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Air Pressure Regulation in Air Bladders of *Ascophyllum nodosum* (Fucales, Phaeophyceae)

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Diurnal and age-related changes in air pressure were measured in air bladders of *Ascophyllum nodosum* from the Atlantic coast of Nova Scotia. Exterior and interior bladder volumes vary significantly with 4 and 6 y bladders being about 40% larger than 2 y bladders ($p < 0.01$). Freshly collected bladders yielded a mean pressure of 40.8 ± 6.5 cm H_2O. Overnight (20 h) dark treatment at 15°C generated pressure reductions by 80% in 2 y bladders but only by about 30% in 4 and 6 y bladders. Furthermore, in 2 y bladders 8 out of 11 bladders were reduced to atmospheric pressure. Pressure losses were inversely related to pressure recovery after 2.5 h in natural daylight, but after 5 h in daylight there was no significant difference in pressure within the age groups. Even under 25% of full illumination, bladders inflated to full pressure after 5 h. The results show that differences in bladder age and bladder wall thickness have roles in diurnal patterns of bladder inflation and deflation. These results confirm that bladder inflation is based on photosynthetic O_2 production and not on partial pressures of O_2 in the surrounding medium as was suggested for *Sargassum*. Chemical analyses of fluid recovered after the interior of bladders were washed with saline showed no evidence for the occurrence of surfactant that might be responsible for maintaining the air-liquid interface.

Key Words: air bladders, air pressure, *Ascophyllum*, Fucaceae

INTRODUCTION

Ascophyllum nodosum is among the most conspicuous intertidal seaweeds of cold to warm temperate shores of the North Atlantic Ocean. Distributed from New Jersey to Greenland and Iceland and from northern Norway to the Iberian Peninsula, *A. nodosum* forms extensive stands with high frond densities (Baardseth 1970). Through most of its distribution, the species occurs in a wide range of salinities and wave exposures from protected estuaries to moderately exposed rocky shores. Except for specialized forms in salt marshes where plants are loosely attached to sediments, thalli produce erect, regularly branched fronds with air bladders. The air bladders develop at annual intervals following growth of the apex (Baardseth 1970; Cousens 1982). Bladders form a more or less permanent part of the frond morphology and provide a buoyancy mechanism that maintains fronds erect in the water column during high tides (Damant 1937; Fritsch 1945; Tammes 1954; Aleem 1969; Dromgoole 1990). Although Hurka (1974) suggests that bladders are an adaptation against physical damage from wave action, most authors consider bladders an adaptation for light and nutrient acquisition in stands with extremely high frond densities.

Garbary *et al.* (2006) provided a detailed morphological and developmental account of bladders in *Ascophyllum nodosum*. A key observation was that the bladder wall varied in thickness during development with older bladders having thicker walls as previously documented by Damant (1937). This raised the possibility that bladders of different ages were physiologically different. Dromgoole (1990) provided an overview of bladder physiology in diverse brown algae. Here we use *A. nodosum* to examine the time course of bladder inflation in bladders of different ages and volumes following exposure to darkness and different light intensities using natural illumination. The occurrence of an air-liquid interface in the bladder interior suggests that surfactant, a surface tension reducing substance, might be present to maintain this bladder structure (e.g., Brackenbury *et al.* 2001, 2004). Thus we also assessed if surfactant was present within

