Journal of Experimental Marine Biology and Ecology 440 (2013) 176–184

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Interactions between thermal and wave environments mediate intracellular acidity (H_2SO_4), growth, and mortality in the annual brown seaweed *Desmarestia viridis*

Caitlin Blain, Patrick Gagnon *

Department of Ocean Sciences, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada A1C 5S7

ARTICLE INFO

Article history:
Received 25 October 2012
Accepted 28 December 2012
Available online xxxx

Keywords:
Desmarestia viridis
Growth
Mortality
Sea temperature
Sulfuric acid
Waves

ABSTRACT

We used three laboratory experiments as well as measurements, throughout an entire growth season, of the length and survival of sporophytes, sea temperature, and wave height at two subtidal sites in Newfoundland (Canada), to investigate causal relationships between environmental variability, intracellular acidity, growth, and mortality in the highly acidic (H_2SO_4), annual brown seaweed *Desmarestia viridis* (O.F. Müller) J.V. Lamouroux. Light, grazing, and epibionts did not affect acidity. In the absence of waves, acid loss was threefold lower in cold (6.5 °C) than warm (11 °C) water, and threefold higher in the absence than presence of waves in warm (11 to 13 °C) water. There were three phases of change in frond length at both sites: (1) increase [March to late June], (2) no change [July to mid-August], and (3) decrease [mid-August to late October]. Mortality rates and sea temperature in Phase 1 were low, whereas the onset of increasing mortality at the end of Phase 2 coincided well with the end of a 2-week period during which temperature increased rapidly, from 6 to 11 °C. Results strongly suggested the more severe wave climate at the most wave-exposed site in September, when temperature was ~10 °C, facilitated the dispersal of the acid being released, which delayed mortality by ~10 days compared to the other site. All sporophytes had disappeared at both sites in late October. This is the first integrated analysis of seasonal variation in growth and mortality of *D. viridis* sporophytes. The strong connections between laboratory and field data attest to the critical role that thermal and hydrodynamic environments play in the life history of this unique seaweed.

1. Introduction

Seaweeds are one of the most conspicuous and productive components of shallow marine ecosystems worldwide (Duffy and Hay, 2000; Duggins et al., 1990; Steneck et al., 2002). Despite a productivity that can surpass that of the most productive terrestrial forests, fleshy seaweeds are routinely kept in check by herbivores (Carpenter, 1986; Cyr and Pace, 1993; Hay, 1991; Mann, 1973). Like terrestrial and freshwater plants (Dorenbosch and Bakker, 2011; Hanley et al., 2007; Howe and Jander, 2008; Strauss and Agrawal, 1999; Zamora et al., 1999), seaweeds exhibit numerous adaptations to avoid or deter (impede) grazing (Amsler and Fairhead, 2006; Amsler et al., 2009; Duffy and Hay, 1990; Hay, 1996; Jormalainen and Honkanen, 2008). In general, avoidance involves recruiting in habitat patches without grazers (spatial avoidance) or exhibiting a complex life history that may include a microscopic stage (alternation of generations), which is not accessible to grazers (Lubchenco and Cubit, 1980; Milchunas and Noy-Meir, 2002). Avoidance is common in habitats with low grazer abundance, whereas deterrence is characteristic of herbivore-dominated communities (Bolser and Hay, 1996; Duffy and Hay, 1990; Hay and Steinberg, 1992; Lubchenco and Gaines, 1981). Deterrence mechanisms include the production of structural and chemical defenses, and hence are generally regarded as energetically more costly strategies than avoidance (Agrawal, 2000, 2005; Gagnon et al., 2006; Hay, 2009; Konar, 2000; Konar and Estes, 2003; Steneck, 1986).

The annual, brown seaweed *Desmarestia viridis* (O.F. Müller) J.V. Lamouroux (Desmarestiales), is one of the very few fleshy macrophytes commonly found in shallow (<15 m deep) urchin-dominated barren grounds in the northwestern Atlantic. The ability of *D. viridis* to produce and store high concentrations of sulfuric acid (H_2SO_4) in intracellular vacuoles (down to a pH of 0.5, Gagnon et al., 2013; McClintock et al., 1982; Sasaki et al., 1999) is unusual among seaweeds. Using *D. viridis* sporophytes from Newfoundland (eastern Canada), Gagnon et al. (2013) showed the acid is continuously and irreversibly accumulated as the seaweed grows from a recruit in March, to adult, in June, and that rising sea temperatures during summer coincide with the release of the acid to the environment. The latter study uncovers synergistic, pervasive effects of changes in water temperature and salinity on the ability of *D. viridis* to produce and accumulate the acid. Yet, these two factors may compound with others in affecting acid production, growth, and survival. Specifically, light, wave action, and grazing can markedly affect the distribution, growth, morphology, and chemical makeup of seaweeds (Barko et al., 1982; Blanchette, 1997; Chapman and Craigie, 1977; Gagné et al., 1982; Twilley and Barko, 1990; Yñiguez et al., 2010).

* Corresponding author. Tel.: +1 709 864 7663; fax: +1 709 864 3220.
E-mail address: pgagnon@mun.ca (P. Gagnon).

0022-0981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jembe.2012.12.013

Effects of light and temperature on photosynthesis, growth, and reproduction in Desmarestiales are relatively well documented for species endemic to the Southern Hemisphere, especially the Antarctic region. For example, in the perennials *Desmarestia anceps* Montagne, *Himantothallus grandifolius* (A. Gepp & E.S. Gepp) Zinova, and *Phaeurus antarcticus* Skottsberg, gametophyte and sporophyte development is chiefly controlled by light, temperature, and nutrient levels (Wiencke and Clayton, 1990; Wiencke and tom Dieck, 1989; Wiencke et al., 1996), whereas thermal tolerances are generally lower in sporophytes than gametophytes (Wiencke et al., 1994). Growth and survival in the phylogenetically related, though amphiequatorial, *D. viridis* are poorly documented (but see Gagnon et al., 2003b, 2004). Furthermore, experimental testing of effects of light, wave action, and their interaction with temperature on acidity is lacking. A few studies suggest that sulfuric acid in *D. viridis* and a few sister species reduces grazing by urchins (Gagnon et al., 2006; Molis et al., 2009; Pelletreau and Muller-Parker, 2002). However, it is unclear whether grazing and epibionts affect acidity. An integrated study of the factors that may affect acidity in *D. viridis* and the relationships between growth, survival, and environmental variability throughout its existence as a sporophyte, is required to further identify the ecological causes and consequences of acid production.

In the present study, which builds upon findings by Gagnon et al. (2013), we use three laboratory experiments with *D. viridis* sporophytes from Newfoundland to investigate causal relationships between abiotic and biotic factors and intracellular acidity in *D. viridis*. Specifically, we (1) examine individual and combined effects of water temperature and light on acidity during senescence, (2) determine whether wave action affects acidity and how this effect may change over time, and (3) test the postulate that grazing and epibionts augment acidity. Furthermore, we use measurements, throughout an entire growth season, of the length and survival of *D. viridis* sporophytes, sea temperature, and wave height at two sites, to (4) characterize growth and mortality patterns and their relationship with natural environmental variability.

2. Materials and methods

2.1. Study sites

This study was conducted with *D. viridis* at two sites in Bay Bulls on the eastern side of the Avalon Peninsula, Newfoundland and Labrador (Canada): Bread and Cheese Cove (BCC, 47°18′35″ N, 52°47′30″ W) and Keys Point (KP, 47°18′15″ N, 52°48′24″ W). BCC is a small (~0.1 km^2) semi-protected cove on the northern shore of Bay Bulls, whereas KP is located along the southern shore and is fully exposed to offshore waves and swell. The two sites are ~1.4 km apart. The seabed at both sites is composed of gently sloping bedrock to a depth of ~15 m (chart datum). Patchy kelp beds (mainly *Alaria esculenta* (Linnaeus) Greville and *Laminaria digitata* (Hudson) J.V. Lamouroux) dominate the 0–2 m depth range, followed in deeper water by extensive urchin (*Strongylocentrotus droebachiensis*, O. F. Müller) barrens to a depth of ~15 m. Dense populations of *D. viridis* establish every year in these urchin barrens, as well as scattered patches of the perennial conspecific *Desmarestia aculeata* (Linnaeus) J.V. Lamouroux and grazing resistant kelp *Agarum clathratum* Dumortier (Gagnon et al., 2005). The latter species develops dense beds at depths >15 m, where urchin abundance is low.

