ACID (H_2SO_4) PRODUCTION, PERSISTENCE, AND FUNCTIONAL IMPORTANCE OF THE ANNUAL, BROWN SEAWEED DESMARESTIA VIRIDIS IN NEWFOUNDLAND, CANADA

CAITLIN O'HARA BLAIN

Acid (H_2SO_4) production, persistence, and functional importance of the annual, brown seaweed *Desmarestia viridis* in Newfoundland, Canada

by

Caitlin O'Hara Blain

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ABSTRACT

Current models of shallow rocky community organization and stability in the northwestern Atlantic (NWA) emphasize kelps and their vulnerability to grazers and other mortality agents. This paradigm may overshadow the possible contribution of other groups of less studied seaweeds with overlapping distribution to ecosystem resilience. The annual, brown seaweed *Desmarestia viridis* is one of only a few species of fleshy seaweeds commonly found in urchin barrens in the NWA. The exceptional ability of *D. viridis* sporophytes to produce and store sulfuric acid (H_2SO_4) in intracellular vacuoles makes the species a compelling model for studies of controls and importance of acid production in seaweeds at the individual, population, and community levels. This research used laboratory experiments and surveys of individuals and populations throughout an entire growth season (February to October 2011) at two subtidal sites on the southeastern tip of Newfoundland (Canada) to determine controls of acid production in, as well as the functional importance and persistence of, *D. viridis* sporophytes. Results showed that light, grazing, and epibionts have no perceptible effects on intracellular acidity, whereas temperature and wave action exert strong, synergistic effects. Mortality rates and sea temperature from March to late June were relatively low, whereas the onset of increasing mortality in mid-August coincided with marked increases in sea temperature. The quick development of "*Desmarestia* beds" in urchin barrens created biological structure for major recruitment pulses in characteristic invertebrate and fish assemblages. These findings provide novel insights into the ecological and evolutionary causes and consequences of acid production in Desmarestiales, while elevating the importance of *D. viridis* as a foundation species in urchin barrens in the NWA.

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cover and the amplitude of the wave-induced sweeping motion are lowest (Min), and mortality and grazing by urchins are moderate. As recruits grow to adult-size (~50 to 60 cm) sporophytes in July, AGR and intracellular pH decrease (the latter being indicative of sulfuric acid production and accumulation) at a decelerating rate, whereas mortality decreases as a result of increasing cover and sweeping further reducing urchin grazing. The rapid increase in mean sea temperature in June and July to above 10°C marks the onset of mass release of acid to the environment (as shown by increasing pH) and senescence (as shown by sudden declines in cover and AGR) until all sporophytes disappear in October. The sudden increase in the frequency of urchin grazing on decaying sporophytes in August and September precipitates mortality and decline in cover. Throughout its existence as a highly branched, sweeping sporophyte, *D. viridis* provides a suitable surface for deposition of eggs by fish (May to September), as well as to distinct assemblages of mobile epifauna to recruit, cling, graze, and capture prey, as notably shown by dramatic increases of up to >150 000 individuals kg^{-1} *D. viridis* in herbivorous gastropods (G), and predatory copepods (C), amphipods (A), and isopods (I) in September (refer to the discussion for proposed cascades resulting from acid build-up and release in *D. viridis*).

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CO-AUTHORSHIP STATEMENT

The work described in this thesis was conducted by Caitlin Blain with guidance from Patrick Gagnon, William Driedzic, David Schneider, and Raymond Thompson. Caitlin Blain was responsible for field and laboratory data collection and analysis (with assistance by Patrick Gagnon) and contributed to modifications brought to the original design by Patrick Gagnon. All chapters were written by Caitlin Blain with intellectual and editorial input by Patrick Gagnon. An abridged version of Chapter II is published in *Journal of Experimental Marine Biology and Ecology* (full reference below). Part of the data in Chapter II (section 2.3.4) is published in *Marine Biology* (full reference below) to supplement data collected by Patrick Gagnon, Caitlin Blain, and research intern Johanne Vad (École Normale Supérieure de France, ENS) in a foundation study. A shortened version of Chapter III is currently being prepared for publication in the primary literature. Any additional publication in the primary literature resulting from this work will be co-authored by Caitlin Blain and Patrick Gagnon.

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CHAPTER I

GENERAL INTRODUCTION

Some of the most productive marine communities worldwide are found in the shallow, rocky, subtidal zone of cold and temperate seas, where large, erect, fleshy seaweeds markedly increase habitat complexity (Duggins et al. 1990, Amsler et al. 1995, Steneck et al. 2002, Clark et al. 2004, Jormalainen and Honkanen 2008). A prime example of highly productive and biodiverse communities is that of kelp beds (forests), which thrive in polar and subpolar zones in both hemispheres, as well as in upwelling zones in tropical and subtropical regions (Dayton 1985a). In addition to providing food and habitat for reproduction to a host of marine invertebrates, fish, and mammals, kelps and the export of detritus they generate are important contributors to secondary production in coastal marine ecosystems, whether aquatic or terrestrial, and shallow or deep (Duggins et al. 1989). Kelp bed productivity is largely due to trophic level complexity characterized by high species abundance with overlapping ecological niches (Duffy 2002, Worm and Duffy 2003). Yet, such balance can be altered by natural phenomena and anthropogenic stressors operating over a range of spatial and temporal scales, including wave storms, decrease or increase in predator populations, overfishing, and species introductions (Ruiz et al. 1997, Coleman and Williams 2002, Bax et al. 2003). One key factor affecting the distribution and abundance of kelps is grazing by sea urchins, which has been shown to drive shifts between two iconic, alternative community states, "kelps beds" and "urchin barrens" (*sensu* Dayton 1985a), in many seas including the northwestern Atlantic (Himmelman and Steele 1971, Mann 1977, Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay et al. 2009).

The ability of fleshy seaweeds to deter or outcompete herbivores, epibionts, and other seaweeds for nutrients and space is central to their establishment (Dayton 1971,

Menge 1976, Sousa 1979, Konar 2000). Moreover, to cope with the challenges of a sessile lifestyle, seaweeds have evolved avoidance and deterrence mechanisms such as temporal, spatial, structural, associational, and chemical defenses (Duffy and Hay 1990, Hay 1996, Gagnon et al. 2003a, Amsler and Fairhead 2006, Jormalainen and Honkanen 2008, Amsler et al. 2009b). Spatial and temporal herbivore-avoidance "tactics" are especially common in seaweeds of temperate, seasonal seas. Such seaweeds may temporarily escape effects of herbivory by exhibiting a heteromorphic life history (e.g. *Porphyra* sp., Lubchenco and Cubit 1980) or by recruiting in areas of lower macrograzer density, including topographic depressions (Lubchenco and Gaines 1981, Menge et al. 1985). In contrast, seaweed structural and chemical defenses are widespread in communities in which herbivory is constant and intense to withstand, rather than avoid, grazers (see reviews by Steneck and Watling 1982, Hay and Fenical 1988, Duffy and Hay 1990, Hay 1996). One good example of structural defense is that of flat, solid crusts of red coralline seaweeds commonly found in urchin barrens, which are difficult for invertebrate and fish grazers to grasp and detrimental to their mouth parts and digestive systems (Steneck 1983, 1986). Chemical deterrents, such as polyphenolics, complex carbon compounds, and other secondary metabolites are widespread and phylogenetically diverse among seaweeds (Van Alstyne et al. 1999, Fairhead et al. 2005, Amsler and Fairhead 2006, Long and Trussell 2007).

Although grazing affects seaweed structural, morphological, and chemical traits, so do other biotic (e.g. epibionts) and abiotic (e.g. nutrients, salinity, temperature, light, and hydrodynamic conditions) factors, which also regulate growth and reproduction (e.g. Chapman and Burrows 1971, Barko et al. 1982, Gagné et al. 1982, Twilley and Barko

1990, Blanchette 1997, Yñiguez et al. 2010). In particular, salinity and temperature, which control intracellular pH and metabolic rates, as well as light, which is critical to the initiation of developmental processes and photosynthesis (Lobban and Harrison 1994), can vary greatly in inshore marine habitats due to freshwater runoff, tidal flushing, and attenuation of light by the water column (Pinet 2012), and hence interact in complex ways to regulate the vertical and geographic range of seaweeds, both gametophytes (microscopic, sexually reproducing individuals) and sporophytes (macroscopic, asexually reproducing individuals) (Chapman and Burrows 1970, Lüning and Dring 1985, Lüning 1990, Kübler and Davison 1993, Lobban and Harrison 1994, Cronin and Hay 1996, Pakker and Breeman 1996, Strand and Weisner 2001, Lee et al. 2007). The occurrence of seaweed in a particular area at a given time is, therefore, the outcome of a complex suite of interactions between abiotic and biotic controls.

The phaeophyte (brown seaweed) family Desmarestiaceae, which contains species endemic to the Southern Hemisphere (Peters et al. 1997), is the dominant group of large fleshy seaweeds in the Antarctic (Moe and Silva 1977). Like kelps of temperate seas, Antarctic Desmarestiales, mainly *Desmarestia anceps*, *Desmarestia menziesii*, and *Himantothallus grandifolius*, which vary in frond morphology from thin and finely branched to thick and leathery, markedly increase habitat complexity, providing a number of vegetal and animal species with perennial canopy and substratum for recruitment, growth, and protection (Wiencke and Clayton 1990, Amsler et al. 1995, Wiencke et al. 1995, Wiencke et al. 2007, Wiencke and Amsler 2012). Together, these three species can cover up to 80% of the seabed and support highly diverse invertebrate assemblages dominated by amphipods (DeLaca and Lipps 1976, Brey and Clarke 1993, Amsler et al.

1995, Iken 1999, Huang et al. 2007, Amsler et al. 2008, Wiencke and Amsler 2012). Studies also suggest that the bulk of their primary production is exported to benthic and detrital food webs (Dawson et al. 1985, Dieckmann et al. 1985, Reichardt 1987, Fischer and Wiencke 1992, Dunton 2001).

The annual, amphiequatorial, Desmarestiales *Desmarestia viridis* can be a seasonally dominant community component in urchin barrens in the northwestern Atlantic (NWA; Nova Scotia and northwards) and other cold seas in the Northern Hemisphere (Konar 2000, Gagnon et al. 2003a, Gagnon et al. 2006, Molis et al. 2009). The occurrence of *D. viridis* sporophytes in such intensively grazed environments is, at a first glance, surprising considering the small holdfast, thin and highly flexible stipe, and profusely-branched frond, which can measure up to 1.5 m in length (Fig.1.1, Gagnon et al. 2006). Studies suggest that this particular morphology, which allows sporophytes to sweep back and forth over the bottom with even slight wave action, together with the exceptional ability to produce and store high concentrations of sulfuric acid (H_2SO_4) in intracellular vacuoles (McClintock et al. 1982, Sasaki et al. 2004, Gagnon et al. 2013), provide *D. viridis* with mechanical and chemical defenses against urchin grazing (Konar 2000, Pelletreau and Muller-Parker 2002, Gagnon et al. 2006, Molis et al. 2009). Yet, a recent study of *D. viridis* in Newfoundland (eastern Canada) indicates that the acid is produced and accumulated continuously and irreversibly as sporophytes grow to adult size, and that this build-up inevitably culminates in dramatic mass releases of acid and die-offs (Gagnon et al. 2013). Therefore, the significance of sulfuric acid may be greater than just a chemical deterrent. Casual observations of *D. viridis* sporophytes in the

Figure 1.1. *Desmarestia viridis* sporophytes in urchin (*Strongylocentrotus droebachiensis*) barrens on 13 June, 2011 at a depth of 6 m in Bay Bulls, southeastern Newfoundland (Photo: Patrick Gagnon).

northern Gulf of St. Lawrence and Atlantic costs of Nova Scotia and Newfoundland (Patrick Gagnon, personal observations over the last 15 years), as well as one study in the northern Gulf of St. Lawrence (Bégin et al. 2004), suggest *D. viridis* supports distinct epifaunal assemblages throughout its distributional range in the NWA. However, such information is fragmentary, covering only a few months of the lifespan of *D. viridis* and larger epifauna, and hence not sufficiently detailed to draw accurate conclusions about the functional importance of the species in urchin barrens. A better understanding of abiotic and biotic controls of acid production in, as well as spatial and temporal dynamics of, *D. viridis* sporophytes and associated epifauna, is required to determine the vulnerability of this species to environmental variability and contribution to community and ecosystem stability.

This thesis aims to determine causal relationships between abiotic and biotic factors and intracellular acidity in *D. viridis* sporophytes to gain a better understanding of the sensitivity of the species to environmental variability (Chapter II), while characterizing the persistence and functional importance of sporophytes in urchin barrens to test the overall postulate that *D. viridis* is functionally comparable to a foundation species (*sensu* Bruno and Bertness 2001) facilitating recruitment in, and supporting distinct assemblages of, invertebrates (Chapter III). Work involves laboratory experiments in aquaria and wave tanks at the Ocean Sciences Centre of Memorial University of Newfoundland, as well as surveys of individuals and populations (via SCUBA diving) throughout an entire growth season (February to October 2011) in *D. viridis* sporophytes at two subtidal sites, Bread and Cheese Cove (BCC) and Keys Point (KP), in Bay Bulls, on the southeastern tip of Newfoundland.

Chapter II uses four laboratory experiments, in which the intracellular acidity of *D. viridis* sporophytes from KP exposed to various temperature, light, and wave conditions, as well as to the presence and absence of grazers and epibionts, is measured and compared. Specifically, it 1) examines individual and combined effects of water temperature and light on acidity during senescence, 2) determines whether wave action affects acidity and how this effect may change over time, 3) tests the hypothesis that grazing and epibionts augment acidity, and 4) tests the hypothesis that tissue loss during the natural senescence phase is lower in cold than warm water. It also uses measurements of the length and survival of *D. viridis* sporophytes, sea temperature, and wave height at BCC and KP to 5) characterize growth and mortality patterns and their relationship with natural environmental variability.