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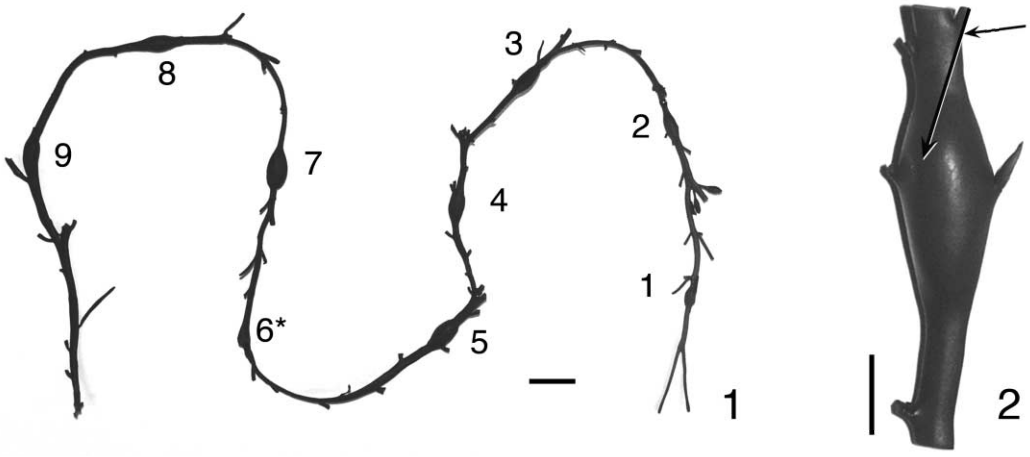

Fig. 1. *Ascophyllum nodosum*. Primary axis of sample frond with lateral branches removed indicating annual air bladders 1-9. (* indicates damaged bladder). Scale = 2 cm.

Fig. 2. Sample air bladder of *Ascophyllum nodosum* used in pressure experiments showing location of needle insertion into axis (short arrow) and direction of insertion into air bladder (large arrow). Scale = 1 cm

the air bladders of *A. nodosum*.

MATERIALS AND METHODS

Ascophyllum nodosum (Fig. 1) was collected at Tor Bay, Nova Scotia (45.18°N, 61.35°W) in early August 2005. This site is exposed to moderate wave action and full salinity (> 30 psu) (Garbary *et al.* 2006). Specimens were collected at midday during low tide and clear sunny conditions. After collection, *A. nodosum* was transported to the laboratory in plastic bags to retain hydration. One group of fronds was separated for the immediate determination of air bladder pressures as described below (completed within 4 h of collection, **FRESH** control). An additional 16 randomly selected fronds were separated for characterization of conspicuous morphological features of the population. After Back (1993), the following measurements were made on each frond: length, mass, number of vesicles, number of dichotomies in longest axis, and length of basal branch segment. The remaining fronds were left intact in plastic bags in a 15°C growth chamber.

The following morning (ca. 20 h after collection) air bladders were subsequently isolated by excising bladders with a razor blade and retaining approximately one centimetre of branch axis on each side of the bladder (Fig. 2). Isolation procedures were completed under dim lighting conditions in the laboratory (curtains drawn and overhead lights off, in a room with west facing windows) according to bladder age along the main frond. Two and 4 year old bladders as well as those 6 years and older (maximum of 8 y; hereafter referred to as 6 y bladders) were immediately placed into translucent, plastic containers (7 x 31 x 23 cm) in approximately 2.0 L of seawater in the experimental conditions described below with about 150 bladders in each container. Approximately 15-20 bladders of each of the three age groups were randomly selected for each exposure condition. Some bladders representing the three age categories (i.e. 2, 4, and 6 y) were immediately tested for bladder pressure. These bladders were considered the **DARK** control. Only undamaged, inflated bladders were used in the experiments.

Table 1. Temperatures changes in incubation chambers used in outdoor experiment.

Time	Full sun (°C)	25% of full sun (°C)	Dark control (°C)
11 am	18	18	18
12 am	19	19	19
1 pm	21	21	20
2 pm	22	20	20
3 pm	22	22	22
4 pm	22	21	21

The experimental design consisted of the two control conditions described above (i.e. **FRESH** and **DARK**) and four treatment conditions. Four of the exposure groups were placed outside under either full or partial natural light conditions. Seawater temperature in each container was recorded hourly over the duration of the exposure to ensure conditions were comparable between the various groups (Table 1). Photosynthetically active radiation

