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Effects of land-based fish farm effluent on the morphology and growth of *Ascophyllum nodosum* (Fucales, Phaeophyceae) in southwestern Nova Scotia

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Phenotypic plasticity was examined in the economically and ecologically important brown alga *Ascophyllum nodosum* in southwestern Nova Scotia, considering specifically how nutrient loading affected its vegetative and reproductive features. To determine this, we examined morphometric changes in *A. nodosum* from two sites receiving direct effluent impacts from a land-based finfish aquaculture facility and from two control sites, approximately 2 km away from the aquaculture facility in opposite directions. Fronds from test sites were significantly younger than from control sites (5 y vs. 8 y); however, fronds from farm sites were significantly larger (219 g vs. 90 g) because of their higher growth rates. Thalli from farm sites had greater reproductive potential, as shown by numbers of receptacle initials (797 initials vs. 281 initials). These results suggest limited nutrient inflows from land-based aquaculture may positively affect adjacent *Ascophyllum* populations by inducing higher growth rates. We conclude that the coordination of effluent management from land-based aquaculture with natural resource harvesting of *A. nodosum* may be beneficial. Further study is necessary to determine the limits of nutrient loading for this potentially beneficial outcome.

Key Words: aquaculture; *Ascophyllum nodosum*; morphology; nutrient enrichment; plasticity; tidal heights; wave exposure

INTRODUCTION

Ascophyllum nodosum (Linnaeus) Le Jolis (hereafter referred to as *Ascophyllum*) is a brown, fucoid macroalga. *Ascophyllum* is perennial and grows with *Fucus vesiculosus* Linnaeus, where they dominate the intertidal zone over a range of environments, from moderately exposed to sheltered shores, in the temperate North Atlantic (David 1943, Baardseth 1970, Åberg 1992, Eckersley and Garbary 2007, Gollety et al. 2011). *Ascophyllum* is a modular organism, with a holdfast that produces many fronds (Cousens 1984, Eckersley and Garbary 2007). It is a long-lived organism with individual fronds surviving up to 20 years and the thalli surviving up to a century (Keser and Larson 1984, Åberg 1992). It forms dense populations in southwestern Nova Scotia, Canada, producing stands that are up to 8 kg dry weight m^{-2} (MacFarlane 1952, 1964, 1966, Lazo et al. 1994). Its long lifespan and abundance may be partly attributable to its resistance to both grazing (Pavia et al. 1999) and epiphyte colonization (Garbary et al. 2009), and to a temperature tolerance down to -20°C (Åberg 1992).

Dense *Ascophyllum* growths form canopies that play an important role in community structure through their influence on the physical environment, which in turn influences the biological community; e.g., *Fucus vesicu-*

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losus often grows in association with *Ascophyllum* (Bertness et al. 1999). It also forms multiple symbioses, most prominently with a fungal endophyte, *Mycophycias ascophylli* (Cotton) Kohlmeyer and Volkman-Kohlmeyer, and with a red algal epiphyte, *Vertebrata lanosa* (Linnaeus) T. A. Christensen (Garbary and McDonald 1995, Garbary and Deckert 2001, Xu et al. 2008). *Ascophyllum* is an important commercial crop that is harvested for a variety of applications in southwestern Nova Scotia and New Brunswick (e.g., Acadian Seaplants Ltd.: http://www.acadianseaplants.com/) (Sharp and Semple 1990).

Researchers have performed extensive studies on *Ascophyllum* growth rates, sexual recruitment, and the environmental conditions that affect these. *Ascophyllum* grows slower than *Fucus vesiculosus* does (Mathieson et al. 1976, Keser and Larson 1984). *Ascophyllum* has an apical meristem in each branch and a growth rate of 70-130 mm y^{-1}, with most populations averaging 9-10 cm y^{-1} in Nova Scotia (MacFarlane 1933, Cousens 1984, Eckersley and Garbary 2007). Growth rate of fronds begins to increase in April, with the formation of a new air bladder at the apex of each branch (e.g., MacFarlane 1933, David 1943, Mathieson et al. 1976). Air bladders mostly complete their growth by June (Cousens 1985), but enlargement can continue for several years (Garbary et al. 2006). Except for during the first two years after frond initiation, these air bladders form annually (Keser and Larson 1984).

Many factors affect growth rates, including frond size, competition, the algal canopy (Eckersley and Garbary 2007), nutrient concentrations, temperature, sunlight (Mathieson et al. 1976, Keser et al. 2005), wave exposure (Vadas and Wright 1986), position on the shoreline and substratum (David 1943), and harvesting (Ugarte and Sharp 2001, Ugarte et al. 2006). These factors may also cause morphological differences in *Ascophyllum* fronds. Frond lengths are shorter in areas with greater wave action; thalli in full sun grow less than partially shaded plants do, and plants with intermediate wave exposure produce larger air bladders and more receptacles (David 1943). Vadas and Wright (1986) found a relationship between frond age and wave exposure, with the longest-lived fronds occurring in sheltered habitats.