2.2. Temperature and light (Experiment 1)

Tissue loss in *D. viridis* sporophytes during senescence is consistently lower and slower in cold than warm water (Gagnon et al., 2013). To further understanding about effects of temperature on intracellular acidity during senescence and how these may interact with another key environmental variable, light, we used a factorial experiment, Experiment 1, in which frond tissues of *D. viridis* were exposed to either of four treatments in glass tanks: (1) cold water and low light intensity [C/L], (2) cold water and high light intensity [C/H], (3) warm water and low light intensity [W/L], and (4) warm water and high light intensity [W/H]. Temperatures in the cold and warm water treatments were 6.6 ± 0.2 °C and 11.1 ± 0.1 °C, respectively, which reflects low-stress (cold) and sub-lethal (warm) thermal conditions for the species (Gagnon et al., 2013). Light intensity in the low and high light treatments was 0.1 and 25 μmol photons m^{-2} s^{-1}, respectively, and was applied uninterruptedly (24 h day^{-1}) throughout the experiment. These intensities paralleled those in Fortes and Lüning (1980), while corresponding to intermediate values at the deeper end of *D. viridis* distribution at our study sites (Blain and Gagnon, unpubl. data).

We ran the experiment from 2 to 12 September, 2011 with tissues from 24 *D. viridis* sporophytes (~50 cm in length) collected (via SCUBA diving) on 1 September at depths between 6 and 12 m at KP. One piece of ~25 g was cut with scissors from the distal end of each sporophyte and placed in rigid, 4-L plastic containers (one piece per container) sealed under water to prevent contact of tissues with air at the surface (which could have caused acid loss and tissue death). Tissues from only those sporophytes that showed no sign of deterioration (e.g. discoloration and sloughing) were collected. Containers were put in large, opaque plastic bins and transported to the Ocean Sciences Centre (OSC, Memorial University of Newfoundland) where they were transferred to large holding tanks supplied with ambient (8.9 ± 0.6 °C), flow-through seawater pumped in from the adjacent embayment, Logy Bay. On 2 September (within less than 24 h of collection), we cut ~5 g of tissues from each piece in the tanks to determine their intracellular pH (see below) at the beginning of the experiment. Each remaining piece (~20 g) was transferred to either of 24, 75-L glass aquaria and secured to the bottom with 12-g weights attached to the stipe with a plastic cable tie. The aquaria were grouped in six blocks of four aquaria each. Each tank in each block was randomly assigned to one of the four experimental treatments (C/L, C/H, W/L, and W/H), for a total of six replicates per treatment.

Each tank was surrounded by a thick, opaque canvas to standardize light conditions. The desired light intensities were created with an incandescent, 100-Watt light bulb (Soft White, General Electric) positioned 45 cm above the water surface and controlled with dimmers. We used a chiller to cool the water in the cold water treatments, whereas seawater pumped in from the bay was used in the warm water treatments. Temperature and light intensity in one randomly chosen tank of each treatment was monitored with a temperature and light logger (±0.5 °C, HOBO Pendent; Onset Computer Corporation) throughout the 10 days that the experiment lasted. Tissues in the tanks were gently agitated twice a day to prevent the accumulation of sediments on their surface. Water was delivered to each tank at a rate of 1 L min^{-1}. The intracellular pH of *D. viridis* in each tank was determined again at the end of the experiment. Initial and final pH values were used to determine changes in pH in each treatment. To increase sample size, we reran the experiment (using the approach described above) from 13 to 23 September, 2011 with tissues from 24 sporophytes collected on 12 September. Therefore, each treatment was replicated 12 times in total.

2.3. Wave action (Experiment 2)

To determine whether wave action affects intracellular acidity, we conducted an experiment, Experiment 2, in an oscillatory wave tank (a modified version of that shown in Gagnon et al., 2003a), which mimicked the wave-induced sweeping motion of *D. viridis* fronds in natural habitats. We inserted a panel in the center of the tank to create two working sections (220 cm [L] × 90 cm [W] × 62 cm [H] each), one with and one without waves. Each trial lasted 1 week (7 days) during which six sporophytes (~50 cm in length), three in each of the two sections, were exposed to either no waves (still water) or 20 wave cycles per minute, each with a peak horizontal velocity of 0.2 m/s (measured with a Doppler current meter [Vector Current

Meter, Nortek]). Each sporophyte was assigned randomly to either of the two wave treatments (with and without) and secured to the tank bottom with a 2-kg weight attached to the stipe (~1 cm above the holdfast) with a plastic cable tie. Sporophytes were distributed in each working section so to eliminate contact with one another and with the sides of the tank.

We ran 15 trials from 7 April to 25 September, 2011 (~6 months) with freshly (<48 h) collected sporophytes (see Experiment 1 for collection and maintenance procedures prior to experimentation). Each trial was run with flow-through seawater from Logy Bay and sporophytes were exposed to indirect, natural light entering the lab through two large (0.5 m in diameter) circular windows. Water temperature in the tank was measured throughout with a temperature logger (±0.5 °C, HOBO Pendent; Onset Computer Corporation). Tissue acidity was determined for each sporophyte (see below) prior to start and at the end of each trial. Initial and final tissue pH values were used to calculate mean changes in pH for each group of three sporophytes in each trial. Subsequent examination of temperature data indicated marked differences in temperature ranges among blocks of 2 months: (1) 2.1 to 4.0 °C in April–May [late spring], (2) 4.5 to 10.4 °C in June–July [early summer], and (3) 11.2 to 12.8 °C in August–September [late summer], which corresponded relatively well with pre- and post-onset-of-acid release and senescence phases described by Gagnon et al. (2013). To detect possible interactions between wave action and seasonality on acidity, trials were divided into three groups based on the time they were run, leading to five replicates for each wave treatment in each of late spring, early summer, and late summer (see Statistical analysis).

2.4. Grazing and epibionts (Experiment 3)

To test the postulate that grazing and epibionts augment acidity, we used a factorial experiment, Experiment 3, in which frond tissues of *D. viridis* were exposed in glass tanks (same as those in Experiment 1) to the presence (+) or absence (−) of grazers (G) and epibionts (E) in four treatments: (1) absence of grazers and epibionts [G−E−], (2) presence of grazers only [G+E−], (3) presence of epibionts only [G−E+], and (4) presence of grazers and epibionts [G+E+]. We used the green sea urchin, *S. droebachiensis*, and caprellid amphipod *Caprella* sp., as the grazer and epibiont, respectively, since they are natural consumers or biofoulers of *D. viridis* in eastern Canada (Gagnon et al., 2005, 2006), including at our study sites. Ten urchins (4-to-6 cm in test diameter) and 20 caprellids (1-to-3 cm in length) were used in treatments necessitating one or the other.

Urchins and caprellids were collected (via SCUBA diving) on 2 June at depths between 2 and 12 m at KP. They were transferred to holding tanks supplied with ambient, flow-through seawater upon arrival at the OSC and starved for 1 week (urchins) or 2 days (caprellids) to standardize hunger levels in each species. We ran the experiment from 9 to 19 June, 2011 with tissues from 24 *D. viridis* sporophytes (~50 cm in length) collected on 8 June at depths between 6 and 12 m at KP (tissues were collect and transported to the OSC as described in Experiment 1). Water temperature during acclimation in the holding tanks was 6.2±0.8 °C. Tissues were gently groomed to remove epifauna, if any, prior to conducting the experiment. On 9 June (within less than 24 h of *D. viridis* collection), we cut ~5 g of tissues from each piece in the tanks to determine intracellular pH (see below) at the beginning of the experiment. Each remaining piece (~20 g) was weighted (blotted wet weight) and secured to the bottom of either of the 24 glass tanks with a 12-g weight attached to the stipe with a plastic cable tie. Tissue weight was determined in less than 20 s following emersion to minimize exposure to air. The aquaria were grouped in six blocks of four aquaria each. Each tank in each block was randomly assigned to one of the four experimental treatments (G−E−, G+E−, G−E+, and G+E+), for a total of six replicates per treatment.