Chapter III uses measurements of the cover of *D. viridis* on the seabed and urchin density at BCC and KP, as well as epifauna on *D. viridis* and two other conspicuous seaweeds in urchin barrens, the non-acidic sister species *Desmarestia aculeata* and grazing-resistant kelp *Agarum clathratum*, at KP. Specifically, it 6) characterizes and relates temporal variability in the abundance of *D. viridis* and green sea urchin at multiple depths, as well as 7) compares intra- and interspecific changes in epifaunal assemblages. It further uses two complementary laboratory experiments to 8) gain insights into factors contributing to the observed variability in the distribution of *D. viridis* and epifaunal assemblages of *D. viridis*, *D. aculeata*, and *A. clathratum*. Results of this and other studies of *D. viridis* are assimilated into a comprehensive, empirical model of ecological interactions for the species. They are also used to propose an important change to the study of shifts among community states in shallow, rocky ecosystems in the NWA.

Chapters II and III are written in a format compatible with the publication of research articles, which explains the repetition of information where appropriate, as well as the use of first-person plural pronoun ("we") and possessive determiner ("our") throughout. Chapter IV presents a summary of main findings and their contribution to advancing knowledge about the ecophysiology of *D. viridis*, while proposing research lines to build upon the proposed, revised framework to the study of shifts among community states in shallow, rocky ecosystems in the NWA.

CHAPTER II

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

2.1 INTRODUCTION

Seaweeds are one of the most conspicuous and productive components of shallow marine ecosystems worldwide (Duggins et al. 1990, Duffy and Hay 2000, Steneck et al. 2002). Despite a productivity that can surpass that of the most productive terrestrial forest, fleshy seaweeds are routinely kept in check by herbivores (Mann 1973, Carpenter 1986, Hay 1991, Cyr and Pace 1993). Like terrestrial and freshwater plants (Strauss and Agrawal 1999, Zamora et al. 1999, Hanley et al. 2007, Howe and Jander 2008, Dorenbosch and Bakker 2011), seaweeds exhibit numerous adaptations to avoid or deter (impede) grazing (Duffy and Hay 1990, Hay 1996, Amsler and Fairhead 2006, Jormalainen and Honkanen 2008, Amsler et al. 2009a). 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 ecosystems with low grazer abundance, whereas deterrence is characteristic of herbivore-dominated communities (Lubchenco and Gaines 1981, Duffy and Hay 1990, Hay and Steinberg 1992, Bolser and Hay 1996). Deterrence mechanisms include the production of structural and chemical defenses, and hence are generally regarded as energetically more costly strategies than avoidance (Steneck 1986, Agrawal 2000, Konar 2000, Konar and Estes 2003, Agrawal 2005, Gagnon et al. 2006, Hay 2009).

The annual, brown seaweed *Desmarestia viridis* (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 remarkable 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, McClintock et al. 1982, Sasaki et al. 1999, Gagnon et al. 2013) 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 coincides with the release of the acid to the environment. The latter study uncovered 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 (Chapman and Craigie 1977, Barko et al. 1982, Gagné et al. 1982, Twilley and Barko 1990, Blanchette 1997, Yñiguez et al. 2010).

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*, *Himantothallus grandifolius*, and *Phaeurus antarcticus*, gametophyte and sporophyte development is chiefly controlled by light, temperature, and nutrient levels (Wiencke and tom Dieck 1989, Wiencke and Clayton 1990, 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. 2003a, Gagnon et al. 2004). Furthermore, experimental testing of effects of light, wave action and their interaction with temperature on acidity is lacking, which limits knowledge about the

vulnerability of *D. viridis* sporophytes to chages in biotic and abiotic environments. A few studies suggest that sulfuric acid in *D. viridis* and a few sister species reduces grazing by urchins (Pelletreau and Muller-Parker 2002, Gagnon et al. 2006, Molis et al. 2009). 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, we use four 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, (3) test the hypothesis that grazing and epibionts augment acidity, and (4) test the hypothesis that tissue loss during the natural senescence phase is lower in cold than warm water. Furthermore, we use measurements, throughout an entire growth season, of sea temperature, wave height, and the length and survival of *D. viridis* sporophytes at two sites, to (5) characterize growth and mortality patterns and their relationship with natural environmental variability.

2.2 MATERIALS AND METHODS

2.2.1 Study sites

This study was conducted with *Desmarestia 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* and *Laminaria digitata*) dominate the 0-2 m depth range, followed in deeper water by extensive urchin (*Strongylocentrotus droebachiensis*) 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 *D. aculeata* and grazing-resistant kelp *Agarum clathratum* (Gagnon et al. 2005). The latter species develops dense beds at depths >15 m, where urchin abundance is low.

2.2.2 Experiment 1: Temperature and light

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 & Lüning (1980), while corresponding to average values at the deeper end of *D. viridis* distribution at our study sites (Blain and Gagnon, unpublished 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 holding tanks to determine their intracellular pH (see Section 2.2.6) at the beginning of the experiment. Each remaining piece (~20 g) was transferred to either of 24, 75-L glass tanks and secured to the bottom with 12-g weights attached to the stipe with a plastic cable tie. The tanks were grouped in six blocks of four tanks each. Each tank in each block was

randomly assigned one of the four experimental treatments (C/L, C/H, W/L, 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.

Complementary trials in which we measured the change, over seven days, in intracellular pH of 15 sporophytes of *D. viridis* collected at KP on 23 July 2012 and transported to and maintained in our laboratory the same way they were in Experiments 1 and 2 (see below), as well as that of 15 sporophytes that remained at KP during the same period, showed similar decreases in pH of 0.0002±0.01 (those maintained in the lab) and 0.005±0.01 (those maintained in the field) ($F_{1,27}$=0.076, p=0.78). Therefore, we assumed

that our approaches to collection, transportation, and maintenance of *D. viridis* sporophytes in the laboratory did not alter intracellular acidity prior to running trials in all four experiments (see Sections 2.2.2 to 2.2.5).

2.2.3 Experiment 2: Wave action

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. 2003b; Appendix A), which mimicked the wave-induced sweeping motion of *D. viridis* fronds in natural habitats. We inserted a panel in the centre of the tank to create two working sections (220[L]×90[W]×62[H] cm each), one with and one without waves. Each trial lasted one week (seven 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^{-1} (measured with a Doppler current meter [Vector Current Meter, Nortek)]. Each sporophyte was assigned haphazardly 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 prior to start and at the end of each trial (see Section 2.2.6). 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 two 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 Section 2.2.8).

2.2.4 Experiment 3: Grazing and epibionts

To test the hypothesis 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 used 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, *Strongylocentrotus droebachiensis*, and caprellid amphipod *Caprella* spp., as the grazer and epibiont, respectively, since they are natural consumers or biofoulers of *D. viridis* in

eastern Canada (Gagnon et al. 2005, Gagnon et al. 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 one week (urchins) or two days (caprellids) to standardize hunger levels in each species (Wessels et al. 2006). 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 collected 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 hours of *D. viridis* collection), we cut ~5 g of tissues from each piece in the tanks to determine intracellular pH (see Section 2.2.6) at the beginning of the experiment. Each remaining piece (~20 g) was weighed (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 tanks were grouped in six blocks of four tanks each. Each tank in each block was randomly assigned 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 L min^{-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 plants collected on 24 June, as well as urchins and caprellids collected on 18 June. Therefore, each treatment was replicated 12 times in total.

To determine whether exposure of *D. viridis* tissues to air (which was inevitable in all four experiments, see Sections 2.2.2 to 2.2.5) affected acidity, we measured the change, over seven 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 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. Sporophytes exposed to air for 0 s followed the same procedure, yet were immediately 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.

2.2.5 Experiment 4: Tissue loss during senescence

Sporophytes during the observed July-October senescence phase decayed and disappeared more rapidly in shallow than deep water (see Sections 2.3.5 and 2.4). To test the hypothesis that tissue loss in *D. viridis* sporophytes during the natural senescence phase is lower in cold than warm water, we conducted an experiment (Experiment 4) in glass tanks (same as in Experiments 1 and 3) in which frond tissues were exposed to either cold (2.5±0.1°C) or warm (10.8±0.3°C) seawater. Our objective was to determine temperature-mediated degradation rate in senescing sporophytes. However, in the absence of prior knowledge about the timing of acid release, it was not possible to determine with certainty if any given sporophyte had entered senescence before the end of September, when it became obvious that all sporophytes were sloughing tissues. To maximize the likelihood that all sporophytes used in the experiment were indeed senescing, we conducted the experiment with some of the last survivors of the 2011 growth season.

We ran the experiment from 14 October to 13 November, 2011 with tissues from 24 *D. viridis* plants (~40 cm in length) collected on 13 October at depths between 6 and 12 m at KP. One piece of ~60-70 g of tissues was cut with scissors from the distal end of each sporophyte (tissues were collected and transported to the OSC as described in Experiment 1). Water temperature during acclimation in the holding tanks was 10.4±0.1°C. On 14 October (<24 h from collection), we trimmed each sporophyte with scissors to standardize frond weight to ~40 g. Each sporophyte was then secured to the

bottom of either of the 24, 75-L glass tanks, with a 12-g weight attached to the stipe with a plastic cable tie, and assigned to one of the two experimental treatments (cold and warm water), for a total of 12 replicates for each treatment.

The light environment in each tank was created and controlled as per the procedures described in Experiment 1, with alternations of 12 h of light (25 μmol photons $m^{-2}s^{-1}$) and 12 h of darkness (similar to Experiment 3). Raw water from Logy Bay was used in the warm water treatment, whereas a chiller was used to cool the water from Logy Bay in the cold water treatment. To reduce the risk of thermal stress at the onset of experiment, the chilled seawater for cold water tanks was introduced gradually with ambient seawater over ~4 hours. Water was delivered to each tank at a rate of 1 L min^{-1} and temperature in one tank of each treatment, chosen randomly by drawing a number corresponding to each of the tanks, was monitored at the bottom with temperature loggers throughout the 30 days that the experiment lasted. Sporophytes in the tanks were gently agitated twice a day to prevent the accumulation of sediments on their surface. Tissues that detached from the sporophytes were siphoned out of the tanks every two or three days. Tissue weight in each tank was determined every five days until the end of the experiment.

2.2.6 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 (±0.01 pH units, model Accumet AP72; Fisher Scientific). The three values were averaged to estimate the raw pH of the tissue sample. The following equation (Sasaki et al. 2005) was used to estimate the intracellular pH of the tissue sample:

$$\text{Intracellular pH} = -\log(\alpha \times \text{Dilution factor})$$

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:

$$\text{Dilution factor} = \frac{(\text{distilled water (g)} + \text{tissue fresh weight (g)}) \times \text{water content (mL)}}{\text{tissue fresh weight (g)} \times \text{water content (mL)}}$$

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.

2.2.7 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 two months, we tagged, on 18 March, 1 April, and 16 May, an additional 15, 16, and 20 plants at BCC and 10, 15, and 16 plants 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 an arithmetic growth rate, AGR, 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, AGR 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. The pressure of the water column on the seabed, which we converted into wave height 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

pressure readings were aggregated into hourly averages. Each hourly average was corrected for barometric pressure by subtracting the atmospheric pressure (psi) at the date and time of measurement (http://www.Isdm-gdsi.gc.ca/meds/Prog_Nat/applications-eng.htm, Station St. John's, HLY01 403506). The corrected pressure was converted into a raw depth (m) by multiplying it by a conversion factor of 0.68 m psi^{-1} (NOAA diving manual: diving for science and technology) to account for the depth of the logger. The raw depth was corrected for tidal elevation by subtracting the elevation at the date and time of measurement (http://www.tides.gc.ca/eng, Station 905). 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, AGR and mean daily sea temperature and SWH within and between sites and phases (see Section 2.2.8).

2.2.8 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 the heteroscedasticity of the residuals when using the raw data (see Appendix B for details about analysis of residuals and data

transformation). 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. The latter analyses were also applied to the square-root transformed data to correct for the heteroscedasticity of the residuals when using the raw data. 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 the residuals generated by the ANOVA met the assumptions.

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 the residuals generated by the ANOVAs met the assumptions. 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. The latter analyses were also

applied to the raw data since the residuals generated by the ANOVAs met the assumptions. 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 a two-way repeated measures ANOVA (Hand and Taylor 1987, Crowder and Hand 1990) with the factors Temperature (cold and warm water) and Time (after 5, 10, 15, 20, 25, and 30 days), to examine effects of water temperature on the loss of frond tissues of *D. viridis* during the last part of the senescence phase (Experiment 4). Raw data were used since the residuals generated by the ANOVA met the assumptions.

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 arithmetic growth rate (AGR) of *D. viridis* sporophytes and mean daily sea temperature and significant wave height (SWH) between sites over time. Since no transformation corrected for the heteroscedasticity of the residuals from raw data on AGR and temperature, the ANOVAs were also applied to the rank transformed data (Appendix B). Because analyses on both raw and ranked-transformed data gave similar conclusions about the significance of each factor, we present results from analyses of the raw data as suggested by Conover (1980). We applied the analysis of SWH to the raw data since the residuals generated by the ANOVA met the assumptions.

In all analyses, homogeneity of the variance was verified by examining the distribution of the residuals and referring to the Levene tests, whereas normality of the residuals was verified by examining the normal probability plot of the residuals and referring to the Shapiro-Wilk's statistics (Appendix B) (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.

2.3 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. We also summarize these changes in terms of molarity, which can be used interchangeably with pH (Appendix C).

2.3.1 Experiment 1: Temperature and light

Analysis of data from Experiment 1 with frond tissues of *D. viridis* exposed for 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 2.1). Tissues released acid to the environment in all four treatments as shown by increases in pH of 0.13±0.02 (Cold/High) to 0.40±0.02 (Warm/High) units (Fig. 2.1). 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. 2.1). Overall these results indicate that tissues were less stressed in cold than warm water.

Table 2.1 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 Section 2.2.2 for a description of the experiment).