In areas where nutrients are either limiting or in excess, growth can slow, and individual species may become noncompetitive or reduce their reproductive rates. When excessive nutrient levels occur, eutrophication may change the community composition significantly. Nitrogen is usually the most limiting nutrient in temperate marine ecosystems (Pavia et al. 1999). One experimental nitrogen enrichment of *Ascophyllum* resulted in increased growth rates, as well as an increase in tissue nitrogen (Pavia et al. 1999). In this study, we examined how high nutrient loads in the effluent from a finfish aquaculture facility, which had been in operation for about 10 years, would affect *Ascophyllum*. We used quantitative measures of thallus growth, morphology, and reproduction at two impacted sites with varying wave exposures and two control sites without effluent inputs. In addition, at each site we differentiated between thalli collected at two shore elevations. Our primary objectives were to evaluate the thallus morphology and reproduction associated with high nutrient levels, wave exposure, and shore elevation, and to characterize how these factors affect the phenotypic plasticity of this important fucoid species.

MATERIALS AND METHODS

Site descriptions

We collected fronds from two intertidal elevations, at two test and two control sites (Fig. 1). The test sites were at the outflow of a land-based finfish farm raising Atlantic Halibut, *Hippoglossus hippoglossus* (Linnaeus), at Woods Harbour, Nova Scotia (43°31′34″ N, 65°43′43″ W). During the previous year, this aquaculture facility had used a microbial filter to reduce ammonium levels, passing one-third of the effluent stream through tanks containing extensive growths of *Chondrus crispus* Stackhouse and *Palmaria palmata* (Linnaeus) Weber et Mohr. For the ten years preceding use of this filter, the facility had released the entire effluent stream into the intertidal zone. Currently, the effluent stream contains 300 μM NO_3^- and has a 10 : 1 N : P ratio, although year-round ambient seawater concentrations are less than 3 μM NO_3^- in southwest Nova Scotia Peter Corey personal communication. The effluent stream entered the surrounding intertidal zone via two pipes, on a wave-exposed shore and a small, wave-protected man-made harbor, "farm-out" and "farm-in," respectively. Their combined outflow varied between 500 and 2,500 m^3 per day (Peter Corey personal communication), and we estimated total nitrogen released into the farm sites at 1,500 kg y^{-1}. The *Ascophyllum* thalli at these experimental sites grew within 75 m of the outflow pipes. At the farm-out site, the intertidal zone substratum consisted of large boulders, whereas the farm-in site had a gravel and small boulder / sand substratum in the low intertidal zone, while its upper intertidal zone comprised rocks and a wooden wharf.

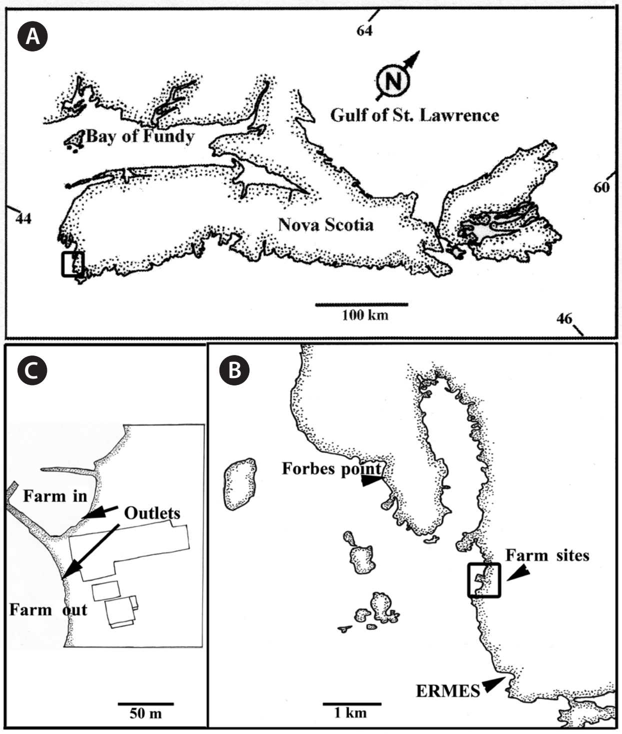

Fig. 1. Map of Nova Scotia (A) with boxed area shown enlarged in (B), showing the portion of southwestern Nova Scotia with sample sites, with boxed area shown enlarged in (C), to give details of the farm sites. Arrows in B indicate collection sites. C indicates position of farm in and farm out as well as locations of effluent outlets. ERMES, Evelyn Richardson Memorial Elementary School.