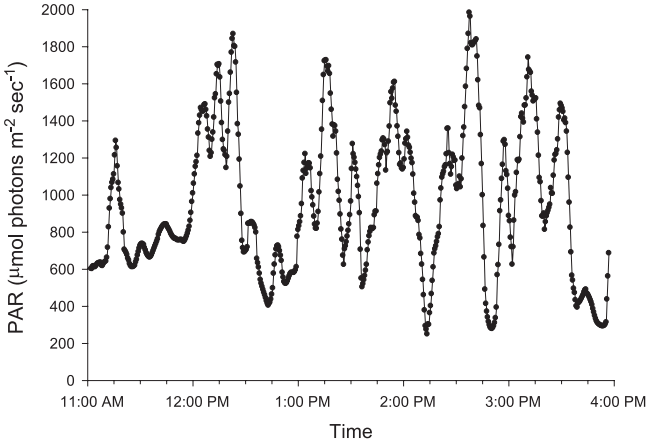

Fig. 3. Changes in photosynthetically active radiation between 11 am and 4 pm on August 2, 2005 when the experiment was conducted using natural light. Points represent the running mean of 10 readings taken at 30 s intervals during the experimental period. The initial values during the first and last 2.5 min are excluded.

(PAR) was measured at 30 sec intervals during the five-hour outdoor treatment period. PAR was measured using a LI-COR (Li-1400) meter equipped with the Quantum sensor LI-190SA (Li-COR Biosciences, Lincoln, NE). PAR varied considerably during the experiment with a mean of 953 μmol photons m^{-2} sec^{-1} (Fig. 3). Trays with bladders were placed in direct sunlight for 2.5 h (**FULL2.5**) or 5 h (**FULL5**). For partial light conditions (**PART**), mesh screens were placed over the plastic containers in order to reduce light exposure to approximately 25% of the full exposure groups. Similar exposure time periods were conducted, resulting in **PART2.5** and **PART5** groups. The remaining exposure group was placed outside covered with dark plastic to prevent light exposure for a 5 hour time period. After the light exposure, volume and pressure measurements were completed as described below. To determine time course changes during the first 2.5 h of exposure, 2 and 4 y old bladders were exposed to full light conditions as described above for 1, 1.5, 2, and 2.5 h. Pressure and volume measurements were completed at each time period.

Prior to determining internal pressures, bladder dimensions were measured with a vernier caliper in order to calculate the exterior and interior volume of each bladder. On each bladder, length (L), width (W) and height (H) were determined to the nearest 0.1 mm, and exterior volume was calculated based on the assumption that the shape was ellipsoid (V = 4/3 pi x L/2 x H/2 x W/2). Age-dependant wall thickness measurements were completed by sectioning a transverse ring through the midpoint of each bladder and measuring using a calibrated stereomicroscope at 32x magnification. Interior bladder volume was calculated by subtracting values for bladder wall thickness (determined after measuring pressure) from the L, W and H values.

Bladder pressure was assessed using a pressure transducer (Potometer; Qubit Systems, Kingston, Ontario) connected to a stopcock and a 23 gauge bevelled needle. The needle was allowed to penetrate from the frond axis into the interior of the air bladder and the peak pressure was digitized via a Serial box interface (Vernier software; Portland Oregon). Data were recorded by Data Logger software (Vernier Software; Portland, Oregon) on a Power MacIntosh 7200/120 system (Apple, Cupertino, California). Prior to pressure measurements, the transducer was calibrated by a U-manometer apparatus in order to convert voltage signals into centimetres of water pressure (cm H_2O). Between each bladder pressure measurement, the transducer was equilibrated to atmospheric pressure by opening the stopcock to room air.

Analysis of the interior environment of the air bladders for phospholipids and proteins, indicative of surfactant, was carried out by injecting 3% seawater or 0.85% saline into 2, 4, and 6 y bladders. Two bladders of each age group were washed twice by filling the airspace with approximately 0.5-1 mL and withdrawing contents using a 3 mL syringe connected to an 18-gauge needle. Each volume was reinstilled a total of three times and the 2 volumes combined. Details of analysis for possible phospholipid-phosphorus to quantify surfactant are described in Brackenbury *et al.* (2001, 2004). An SDS-PAGE gel was completed for protein analyses and stained using silver stain.

A one-way analysis of variance (ANOVA) with a Bonferroni post-hoc test was used to analyse statistical differences among bladders within an age group. Significant differences in volume between the bladder ages were also assessed by a one-way ANOVA. A probability value of less than 0.05 was considered significant.