Source of variation	***df***	**MS**	***F*-value**	***p***
Temperature	1	0.95	88.32	<0.01
Light	1	0.0022	0.20	0.66
Temperature × Light	1	0.0037	0.37	0.55
Error	43	0.46		
Corrected total	46			

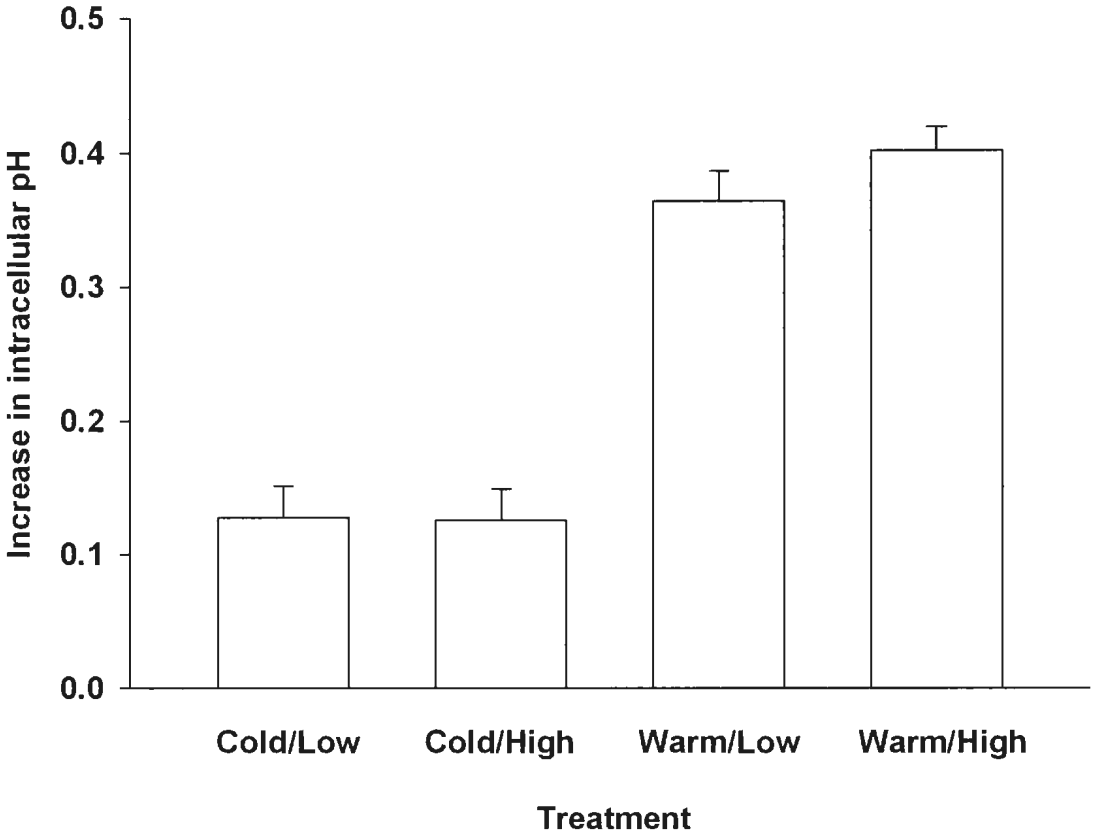


Figure 2.1. Increase in mean (+SE) intracellular pH (denoting a decrease in acidity or loss of acid) of frond tissues of *Desmarestia viridis* (from initial mean value of 0.81±0.02) exposed 10 days to either of four combinations of water temperature (Cold [6.6°C ± 0.2] and Warm [11.1°C ± 0.1]) and light 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).

2.3.2 Experiment 2: Wave action

Analysis of data from Experiment 2 with frond tissues of *D. viridis* exposed seven 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 2.2). 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. 2.2). 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. 2.2).

2.3.3 Experiment 3: Grazing and epibionts

Analysis of data from Experiment 3 with frond tissues of *D. viridis* exposed 10 days to either of four combinations of grazers (presence and absence of urchins) and epibionts (presence and absence of caprellids) treatments indicated that changes in intracellular pH occurred independently of both factors (Table 2.3). Tissues lost acid in all treatments, as shown by increases in pH of 0.005±0.022 (G+E-) to 0.045±0.019 (G-E+) pH units (Fig. 2.3). There was a general trend towards smaller pH increases in treatments with than without grazers, but these differences were not significant (Table 2.3, Fig. 2.3). Tissues were lost in all treatments, as shown by decreases in wet

Table 2.2. 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 seven days in Experiment 2 (see Section 2.2.3 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			

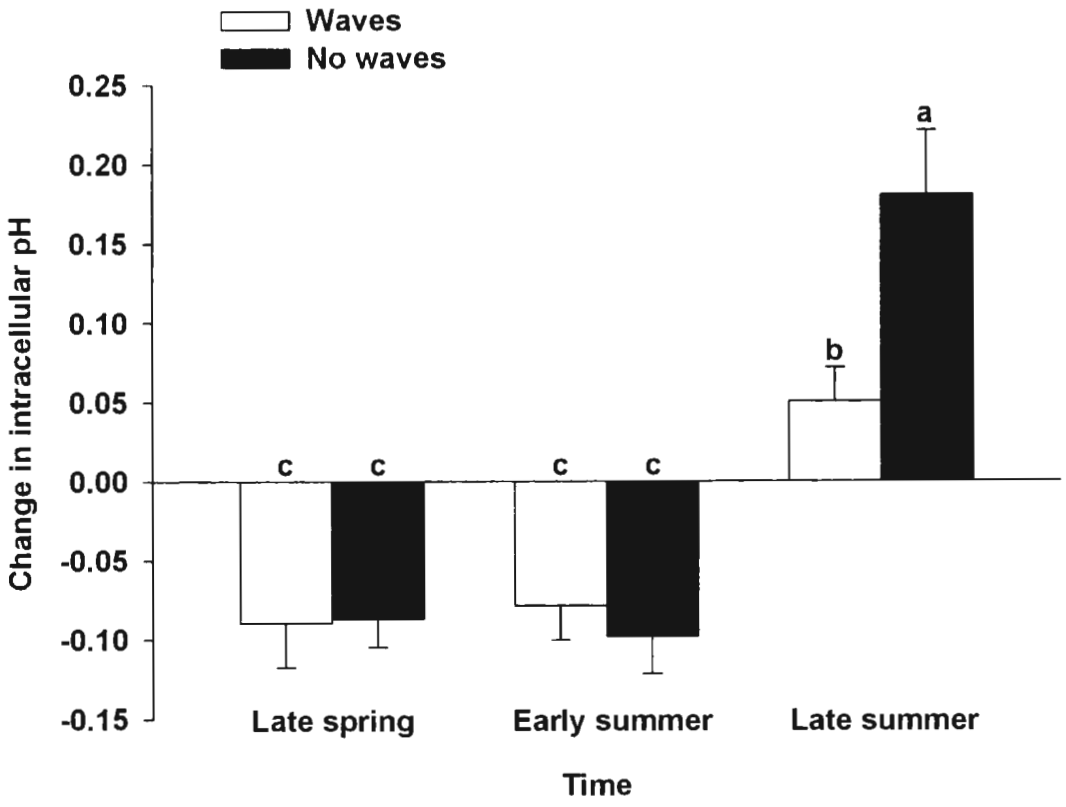


Figure 2.2. Change in mean (+SE) intracellular pH of frond tissues of *Desmarestia viridis* (from initial mean value of 0.77±0.01) exposed seven days to either the presence (0.2 m s^{-1}) or absence (0 m s^{-1}) of waves in late spring (Apr-May), early summer (Jun-Jul), and late summer (Aug-Sep) (Experiment 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).

Table 2.3. Summary of two-way ANOVA (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 Section 2.2.4 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	0.0000022	<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			

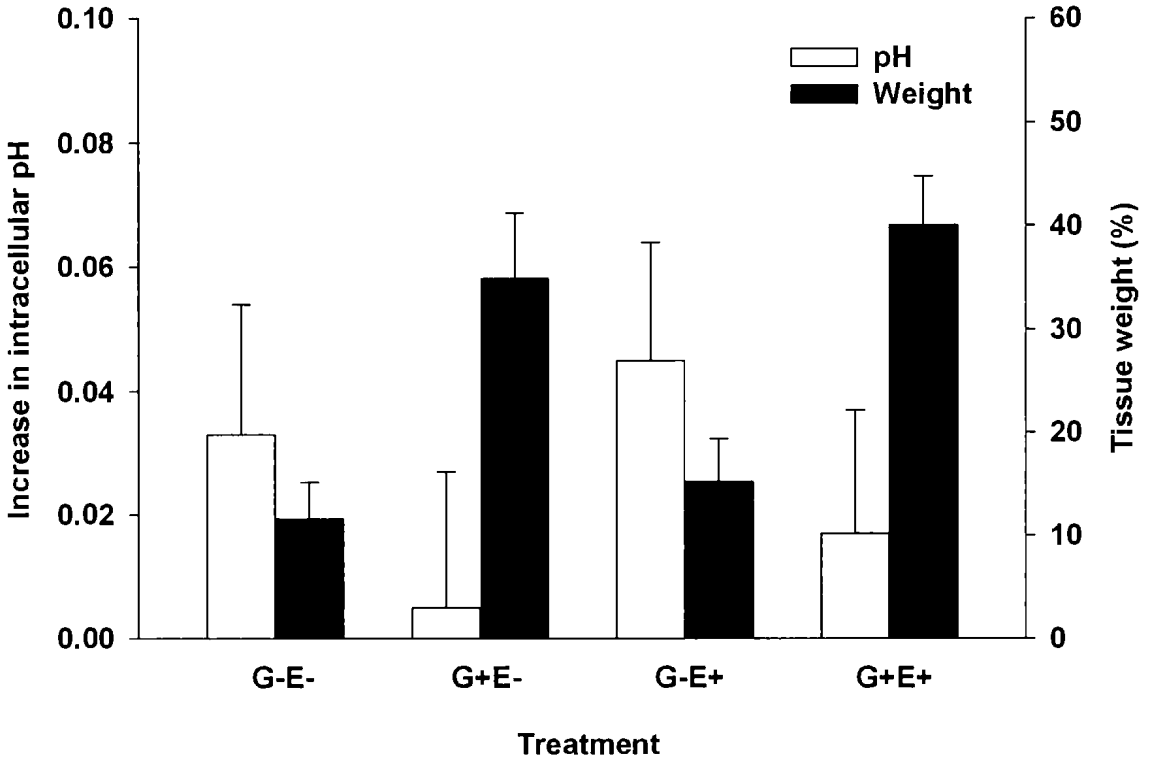


Figure 2.3. Increase (denoting a decrease in acidity or loss of acid) in mean (+SE) intracellular pH (from initial mean value of 0.59±0.01) and decrease in mean (+SE) wet weight as a percentage of initial wet weight, of frond tissues of *Desmarestia viridis* exposed 10 days to the presence (+) or absence (-) of grazers (G) [10 green sea urchins, *Strongylocentrotus droebachiensis*] and epibionts (E) [20 caprellid amphipods *Caprella* spp.] (Experiment 3) (n=12 for each treatment).

weight of 11.6±3.5% (G-E-) to 40.1±4.7% (G+E+). Yet, losses were at least twice as high in treatments with, than without grazers (LS means, $p<0.001$, data pooled across grazers treatments; Fig. 2.3). Tissue loss in the presence of epibionts only (G-E+) was not different than that in the control treatment (G-E-) (Table 2.3). Overall, these results indicate that epibionts had considerably less effect, if any, on the chemistry and physical integrity of *D. viridis*, than grazers.

2.3.4 Experiment 4: Tissue loss during senescence

Analysis of data from Experiment 4 with frond tissues of *D. viridis* exposed to either of two water temperatures (cold and warm) for 30 days indicated tissues were lost throughout (as shown by declining tissue weight) in cold and warm water (Table 2.4, Fig. 2.4). However, the difference in tissue weight after 5, 10, and 15 days was 30-32% lower in warm than cold water and it took >15 days and <10 days to observe a tissue weight reduction ≥50% in cold and warm water, respectively (Fig. 2.4). The number of days to complete sloughing of tissues off the stipe and laterals was twice as high in cold (~30 days) than warm (~15 days) water.

2.3.5 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. 2.5). Frond length at BCC and KP increased at decelerating, though comparable rates, from March, when mean length was 11.1±2.0 [BCC] and 7.6±0.8 [KP] cm, to early

Table 2.4. Summary of two-way repeated measures ANOVA (applied to raw data) examining the effect of Temperature (cold and warm) and Time (after 5, 10, 15, 20, 25, and 30 days) on the loss of frond tissues of *Desmarestia viridis* during the last part of the senescence phase in Experiment 4 (see Section 2.2.5 for a description of the experiment).