For the two control sites, we selected locations approximately 2 km from the test sites, one north, one south, with no associated industrial or domestic effluent sources that could affect water quality. The first site was adjacent to Evelyn Richardson Memorial Elementary School (ERMES) in Shag Harbour (43°29′50″ N, 65°43′45″ W). A shallow inlet provided some shelter. The site had a rocky substratum dominated by *Ascophyllum*. The low intertidal substratum was sandy, with large rocks supporting populations of *Ascophyllum* and *Fucus*. The second site, at Forbes Point (43°33′06″ N, 65°45′43″ W), had open exposure to the waves, and *Ascophyllum* again dominated the rocky substratum in the upper intertidal zone. The low intertidal zone was sandy, with large boulders harboring *Ascophyllum*. Only the ERMES site had been regularly harvested commercially for *Ascophyllum*. However, we found no indications that any of our sampled fronds had sustained damage from a harvesting procedure (i.e., raking).

Sampling and measurement methods

At each of the four sites, the elevation gradient occupied by *Ascophyllum* was divided into two elevations (high and low). During low tide, in late September 2010, we harvested 20 whole *Ascophyllum* thalli haphazardly from among the larger thalli at each of the two tidal elevations for each site. Whole thalli having intact holdfasts were harvested, with the longest frond on each thallus having an unbroken primary axis, to allow for a better approximation of maximum frond age. We transported the thalli in plastic bags, to prevent desiccation, and stored them at 5°C, for those to be processed within four days, or froze them at -20°C, for later processing.

For each thallus, we determined biomass, along with vegetative biomass (after removing the receptacle initials), and counted the number of receptacle initials. For the longest frond from each thallus, we measured frond length, number of dichotomies and air bladders on the longest axis, maximum frond width not associated with an air bladder, and greatest air bladder length. To measure frond width and air bladder length, we used vernier calipers, measuring to the nearest 0.1 mm. The other measurements used a meter ruler and measured to the nearest millimeter. Since air bladder size can vary significantly along a given axis (Brackenbury et al. 2006, Garbary et al. 2006), we measured only the largest air bladder on each frond.

Using these same fronds, we also measured the length of the current year's growth (i.e., from the frond tip to the base of the first bladder) and the distance between the bases of the first and second bladders (similar to Vadas et al. 2004). We thus determined the length of the 2009 growth increment, which allowed us to calculate the percentage of the previous year's growth completed by September 2010, when we harvested the thalli.

To determine tissue N and C content, we first collected samples of frond apices monthly, from June through September, at all sites. The 30 frond apices from each tidal height were randomly divided into four samples, dried at 60°C, and then ground in a bead mill (Model MM200 Grinder; Retsch, Haan, Germany). Finally, 1.5-2.5 mg of powder were removed from each sample to determine N and C composition using a Perkin Elmer 2400 series II CHNS/O elemental analyzer (Waltham, MA, USA).

Statistical analysis

One- and two-way analyses of variance (ANOVAs) were conducted for each of the analyzed morphometric features, with sites and tidal heights as the dependent variables using JMP statistical software (SAS Inc., Cary, NC, USA). Subsequently, we used Tukey's post hoc test (Zar 1999) to determine which sites and intertidal heights differed and how these factors interacted with thallus and frond morphologies. A one-way ANOVA of tissue nutrient data determined any significant differences among months or between farm and control conditions, followed by a Tukey's post-hoc analysis to discern any patterns in tissue nutrient changes between June and September.

RESULTS

Comparisons within control and farm sites

When the four sites were considered independently for the 12 morphological and growth features of whole thalli and fronds (Table 1), the sites fell into two groups: the farm sites and control sites. All features showed some significant differences among the sites. The two farm sites did not differ significantly from each other on eight of the characters, while control sites did not differ significantly on seven characters (Table 1). Of the four features that differed significantly different between the farm sites, three had means outside the ranges of control sites (greater thallus mass, greater vegetative mass, and greater number of receptacle initials). Of the five features that differed significantly between control sites (maximum frond width, number of air bladders, length of air bladders, number of air bladders, and mass per receptacle initial), four of these (all but mass per receptacle initial) had more extreme means than those of farm sites.

Comparisons between control and farm sites

The primary objective in this study was to characterize the potential morphological differences of *Ascophyllum* between the farm and control sites. In the field, the *Ascophyllum* populations at various sites differed conspicuously. At control sites, the thalli were a yellowish olive-green, whereas thalli at the experimental sites were dark green to almost black. Fronds at control sites were less robust, with thinner branches, smaller air bladders, and shorter distances between air bladders compared to those

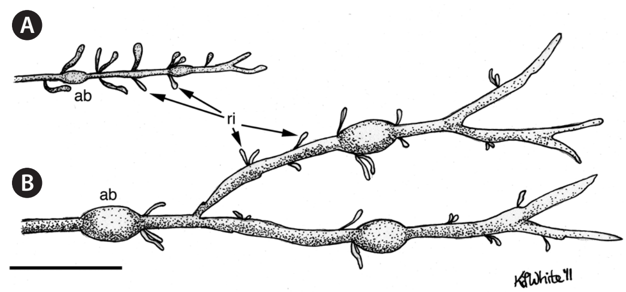

Fig. 2. Portions of *Ascophyllum* fronds from control (A) and farm (B) sites, showing production of receptacle initials (ri) and differences in size of axes and air bladders (ab). Scale bar represent: 5 cm.