RESULTS

The morphology of the Tor Bay population of *A. nodosum* is summarized in Table 2. Frond length ranged from 490 to 960 mm with approximately 2 to 11 dichotomies in the longest axis. All of the fronds had a

Table 2. Morphology of 16 randomly selected *Ascophyllum nodosum* fronds. Values indicate mean ± S.E.M.

Frond Length (mm)	Mass (g)	Vesicles per frond	Dichotomies in long axis	Stipe length (mm)
661 ± 37	45.1 ± 5.7	61 ± 7	6 ± 1	36 ± 9

minimum of four annual bladders with some of the fronds possessing 12 y bladders.

Air bladder volume changed significantly with age in Tor Bay air bladders with exterior volume in 4 and 6 year old bladders being significantly larger than 2 y bladders (40% increase, $p < 0.001$) (Fig. 4). Bladder walls increased in thickness along the same age gradient; however, the 14 % increase was only significant at $p < 0.1$. Despite the small increase in wall thickness, interior bladder volume increased between 2 y bladders and 4 and 6 y bladders ($p < 0.005$). The absence of significant differences in interior bladder volume between 4 and 6 y bladders means that pressure changes can be directly compared.

Air pressure in bladders 4 h after collection (**FRESH**) varied from 20-60 cm H_2O. Although there was no significant difference among bladders of different age classes, 4 y bladders were about 10% higher than 2 y bladders and about 30% higher than 6 y bladders (Fig. 5A). Following overnight dark storage, bladder pressures decreased when compared to freshly collected specimens (Fig. 5B). The amount of pressure change was variable in the different age groups. While all of the pressures in the freshly collected samples were similar for all bladders with an average value of 40.8 ± 6.5 cm H_2O, measurements from the DARK controls revealed an 80% pressure decrease for 2 y bladders, a 30% decrease in 4 y bladders and a 60% loss of pressure in the 6 y bladders (Fig. 5). After dark exposure the majority of air bladders maintained a positive pressure although in the 2 y bladders, 8 of 11 had atmospheric values compared to only 1 bladder in the other age groups.

This pressure decrease was reversible after light exposure as shown in Figs 6-7. Following 2.5 h of full light there was over an 18 fold increase in pressure in the 2 y bladders, while the 4 and 6 y bladders exhibited a 4 fold and 11 fold increase respectively (Fig. 6A). There were no significant changes in air bladder pressures after 5 h of full light (**FULL**) as compared to 2.5 h of exposure (Fig. 6B). Similar results were attained after partial (**PART**) light exposure treatment. There were no

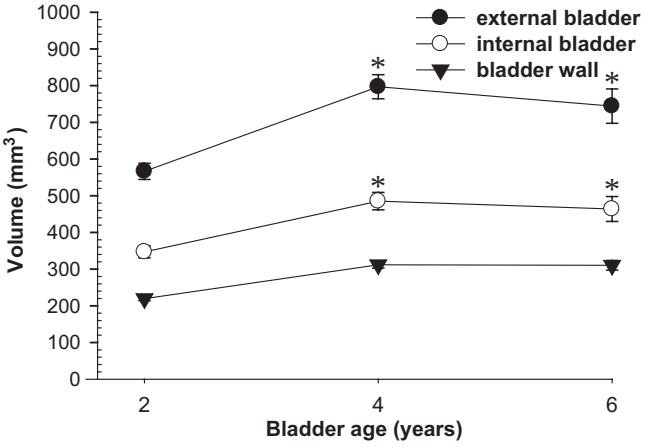

Fig. 4. Volume of air bladders, bladder walls and internal bladder spaces in air bladders of different ages from *Ascophyllum nodosum* collected at Tor Bay. Note: volumes based on formula for ellipsoid. Internal bladder volume based on calculation of external volume minus bladder wall volume. Asterisk (*) denotes significant difference ($p < 0.005$) when compared to 2 y bladders.