Source of variation	***df***	**MS**	***F*-value**	***p***
Time	5	12105.24	167.38	<0.01
Temperature	1	23343.80	322.78	<0.01
Temperature × Time	5	244.94	3.39	<0.01
Error	132	72.32		
Corrected total	143			

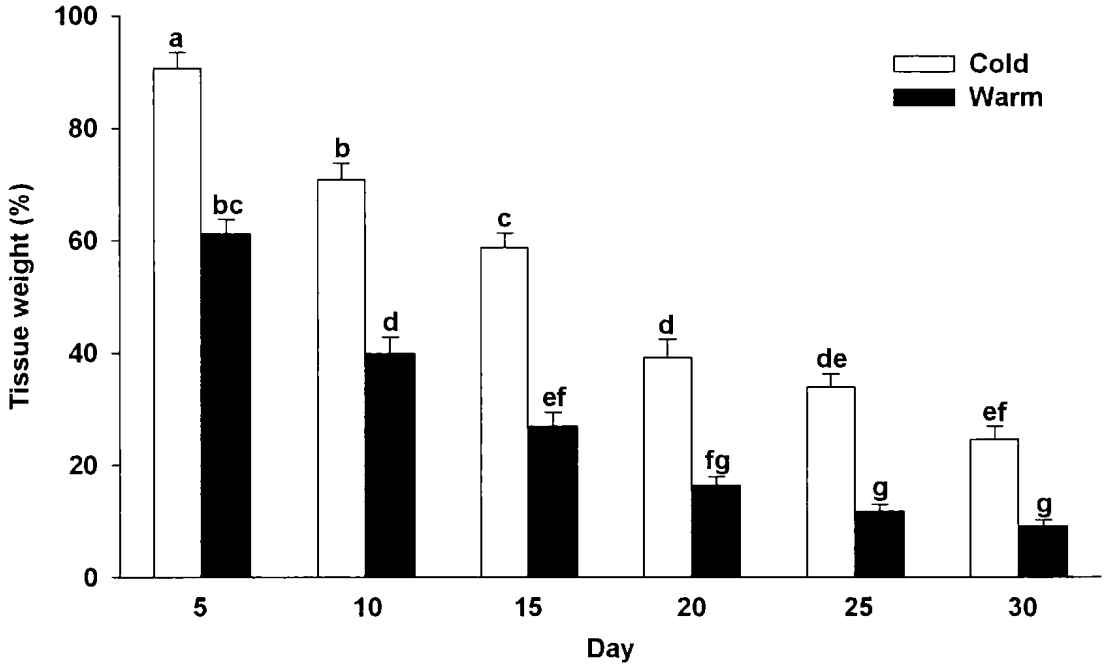
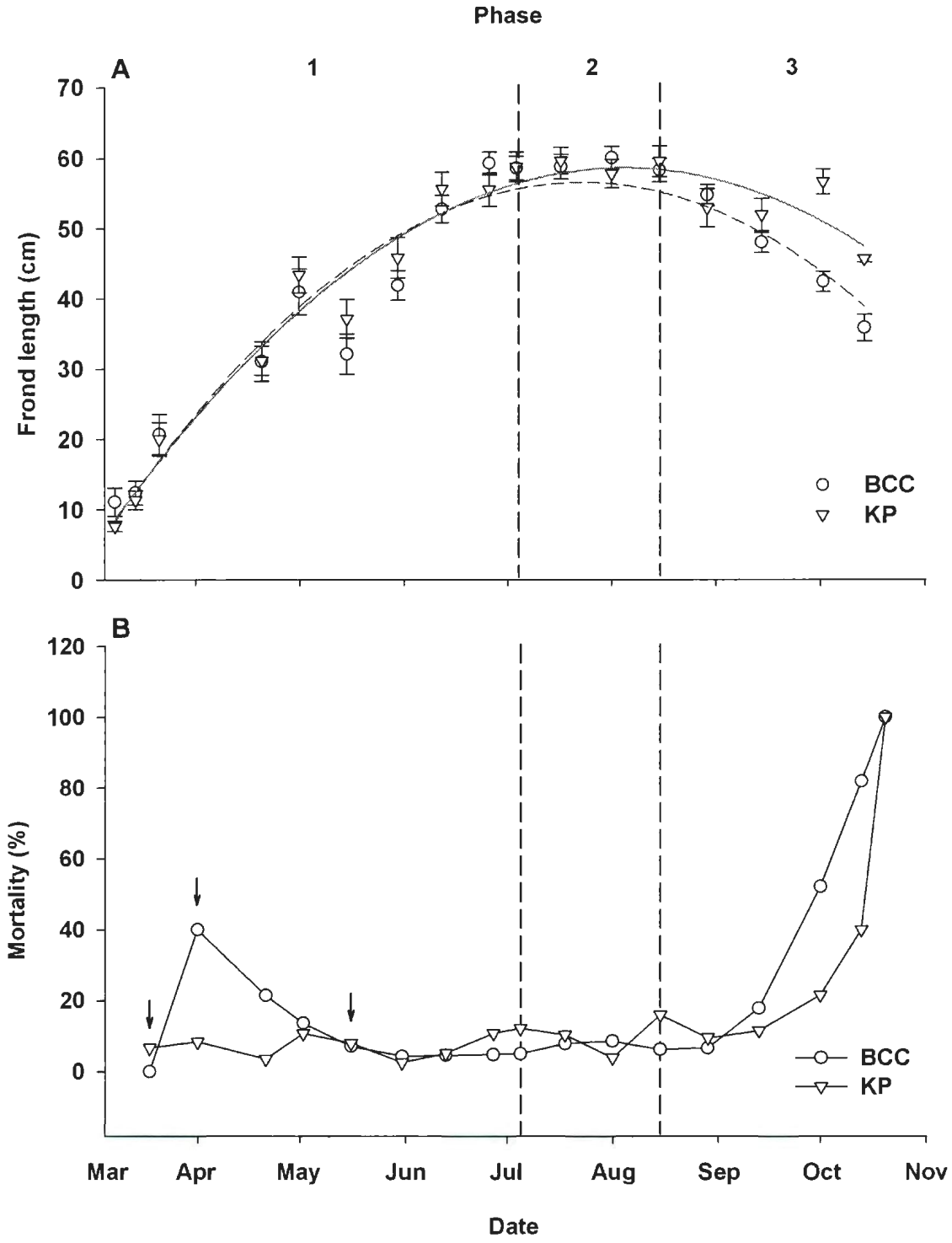


Figure 2.4. Mean (+SE) wet weight as a percentage of initial weight of frond tissues of *Desmarestia viridis* exposed 30 days to cold (2.5±0.1°C] and warm [10.8±0.2°C] water (Experiment 4). Bars not sharing the same letter are different (LS means tests, $p<0.05$; n=12 for each Temperature treatment).


Phase
A
1
2
3
Frond length (cm)
70
60
50
40
30
20
10
0
BCC
KP
B
Mortality (%)
120
100
80
60
40
20
0
BCC
KP
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

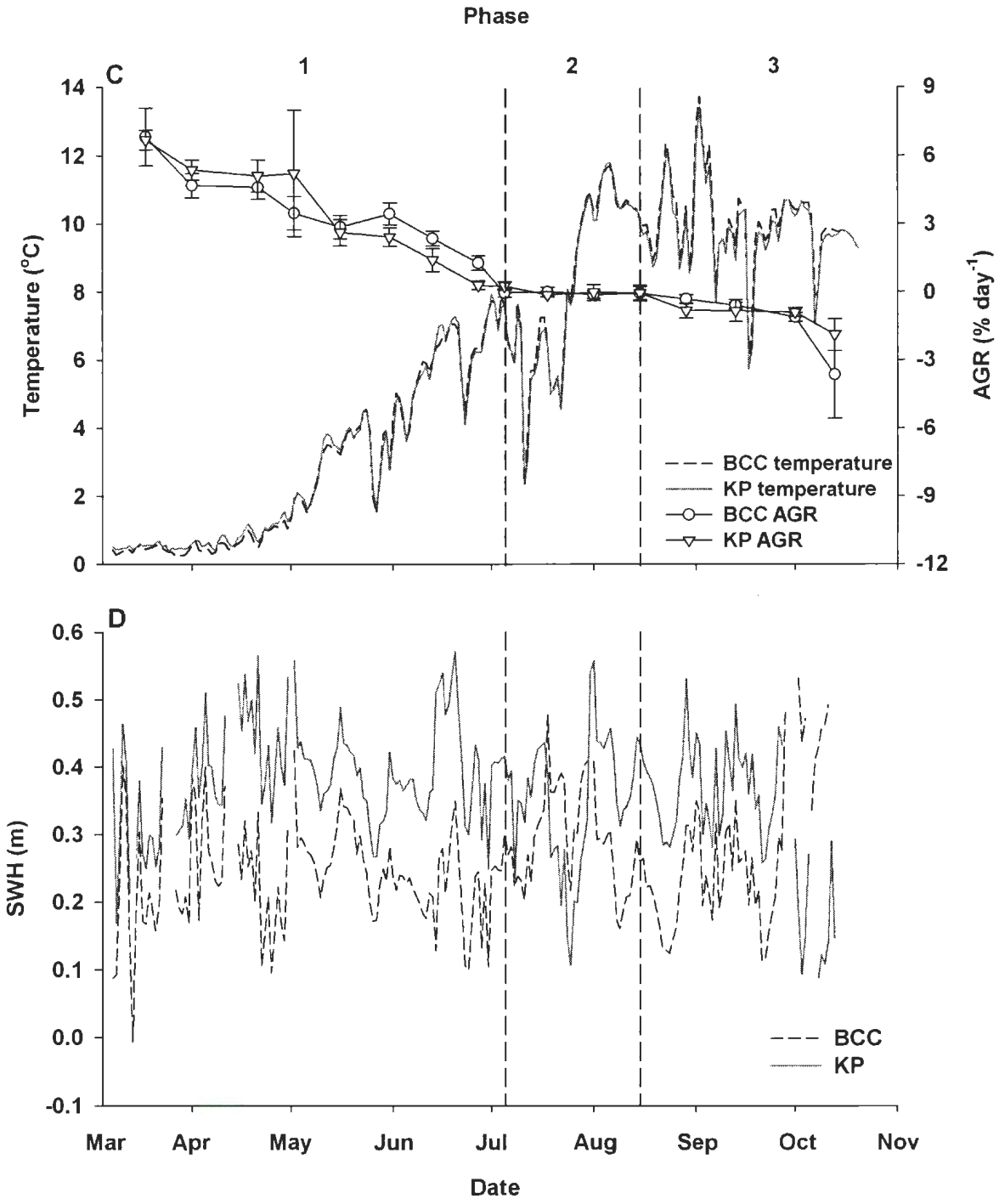

Phase
C
1
2
3
Temperature (°C)
AGR (% day-1)
BCC temperature
KP temperature
BCC AGR
KP AGR
D
SWH (m)
BCC
KP
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Date

Figure 2.5. Change in mean frond length (±SE) of *Desmarestia viridis* sporophytes (A), mortality (B), daily sea temperature, arithmetic growth rate (AGR, ±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 AGR and frond length data point is the average of growth rates and length of all plants measured on the corresponding date (n=3 to 39 [AGR] 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 plants 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), where x is the number of days from the first day of the first sampling month (1 March, 2011). 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).

July, when mean length was 58.6±1.7 [BCC] and 58.8±2.1 [KP] cm (Fig. 2.5). 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 (averaged length: 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. 2.5). 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. 2.5). 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 still relatively tall, ~35 to 45 cm (Fig. 2.5), 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 AGR indicated growth rates varied similarly between sites, while being different between phases (Table 2.5). Accordingly, AGR estimates pooled across sites in Phases 1, 2, and 3, averaged 2.9±0.1 (positive), -0.02±0.08 (virtually null), and -0.7±0.1 (negative) % day^{-1}, respectively, and differed statistically from each other (Table 2.5). Like AGR, mean sea temperature was similar between sites, though differed among phases (Table 2.5), 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. 2.5). Simultaneous onsets of decrease in frond length, increasing mortality, and negative AGR 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. 2.5). Significant wave

Table 2.5. 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 arithmetic growth rate (AGR) 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*
AGR				
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			

height (SWH) differed between sites and phases (a significant interaction between the factors Site and Phase; Table 2.5, Fig. 2.5). It was 63% and 22% higher (LS means, $p<0.001$) at KP than BCC in Phase 1 and Phase 3, respectively, though similar between sites in Phase 2 (LS means, $p=0.068$; Fig. 2.6). Comparable peaks of 0.65 m (BCC, 31 July) and 0.62 m (KP, 28 September) were noted between sites (Fig 2.5).

2.4 DISCUSSION

We demonstrated synergistic effects of sea temperature and the hydrodynamic environment on the ability of the annual, brown seaweed *Desmarestia 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). The latter study also uncovers similar upper thermal tolerances to those in sporophytes of the Antarctic, perennial, and taxonomically related species *Desmarestia anceps*, *Phaeurus antarcticus*, and *Himantothallus grandifolius* (Wiencke and tom Dieck 1989, Wiencke et al. 1994).

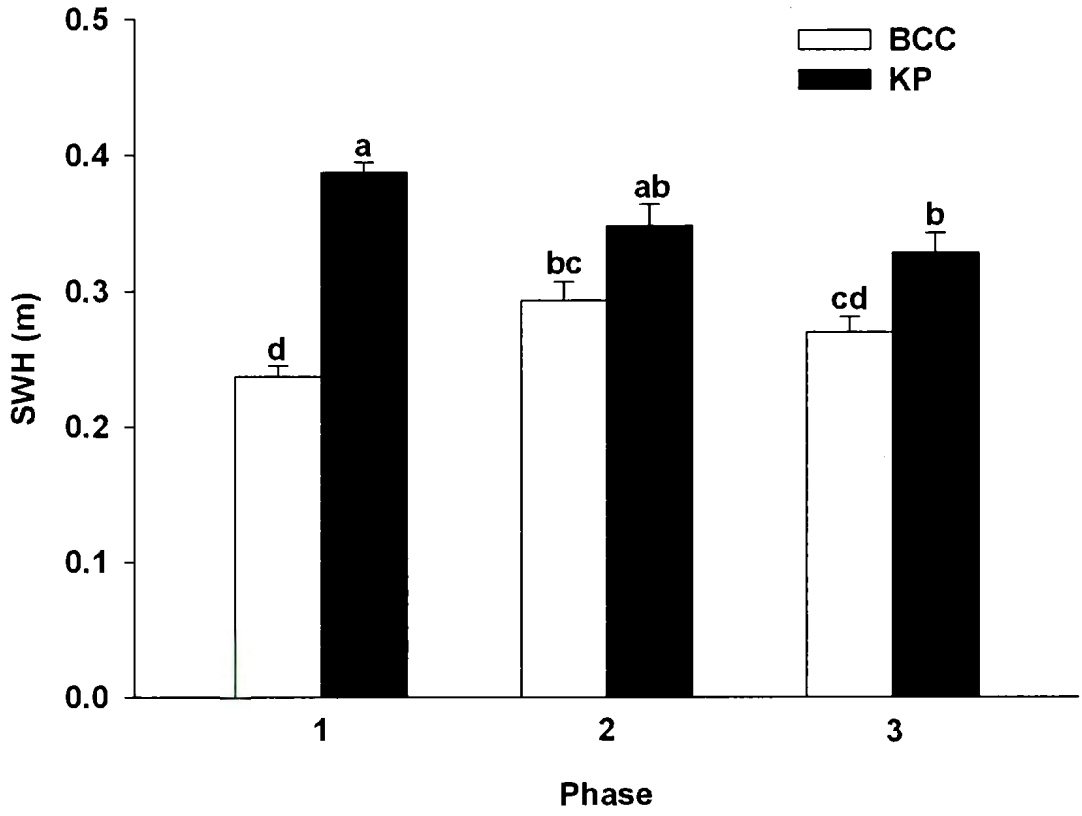


Figure 2.6. Mean (+SE) daily significant wave height (SWH) in each phase of change (Phase 1 [increase], Phase 2 [no change], and Phase 3[decrease]) in frond length of *Desmarestia viridis* sporophytes at the two study sites (BCC and KP). Each bar is the average of daily SWH from 8 March to 7 July, 8 July to 15 August, and 16 August to 13 October, 2011 (see Section 2.2.7 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]).