Table 1. Morphological features of *Ascophyllum* from the four study sites. Different letters associated with each mean ± standard deviation indicate significant differences at $p < 0.05$ based on Tukey's post hoc analysis

Character	Farm-in	Farm-out	ERMES	Forbes
Thallus mass (g)	260.7 ± 20.1^{a}	177.7 ± 20.1^{b}	104.3 ± 20.1^{bc}	76.2 ± 20.1^{c}
Vegetative mass (g)	209.2 ± 17.7^{a}	126.5 ± 17.7^{b}	79.1 ± 17.7^{b}	64.3 ± 17.7^{b}
Reproductive mass	51.5 ± 6.4^{a}	51.2 ± 6.4^{a}	25.1 ± 6.4^{b}	11.9 ± 6.4^{b}
No. Receptacle initials	968.4 ± 77.8^{a}	625.2 ± 77.8^{b}	381 ± 77.8^{bc}	180.2 ± 77.8^{c}
No. Initials / Vegetative thallus	5.6 ± 1.3^{ab}	8.8 ± 1.3^{a}	4.0 ± 1.3^{b}	3.1 ± 1.3^{b}
Frond length (cm)	78.1 ± 2.7^{b}	94.9 ± 2.7^{a}	85.4 ± 2.7^{ab}	87.2 ± 2.7^{ab}
Max. width (cm)	0.56 ± 0.02^{a}	0.50 ± 0.02^{a}	0.35 ± 0.02^{b}	0.19 ± 0.02^{c}
No. Dichotomies	5.1 ± 0.3^{b}	5.0 ± 0.3^{b}	6.3 ± 0.3^{a}	5.2 ± 0.3^{ab}
No. Air bladders	4.8 ± 0.34^{c}	5.0 ± 0.34^{c}	7.2 ± 0.34^{b}	9.0 ± 0.34^{a}
Maximum length air bladders (cm)	2.8 ± 0.08^{a}	2.6 ± 0.08^{a}	1.9 ± 0.08^{b}	1.4 ± 0.08^{c}
No. Air bldder / cm	0.06 ± 0.08^{c}	0.05 ± 0.08^{c}	0.08 ± 0.08^{b}	0.1 ± 0.08^{a}
Mass per receptacle initial (g)	0.08 ± 0.03^{ab}	0.05 ± 0.03^{b}	0.18 ± 0.03^{a}	0.03 ± 0.03^{b}

ERMES, Evelyn Richardson Memorial Elementary School.

at control sites (Table 1, Fig. 2). These field observations were extended with more comprehensive morphometric evaluations (Table 2). Whereas frond lengths at control and experimental sites were similar, thallus biomass was over twice as great at farm sites, and this biomass showed a similar distribution between vegetative and reproductive tissues (79 and 77% vegetative for control and farm sites, respectively). This difference in biomass resulted from farm sites having much wider thalli (5.3 mm vs. 2.7 mm; significant at $p < 0.05$), which were also thicker (not measured). At farm sites, the longest frond on each thallus had fewer air bladders on its primary axis, and these farm site air bladders were longer (2.7 cm long vs. 1.6 cm long; significant at $p < 0.05$). The difference in air bladder numbers reflects differences in frond age, with the large fronds at control sites being three years older than those at farm sites (8.1 y vs. 4.9 y, respectively; significant at $p < 0.05$).

With respect to reproductive features, thalli from farm sites had more receptacle initials per thallus (797 vs. 280), and this corresponded to more initials per unit mass of vegetative thallus (7.2 receptacles g^{-1} vs. 3.5 receptacles g^{-1} for farm and control sites, respectively, significant at $p < 0.05$). Average receptacle biomass was significantly lower at farm sites (0.06 g vs. 0.10 g; significant at $p < 0.05$). The 21 and 23% of thallus biomasses comprising receptacle tissue suggests a standard reproductive : vegetative biomass ratio for these populations at this stage in development (Fig. 2).