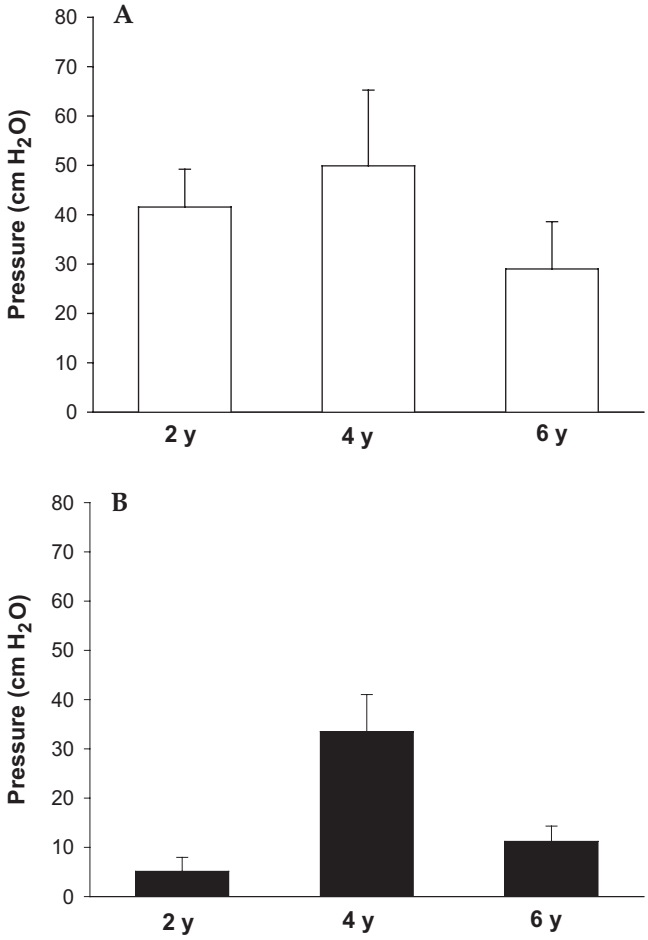

Fig. 5. *Ascophyllum nodosum*. A) Air pressures in 2, 4 and 6 y bladders collected during mid-day low tide and measured within four hours (FRESH control). B) Bladder pressures measured after fronds placed for 18 h in darkness and up to 2 h in dim light (DARK control).

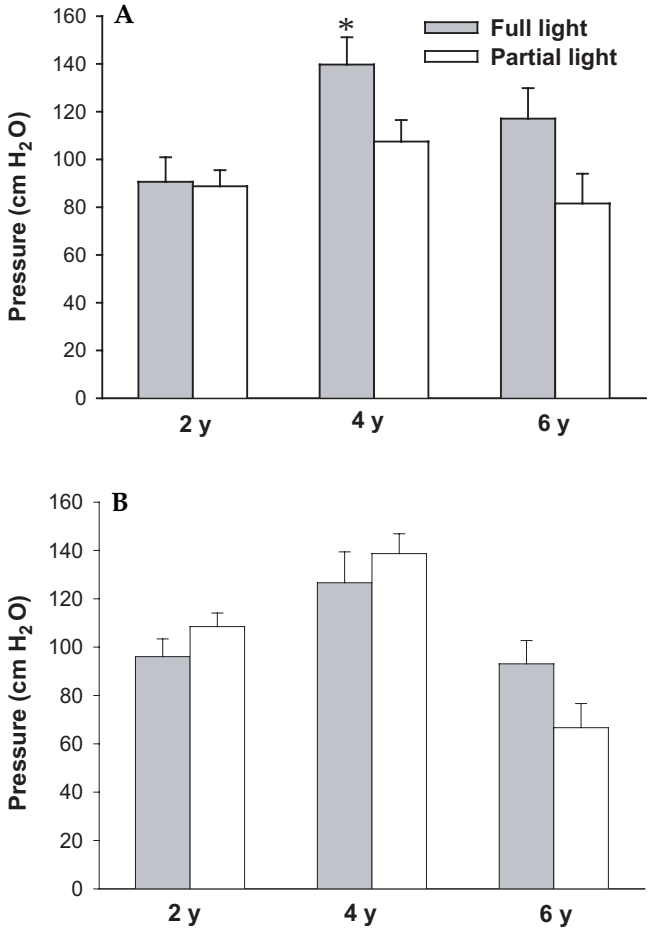

Fig. 6. *Ascophyllum nodosum*. Air pressures of 2, 4, and 6 y air bladders placed in full and 25% natural sunlight for 2.5 h (A) and 5 h (B). Asterisk (*) denotes significant difference ($p < 0.05$) between 4 y bladders at full and partial light.