The light environment in each tank was created and controlled as per the procedures described in Experiment 1, with the exception that a daily cycle of 12 h of light (25 μmol photons m^{-2} s^{-1}), followed by 12 h of darkness, was used. Water from Logy Bay was delivered to each tank at a rate of 1 $Lmin^{-1}$ and temperature (uncontrolled) in one randomly chosen tank of each treatment was monitored with temperature loggers throughout the 10 days that the experiment lasted. Temperature in the tanks during the experiment was 6.5±0.2 °C. The intracellular pH and blotted wet weight of *D. viridis* in each tank was again determined at the end of the experiment. Initial and final pH and weight values were used to determine changes in pH and amounts of tissues lost to grazing in each treatment. To increase sample size, we reran the experiment from 25 June to 5 July, 2011, with tissues from 24 sporophytes collected on 24 June, as well as urchins and caprellids collected on 18 June. Therefore, each treatment was replicated 12 times in total.

Procedures for collection, transportation, and maintenance of *D. viridis* sporophytes in the laboratory described in the three experiments were tested in a previous study with no perceptible effects on intracellular acidity (Gagnon et al., 2013). To determine whether exposure of *D. viridis* tissues to air (which was inevitable in all three experiments) affected acidity, we measured the change, over 7 days, in the intracellular pH of sporophytes exposed to air for either 0, 10, or 20 s (n=10 for each exposure time). Sporophytes were collected at KP on 24 July, 2012 (when the intracellular pH was fairly stable, Gagnon et al., 2013) and transported to and maintained in holding tanks the same way they were in Experiments 1, 2, and 3. After determining intracellular pH, each sporophyte was taken out of the tank, gently squeezed with fingers to remove excess water, exposed to air for the assigned duration, and reintroduced in the water. The intracellular pH of each sporophyte was again determined on 31 August, 2012. We found only very slight decreases in pH ranging from 0.0005±0.0001 (10 s) to 0.002±0.001 (0 s) ($F_{1,29}=0.45$, $p=0.64$). Therefore, we assumed that intracellular acidity was not affected by our approaches to handling and weighing of sporophytes in the laboratory.

2.5. Intracellular pH

Every determination of intracellular pH of *D. viridis* sporophytes in Experiments 1, 2, and 3 required the use of 4.8 to 5.3 g of tissues (blotted wet weight, measured with a balance with a precision of ±0.01 g [model PB-3002-S/FACT; Mettler Toledo]). Tissues were crushed for 60 s in 100 mL of distilled water with a high-speed blender (model Magic Bullet; Homeland Housewares). The blend was suctioned through a 25-μm filter paper (model 1004-070; Whatman) to remove particulates and subsequently divided into three parts of 25 mL each. The pH of each part was measured with a pH electrode (±0.02 pH units, model Accumet AP72; Fisher Scientific). The three values were averaged to estimate the raw pH of the tissue sample. The following equation was used to estimate the intracellular pH of the tissue sample (Gagnon et al., 2013; Sasaki et al., 2005): Intracellular pH = −log (α × Dilution factor), where α is the concentration of protons in the filtered blend calculated from the raw pH value ($[H^+]$ = antilog(-pH)) and Dilution factor is obtained from the equation: Dilution factor = ((distilled water [g] + tissue fresh weight [g]) × water content [mL])/(tissue fresh weight (g) × water content (mL)), where distilled water is the weight (100 g) of distilled water in which *D. viridis* tissues were blended, tissue fresh weight is the wet weight (between 4.8 and 5.3 g) of tissues, and water content is the volume (100 mL) of distilled water in which tissues were blended.

2.6. Growth and mortality patterns

To characterize growth and mortality patterns and their relationship with natural environmental variability, we measured, throughout an entire growth season, the length and survival of *D. viridis* sporophytes, sea temperature, and wave height at the two study sites. On 8 March,

2011 we tagged (with numbered disks and eyebolts secured to the seabed with marine epoxy [A-788 Splash Zone Compound; Carboline Company]), and measured the length (±0.5 cm) from the base of the holdfast to the distal end of the frond, of 10 (BCC) and 15 (KP) small (<10 cm) recruits at depths between 2 and 12 m. Subsequent length measurements of surviving sporophytes were done biweekly from 8 March to up to 20 October, 2011, when all sporophytes had disappeared. To compensate for mortalities, which were relatively frequent within the first 2 months, we tagged, on 18 March, 1 April, and 16 May, an additional 15, 16, and 20 sporophytes at BCC and 10, 15, and 16 sporophytes at KP, respectively, for a total of 61 (BCC) and 56 (KP) sporophytes. A sporophyte was considered dead when only the holdfast was still present. Using length data, we calculated a specific growth rate, SGR, for each sporophyte during each interval separating two consecutive measurements with the equation: $((L_f - L_o)/L_o)/t$, where L_o and L_f are the initial and final lengths of the frond (from the holdfast to the distal end of the frond), respectively, and t is the number of days between the two length measurements. Accordingly, SGR is expressed as a percentage of frond length per day ($\%day^{-1}$) and reflects the change in length over a specific period, relative to the previous period of assessment.

At each site, temperature was recorded every hour throughout the survey by each of three temperature loggers (±0.5 °C, model HOBO Pendent; Onset Computer Corporation) attached to eye bolts drilled into the seabed at depths of 3, 6, and 9 m. Wave height, which we used to estimate the severity of the hydrodynamic environment, was also recorded every minute by a water level logger (±0.05 cm, model HOBO U20 Water Level Logger; Onset Computer Corporation) secured to the seabed with eyebolts at a depth of 12 m. Raw wave heights were corrected for tidal elevation, barometric pressure, and depth of the logger. Temperature data pooled across the three depths, as well as significant wave height (SWH), defined as the average height of the highest one-third of the wave data, were aggregated into mean daily averages. Preliminary data inspection indicated sporophyte length at both sites generally increased (Phase 1) from March to early July, remained relatively unchanged (Phase 2) from July to mid-August, and decreased (Phase 3) afterwards until the end of the survey in mid-October. Accordingly, we aggregated, and analyzed differences in, SGR and mean daily sea temperature and SWH within and between sites and phases (see Statistical analysis).

2.7. Statistical analysis

We used a two-way ANOVA with the factors Temperature (cold and warm water) and Light (low and high intensity) to examine effects of temperature and light intensity on the intracellular acidity of *D. viridis* (Experiment 1). We applied the analyses to the square-root transformed data to correct for heteroskedasticity. As mentioned above, we ran the experiment twice in September, 2011. Prior to running this analysis, we had used a four-way ANOVA with the factors Run (each of the two September runs), Block (each of the six blocks of tanks in each run), Temperature (cold and warm water), and Light (low and high intensity), to determine whether results differed between blocks within trials. There was no significant interaction between the factors Run and Block ($F_{5,32} = 0.63$; $p = 0.68$), and hence we applied the two-way ANOVA to the pooled data from both runs. We used a two-way ANOVA with the factors Waves (with and without waves) and Time (late spring, early summer, and late summer) to analyze effects of wave exposure and seasonality on the intracellular acidity of *D. viridis* (Experiment 2). The analysis was applied to the raw data since they met the ANOVA postulates.