We found no convincing evidence that light retards or accelerates acid loss 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 (Brown and Richardson 1968, Chapman and Craigie 1977, Fortes and Lüning 1980, Barko et al. 1982, Twilley and Barko 1990, Blanchette 1997). 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 (Karban and Myers 1989, Hay 1996, Karban and Baldwin 1997, Karban et al. 1999, Ceh et al. 2005). Accordingly, in ecosystems with consistently high herbivore abundances, as 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, *Strongylocentrotus droebachiensis*, and fouling by up to 20 caprellids *Caprella* spp., the change in intracellular pH of the remaining *D. viridis* tissues remained similar to that of ungrazed or unfouled plants

(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 1985b, Himmelman and Nédélec 1990, Pelletreau and Muller-Parker 2002, Gagnon et al. 2006, Molis et al. 2009), 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 plants 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 (Himmelman and Nédélec 1990, Gagnon et al. 2003a, Gagnon et al. 2004), also implies that *D. viridis* survival depends on some other factors (see below and Gagnon et al. 2006, Gagnon et al. 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 arithmetic growth rates (AGR) 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 1972b, Konar 2000), northwestern Atlantic (van den Hoek 1982, Gagnon et al. 2003a), 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 sporophyte that initiated such changes irreversibly worsened over time, with a few sporophytes changing and disappearing in shallower, warmer water, as early as mid-July.

We experimentally demonstrated that tissue loss in *D. viridis* at the end of the natural senescence phase was about twice as low in cold (~3°C) than warm (~11°C) water (Experiment 4). This pattern, together with our finding 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, 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 trade-offs 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.

CHAPTER III

Changing perceptions: evidence of a third, transient community state in kelp-urchin-dominated ecosystems in the northwestern Atlantic

3.1 INTRODUCTION

Erect fleshy seaweeds are a dominant component of shallow, rocky benthic communities in polar, subpolar, and cold-temperate seas (Duggins et al. 1990, Irlandi and Peterson 1991, Amsler et al. 1995, Schiel and Hickford 2001, Wiencke et al. 2007, Wiencke and Amsler 2012). By creating vertical structure that modifies the light (Lüning and Dring 1985, Kennelly 1989, Clark et al. 2004) and hydrodynamic environments (Eckman et al. 1989, Duggins et al. 1990, Ackerman and Okubo 1993), seaweeds provide substrate and food for benthic and pelagic organisms (Duggins et al. 1989, Duggins et al. 1990, Iken et al. 1997, Iken 1999, Steinberg and de Nys 2002), ultimately modulating predator-prey interactions (Menge 1978, Peterson 1982, Orth et al. 1984, Dean and Connell 1987a, Duffy and Hay 1991, Irlandi and Peterson 1991, Gagnon et al. 2003a). Because seaweeds generally contribute to increasing marine biodiversity (Stoner 1980, Gilinsky 1984, Dean and Connell 1987a, b, c, Taylor and Cole 1994), any factor that alters their abundance is likely to trigger bottom-up cascades (see reviews by Bruno and Bertness 2001, Duffy 2006, Wiencke and Amsler 2012).

A prime example of cold, marine benthic communities organized around the high productivity of erect, fleshy seaweeds is that of the western Antarctic Peninsula, where three perennial species with contrasting morphologies in the order Desmarestiales, *Desmarestia anceps*, *Desmarestia menziesii*, and *Himantothallus grandifolius*, form thick canopies that cover up to 80% of the seabed (Amsler et al. 1995, Wiencke and Amsler 2012). These foundation species (*sensu* Bruno and Bertness 2001) support highly diverse and relatively stable assemblages of invertebrates for the three seaweeds chemically deter dominant grazers, including amphipods (Ankisetty et al. 2004, Huang et al. 2006) and

omnivorous fishes and sea stars (Amsler et al. 2005), and hence provide continuous access to vertical structure for recruitment and growth (Amsler et al. 2005, Amsler et al. 2008, Aumack et al. 2011, Zamzow et al. 2011). This situation contrasts with many shallow marine ecosystems at lower latitudes in both Hemispheres where macroherbivores largely control the structure and dynamics of benthic communities (Lawrence 1975, Lubchenco and Gaines 1981, Fletcher 1987, Hay and Fenical 1988, Watanabe and Harrold 1991, Hagen 1995, Ruitton et al. 2000, Shears and Babcock 2002).

To date, empirical and analytical models of benthic community dynamics for the extensively studied, shallow rocky ecosystems in the northwestern Atlantic (NWA) have largely focused on kelp-urchin interactions, with shifts in distribution and abundance of kelp (mainly *Saccharina longicruris* and *Alaria esculenta*) that can be predicted reasonably well from population shifts in their main predator, the omnivorous green sea urchin, *Strongylocentrotus droebachiensis* (Scheibling et al. 1999, Gagnon et al. 2004, Steneck et al. 2004, Lauzon-Guay and Scheibling 2010). The traditional view that these ecosystems alternate between two community states, kelp bed and urchin barrens, was recently broadened to include, for the Nova Scotia region, a likely multiyear, transient state dominated by the introduced green seaweed *Codium fragile* ssp. *fragile* (Scheibling and Gagnon 2006, Kelly et al. 2011). The possibility of another community state in regions invaded by *C. fragile*, together with improving knowledge about the stability and functional importance of less studied, yet conspicuous, canopy-forming, indigenous seaweeds in urchin barrens (e.g. Scheibling et al. 1999, Gagnon et al. 2003a, Bégin et al. 2004, Gagnon et al. 2004, Gagnon et al. 2005), call for a critical reassessment of the generality of phase-shifts and their mechanisms in shallow rocky communities in the

NWA. Recent studies of acid (H_2SO_4) production and mortality in the annual Desmarestiale *Desmarestia viridis* in urchin barrens in Newfoundland (eastern Canada) show the acid is continuously and irreversibly accumulated within vacuoles (resulting in a pH as low as 0.53 upon homogenization of tissues) as sporophytes grow from recruit to adult (March-June) and that this build-up inevitably culminates into mass releases of acid (July-August) and die-offs (September-October), when mean sea temperature raises to above ~12°C (Gagnon et al. 2013, Chapter II). These and other studies in the northern Gulf of St. Lawrence (Gagnon et al. 2003a, Gagnon et al. 2004) and Nova Scotia (Scheibling et al. 1999, Kelly et al. 2011, P. Gagnon, unpublished data) suggest *D. viridis* forms large (10s to 100s of m^2), ephemeral, grazing-resistant stands that may limit urchin movement, while facilitating recruitment in invertebrates and other seaweeds, including kelp.

In the present study, we test the overall hypothesis that *D. viridis* functions as a foundation species facilitating recruitment in, and supporting distinct assemblages of, invertebrates by examining the persistence and functional importance of sporophytes in urchin barrens at two subtidal sites in Newfoundland throughout an entire growth season (February to October). Specifically, we 1) characterize and relate temporal variability in the abundance of *D. viridis* and green sea urchin at multiple depths, 2) compare changes in epifaunal assemblages in *D. viridis* and two other conspicuous, canopy-forming seaweeds in urchin barrens, the non-acidic Desmarestiale *Desmarestia aculeata* and the grazing-resistant kelp *Agarum clathratum,* and 3) conduct two complementary laboratory experiments to gain insights into factors contributing to the observed variability in the

distribution of *D. viridis* and epifaunal assemblages of *D. viridis*, *D. aculeata*, and *A. clathratum*. We assimilate results of this and other studies of *D. viridis* into a comprehensive model of ecological interactions for the species. Furthermore, we propose an important change to the study of community dynamics associated with shifts between alternative stable states in shallow, rocky ecosystems in the NWA: the need to regard certain ephemeral, canopy-forming seaweeds as structuring forces interfering with top-down processes rather than simple prey for keystone grazers.

3.2 MATERIALS AND METHODS

3.2.1 Study sites

This study was conducted with *Desmarestia viridis*, *Desmarestia aculeata*, *Agarum clathratum*, and the kelp *Alaria esculenta* (see below) at two gently sloping, rocky subtidal sites located ~1.4 km apart in Bay Bulls, on the southeastern tip of Newfoundland: 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). Wave action is significantly less at BCC than KP (Chapter II). Seaweed assemblages at both sites are dominated by the kelps *A. esculenta* and *Laminaria digitata* to a depth of ~2 m, followed by extensive pavements of red coralline seaweeds, mainly *Lithothamnion glaciale* and *Clathromorphum compactum*, to a depth of ~15 m. These pavements, hereafter termed "barren zone" or "barrens" to follow the convention, are colonized year round by green sea urchin, *Strongylocentrotus droebachiensis*, as well as *D. aculeata*, which forms small (a few m^2), scattered patches on boulder tops and ridges at depths between 2 and 10 m. *Desmarestia*

viridis sporophytes establish annually in both barrens from March to October (Chapter II). The grazing-resistant kelp *A. clathratum* (Gagnon et al. 2005) forms small, scattered patches throughout the barrens and large (up to several tens of m^2) stands at depths >15 m.

3.2.2 Distribution and abundance of *D. viridis* and urchins

To evaluate the variability in the distribution and abundance of *Desmarestia viridis* and green sea urchins, we monitored changes in the cover (*D. viridis*) and density (urchin) in the barrens at BCC and KP during the entire 2011 sporophyte growth season in *D. viridis*. At each site, we permanently marked, in January 2011, both ends of one 20- to 25-m transect running parallel to the shoreline at 2, 3, 4, and 8 m depths with bolts set into the bedrock. These depths were chosen to cover the vertical range of *D. viridis* at both sites based on surveys in previous years, while focusing on the shallower portion of that range (2 to 4 m), which is where *D. viridis* and urchin abundances were most likely to vary in response to generally more variable temperature and wave conditions than in deeper (8 m) water (Gagnon et al. 2013, Chapter II). On 8 March, 2011, a 0.5-m (for the 2 m deep transect) or 1-m (for transects deeper than 2 m) swath of seabed was filmed on each side of each transect with a submersible, digital video camera (Sony HVR-V1 with an Amphibico Endeavor housing) propelled by a diver at a speed of ~0.1 m sec^{-1} at a fixed distance (0.5 m or 1.5 m depending on transect depth and tidal elevation) above bottom. This procedure was repeated biweekly until 13 October, 2011, after which all *D. viridis* sporophytes had deteriorated to a point where they were too small (~1 cm) to be detected on the imagery (all sporophytes had disappeared by 20

October, Chapter II). Each footage on each side of each transect was converted into a single image with PanoraGen.DV V1.0. Depending on image clarity and transect depth and length, each image (two per transect) was segmented into 20 to 50 frames of 0.4 m^2 or 12 to 25 frames of 0.8 m^2 with PhotoImpact V6.0. The percentage cover of *D. viridis* was estimated within each of five randomly selected frames using a digital grid with 100 point intersects, whereas urchin density was obtained by dividing the number of urchins >1 cm in test diameter (the smallest detectable size on the imagery) in each of five haphazardly selected frames without fleshy seaweeds by the surface area of the frame. Mean *D. viridis* cover and urchin density at each depth on each sampling day (14 days in total) for each site were determined by averaging the 10 corresponding measures for each variable.

3.2.3 Epifaunal assemblages

Of the three seaweeds in urchin barrens we investigated for epifaunal assemblages, only one, *Desmarestia viridis*, has an annual life cycle, with sporophytes exhibiting three phases of change in length at BCC and KP: (1) increase [March to late June], (2) no change [July to mid-August], and (3) decrease [mid-August to late October] (Chapter II). The two other species, *Desmarestia aculeata* and *Agarum clathratum*, are perennials with sporophytes that can live up to at least a few years (Vadas 1968, Chapman and Burrows 1971) and, in the case of *A. clathratum*, form highly stable patches in urchin barrens (Gagnon et al. 2005). Sporophytes in both *D. viridis* and *D. aculeata* have a highly flexible stipe and profusely branched fronds, which sweep back and forth over the bottom with wave action (Gagnon et al. 2003a, Gagnon et al. 2006).

This effect is less pronounced in *D. aculeata* due to cortication, during summer, of new tissues added annually (Chapman and Burrows 1971, Chapman 1972a). Sporophytes of *A. clathratum* have a semi-rigid stipe and a large crinkled frond, which becomes thicker and tougher with age (Vadas 1968), thereby limiting frond movement compared to *D. viridis* and *D. aculeata*. The relatively low palatability of *D. viridis* and *A. clathratum* to urchins is thought to be due to chemical deterrents in frond tissues, sulfuric acid and phenolics, respectively (Vadas 1977, Himmelman 1984, Himmelman and Nédélec 1990, Pelletreau and Muller-Parker 2002, Molis et al. 2009). There is no known anti-grazing substance in *D. aculeata* (McClintock et al. 1982, Sasaki et al. 1999, Pelletreau and Muller-Parker 2002, Lyons et al. 2007). To assess the functional importance of each of these three ontogenetically, morphologically, and chemically different seaweeds, we tracked changes in epifaunal assemblages of each species at KP during the entire 2011 growth season in *D. viridis*. Given the interspecific differences outlined above and marked changes in length, bushiness, and intracellular acidity of *D. viridis* sporophytes within only a few months (Gagnon et al. 2013, Chapter II), we predicted that epifaunal assemblages in *D. viridis* would be more variable and less diverse than those in the longer-lived, morphologically comparable, non-acidic *D. aculeata.* We also predicted that epifaunal assemblages in *D. viridis* would be more variable than those in the perennial *A. clathratum*, yet more diverse due to the greater morphological complexity of the former.

On 18 February, 2011, and every 26 to 35 days until 9 October, 2011, we hand collected (via SCUBA diving) frond tissues from 10 sporophytes in each three seaweeds at depths between 6 and 10 m (*D. aculeata*) and 8 and 12 m (*D. viridis* and *A. clathratum*). Tissues from only those sporophytes that showed no or the least

pronounced external signs of deterioration were collected (frond discoloration and sloughing in *D. viridis* began at shallower depths in late July). Approximately 10 g of tissues was cut with scissors from the distal end of each sporophyte and placed immediately in rigid 4-L plastic containers (one piece per container) sealed under water to prevent the loss of epifauna. Containers were transported to the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland where the content of each container was sieved and each piece of seaweed gently groomed to ensure all epifauna ≥250 μm were collected. Encrusting or gelatinous invertebrates such as bryozoans, cnidarians, and egg masses were identified and counted (individuals or colonies) within 24 hours from arrival at the OSC. All other epifauna were immersed in a 5% formalin-seawater solution for 24 hours and transferred into glass vials with a 70% ethanol-freshwater solution for preservation and later identification and counting. Organisms were identified to species where practical, and to genus (e.g. *Mytilus* sp.) or family (e.g. Halacaridae) otherwise (see Appendix D for taxonomic keys and guides used). Seaweed tissue wet weight was determined with a balance (±0.01 g, model PB-3002-S/FACT; Mettler Toledo).