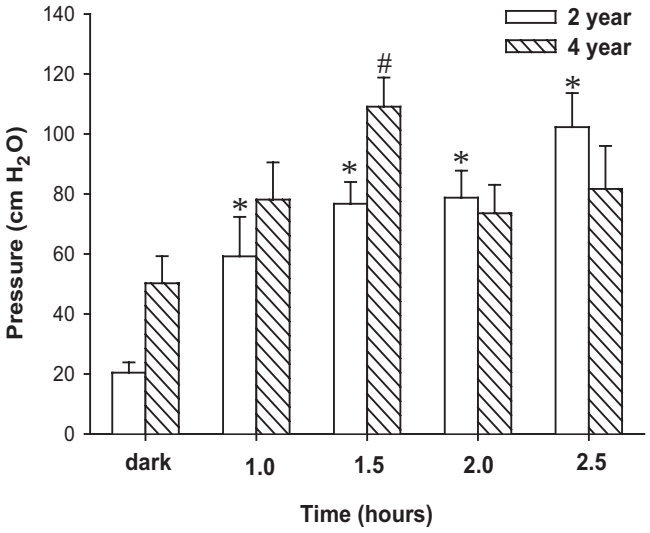

Fig. 7. *Ascophyllum nodosum*. Time course for inflation of 2 and 4 y air bladders exposed to full and 25% sunlight. *Denotes significant difference compared to dark exposed 2 y bladders; # denotes significant difference compared to dark and 1 h exposed 4 y bladders.

significant differences in bladder pressures between the two light conditions for the 2 and 6 y bladders following 2.5 h of exposure (Fig. 6A). However, the 4 y bladders exposed to partial light showed only a 3 fold increase in bladder pressure, significantly lower than the values obtained after full light ($p < 0.03$). After 5 h of exposure, there were no significant differences in bladder pressures within any of the age groups compared to the FULL 5 h group (Fig. 6B). There were also no significant differences in the pressure values between the baseline dark controls and the 5 h dark exposed bladders of similar ages.

For the time course experiment, only 2 and 4 y bladders were analyzed for pressure changes over a 2.5 h period (Fig. 7). These bladders were chosen due to the nature of the differences between these groups in the experiments described above. In 2 y bladders, pressures significantly increased after 1 hr of full light exposure as shown in Fig. 7 compared to dark control values ($p < 0.001$). These remained significantly higher than the dark controls for the remainder of the time course with no significant differences among any of the light exposure times. The 4 y bladders showed a similar trend, with pressure increases after light exposure. After 1.5 h, pressures were significantly higher than dark controls and the 1 h exposure group ($p < 0.03$); however, after 2-2.5 h there were no significant differences from baseline, dark control values.

Analyses of fluid obtained after washing the bladder airspace were negative for phospholipids or proteins that might serve as a surfactant.

DISCUSSION

The purpose of the current study was to elucidate age-related physiological differences in air bladders of *A. nodosum*. Previous studies by Damant (1937) showed that bladder position, which correlates with age, generated distinct pressure changes. Furthermore Garbary *et al.* (2006) showed a developmental change in bladder size and wall thickness with age in *A. nodosum* from Tor Bay and nearby sites. Given these results, differences in air bladder physiology might be expected.