We used two two-way ANOVAs with the factors Grazers (presence and absence of urchins) and Epibionts (presence and absence of caprellids) to examine effects of grazing and epibionts on the intracellular acidity and loss of tissues of *D. viridis* (Experiment 3). The two analyses were applied to the raw data since they both met the ANOVA postulates. As mentioned above, we ran the experiment twice in June, 2011. Prior to running these two-way ANOVAs, we had used two four-way ANOVAs (one for acidity and one for tissue loss) with the factors Run (each of the two June runs), Block (each of the six blocks of tanks in each run), Grazers (presence and absence of urchins) and Epibionts (presence and absence of caprellids), to determine whether results differed between runs and blocks. There were no significant interactions between the factors Run and Block in both analyses ($F_{5,33} = 0.61$; $p = 0.63$ and $F_{5,33} = 0.82$; $p = 0.54$, respectively), and hence we applied the two two-way ANOVAs to the pooled data from both runs.

We used three two-way ANOVAs with the factors Site (BCC and KP study sites) and Phase (the three phases of change in frond length) to investigate differences in specific growth rate (SGR) of *D. viridis* sporophytes and mean daily sea temperature and significant wave height (SWH) between sites over time. Since no transformations corrected for the heteroskedasticity detected in the raw data on SGR and temperature, the ANOVAs were also applied to the rank transformed data. Because analyses on both raw and ranked-transformed data gave similar results, we present results from analyses of the raw data as suggested by Conover (1980). We applied the analysis of SWH to the raw data, which met the ANOVA postulates.

In all analyses, normality was verified using Shapiro–Wilk's statistics and homogeneity of variance by examining the graphical distribution of residuals and using Levene tests (Snedecor and Cochran, 1989). To detect differences among levels within a factor, we used Tukey HSD multiple comparison tests (comparisons based on least-square means) (Sokal and Rohlf, 2012). A significance threshold of 0.05 was used for all statistical tests. All analyses were conducted with JMP 7.0 and Minitab 16.

3. Results

As shown by Gagnon et al. (2013), an increase in intracellular pH in *D. viridis* is always accompanied by a decrease in pH of the surrounding water. Such simultaneous, opposite changes in pH are indicative of acid loss to the environment. Therefore, where appropriate, we report changes in mean intracellular pH of *D. viridis* over time in raw pH units, or in terms of gain (decrease in pH) or loss (increase in pH) of acid.

3.1. Temperature and light

Analysis of data from Experiment 1 with frond tissues of *D. viridis* exposed 10 days to either of four combinations of water temperature (cold and warm) and light intensity (low and high) indicated changes in intracellular pH varied with temperature only (Table 1). Tissues released acid to the environment in all four treatments as shown by increases in pH of 0.13 ± 0.02 units (Cold/High) to 0.40 ± 0.02 units (Warm/High) (Fig. 1). Yet, mean loss was approximately threefold higher in warm than cold water, regardless of light levels (LS means, $p < 0.001$, data pooled across temperature treatments) (Fig. 1). Overall, these results indicate that tissues were less stressed in cold than warm water.

Table 1
Summary of two-way ANOVA (applied to square-root transformed data) examining the effect of Temperature (cold and warm water) and Light (low and high intensity) on changes in intracellular acidity of frond tissues of *Desmarestia viridis* over 10 days in Experiment 1 (see Materials and methods for a description of the experiment).

Source of variation	*df*	MS	F-value	*p*
Temperature	1	0.95	88.32	<0.01
Light	1	0.00	0.20	0.66
Temperature × Light	1	0.00	0.37	0.55
Error	43	0.46		
Corrected total	46			

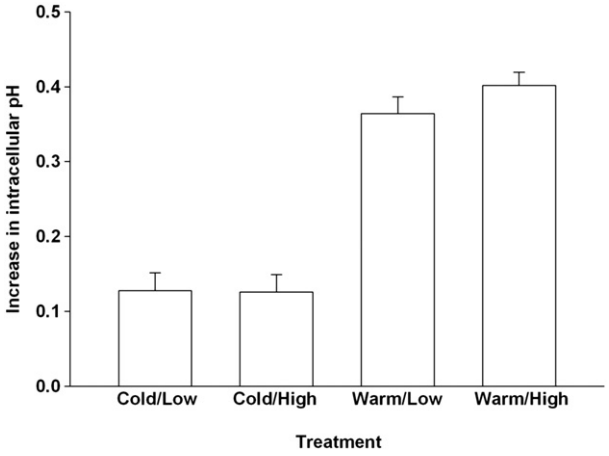

Fig. 1. Increase in mean (+SE) intracellular pH (denoting a decrease in acidity or loss of acid) of frond tissues of *Desmarestia viridis* (from initial mean value of 0.81 ± 0.02) exposed for 10 days to either of 4 combinations of water temperature (Cold [6.6 °C ± 0.2] and Warm [11.1 °C ± 0.1]) and light intensity (Low [0.1 μmol photons m^{-2} s^{-1}] and High [25 μmol photons m^{-2} s^{-1}]) (Experiment 1) (n = 12 for each treatment, except for Warm/High where n = 11).

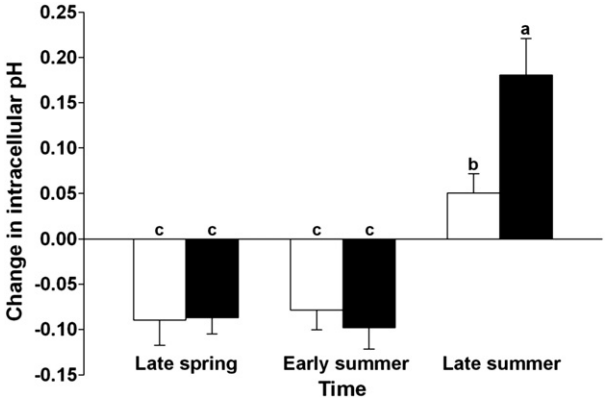

Fig. 2. Change in mean (+SE) intracellular pH of frond tissues of *Desmarestia viridis* (from initial mean value of 0.77 ± 0.01) exposed 7 days to either the presence (0.2 m/s) or absence (0 m/s) of waves in late spring (Apr–May), early summer (Jun–Jul), and late summer (Aug–Sep) (Experiment 2). Negative and positive changes in pH denote increases and decreases in acidity, respectively. Bars not sharing the same letter are different (LS means tests, $p < 0.05$; n = 5 for each Wave × Time treatment, except for early summer where n = 4). □ waves, ■ no waves.

3.2. Wave action

Analysis of data from Experiment 2 with frond tissues of *D. viridis* exposed for 7 days to either the presence or absence of waves, indicated that changes in intracellular pH varied with time (late spring, early summer, and late summer) between wave treatments (a significant interaction between the factors Waves and Time, Table 2). Whether waves were present or not, tissues consistently produced and accumulated acid in late spring and early summer, when temperature ranged from 2.1 °C to 10.4 °C, as shown by increases in intracellular acidity of 0.08 ± 0.02 (Waves/Early summer) to 0.10 ± 0.02 (No waves/Early summer) pH units (Fig. 2). Acid loss occurred in late summer only, when temperature ranged from 11.2 °C to 12.8 °C, and was on average 0.13 pH units higher in the absence (0.18 ± 0.04) than presence (0.05 ± 0.02) of waves (LS means, $p = 0.0021$, Fig. 2).

3.3. Grazing and epibionts

Analysis of data from Experiment 3 with frond tissues of *D. viridis* exposed for 10 days to either of four combinations of grazers (presence and absence of urchins) and epibionts (presence and absence of caprellids) treatment, indicated that changes in intracellular pH occurred independently of both factors (Table 3). Tissues lost acid in all treatments, as shown by increases in pH of 0.005 ± 0.022 units (G + E −) to 0.045 ± 0.019 units (G − E +) (Fig. 3). There was a general trend towards smaller pH increases in treatments with than without grazers, but these differences were not significant (Table 3, Fig. 3). Tissues were lost in all treatments, as shown by decreases in wet weight of 11.6 ± 3.5% (G − E −) to 40.1 ± 4.7% (G + E +). Yet, losses were at least twice higher in treatments with than without grazers (LS means, $p < 0.001$, data pooled across grazers treatments; Fig. 3). Tissue loss in the presence of epibionts only (G − E +) was not different than that in the control treatment (G − E −) (Table 3). Overall, these results indicate that epibionts had considerably less effect, if any, on the chemistry and physical integrity of *D. viridis*, than grazers.