3.2.4 Vulnerability to grazing

To gain insights into factors contributing to the observed variability in the distribution of *Desmarestia viridis* (section 3.2.2) and epifaunal assemblages in *D. viridis*, *Desmarestia aculeata*, and *Agarum clathratum* (section 3.2.3), we conducted two complementary experiments in which we exposed sporophytes (Experiment 1) and gelatinized sporophyte extracts (Experiment 2) to grazing. We used the green sea urchin as the grazer since it is the dominant consumer of all three seaweeds in eastern Canada

(Himmelman 1984, Keats et al. 1990, Gagnon et al. 2003a, Gagnon et al. 2005), including at our study sites.

Experiment 1 tested the hypothesis that in the absence of waves and currents, vulnerability (tissue loss) to grazing is lowest in seaweeds with both anti-grazing substances and tough tissues, that is the vulnerability of *A. clathratum* (phenolics and semi-rigid frond) is lower than that of *D. viridis* (sulfuric acid and soft, delicate branches), which in turn is lower than that of *D. aculeata* (no known chemical deterrent and soft, delicate branches) (see Section 3.2.3 for details about the morphology and chemical makeup of each species). We used the kelp *Alaria esculenta*, which is one of the most heavily consumed seaweeds by *S. droebachiensis* in the NWA (Himmelman 1984, Himmelman and Nédélec 1990), as a reference to test the validity of the results, with the expectation that vulnerability is highest in *A. esculenta*. We ran the experiment from 15 to 19 July, 2011, with tissues from five sporophytes in each four species collected (via SCUBA diving) on 14 July at depths between 2 and 15 m at KP. One piece of ~30 g of tissues was cut with scissors from the distal end (*D. viridis* and *D. aculeata*) or middle of the frond (*A. clathratum* and *A. esculenta*) 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. Tissues from only those sporophytes that showed no or the least pronounced external signs of deterioration were collected. Containers were transported to the OSC where they were transferred to large holding tanks supplied with ambient (7.8±0.5°C), flow-through seawater pumped in from the adjacent embayment, Logy Bay.

On 15 July (within less than 24 hours of seaweed collection), we cut two pieces of ~10 g (blotted wet weight) from each original sample of ~30 g. Tissue weight was determined with precision with a balance (same model as above) in less than 20 s following emersion. Such inevitable exposure of *D. viridis* tissues to air had no effect on acidity (Chapter II). One piece of each pair was transferred to either of 20, 75-L glass tanks supplied with flow-through sea water (1 L min^{-1}) and secured to the bottom with 12-g weights attached to the stipe or frond with a plastic cable tie. Ten urchins (3 to 6 cm in test diameter) collected on 7 July, 2011 at KP at depths between 2 and 12 m and starved for one week to standardize hunger levels were introduced to each tank and allowed to graze seaweeds for 48 hours. Each piece of seaweed was reweighed at the end. Tissue loss to grazing was corrected for autogenic loss or gain, as determined by applying the procedures above to the second set of ~10-g pieces of sporophytes over the following 48 hours, except no urchins were introduced to the tanks. We used the following equation to obtain the corrected tissue loss in each tank (Wessels et al. 2006):

$$\text{Tissue loss} = [(T_o \times C_f / C_o) - T_f]$$

where T_o and T_f are the initial and final weights of seaweed tissues exposed to urchins, respectively, and C_o and C_f are the mean initial and final weights of the corresponding autogenic control, respectively.

The 20 tanks were grouped in five blocks of four tanks. Each tank in each block was randomly assigned one of the four seaweeds, for a total of five replicates per treatment. Each tank was surrounded by a thick, opaque canvas to standardize light conditions. The standardized 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 on a 12-hour light/dark cycle. To increase the sample size, we reran the experiment (including the autogenic control) from 19 to 23 and 23 to 27 July, 2011, with tissues from two additional groups of 20 sporophytes (five in each of the four species in each group) collected on 18 and 22 July, respectively, and urchins collected on 12 and 14 July and starved for one week. As in the first run, treatments in each of these two additional runs were reassigned randomly to tanks in each block to eliminate confounding effects of treatment and block, as well as tank and treatment. Therefore, each treatment was replicated 15 times in total. Temperature in one randomly chosen tank of each treatment was monitored with a temperature logger (±0.5°C, HOBO Pendent; Onset Computer Corporation) throughout the experiment.

Experiment 2 examined the unique contribution of seaweed chemical makeup to grazer deterrence. We tested the prediction that vulnerability to grazing is lowest in seaweeds with anti-grazing substances, that is the vulnerability of *D. viridis* (sulfuric acid) and *A. clathratum* (phenolics) is about the same and lower than *D. aculeata* (no known chemical deterrent). As in Experiment 1, we used *A. esculenta* as a reference with the expectation that it is the most vulnerable of all four species. We ran the experiment from 27 to 31 July, 2011 with tissues from four sporophytes in each four species collected (via SCUBA diving) on 25 July at depths between 2 and 15 m at KP. Procedures for collection, transportation, and maintenance of sporophytes in the laboratory were similar to those in Experiment 1. Water temperature during acclimation in the holding tanks was 8.8±0.5°C.

On 27 July (within less than 48 hours of seaweed collection), we cut one piece of ~10 g (blotted wet weight, measured with the same balance as above) from each original

sample of ~30 g. Each piece, weighing between 9.8 and 10.2 g, was 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. Eighty (80) mL of the filtrate (extract) were agitated and warmed on a heating plate (model VMS-C7 S1; VWR International) after adding 2 g of granulated agar (product BP1423-500; Fisher Scientific) to it. The resulting extract-agar solution was poured into two circular, 50-mL Petri dishes and allowed sufficient time to cool. Each solidified disk was removed from its dish and weighed with a balance (same model as above). We prepared eight additional disks each made up of 40 mL of distilled water and 1 g of agar (no seaweed extract) to verify whether the disks were consumed by urchins. One disk of each pair with seaweed extract and four disks with no extract were each transferred to one of 20, 75-L glass tanks supplied with flow-through sea water (1 L min^{-1}) and secured to the bottom with 5-g weights. Ten urchins (3 to 6 cm in test diameter) were collected on 18 July, 2011 at KP at depths between 2 and 12 m, starved for one week, and introduced to each tank in which they were allowed to graze disks for 48 hours. Each disk was reweighted at the end. Disk loss to grazing was corrected for artificial loss or gain, as determined by applying the procedures above to the second set of disks over the following 48 hours in the absence of urchins. We used the same equation as in Experiment 1 to obtain the corrected disk loss in each tank.

The 20 tanks were grouped in four blocks of five tanks. Each tank in each block was randomly assigned one of the five treatments, for a total of four replicates per treatment. Light conditions in each tank were similar to Experiment 1. To increase the sample size, we reran the experiment (including the artificial control) four times between

31 July and 20 August, 2011 with additional groups of 16 sporophytes (four in each of the four species) and urchins collected <72 hours and <10 days prior to each run, respectively. As in the first run, treatments in each of these four additional runs were reassigned randomly to tanks in each block to eliminate confounding effects of treatment and block, as well as tank and treatment. Therefore, each treatment was replicated 20 times in total. Like in Experiment 1, temperature in one randomly chosen tank of each treatment was monitored with a temperature logger (same model as above) throughout the experiment. Mean water temperature during trials was comparable between Experiment 1 (6.1±0.2°C) and Experiment 2 (8.3±0.1℃).

3.2.5 Statistical analysis

We used a two-way ANOVA with the factors Site (two study sites: BCC and KP) and Depth (four sampling depths: 2, 3, 4, and 8 m) to examine effects of site and depth on the mean ratio of *D. viridis* cover to urchin density from 8 April to 13 October, 2011. We excluded from the analysis data acquired on 8 March, 2011 since null *D. viridis* cover values on this date artificially skewed the mean ratio. The analysis was applied to the raw data since the residuals generated by the ANOVA met the assumptions (see Appendix B for details about analysis of residuals and data transformation) Because there was a significant interaction between the factors Site and Depth (see Section 3.3.1) we further examined the relationship between *D. viridis* cover and urchin density for each depth at each site using simple linear regression analysis.

We used non-metric multidimensional scaling (MDS) on 4^{th}-root transformed density of each taxon (since density differed by of up to three orders of magnitude within and between taxa) in each month (February to October) to classify epifaunal assemblages from each seaweed (*D. viridis*, *D. aculeata*, and *A. clathratum*) (Clarke and Gorley 2006). We examined differences in assemblages between months and seaweeds using analysis of similarity (ANOSIM), followed by analyses of similarity percentages (SIMPER) to identify taxa contributing the most (at least 5%) to similarities and dissimilarities within and between seaweeds (Clarke and Gorley 2006).

We used two-way ANOVAs (one for each group of organisms) with the factors Seaweed (three seaweeds: *D. viridis*, *D. aculeata*, and *A. clathratum*) and Month (random factor with nine levels: February to October) to examine temporal changes within and between seaweeds in the density of individuals in the six numerically dominant (i.e. with a peak density $\geq$900 individuals kg^{-1} seaweed in any month) invertebrate taxa (Bivalvia, Gastropoda, Copepoda, Amphipoda, Polychaeta, and Isopoda) and fish and gastropod egg masses. No transformation corrected for the heteroscedasticity of the residuals in the eight analyses on the raw data, and hence the ANOVAs were also run with the rank-transformed data. Because analyses on both raw and ranked-transformed data gave similar conclusions about the significance of each factor, we presented the results from analyses of the raw data, as suggested by Conover (1980). Likewise, we used two two-way ANOVAs with the factors Seaweed (three seaweeds: *D. viridis*, *D. aculeata*, and *A. clathratum*) and Month (random factor with nine levels: February to October) to examine temporal changes in the Shannon diversity index (H') and Pielou's evenness

index (J') of epifauna for each seaweed in each month. Both analyses were applied to the raw data since the residuals generated by each ANOVA met the assumptions.

We used a one-way ANOVA with the factor Seaweed (four seaweeds: *D. viridis*, *D. aculeata*, *A. clathratum*, and *Alaria esculenta*) to examine differences in proportions of seaweed tissue weight loss (relative to initial weight) to urchin grazing (Experiment 1), as well as a one-way ANOVA with the factor Seaweed (the same four seaweeds as above and one procedural control) to examine differences in proportions of agar-embedded seaweed extracts weight loss (relative to initial weight) to urchin grazing (Experiment 2). We treated both analyses as a particular case of the generalized linear models (McCullagh and Nelder 1989, Bolker et al. 2008), which assumed a binomial distribution of the response variable (ratio of final to initial weight). Thus, we did not test for normality and homoscedasticity in the data. Prior to running the above mentioned one-way ANOVAs, we used a three-way ANOVA (generalized linear model assuming a binomial distribution) with the factors Seaweed (the four seaweeds), Run (three runs of four replicates each), and Block (five blocks of four tanks in each run) to determine whether results differed between runs and blocks in Experiment 1. Likewise, we used a three-way ANOVA with the factors Seaweed (the four seaweeds and one procedural control), Run (five runs of four replicates each), and Block (four blocks of five tanks in each run) to determine whether results differed between runs and blocks in Experiment 2. We found no statistical differences in both three-way ANOVAs (Experiment 1: Factor = Run, χ^2=0.38, p=0.83; Factor = Block, χ^2=0.62, p=0.96; Factor = Run x Block, χ^2=2.01, p=0.98; Experiment 2: Factor = Run, χ^2=0.41, p=0.98; Factor = Block, χ^2=0.18, p=0.98;

Factor = Run x Block, χ^2=0.83 p=1.00), and hence, we applied the one-way ANOVAs to data pooled across runs.

In all ANOVAs and regression analyses, homogeneity of the variance was verified by examining the distribution of the residuals and referring to the Levene tests, whereas normality of the residuals was verified by examining the normal probability plot of the residuals and referring to the Shapiro-Wilk's statistics (Appendix B) (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. All analyses were conducted with JMP 7.0 and Minitab 16, except multivariate analyses, which were carried out with Plymouth Routines in Multivariate Ecological Research (PRIMER) software, v6.1.10.