Table 2. Values of morphometric characters at control and test sites

Character	Control site	Test site
Thallus mass (g)	90.2 ± 14.8	219.2 ± 14.8*
Thallus vegetative mass (g)	71.71 ± 12.8	167.86 ± 12.8*
Thallus reproductive mass	18.5 ± 28.9	51.4 ± 49.7*
No. Receptacle initials per thallus	280 ± 58	797 ± 58*
No. Initials / g of vegetative thallus	3.5 ± 4.8	7.2 ± 10.9*
Frond length (cm)	86.4 ± 2.4	86.5 ± 2.4
Maximum width (cm)	0.27 ± 0.02	0.53 ± 0.02*
No. Dichotomies	6.0 ± 0.2	5.1 ± 0.2
No. Air bladders	8.1 ± 0.3	4.9 ± 0.3*
Length air bladders (cm)	1.6 ± 0.1	2.7 ± 0.1*
Air bladders / cm	0.095 ± 0.003	0.058 ± 0.003*
Receptacle initial biomass (g)	0.10 ± 0.29	0.06 ± 0.03

Figures indicate mean ± standard error (n = 20).
*Significant at $p < 0.05$.

Impact of tidal height on farm vs. control features

At each study site, we collected *Ascophyllum* thalli at two tidal elevations: high and low. For this analysis, data for the two farm sites and for the two control sites were combined (Fig. 3A-H). The most conspicuous feature of these data is the emergence of clear morphological differences that are independent of tidal elevation. Only one of the eight characters (frond length) showed significant differences between high and low shore fronds, with longer fronds at the higher elevation at the farm sites; whereas at the control sites, frond length was significantly greater in low shore thalli (Fig. 3D). Only three morphological characteristics (reproductive mass, frond length, and number of reproductive initials per gram of vegetative mass) had overlapping means between control sites and farm sites. The two-way ANOVAs on these eight characteristics, which included both site and tidal elevation as variables, found sites always had a highly significant effect. Tidal height was significant for five characteristics, and the interactions of the two effects were significant for four (Table 3).

Growth rates

To evaluate annual growth among sites and tidal heights, the current year's growth was measured and compared to the length of the previous year's growth segment (see Fig. 2). Using the entire data set, we compared differences between high and low shore thalli and between control and farm sites (Table 4). While all populations initiated growth simultaneously in March (Kim unpublished observations), by the September data collection, the current year's growth was 20% greater in thalli from the lower shores. This difference resulted from the slower summer growth of upper shore thalli, which had completed only 69% of their growth by the September collection, whereas lower shore thalli had completed almost 80% of their annual growth during the six months prior to collection. Given the absence of a significant difference in the previous year's growth (i.e., means of 10.1 and 10.5 cm for high and low shores, respectively), high shore plants must grow faster during the second six months of their annual growth cycle.

The differences in annual growth increments between control and farm sites were much greater than between high and low shores. Thus, apical segments at control sites had grown only 5.2 cm, whereas those at farm sites had grown 10.1 cm (Table 4). This corresponded to a major difference in size for the previous year's segments, i.e., 7.6 and 13.1 cm, respectively (significant at $p < 0.05$). There was also a significant difference in the percentage of the previous year's growth, 70.4 and 81.4% ($p < 0.05$) at control and farm sites, respectively. Thus, control sites must undergo a greater percentage of their growth during the final six months of their annual cycle relative to farm sites (i.e., 30% vs. 20%).

Tissue carbon-nitrogen analysis

Tissue carbon and nitrogen content and C : N ratios varied from June to September (Table 5). Carbon showed a significant decline over the summer, from 38.5 to 36.9%. Nitrogen content varied from a high of 0.9% in June to a low of 0.7% in August. This difference in nitrogen content between August and all the other months was significant. The resulting C : N ratios varied from 48.2 in June to a high of 62.5 in August, with the August ratio differing significantly from those of the other months.