3.4. Growth and mortality patterns

Analysis of field data collected throughout the 2011 growth season showed unimodal patterns of change in the length of fronds of *D. viridis* at the two study sites (Fig. 4). Frond length at BCC and KP increased at decelerating, though comparable rates, from March, when mean length was 11.1 ± 2.0 cm [BCC] and 7.6 ± 0.8 cm [KP], to early July, when mean length was 58.6 ± 1.7 cm [BCC] and 58.8 ± 2.1 cm [KP] (Fig. 4). This phase of increase (Phase 1) was followed by a period of ~1.5 months (early July to mid-August) during which length remained relatively unchanged (58.9 ± 0.8 and 58.9 ± 1.0 cm at BCC and KP, respectively) (Phase 2). The last phase (Phase 3) was marked by an accelerating decrease in length that seemed more pronounced at BCC than KP (Fig. 4). Despite a greater variation in March and April, mortality rates at both sites were relatively low (<15%) and stable throughout Phases 1 and 2 (Fig. 4). The dramatic increase in mortality characterizing Phase 3 was delayed by ~10 days at KP throughout much of September. There were only a few sporophytes (three at BCC and five at KP) left on the last sampling day (13 October). Although these sporophytes were

Table 2
Summary of two-way ANOVA (applied to raw data) examining the effect of Waves (with and without waves) and Time (runs conducted in late spring, early summer, and late summer) on changes in intracellular acidity of frond tissues of *Desmarestia viridis* over 7 days in Experiment 2 (see Materials and methods for a description of the experiment).

Source of variation	*df*	MS	F-value	*p*
Waves	1	0.0099	2.85	0.11
Time	2	0.13	38.25	<0.01
Waves × Time	2	0.015	4.43	0.024
Error	22	0.0035		
Corrected total	27			

Table 3
Summary of two-way ANOVAs (applied to raw data) examining the effect of Grazers (presence and absence of urchins) and Epibionts (presence and absence of caprellids) on changes in intracellular acidity, and wet weight as a percentage of initial wet weight, of frond tissues of *Desmarestia viridis* over 10 days in Experiment 3 (see Materials and methods for a description of the experiment).

Source of variation	*df*	MS	F-value	*p*
Intracellular pH				
Grazers	1	0.0095	1.87	0.18
Epibionts	1	0.0017	0.33	0.57
Grazers × Epibionts	1	<0.001	<0.01	0.98
Error	44	0.0051		
Corrected total	47			
Weight				
Grazers	1	6948.53	25.14	<0.01
Epibionts	1	228.73	0.83	0.37
Grazers × Epibionts	1	7.13	0.03	0.87
Error	44	12159.58		
Corrected total	47			

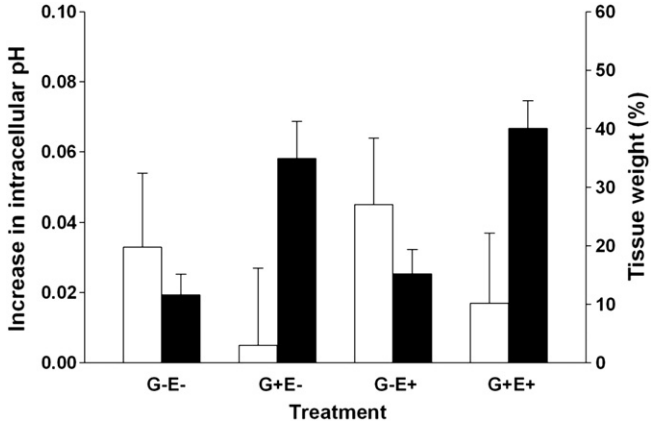

Fig. 3. Increase (denoting a decrease in acidity or loss of acid) in mean (+SE) intracellular pH (from initial mean value of 0.59±0.01) and decrease in mean (+SE) wet weight as a percentage of initial wet weight, of frond tissues of *Desmarestia viridis* exposed 10 days to the presence (+) or absence (−) of grazers (G) [10 green sea urchins, *Strongylocentrotus droebachiensis*] and epibionts (E) [20 caprellid amphipods *Caprella* sp.] (Experiment 3) (n=12 for each treatment). □ pH, ■ weight.

still relatively tall, ~35 to 45 cm (Fig. 4), most tissues had sloughed, leaving only dying, bare stipes and laterals. The seaweed had completely disappeared at both sites on our final visit on 20 October.

The two-way ANOVA on SGR indicated growth rates varied similarly between sites, while being different between phases (Table 4). Accordingly, SGR estimates pooled across sites in Phases 1, 2, and 3, averaged $2.9\pm0.1\%day^{-1}$ (positive), $-0.02\pm0.08\%day^{-1}$ (virtually null), and $-0.7\pm0.1\%day^{-1}$ (negative), respectively, and differed statistically from each other (Table 4). Like SGR, mean sea temperature was similar between sites, though differed between phases (Table 4), being 7 °C warmer in Phase 3 (9.9±0.1 °C) than Phase 1 (2.9±0.2 °C) (LS means, $p<0.001$, data pooled across sites; Fig. 4). Simultaneous onsets of decrease in frond length, increasing mortality, and negative SGR values at both sites coincided well with the end of a 1- or 2-week period during which sea temperature increased rapidly from ~6 to ~11 °C (Fig. 4). Significant wave height (SWH) differed between sites and phases (a significant interaction between the factors Site and Phase; Table 4, Fig. 4). It was 63% and 22% higher (LS means, $p<0.001$) at KP

Table 4
Summary of two-way ANOVAs (applied to raw data) examining the effect of Site (BCC and KP study sites) and Phase (the three phases of change in frond length) on differences in specific growth rate (SGR) of *Desmarestia viridis* sporophytes and mean daily sea temperature and significant wave height (SWH), from 8 March to 13 October, 2011.

Source of variation	*df*	MS	F-value	*p*
SGR				
Site	1	3.077	0.58	0.45
Phase	2	910.21	171.055	<0.01
Site × Phase	2	3.15	0.59	0.55
Error	717	3815.25		
Corrected total	722			
Temperature				
Site	1	1.023	0.20	0.65
Phase	2	2275.44	455.88	<0.01
Site × Phase	2	1.10	0.22	0.80
Error	430	4.99		
Corrected total	435			
SWH				
Site	1	0.65	84.35	<0.01
Phase	2	0.012	1.49	0.23
Site × Phase	2	0.11	14.47	<0.01
Error	407	0.0077		
Corrected total	412			

