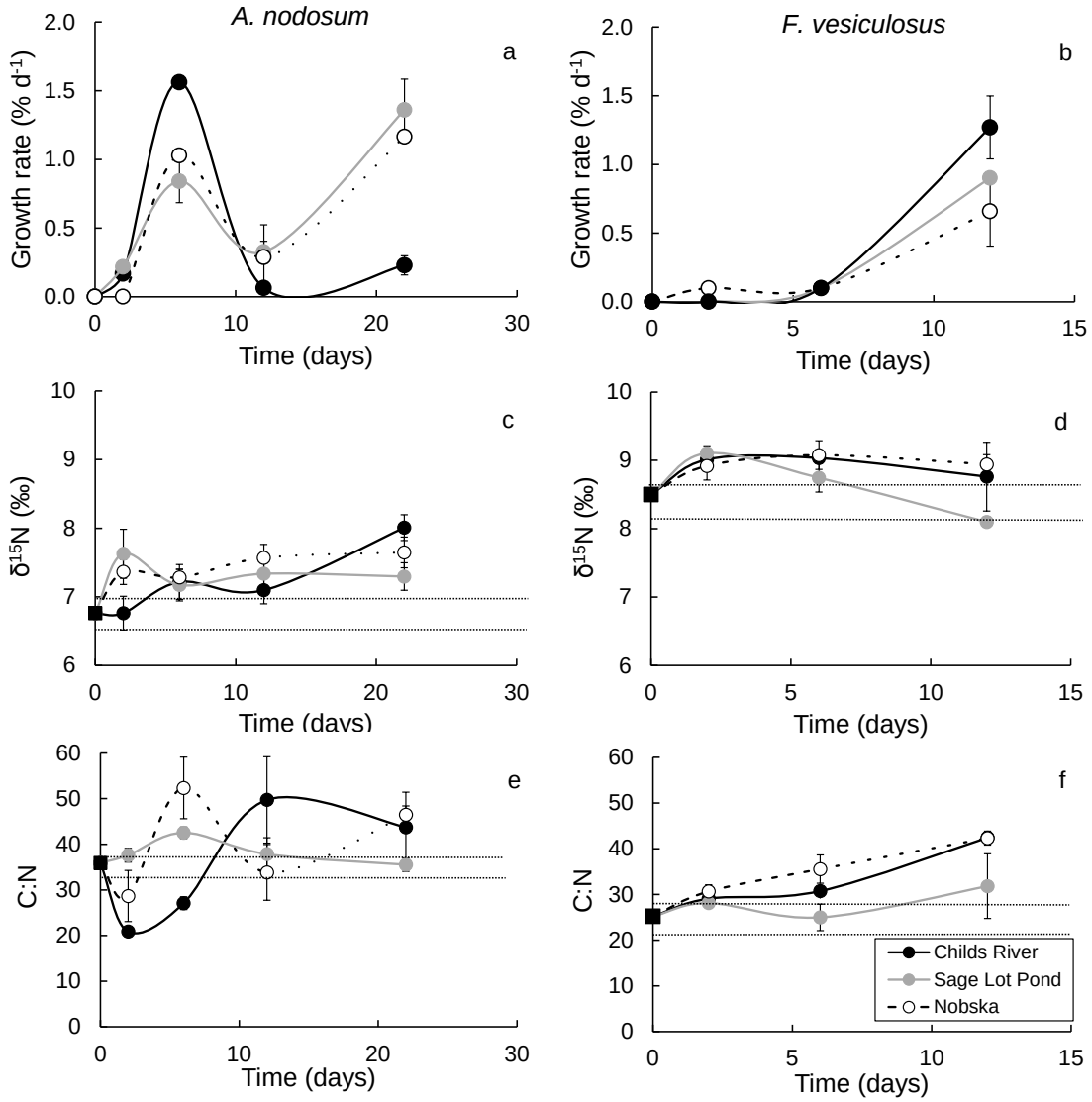
A. nodosum
F. vesiculosus
a
b
c
d
e
f
Growth rate (% d-1)
δ15N (‰)
C:N
Time (days)
Childs River
Sage Lot Pond
Nobska

Fig. 2.