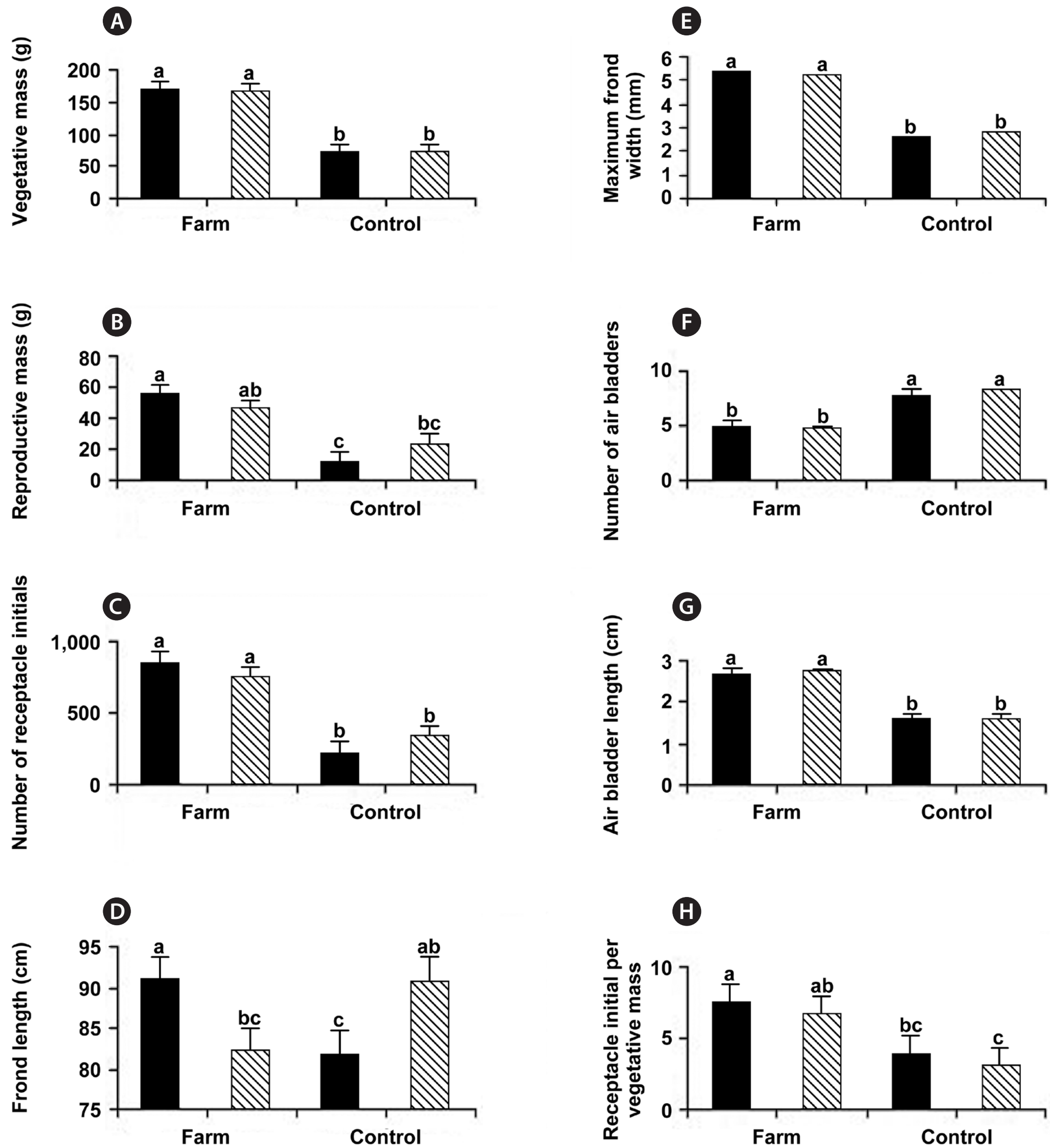

Fig. 3. Morphological variation in *Ascophyllum* at high (black) and low (striped) tidal elevations at farm and control sites. Bars with different letters indicate significant differences, based on one-way ANOVAs, followed by Tukey's post hoc test at $p < 0.05$. (A) Vegetative mass. (B) Reproductive mass. (C) Number of receptacle initials. (D) Frond length. (E) Maximum frond width. (F) Number of air bladders on longest frond axis. (G) Length of longest air bladder. (H) Number of receptacle initials per g of vegetative thallus mass.

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Table 3. Two-way ANOVA tables indicating significance of *Ascophyllum nodosum* morphological features at four study sites and two tidal heights

Morphological feature	Effect	Sum of squares	F ratio	Probability > F
Vegetative mass	Sites	510,703.37	13.941	< 0.001
	Tidal heights	85,488.45	7.001	0.009
	Sites × Tidal heights	4,197.18	0.115	0.952
Reproductive mass	Sites	46,684.80	9.950	< 0.001
	Tidal heights	3,570.494	2.283	0.133
	Sites × Tidal heights	11,960.937	2.549	0.058
No. of receptacle initials	Sites	13,822,243	19.048	< 0.001
	Tidal heights	1,549,111	6.404	0.012
	Sites × Tidal heights	1,643,637	2.265	0.083
Frond length	Sites	5,740.925	6.713	< 0.001
	Tidal heights	12,180.100	42.729	< 0.001
	Sites × Tidal heights	11,672.450	13.650	< 0.001
Maximum frond width	Sites	3.615	91.612	< 0.001
	Tidal heights	0.267	21.856	< 0.001
	Sites × Tidal heights	0.484	13.202	< 0.001
No. of air bladders	Sites	478.619	34.172	< 0.001
	Tidal heights	10.506	2.250	0.136
	Sites × Tidal heights	209.169	14.934	< 0.001
Air bladder length	Sites	54.843	65.634	< 0.001
	Tidal heights	9.543	33.938	< 0.001
	Sites × Tidal heights	5.564	6.659	< 0.001
Receptacle initials / g vegetative tissue	Sites	740.677	3.453	0.018
	Tidal heights	65.608	0.918	0.340
	Sites × Tidal heights	250.883	1.170	0.323