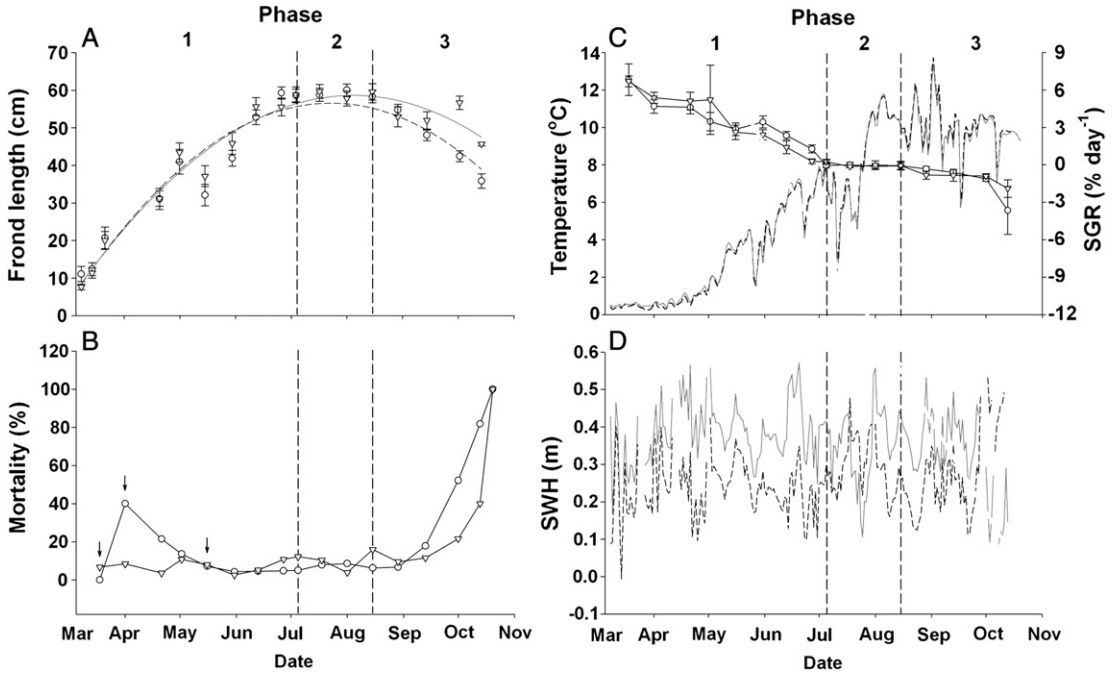

Fig. 4. Change in mean frond length (±SE) of *Desmarestia viridis* sporophytes (A), mortality (B), daily sea temperature and specific growth rate (SGR, ±SE) (C), and daily significant wave height (SWH) (D) at the two study sites (BCC and KP), from 8 March to 13 October, 2011. Sea temperature and wave height data were acquired every hour or minute, respectively, with three temperature loggers and a water level logger secured to the seabed at each site. Each SGR and frond length data point is the average of growth rates and length of all sporophytes measured on the corresponding date (n=3 to 39 [SGR] and 3 to 40 [frond length]). Each mortality data point is the proportion (%) of sporophytes that disappeared relative to the previous sampling date (n=5 to 46). Arrows (B) indicate days on which new sporophytes were tagged at each site to maintain sample sizes. Frond length curves (A) are the quadratic fits to these data: BCC; $y=2.506+0.745x-0.003x^2$ ($r^2=0.964$, $p<0.0001$, n=17) and KP; $y=3.063+0.705x-0.002x^2$ ($r^2=0.980$, $p<0.0001$, n=17).Vertical dashed lines indicate the commencement or end of each of the three phases of change in frond length suggested by preliminary data inspection: Phase 1 (increase), Phase 2 (no change), and Phase 3 (decrease). ○ BCC, ▽KP, - - - BCC, – KP.

than BCC in Phase 1 and Phase 3, respectively, though similar between sites in Phase 2 (LS means, $p = 0.068$; Fig. 5). Comparable peaks of 0.65 m (BCC, 31 July) and 0.62 m (KP, 28 September) were noted between sites (Fig. 4).

4. Discussion

We demonstrated synergistic effects of sea temperature and the hydrodynamic environment on the ability of the annual, brown seaweed *D. viridis* to accumulate and retain sulfuric acid (H_2SO_4) throughout its existence as a sporophyte. A first indication of this was provided by our findings that in the absence of waves, acid loss to the environment (which is an indication that the seaweed is stressed, Gagnon et al., 2013), was threefold lower in cold (~6.5 °C) than warm (~11 °C) water (Experiment 1), while being more than threefold higher in the absence than presence of waves in warm (11 to 13 °C) water (Experiment 2). These results agree with our finding, in a concurrent study, that *D. viridis* is intolerant to temperatures above ~12 °C, while reinforcing the suggestion of a snowball effect whereby the seaweed is vulnerable to the release of its own acid, unless waves and currents dissipate the discharged acid before it deteriorates the remaining healthy tissues (see below and Gagnon et al., 2013). They also uncover similar upper thermal tolerances to those in sporophytes of the Antarctic, perennial, sister species *D. anceps*, *P. antarcticus*, and *H. grandifolius* (Wiencke and tom Dieck, 1989; Wiencke et al., 1994).

We found no convincing evidence that light retards or accelerates acidity in *D. viridis* during senescence (Experiment 1). This result may seem counterintuitive in view of the predominant influence of abiotic controls such as nutrients, salinity, temperature, light, and hydrodynamic conditions, on key biological aspects of seaweeds (e.g. Barko et al., 1982; Blanchette, 1997; Brown and Richardson, 1968; Chapman and Craigie, 1977; Fortes and Lüning, 1980; Twilley and Barko, 1990). Yet, it may simply mean that metabolic pathways for acid production or release in *D. viridis* are independent of light, as also suggested by McClintock et al. (1982). Alternatively, the two artificial light regimes we used may have been too similar to induce different responses, or too different from those in natural environments to induce light-related changes in acidity. Further experimental work with sporophytes collected prior to senescence and exposed to a broader range of light intensities are required to clarify effects of light on acid production and release in *D. viridis*.

The Induced Defense Model (IDM) predicts that the production of defenses in primary producers will increase with increasing threat from grazers (Ceh et al., 2005; Hay, 1996; Karban and Baldwin, 1997; Karban and Myers, 1989; Karban et al., 1999). Accordingly, in habitats with consistently high herbivore abundances, like at our two study sites, defenses should be constitutive, as opposed to inducible, even though this may result in higher energetic costs and risks of autotoxicity. Our hypothesis that grazing and epibionts augment acidity was not supported. Indeed, despite tissue losses of up to 40% (wet weight) to grazing by the green sea urchin, *S. droebachiensis*, and fouling by up to 20 caprellids *Caprella* sp., the change in intracellular pH of the remaining *D. viridis* tissues remained similar to that of ungrazed or unfouled sporophytes (Experiment 3). This finding has important implications for our understanding of the chemical ecology of *D. viridis*. Firstly, it provides the first clear evidence that (heavy) grazing does not induce a higher production of acid, and hence that the alleged anti-grazing function of sulfuric acid in *D. viridis*, and perhaps other acid-producing Desmarestiales (Anderson and Velimirov, 1982; Dayton, 1985; Gagnon et al., 2006; Himmelman and Nédélec, 1990; Molis et al., 2009; Pelletreau and Muller-Parker, 2002), is a fixed, inherited trait with no relationship to grazing intensity, i.e. that the acid is indeed produced constitutively. Secondly, it demonstrates that localized tissue death does not extend to remaining tissues as only a thin (<1 mm) band of tissues around grazed portions immediately turned green, which is indicative of acid discharge. Lastly, that heavily grazed sporophytes did not increase acid production over 10 days (duration of trials in Experiment 3), which is ample time for complete destruction by denser urchin populations in natural habitats where *D. viridis* occurs (Gagnon et al., 2003b, 2004; Himmelman and Nédélec, 1990), also implies that *D. viridis* survival depends on some other factors (see below and Gagnon et al., 2006, 2013).

There was a loss of acid in all treatments in Experiment 3, which was conducted in June, when acid concentration normally increases within sporophytes (Gagnon et al., 2013). Sporophytes were exposed to only low water turbulence created by the seawater entering each tank. This pattern, along with our field data and finding that sporophytes, in June, in a much larger volume of seawater (wave tank), gained acid both in the presence and absence of waves (Experiment 2), reinforces the notion that water circulation is critical for the buildup and prevention of release of acid in *D. viridis* (Gagnon et al., 2013). Interestingly, sporophytes at our two study sites, BCC and KP, grew at a decelerating rate from March to late June (Phase 1), during which time the acid was also being produced and accumulated at a decelerating rate (Gagnon et al., 2013) and sea temperature was low (<8 °C). It is not until mid-August (onset of Phase 3), when sea temperature reached and surpassed, for a few days, the lethal 12 °C for the species (Gagnon et al., 2013), that mean specific growth rates (SGR) of sporophytes became negative (indicative of tissue loss) and mortality rates dramatically increased from highest lows of ~15%, to complete eradication (100% mortality) in late October. Similar seasonal patterns of degradation in *D. viridis* were reported or suggested in the northeastern Pacific (Chapman, 1972; Konar, 2000), northwestern Atlantic (Gagnon et al., 2003b; van den Hoek, 1982), and northeastern Atlantic (van den Hoek, 1982), though their relationship with environmental variability was not examined.