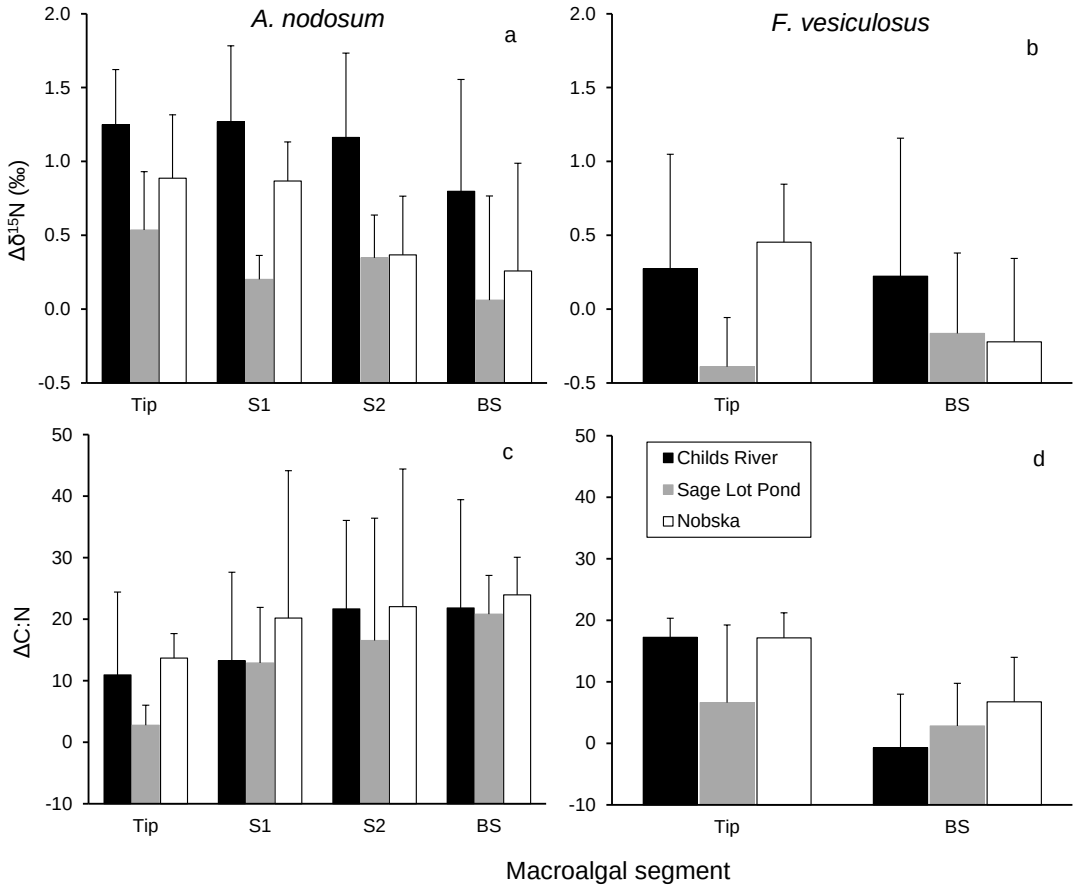
A. nodosum
a
F. vesiculosus
b
c
d
Childs River
Sage Lot Pond
Nobska
Δδ15N (‰)
ΔC:N
Tip
S1
S2
BS
Macroalgal segment

Fig. 3

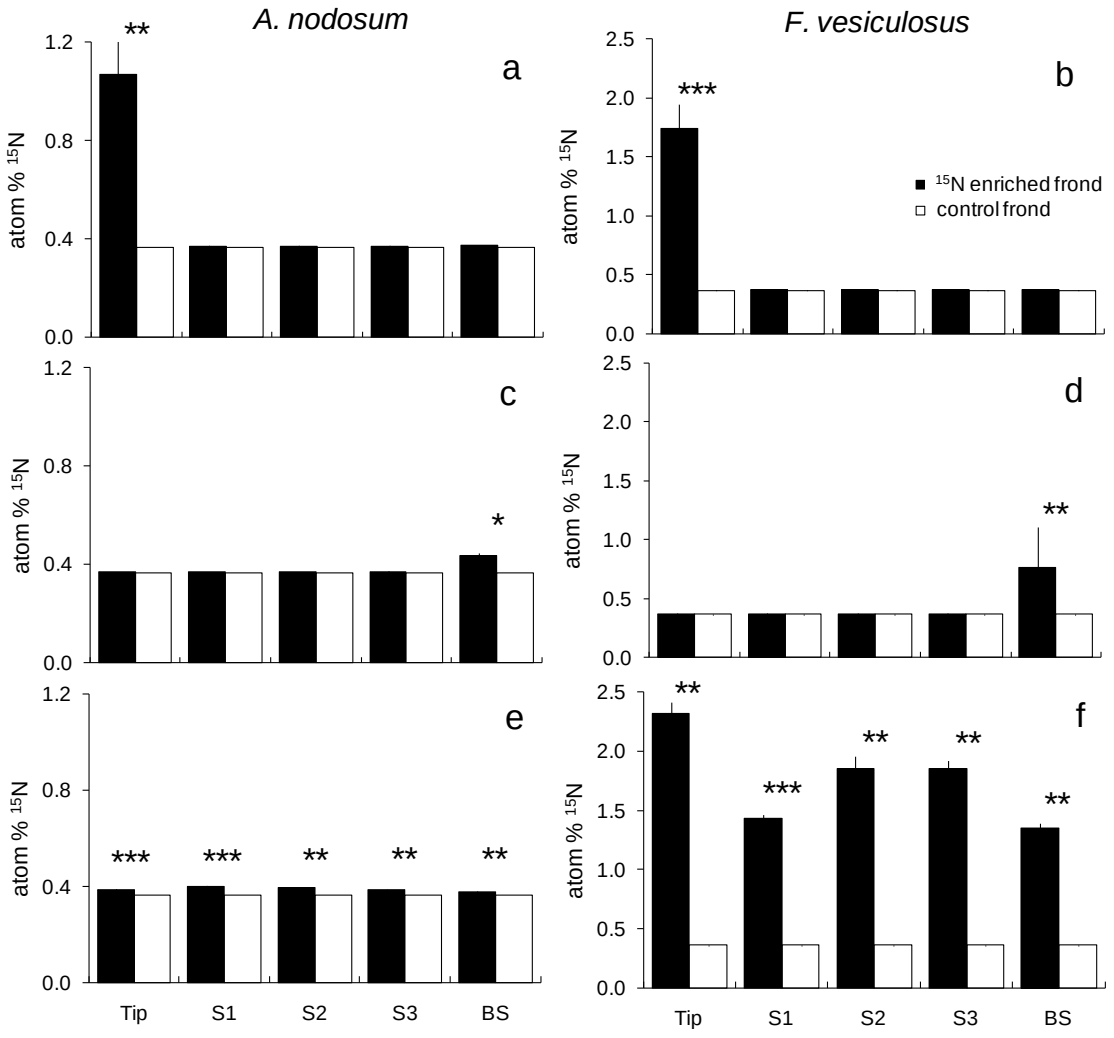
A. nodosum
F. vesiculosus
a
b
c
d
e
f
atom % 15N
15N enriched frond
control frond
Tip
S1
S2
S3
BS

Fig. 4.