Table 4. Annual growth data for *Ascophyllum nodosum* thalli from high vs. low shores, from all four sites and for control versus farm sites

	High shore vs. Low shore	Control vs. Farm
Apical length (cm)	6.9 ± 3.4 vs. 8.3 ± 3.2*	5.2 ± 1.6 vs. 10.1 ± 3.0*
Previous year's growth (cm)	10.1 ± 5.5 vs. 10.5 ± 3.7	7.6 ± 2.5 vs. 13.1 ± 4.7*
Previous year's growth (%)	68.6 ± 22.6 vs. 79.2 ± 23.8*	70.4 ± 21.9 vs. 81.4 ± 23.7*

*Significant at $p < 0.05$.

Table 5. Tissue nutrient analysis for all sites per month. The letter associated with mean ± standard deviation indicates the Tukey's post hoc analysis results showed with the significant difference at $p < 0.05$

	June	July	August	September
Carbon (%)	38.46 ± 0.14^a	38.70 ± 0.14^a	37.48 ± 0.14^b	36.94 ± 0.14^c
Nitrogen (%)	0.94 ± 0.029^a	0.84 ± 0.029^a	0.72 ± 0.029^b	0.89 ± 0.029^a
C : N	48.19 ± 1.87^b	59.67 ± 1.87^a	62.50 ± 1.87^a	56.93 ± 1.87^a

Figures show mean ± standard deviation; sample sizes for June and July, n = 24; for August and September, n = 32.

Position on the shore did not yield significant differences in elemental composition with respect to C and N. Carbon composition values were the same for high and low shore fronds: 38.2 ± 0.4%. Nitrogen content showed greater variation between high and low shore fronds, with 0.82 ± 0.44 and 0.79 ± 0.44% for high and low, respectively; however, these did not differ significantly ($p > 0.05$).

The biggest differences in elemental composition were between farm and control sites (Table 6). While the values for percent carbon showed statistically significant differences (37.3 and 38.3% for farm and control sites respectively, $p < 0.001$), the values were markedly similar. This was not the case for percent nitrogen, where the nitrogen content at farm sites was over twice that of the control sites: 1.2 and 0.5%, respectively (significant at $p < 0.001$). The difference in nitrogen content was responsible for the sharply differing C : N ratios of 32.9 and 81.4 for farm and control sites, respectively (significant at $p < 0.001$).

DISCUSSION

As in other marine algae, *Ascophyllum* populations show considerable morphological variation. Researchers generally consider this a consequence of phenotypic plasticity, in which the fronds respond to differences in their physical, chemical, and / or biological environments. The most extreme form of this plasticity in *Ascophyllum* occurs in the salt marsh ecad *scorpioides*, in which highly entangled mats, are partly incorporated into the marsh sediments, and whose fronds lack holdfasts, air bladders, and receptacles (Chock and Mathieson 1976). To the uninitiated, these thalli may be unrecognizable as the same species that grows on rocky shores. All thalli we examined were obviously *Ascophyllum* and had the typical morphology of thalli from the outer shores of the Bay of Fundy and the Atlantic coast of Nova Scotia (MacFarlane 1933, Cousens 1982, McLean 2007).

The significant differences we found in the morphology of *Ascophyllum nodosum* between farm and control sites indicate that the effluent from the land-based finfish farm has major effects on these populations. The *Ascophyllum* from the farm sites were larger overall (i.e., had greater mass) than were the *Ascophyllum* from the control sites. The farm sites also had more receptacle initials, greater maximum frond width, and greater maximum airbladder length than the control sites had. We conclude that these differences are due to nutrients, not to physical factors in the environment. This is because of the overall similarities in wave exposure and substratum between the farm-out and control sites, and the basic morphological similarity between farm-out and farm-in samples. This is despite the major differences in wave exposure that the latter two populations experience.

The *Ascophyllum* of the two control sites in southwestern Nova Scotia show morphology equivalent to that of specimens on most Nova Scotian shores where the species occurs (MacFarlane 1933, Cousens 1982, Eckersley and Garbary 2007, McLean 2007). The nutrient-induced morphology differences we observed in southwestern Nova Scotia resemble differences between the “typical” morphology and that of high-current areas, e.g., in St. Mary’s Bay and the Captains Pond channel in Antigonish Harbour (McLean 2007, Garbary unpublished observations). These high current areas have widely different wave exposures (e.g., fetches of 5 m to > 5,000 m). Such fronds likely have higher growth rates and a different morphology as a response to an increased ability to absorb nutrients, due to the higher water volume passing over the frond surfaces.