Technically, senescence is the growth phase from full maturity to death. Our data indicated that senescence in *D. viridis* includes a transition phase (Phase 2) of ~1 month (July) during which growth ceases (which we interpret as the attainment of full maturity), and mortality increases slightly before picking up in mid-August. Because we did not measure photosynthetic rates, we cannot ascertain that sporophytes were systematically dying during the transition phase. However, that a large proportion of sporophytes exhibited a slight change in coloration from dark to lighter brown, as well as in toughness from firmly attached branches to increasingly brittle tissues, was indicative that they were under stress. Any sporophytes that initiated such changes irreversibly worsened over time, with a few ones changing and disappearing in shallower, warmer water, as early as mid-July.

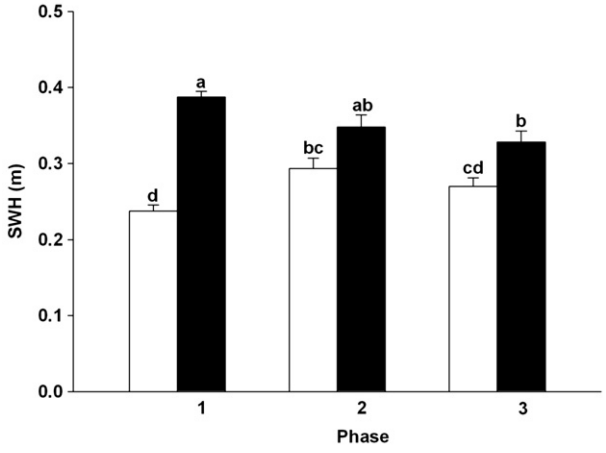

Fig. 5. Mean (+SE) daily significant wave height (SWH) in each phase of change (Phase 1 [increase], Phase 2 [no change], and Phase 3 [decrease]) in frond length of *Desmarestia viridis* sporophytes at the two study sites (BCC and KP). Each bar is the average daily SWH from 8 March to 7 July, 8 July to 15 August, and 16 August to 13 October, 2011 (see Materials and methods for details). Bars not sharing the same letter are different (LS means tests, $p<0.05$; n = 115 [Phase 1], 38 [Phase 2], and 52 [Phase 3]). □ BCC, ■ KP.

We experimentally demonstrated that tissue loss in *D. viridis* at the end of the natural senescence phase is about twice lower in cold (~3 °C) than warm (~11 °C) water (Gagnon et al., 2013). This pattern, together with our finding, in the present study, that significant wave height (SWH) was significantly higher at KP (wave-exposed site) than BCC (semi-protected site) from mid-August to the end of October (Phase 3), help explain the less pronounced decrease in frond length and ~10-day delay in *D. viridis* mortality in September at KP than BCC. We propose that the more severe hydrodynamic environment that characterized KP at that time of year when mean sea temperature neighbored the stressful value of 10 °C (Gagnon et al., 2013) at both sites, facilitated the dispersal of the acid that was being released by the sporophytes, which ultimately slowed down the decay of tissues. This suggestion was also corroborated by results of the experiment in the wave tank (Experiment 2, see above). Additional field studies should include replication of sheltered and exposed sites to strengthen these conclusions.

In summary, this study provides the first detailed analysis of seasonal variation in growth and mortality of *D. viridis* sporophytes and its relationship with abiotic (temperature, light, and wave action) and biotic (grazing and epibionts) factors. The strong connections between laboratory and field data attest to the critical role that thermal and wave environments play in the life history of this unique seaweed. We provided further evidence that the acid is produced constitutively and irreversibly, regardless of grazing, which provides new insights into the ecological and evolutionary causes and consequences of acid production in Desmarestiales. Further studies with members of this intriguing group of seaweeds are required to determine the tradeoffs that may exist between acid production, resource allocation to the various primary and secondary life processes such as photosynthesis and reproduction, and morphological adaptability. Specifically, investigations of how nutrient levels and ongoing changes in ocean temperature and stoichiometry may interfere with these processes must be conducted to better determine and anticipate changes in populations of Desmarestiales and their effects on other components of cold-water coastal ecosystems.

Acknowledgements

We are indebted to Scott Caines, Kyle Matheson, Alicia Morry, Travis Nielsen, Andrew Perry, and Johanne Vad for help with field and laboratory work. This research was funded by NSERC (Discovery Grant) and Canada Foundation for Innovation (CFI-Leaders Opportunity Funds) grants to Patrick Gagnon. **[SS]**