The results of our study are intriguing. On the one hand, in virtually all of the experiments there was a tendency for air pressures to be higher in 4 y bladders relative to 2 y bladders, and for 6 y (and older) bladders

to have lower air pressures than 4 y bladders. On the other hand, 2 y bladders showed more rapid responses and greater magnitudes of change than in 4 y bladders. In addition, during the time course experiment 2 y bladders increased pressure throughout the 2.5 h exposure whereas 4 y bladders reached their maximum at only 1.5 h. The increase in bladder wall thickness between 2 and 4 y bladders might decrease the diffusion of gasses through the bladder wall; however, this does not account for the decline in pressures between 4 and 6 y bladders that had no significant difference in wall thickness. These observations suggest that these are age-related differences in photosynthetic capacity in the bladders themselves. These results correspond to the differences in pigment concentration and net photosynthesis in 1, 4 and 7 y old *A. nodosum* branch segments from the Irish Sea where there was a continual reduction in both physiological measures with age (Stengel and Dring 1998).

Based on experiments on *Sargassum leptopodum* Sonder, Hurka (1971) concluded that O_2 accumulation in brown algal air bladders was not primarily dependent upon photosynthesis, but based on the partial pressure of O_2 in the surrounding medium. A key morphological difference between the bladders of *Sargassum* and those of *Ascophyllum nodosum* (and *Fucus vesiculosus*) is that *Sargassum* does not possess the network of filaments that traverse the airspace of fucacean bladders. Garbary *et al.* (2006) showed that these filaments have chlorophyll *a* similar to adjacent medullary cells in non-bladder portions of the axes where the quantum yield is about 25% of that in the outer cortex (Garbary and Kim 2005). In addition, the filaments traversing the bladder in *Ascophyllum* have a photosynthetic electron transport rate that could supply the bladder with sufficient O_2 to provide the pressures described here (Kang, *personal* communication). Thus even if Hurka is correct in his model for the filling of bladders in *Sargassum*, this mechanism is not consistent with experimental data for Fucaceae shown here or by other authors (e.g., Damant 1937; Tammes 1954; Aleem 1969; Dromgoole 1981).

The pressures that we recorded from air bladders in Nova Scotia are equivalent to those reported previously. Some air bladders after the 20 h of low to dim light exposure showed somewhat collapsed bladders. Most of the values we recorded were above those published by Aleem (1969), with the highest pressures being 60% of the maximum recorded by Damant (1937). Based on the lower seasonal values Damant found in late summer when our experiments were carried out, our values are appropriate for healthy air bladders. Thus, pressures in our experiments were comparable with those for other brown algae (Dromgoole 1990).

Following collection, fronds were placed in plastic bags and subjected to dark or dim lighting conditions for 20 h prior to being exposed to full sunlight. Thus bladders were likely more deflated following our dark exposure than would be the case in nature when the dark and dim natural light period would be about 12 h. Regardless, these bladders reached close to maximum pressures within 2.5 h on a sunny day, even with major fluctuations in natural illumination (Fig. 3).

While the interior of the air bladders remains remarkably dry, Walsby (1972) reported that pressure fluctuations corresponded to fluid in the airspace of the bladder. Considering the presence of the air-liquid interface, increased surface tension may promote bladder collapse especially in younger, more delicate bladders. In the lungs of vertebrates and swim bladders of teleosts, a mixture of phospholipids and/or proteins known as surfactant promotes surface tension reduction and keeps the airspace dry. Analysis of phospholipid-phosphorus and protein content of fluid recovered after washing the airspace did not show the presence of surfactant-like compounds. Hence the air-filled nature of healthy bladders is likely maintained by the slow rate of water diffusion out across the bladder wall and the production of photosynthetic O_2. Dromgoole (1990) suggested that bladders may be capable of removing water, and Garbary *et al.* (2006) provided evidence of bladder repair in several populations of *A. nodosum* from Nova Scotia. These observations suggest that removal of water from bladders is a key component of their physiology.

The regular inflation of air bladders during the light and deflation during the dark was previously shown for both *Ascophyllum nodosum* and *Fucus vesiculosus* (Damant 1937; Tammes 1954; Aleem 1969), although age-related differences were not assessed. The subtlety of the physiological response in bladders of *A. nodosum* shown here could not have been predicted from the apparent homogeneity of anatomy. These results suggest that bladder physiology has a role in growth and survival that reflect ecological factors and developmental constraints that warrant further study.

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