The control site samples had more air bladders. To see if this was a true finding, and not simply a data artifact, we calculated the number of airbladders per unit length. This result shows the control site samples also had more air bladders per unit length of the longest frond. This is significant, as the number of air bladders on the longest frond provides an age estimate for *Ascophyllum* (e.g., Cousens 1982, Garbary et al. 2006, Eckersley and Garbary 2007). Thus, the farm site fronds are, in fact, younger than the control site fronds.

Previous accounts of phenotypic variation emphasize the role of shore elevation in determining growth and morphology. In his extensive review, Chapman (1995) suggests longer fucoid thalli occur lower on the shore because their greater immersion times allow greater growth (e.g., Vadas and Wright 1986, Stengel and Dring 1997). Other data from Nova Scotia (Garbary unpublished) confirm this for the northeastern mainland of the province. Thus, it is curious that we find little support for this in our

Table 6. Tissue nutrient contents for combined data from control and farm sites

	Farm	Control	p-value
Nitrogen (%)	1.18 ± 0.02	0.49 ± 0.02	< 0.001
Carbon (%)	37.29 ± 0.11	38.31 ± 0.10	< 0.001
C : N	32.92 ± 1.59	81.43 ± 1.48	< 0.001

Figures show mean ± standard deviation; for farm sites, n = 48; for control sites, n = 64.

data when we partition it based on tidal elevation (Table 4, Fig. 3). Neither vegetative nor reproductive biomasses, at farm or control sites, show differences attributable to tidal height (Fig. 3A & B). Moreover, at the farm sites, fronds from the lower elevation are significantly shorter in length (Fig. 3D). We confirmed this general observation by measuring the segments' annual growth from the previous year (2009), finding no difference between shore elevations. The apparently contradictory significant difference in apical lengths that we measured in September may simply represent a shift in growth towards later in the season, for upper shore fronds. This anomaly might result from the extensive fogs during summer in southwestern Nova Scotia. These limit desiccation stress, particularly on thalli that have lower surface to volume ratios, such as those at the farm sites.

Our results show that land-based aquaculture facilities, and nutrient enrichment in general, affect the morphology of *Ascophyllum nodosum*. The affected fronds are notably larger (but not longer), and we find no evidence of a detrimental impact on *Ascophyllum*. However, preliminary data on *Fucus vesiculosus* (White and Beveridge unpublished data) suggest that the level of nutrient loading experienced at our farm sites may negatively affect growth of *F. vesiculosus*. At present, however, we cannot distinguish between the possible negative impacts of higher nutrients on *F. vesiculosus* and competitive interactions with *Ascophyllum* as a basis for this result.

Researchers have used tissue N content in seaweeds as an indicator of the local N status (Fong et al. 1994), and studies on tissue N content of macroalgae in coastal environments show strong correlations to N availability (Chopin et al. 2001, Neori et al. 2004). Studies have also employed the C : N ratio as a nitrogen depletion indicator, since tissue N varies depending on the N status in the environment, while tissue C content is rather consistent (Kim et al. 2007). In the present study, the high C : N ratio at the control sites clearly indicates nitrogen depletion in the region. This result is unsurprising, because few significant point or non-point nutrient sources exist in southwest Nova Scotia. At the farm site, the *Ascophyllum* grew faster than did plants at the control sites (meaning *Ascophyllum* used more nitrogen for growth at the farm site), but these fronds still contained more nitrogen in their tissues. This result suggests the land-based finfish aquaculture farm provides sufficient N for the growth of *Ascophyllum* in a limited area (< 2 km from the farm).

Another, potentially more significant impact of the finfish effluent is coastal eutrophication, which often results in blooms of *Ulva* spp. (Ye et al. 2011). We did not observe this at our farm sites at Woods Harbour, although this might reflect high flushing rates, with consequent rapid dilution (farm-in and farm-out), and / or poor substrata for colonization (farm-in). Regardless, other aspects of community structure need to be examined before farm effluents can be considered benign. Measures on such things as the abundance and species composition of algal epiphytes on *Ascophyllum* and *F. vesiculosus*, as well as on species diversity of the algal and invertebrate populations, would be useful to characterize potential impacts.

Ascophyllum is a valuable cash crop in eastern Canada, and the possibility of enhancing its normally slow growth could have positive implications on the southwestern Nova Scotia economy. At the current level of nitrogen in the effluent, i.e., 300 μM nitrate (NO_3^-), as opposed to ambient seawater's maximum of 3 μM nitrate (NO_3^-) in this part of Nova Scotia, the *Ascophyllum* population appears to benefit. The enhanced growth may permit greater commercial harvesting in areas affected by finfish effluent. As one of the fastest growing industries in Atlantic Canada, fish farms are being proposed for coastal waters. The careful placement of finfish aquaculture may benefit currently harvested natural seaweed beds. Such planning could effectively produce integrated, multitrophic aquaculture systems (Chopin et al. 2001, Neori et al. 2004).

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