References

Agrawal, A.A., 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology 81 (7), 1804–1813.
Agrawal, A.A., 2005. Future directions in the study of induced plant responses to herbivory. Entomol. Exp. Appl. 115 (1), 97–105.
Amsler, C.D., Fairhead, V.A., 2006. Defensive and sensory chemical ecology of brown algae. Adv. Bot. Res. 43, 1–91.
Amsler, C.D., Amsler, M.O., McClintock, J.B., Baker, B.J., 2009. Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores. Phycologia 48 (5), 324–334.
Anderson, R.J., Velimirov, B., 1982. An experimental investigation of the palatability of kelp bed algae to the sea urchin *Parechinus angulosus* Leske. Mar. Ecol. 3 (4), 357–373.
Barko, J.W., Hardin, D.G., Matthews, M.S., 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. Can. J. Bot. 60, 877–887.
Blanchette, C.A., 1997. Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. Ecology 78 (5), 1563–1578.
Bolser, R.C., Hay, M.E., 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology 77 (8), 2269–2286.
Brown, T.E., Richardson, R.L., 1968. The effect of growth environment on the physiology of algae: light intensity. J. Phycol. 4, 38–54.
Carpenter, R.C., 1986. Partitioning herbivory and its effects on coral reef algal communities. Ecol. Monogr. 56 (4), 345–363.
Ceh, J., Molis, M., Dzeha, T.M., Wahl, M., 2005. Induction and reduction of antiherbivore defenses in brown and red macroalgae off the Kenyan coast. J. Phycol. 41 (4), 726–731.
Chapman, A.R.O., 1972. Species delimitation in the filiform, oppositely branched members of the genus *Desmarestia* Lamour. (Phaeophyceae, Desmarestiales) in the Northern Hemisphere. Phycologia 11 (3–4), 225–231.
Chapman, A.R.O., Craigie, J.S., 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40, 197–205.
Conover, W.J., 1980. Practical Nonparametric Statistics. John Wiley & Sons, New York.
Cyr, H., Pace, M., 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature 361, 148–150.
Dayton, P.K., 1985. The structure and regulation of some South American kelp communities. Ecol. Monogr. 55, 447–468.
Dorenbosch, M., Bakker, E.S., 2011. Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. Freshw. Biol. 56, 1783–1797.
Duffy, J.E., Hay, M.E., 1990. Seaweed adaptations to herbivory. Bioscience 40 (5), 368–375.
Duffy, J.E., Hay, M.E., 2000. Strong impacts of grazing amphipods on the organization of a benthic community. Ecol. Monogr. 70 (2), 237–263.
Duggins, D.O., Eckman, J.E., Sewell, A.T., 1990. Ecology of understory kelp environments II. Effects of kelps on recruitment of benthic invertebrates. J. Exp. Mar. Biol. Ecol. 143 (1–2), 27–46.
Fortes, M.D., Lüning, K., 1980. Growth rate of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgoländer Meeresun. 34, 15–29.
Gagné, J.A., Mann, K.H., Chapman, A.R.O., 1982. Seasonal patterns of growth and storage in *Laminaria longicruris* in relation to differing patterns of availability of nitrogen in the water. Mar. Biol. 69, 91–101.
Gagnon, P., Wagner, G., Himmelman, J.H., 2003a. Use of a wave tank to study the effects of water motion and algal movement on the displacement of the sea star *Asterias vulgaris* towards its prey. Mar. Ecol. Prog. Ser. 258, 125–132.
Gagnon, P., Himmelman, J.H., Johnson, L.E., 2003b. Algal colonization in urchin barrens: defense by association during recruitment of the brown alga *Agarum cribrosum*. J. Exp. Mar. Biol. Ecol. 290 (2), 179–196.
Gagnon, P., Himmelman, J.H., Johnson, L.E., 2004. Temporal variation in community interfaces: kelp–bed boundary dynamics adjacent to persistent urchin barrens. Mar. Biol. 144 (6), 1191–1203.
Gagnon, P., Johnson, L.E., Himmelman, J.H., 2005. Kelp patch dynamics in the face of intense herbivory: stability of *Agarum clathratum* (Phaeophyta) stands and associated flora on urchin barrens. J. Phycol. 41 (3), 498–505.
Gagnon, P., St-Hilaire-Gravel, L.V., Himmelman, J.H., Johnson, L.E., 2006. Organismal defenses versus environmentally mediated protection from herbivores: unraveling the puzzling case of *Desmarestia viridis* (Phaeophyta). J. Exp. Mar. Biol. Ecol. 334 (1), 10–19.
Gagnon, P., Blain, C., Vad, J., 2013. Living within constraints: irreversible chemical build-up and seasonal temperature-mediated die-off in a highly acidic (H_2SO_4) annual seaweed (*Desmarestia viridis*). Mar. Biol. 160 (2), 439–451. http://dx.doi.org/10.1007/s00227-012-2101-8.
Hanley, M.E., Lamont, B.B., Fairbanks, M.M., Rafferty, C.M., 2007. Plant structural traits and their role in anti-herbivore defense. Perspect. Plant Ecol. 8, 157–178.
Hay, M.E., 1991. Marine-terrestrial contrasts in the ecology of plant chemical defenses against herbivores. Trends Ecol. Evol. 6 (11), 362–365.
Hay, M.E., 1996. Marine chemical ecology: what's known and what's next? J. Exp. Mar. Biol. Ecol. 200, 103–134.
Hay, M.E., 2009. Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. Annu. Rev. Mar. Sci. 1, 193–212.
Hay, M.E., Steinberg, P.D., 1992. The chemical ecology of plant–herbivore interactions in marine vs. terrestrial communities. In: Rosenthal, G., Berenbaum, M. (Eds.), Herbivores: Their Interaction with Secondary Plant Metabolites. Evolutionary and Ecological Processes. Academic Press, San Diego, California, USA, pp. 371–413.
Himmelman, J.H., Nédélec, H., 1990. Urchin foraging and algal survival strategies in intensely grazed communities in eastern Canada. Can. J. Fish. Aquat. Sci. 47 (5), 1011–1026.
Howe, G.A., Jander, G., 2008. Plant immunity to insect herbivores. Annu. Rev. Plant Biol. 59, 41–66.
Jormalainen, V., Honkanen, T., 2008. Macroalgal chemical defenses and their roles in structuring temperate marine communities. In: Amsler, C.D. (Ed.), Algal Chemical Ecology. Springer-Verlag, Berlin, pp. 57–89.
Karban, R., Baldwin, I.T., 1997. Induced Responses to Herbivory. The University of Chicago Press, Chicago.
Karban, R., Myers, J.H., 1989. Induced plant responses to herbivory. Annu. Rev. Ecol. Syst. 20, 331–348.
Karban, R., Agrawal, A.A., Thaler, J.S., Adler, L.S., 1999. Induced plant responses and information content about risk of herbivory. Trends Ecol. Evol. 14 (11), 443–447.
Konar, B., 2000. Seasonal inhibitory effects of marine plants on sea urchins: structuring communities the algal way. Oecologia 125 (2), 208–217.
Konar, B., Estes, J.A., 2003. The stability of boundary regions between kelp beds and deforested areas. Ecology 84 (1), 174–185.
Lubchenco, J., Cubit, J., 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. Ecology 61 (3), 676–687.
Lubchenco, J., Gaines, S.D., 1981. A unified approach to marine plant–herbivore interactions. I. Populations and communities. Annu. Rev. Ecol. Syst. 12, 405–437.
Mann, K.H., 1973. Seaweeds: their productivity and strategy for growth. Science 182, 975–981.
McClintock, M., Higinbotham, N., Uribe, E.G., Cleland, R.E., 1982. Active, irreversible accumulation of extreme levels of H_2SO_4 in the brown alga, *Desmarestia*. Plant Physiol. 70 (3), 771–774.
Milchunas, D.G., Noy-Meir, I., 2002. Grazing refuges, external avoidance of herbivory and plant diversity. Oikos 99 (1), 113–130.
Molis, M., Wessels, H., Hagen, W., Karsten, U., Wiencke, C., 2009. Do sulphuric acid and the brown alga *Desmarestia viridis* support community structure in Arctic kelp patches by altering grazing impact, distribution patterns, and behaviour of sea urchins? Polar Biol. 32 (1), 71–82.
Pelletreau, K.N., Muller-Parker, G., 2002. Sulfuric acid in the phaeophyte alga *Desmarestia munda* deters feeding by the sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 141 (1), 1–9.

Sasaki, H., Kataoka, H., Kamiya, M., Kawai, H., 1999. Accumulation of sulfuric acid in *Dictyotales* (Phaeophyceae): taxonomic distribution and ion chromatography of cell extracts. J. Phycol. 35 (4), 732–739.
Sasaki, H., Murakami, A., Kawai, H., 2005. Seasonal stability of sulfuric acid accumulation in the Dictyotales (Phaeophyceae). Phycol. Res. 53 (2), 134–137.
Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods. Iowa State University Press, Ames.
Sokal, R.R., Rohlf, F.J., 2012. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman, New York.
Steneck, R.S., 1986. The ecology of coralline algal crusts: convergent patterns and adaptative strategies. Annu. Rev. Ecol. Syst. 17, 273–303.
Steneck, R.S., Graham, M.H., Bourque, B.J., Corbett, D., Erlandson, J.M., Estes, J.A., Tegner, M.J., 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ. Conserv. 29 (4), 436–459.
Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14 (5), 179–185.
Twilley, R.R., Barko, J.W., 1990. The growth of submersed macrophytes under experimental salinity and light conditions. Estuaries 13, 311–321.
van den Hoek, C., 1982. Phytogeographic distribution groups of benthic marine algae in the North Atlantic Ocean. A review of experimental evidence from life history studies. Helgoländer Meeresun. 35, 153–214.
Wiencke, C., Clayton, M., 1990. Sexual reproduction, life history, and early development in culture of the Antarctic brown alga *Himantothallus grandifolius* (Desmarestiales, Phaeophycease). Phycologia 29, 9–18.
Wiencke, C., tom Dieck, I., 1989. Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. Mar. Ecol. Prog. Ser. 54, 189–197.
Wiencke, C., Bartsch, I., Bischoff, B., Peters, A.F., Breeman, A.M., 1994. Temperature requirements and biogeography of Antarctic, Arctic and Amphiequatorial seaweeds. Bot. Mar. 37, 247–259.
Wiencke, C., Clayton, M., Langreder, C., 1996. Life history and seasonal morphogenesis of the endemic antarctic brown alga *Desmarestia anceps* Montagne. Bot. Mar. 39 (5), 435–444.
Yñiguez, A.T., McManus, J.W., Collado-Vides, L., 2010. Capturing the dynamics in benthic structures: environmental effects on morphology in the macroalgal genera *Halimeda* and *Dictyota*. Mar. Ecol. Prog. Ser. 411, 17–32.
Zamora, R., Hodar, J.A., Gomez, J.M., 1999. Plant–herbivore interaction: beyond a binary vision. In: Pugnaire, F.I., Valladares, F. (Eds.), Handbook of Functional Plant Ecology. Marcel Dekker, Inc., New York, pp. 677–718.