3.3 RESULTS

3.3.1 Distribution and abundance of *D. viridis* and urchins

Patterns of *Desmarestia viridis* and urchin abundances at 2, 3, 4, and 8 m depths at BCC and KP from March to October, 2011 suggested positive and negative relationships with increasing depth, respectively (Fig. 3.1). Indeed, the highest cover of *D. viridis* ranged from 8.2 (±3.0, SE) % at 2 m to 20.8 (±8.4) % at 8 m at BCC, and from 6.3 (±2.7) % at 2 m to 24.1 (±8.3) % at 8 m at KP, whereas the highest urchin density ranged from 152.3 (±28.2, SE) individuals m^{-2} at 2 m to 70.0 (±8.1) individuals m^{-2} at 8 m at BCC, and from 199.3 (±21.4) individuals m^{-2} at 2 m to 54.2 (±7.5) individuals m^{-2} at 8 m at KP (Fig. 3.1). The overall cover (all depths pooled) of *D. virdis*: 1) increased steadily from March to mid-July at BCC and from March to early August at KP,

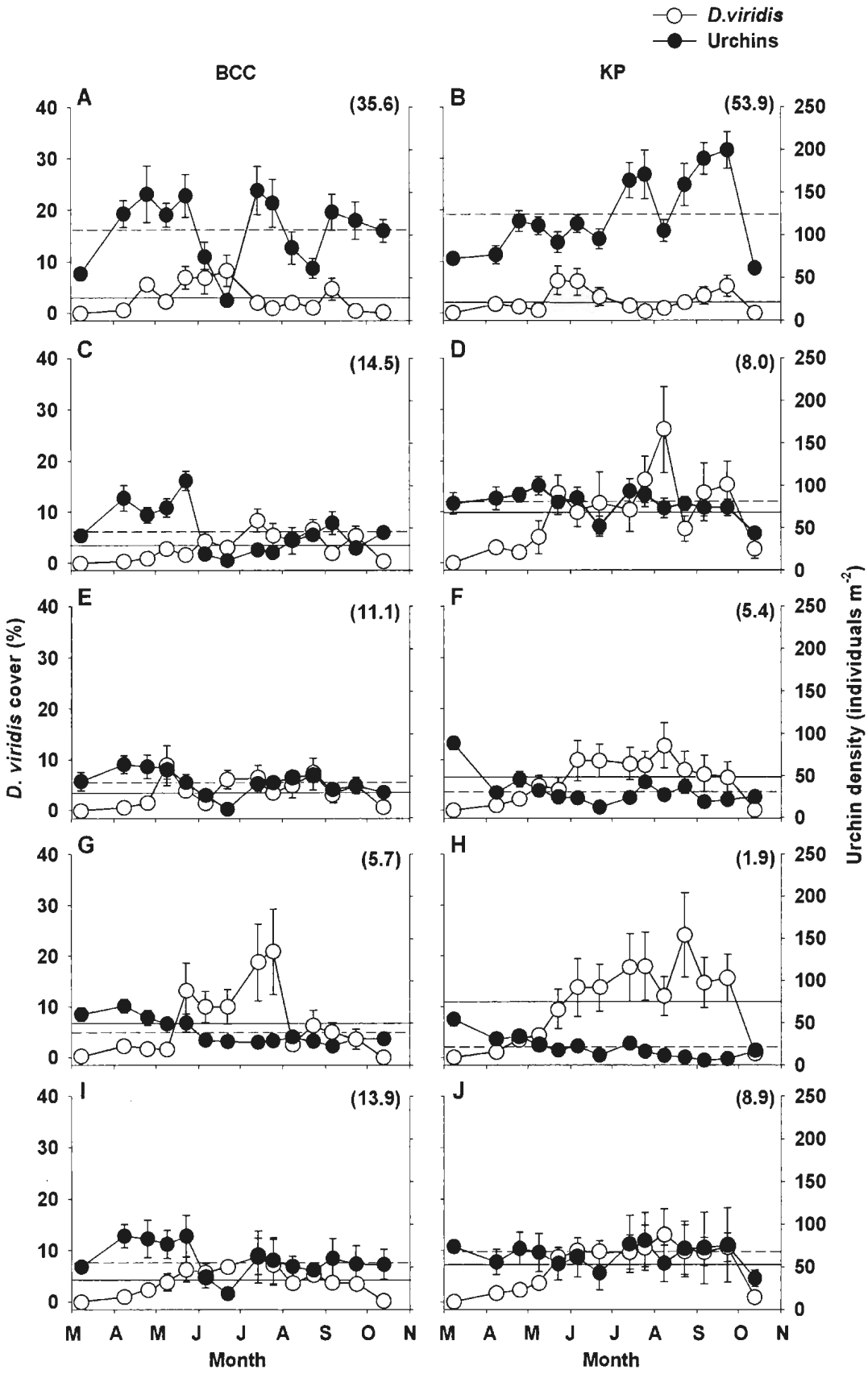

D.viridis
Urchins
BCC
KP
A
(35.6)
B
(53.9)
C
(14.5)
D
(8.0)
E
(11.1)
F
(5.4)
G
(5.7)
H
(1.9)
I
(13.9)
J
(8.9)
D. viridis cover (%)
Urchin density (individuals m-2)
M A M J J A S O N
Month

Figure 3.1. Mean (±SE) *Desmarestia viridis* cover and urchin (*Strongylocentrotus droebachiensis*) density at 2 (A and B), 3 (C and D), 4 (E and F), and 8 (G and H) m depths, and pooled across all depths (I and J) at Bread and Cheese Cove (BCC) and Keys Point (KP) from 8 March to 13 October, 2011. Each data point in panels A to H represents the average cover of *D. viridis* or urchin density in 10 quadrats (0.4 or 0.8 m^2 each) along one transect (20 to 25 m) at each depth (see Section 3.2.2 for sampling details). Each data point in panels I and J is the average *D. viridis* cover or urchin density across all sampling depths. The seeming lack of standard error on some data points is due to low variation in the data. Horizontal solid and dashed lines are the average *D. viridis* cover or urchin density, respectively across all data points (n=14 for each line). The number in parentheses within each panel is the ratio of urchin density to *D. viridis* cover.

2) remained stable until late September at both sites, and 3) decreased rapidly to extinction by mid-October at both sites (Fig. 3.1). The sudden decrease in cover at 8 m at both sites between August and October was when mean sea temperature peaked to above 10°C (Chapter II) and urchin density was below 50 individuals m^{-2} (Fig. 3.1). Decreases in cover during the same period were less pronounced at lesser depths where urchin density was generally higher than at 8 m (Fig. 3.1).

The mean ratio of *D. viridis* cover to urchin density differed between sites and among depths (Table 3.1), being significantly higher at 8 m at KP than BCC (LS means, $p = 0.007$) and any other depths at both sites ($p>0.05$ for all other pairwise comparisons). Regression analysis further uncovered significant, negative relationships explaining between 34% and 61% of the variation between cover and density at 2 and 3 m at BCC and at 4 and 8 m at KP (no significant relationship for any other combination of site and depth; Table 3.2, Fig. 3.2). The urchin density to *D. viridis* cover ratio, which provides a standardized proxy for *D. viridis* vulnerability to urchin grazing (the greater the ratio, the more urchins per unit of *D. viridis* cover and hence higher vulnerability, Gagnon et al. 2006) was 1.5 times greater at KP (53.9) than BCC (35.6) at 2 m, yet between two and three times lower at KP than BCC at greater depths, i.e. a reverse difference between sites that increased from 3 to 8 m (Fig. 3.1). These results together with the 1.5-fold greater overall ratio (all depths pooled) at BCC than KP (Fig. 3.1), indicate that the vulnerability of *D. viridis* to urchin grazing was generally higher and more uniform across depth at the former site. At both sites, urchin grazing on *D. viridis* from March to July was limited to only a few scattered days when wave action, and hence the wave-induced sweeping

Table 3.1. Summary of two-way ANOVA (applied to raw data) examining the effect of Site (BCC and KP) and Depth (2, 3, 4, and 8 m) on the ratio of *D. viridis* cover to urchin (*Strongylocentrotus droebachiensis*) density, from 8 April to 13 October, 2011 (see Section 3.2.2 for details).

Source of variation	***df***	**MS**	***F*-value**	***p***
Site	1	1.25	8.35	< 0.01
Depth	3	1.15	7.70	< 0.01
Site x Depth	3	0.87	5.82	< 0.01
Error	76	0.15		
Corrected total	83			

Table 3.2. Results of linear regression analyses examining the relationships between *D. viridis* cover and urchin (*Strongylocentrotus droebachiensis*) density (x, urchins m^{-2}) at 2, 3, 4, and 8 m depths at each of the two study sites, Bread and Cheese Cove (BCC) and Keys Point (KP) from 8 April to 13 October, 2011 (see Section 3.2.2 for details).

Site	Depth (m)	Equation for *D. viridis* cover (%)	r^2	$F_{(df)}$	p
BCC	2	9.08 - 0.056 x	0.609	$10.90_{(1,7)}$	0.013
	3	6.08 - 0.056 x	0.395	$7.19_{(1,11)}$	0.021
	4	5.54 - 0.053 x	0.143	$1.51_{(1,9)}$	0.25
	8	9.71 - 0.130 x	0.388	$4.40_{(1,7)}$	0.073
KP	2	5.48 - 0.026 x	0.157	$1.50_{(1,8)}$	0.26
	3	24.02 - 0.18 x	0.260	$2.80_{(1,8)}$	0.13
	4	11.46 - 0.17 x	0.448	$5.72_{(1,7)}$	0.049
	8	19.71 - 0.46 x	0.339	$5.61_{(1,11)}$	0.037

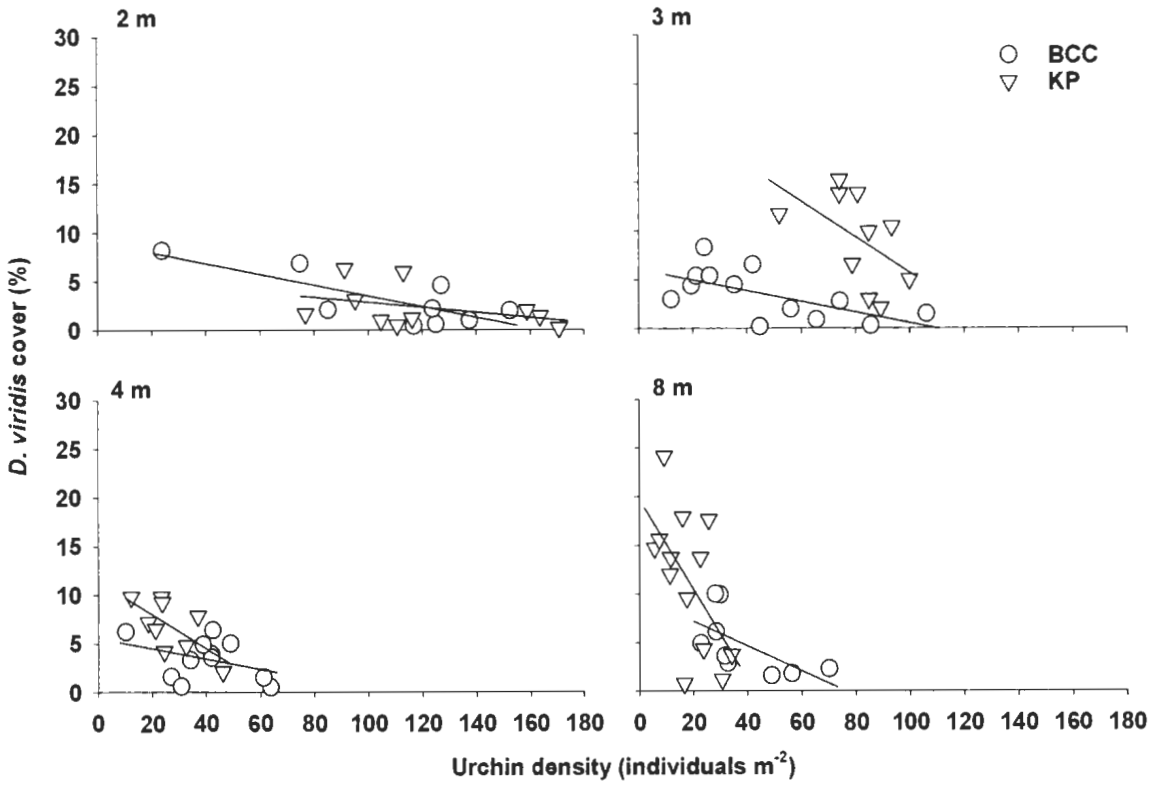


Figure 3.2. Relationship between *Desmarestia viridis* cover and urchin (*Strongylocentrotus droebachiensis*) density at 2, 3, 4, and 8 m depths at Bread and Cheese Cove (BCC) and Keys Point (KP) from 8 April to 13 October, 2011. Solid lines in each panel are the linear regression fits to data for each site (n=9 to 13) (see Table 3.2 for a summary of each regression).

motion of sporophytes, was virtually null. However, we noted dramatic increases in the frequency of urchin grazing on *D. viridis* in early August, which persisted, especially at greater depths, until complete disappearance of *D. viridis* in October.

3.3.2 Epifaunal assemblages

Epifaunal assemblages on *Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum* from 18 February to 9 October, 2011 at KP consisted of 41 taxa, 38 invertebrates and three chordates (all juvenile fish), in eight phyla (Appendix E). For practical reasons, fish (unidentified species) and gastropod (*Lacuna vincta*) egg masses were considered as distinct epifaunal entities (as opposed to real taxa) and included in the MDS, ANOSIM, and SIMPER analyses (one count per mass, see below). However, to minimize data variation and skewing, we excluded from the latter analyses: 1) chordates, which were highly mobile and often moved away while collecting seaweed tissues, 2) copepods, which were relatively abundant on *Desmarestia* spp. throughout the survey, and 3) juvenile crab *Hyas* sp. and nemertean *Tetrastemma* sp., which were rare, on average between zero and one individual per seaweed in any month. MDS using density of the 35 remaining taxa and two types of egg masses separated seaweeds into groups with more similar assemblages between February and August than in September and October, when each seaweed formed a distinct cluster (Fig. 3.3) and *D. viridis* was releasing sulfuric acid to the environment and decaying (Fig. 3.1., Chapter II, Gagnon et al. 2013). Assemblages in *D. viridis* and *D. aculeata* were similar in February and March (ANOSIM: R=0.007, p=0.864) before shifting through April (ANOSIM: R=0.074, p=0.483) towards new, yet similar assemblages from May to August (ANOSIM:

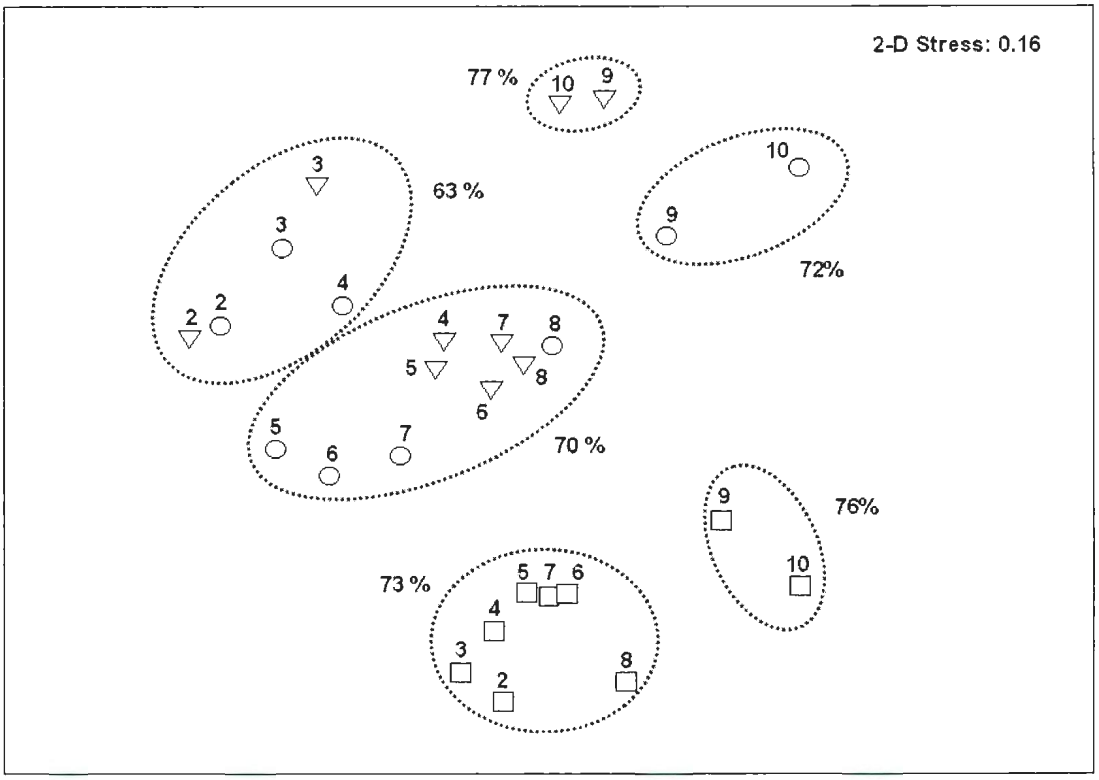


Figure 3.3. Multidimensional scaling (MDS) plot of Bray-Curtis similarities of the seaweeds *Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum* based on associated epifauna (4^{th}-root transformed density, individuals g^{-1} of seaweed) from 18 February to 9 October, 2011 at KP. Ellipses (dashed lines) and corresponding percent similarities indicate homogeneous clusters as determined by ANOSIM ($p<0.001$). Symbols indicate seaweed species: *D. viridis* (○), *D. aculeata* (▽), and *A. clathratum* (□). Numbers next to symbols indicate sampling month: February (2), March (3), April (4), May (5), June (6), July (7), August (8), September (9), and October (10) (n=8 to 10 for each data point, except for *A. clathratum* in February where n=3).

R=0.093, p=0.531) (Fig. 3.3). Assemblages in *A. clathratum* were generally more stable than, and differed significantly from, those in *D. viridis* and *D. aculeata* from February to August (Fig. 3.3; ANOSIM: R=0.702, p<0.001 for Feb-Aug *A. clathratum* cluster vs. Feb-Apr *Desmarestia* spp. cluster; R=0.581, p<0.001 for Feb-Aug *A. clathratum* cluster vs. Apr-Aug *Desmarestia* spp. cluster). SIMPER analysis of data from February to October (entire survey) indicated the snail *Lacuna vincta* contributed the most to similarities in epifaunal assemblages in the three seaweeds, from 23% (*D. viridis*) to 34% (*A. clathratum*), followed by the caprellid amphipod *Ischyrocerus anguipes* and mussel *Mytilus* sp. (Table 3.3). Most of the dissimilarity between *D. viridis* and *D. aculeata* was also caused by the latter three species, which densities were higher (up to two orders of magnitude) on *D. viridis*, except *Mytilus* sp., as well as the snail *Margarites helicinus* and gammarid amphipod *Stenothoe brevicornis*, which were also more abundant on *D. viridis* (Table 3.3). We found comparable patterns of dissimilarity between *D. viridis* and *A. clathratum*, with the exception that *Mytilus* sp. was more abundant on *D. viridis*, as were fish egg masses and the amphipods *Pontogenia inermis* and *Calliopius laeviusculus* (Table 3.3). The polychaete *Spirorbis borealis* and bryozoan *Lichenopora* sp. were generally more abundant on *A. clathratum* than on the two Desmarestiales, thus also contributing to the bulk of the differences in epifaunal assemblages among seaweeds. SIMPER analysis of data in September and October (when seaweeds formed distinct clusters) identified *L. vincta* as the numerically dominant species in *D. viridis*, *Mytilus* sp. in *D. aculeata*, and *S. borealis* and *Lichenopora* sp. in *A. clathratum*.

Table 3.3. Epifauna accounting for ≥5% of the similarity within each of the three seaweed species (diagonal) and for ≥5% of the dissimilarity between two seaweeds from data pooled across months (February to October, 2011). For each grouping, epifaunal taxa are listed in order of decreasing contribution to similarity or dissimilarity among seaweeds. The density of epifauna with an asterisk was higher on the seaweed given at the top of the table.

	Desmarestia viridis	***Desmarestia aculeata***	***Agarum clathratum***
Desmarestia viridis	*Lacuna vincta* *Ischyrocerus anguipes* *Pontogeneia inermis* *Mytilus* sp. *Calliopius laeviusculus* Fish egg masses		
Desmarestia aculeata	*Mytilus* sp. *L. vincta** *I. anguipes** *P. inermis* *C. laeviusculus* *Margarites helicinus** *Stenothoe brevicornis**	*L. vincta* *Mytilus* sp. *C. laeviusculus* *I. anguipes* *M. helicinus*	
Agarum clathratum	*L. vincta** *P. inermis** *I. anguipes** *C. laeviusculus** *Mytilus* sp.* *M. helicinus** *S. brevicornis** Fish egg masses* *Spirorbis borealis*	*Mytilus* sp.* *C. laeviusculus** *I. anguipes** *S. borealis* *Lichenopora* sp. *M. helicinus** *L. vincta**	*L. vincta* *I. anguipes* *S. borealis* *Mytilus* sp. *Lichenopora* sp. *M. helicinus*

The density of copepods, bivalves, gastropods, polychaetes, and egg masses (gastropods and fish) differed among seaweeds over time as shown by the significant interaction between factors Seaweed and Month (two-way ANOVAs, Table 3.4). Although copepods were generally more abundant (up to one order of magnitude in most months) than amphipods, these were the only two common taxa from February to October on *D. viridis*, and to a lesser extent on *D. aculeata* (they were largely absent from *A. clathratum*) (Fig. 3.4). There was a remarkable increase from virtual absence prior to September to >150 000 gastropod and >230 000 bivalve recruits kg^{-1} of *D. viridis* and *D. aculeata* in October, respectively. Likewise, polychaetes increased by three orders of magnitude on *A. clathratum* from August to September, while remaining very low on the two Desmarestiales throughout the survey (Fig. 3.4). Isopod density also increased significantly in the last two months regardless of seaweed (Table 3.4, Fig. 3.4). Gastropod eggs were consistently uncommon on *D. viridis*, being predominantly deposited on *A. clathratum* in May and June, on *D. aculeata* in July and August, and again on *A. clathratum* in October, when their density was twice higher than in any other month (Fig. 3.4). *Desmarestia viridis* was the preferred seaweed for deposition of eggs by fish, with an increase in egg masses of two orders of magnitude from April to May, followed by a steady monthly decline to near absence in September (Fig. 3.4).

The Shannon diversity index differed significantly among seaweeds over time (Table 3.5) and was generally highest in *A. clathratum* from February to May, in *D. aculeata* in June and July, in *D. viridis* in August and September, and again in *A. clathratum* in October (Fig. 3.5). There was a two-fold increase in diversity from

Table 3.4. Summary of two-way ANOVAs (applied to raw data) examining the effect of Seaweed (*Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum*) and Month (each of nine sampling months: February to October, 2011) on the density of individuals in the six numerically dominant invertebrate taxa and gastropod (*Lacuna vincta*) and fish (unknown species) egg masses at Keys Point (see caption of Fig, 3.4 for species in each taxa).

Taxa	Source of variation	*df*	MS	*F*-value	*p*
Bivalvia	Seaweed	2	231405	1.70	0.21
	Month	8	179180	1.11	0.41
	Seaweed × Month	16	164396	4.69	<0.01
	Error	194	35018		
	Total	220			
Gastropoda	Seaweed	2	153513	1.07	0.37
	Month	8	196991	1.17	0.37
	Seaweed × Month	16	170934	3.67	<0.01
	Error	194	46623		
	Total	220			
Copepoda	Seaweed	2	8173.00	4.52	0.026
	Month	8	2091.70	0.96	0.50
	Seaweed × Month	16	2230.50	5.03	<0.01
	Error	193	443.60		
	Total	219			
Amphipoda	Seaweed	2	185.83	22.70	<0.01
	Month	8	13.96	1.65	0.18
	Seaweed × Month	16	8.50	1.14	0.32
	Error	192	7.44		
	Total	218			
Polychaeta	Seaweed	2	6.33	1.79	0.19
	Month	8	5.82	1.42	0.26
	Seaweed × Month	16	4.17	3.36	<0.01
	Error	194	1.24		
	Total	220			

Table 3.4 (continued)

Taxa	Source of variation	*df*	MS	*F*-value	*p*
Isopoda	Seaweed	2	0.28	1.01	0.38
	Month	8	0.68	2.66	0.043
	Seaweed × Month	16	0.25	0.71	0.79
	Error	194	0.36		
	Total	220			
Fish egg masses	Seaweed	2	0.29	3.51	0.052
	Month	8	0.11	1.18	0.37
	Seaweed × Month	16	0.098	4.16	<0.01
	Error	194	0.024		
	Total	220			
Gastropod egg masses	Seaweed	2	0.032	1.62	0.22
	Month	8	0.017	0.81	0.61
	Seaweed × Month	16	0.022	1.75	0.040
	Error	194	0.012		
	Total	220			

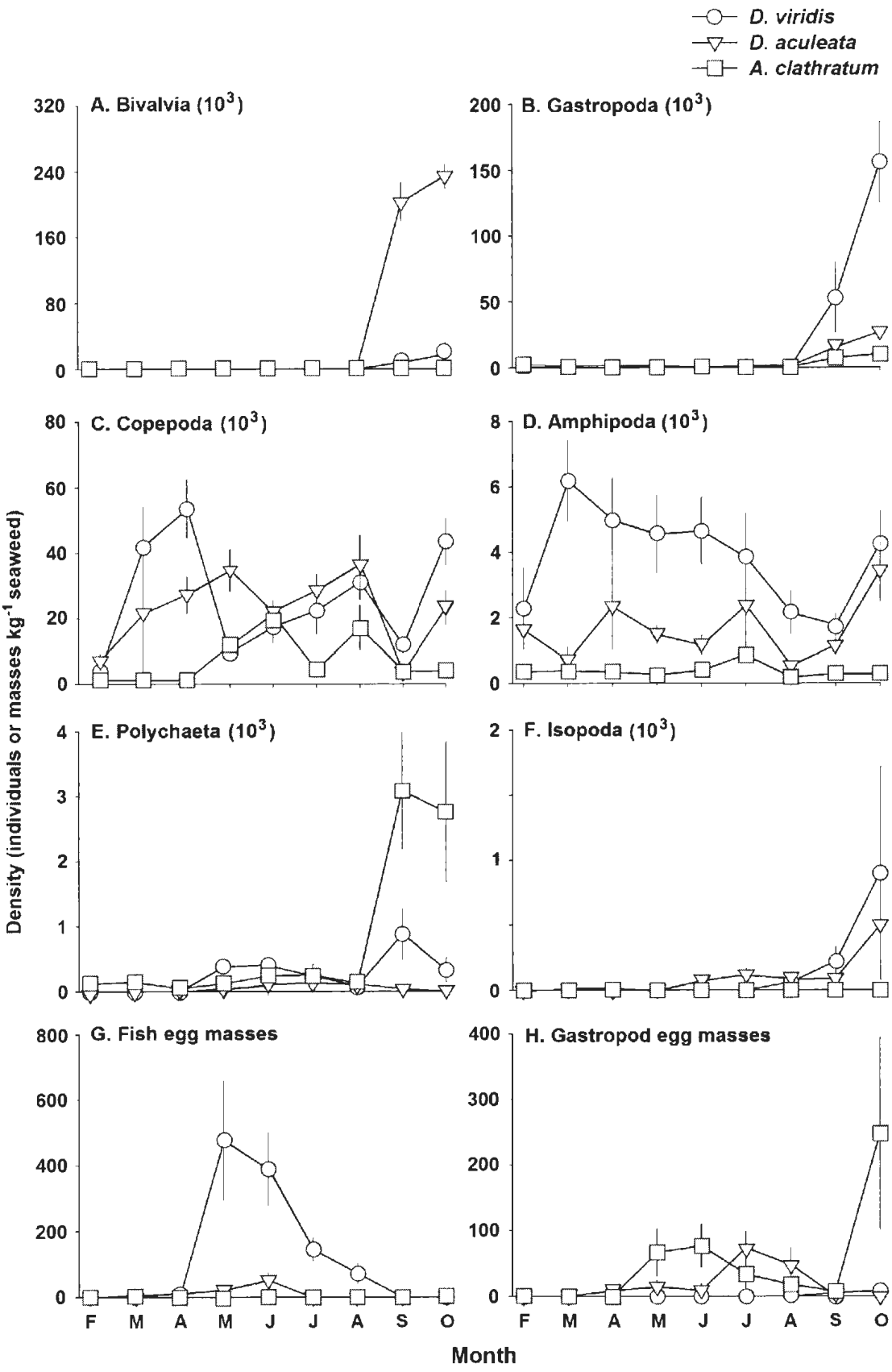

D. viridis
D. aculeata
A. clathratum
A. Bivalvia (10^3)
B. Gastropoda (10^3)
C. Copepoda (10^3)
D. Amphipoda (10^3)
E. Polychaeta (10^3)
F. Isopoda (10^3)
G. Fish egg masses
H. Gastropod egg masses
Density (individuals or masses kg^-1 seaweed)
F M A M J J A S O
Month

Figure 3.4. Mean (±SE) density (note the change in scale) of individuals in the six numerically dominant invertebrate taxa and gastropod (*Lacuna vincta*) and fish (unknown species) egg masses associated with *Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum* from 18 February to 9 October, 2011 at Keys Point (n=8 to 10 for each data point, except for *A. clathratum* in February where n=3). Bivalvia includes *Hiatella arctica, Modiolus modiolus,* and *Mytilus* sp.; Gastropoda includes *Dendronotus frondosus, Lacuna vincta,* and *Margarites helicinus*; Copepoda includes unidentified species in the Order Harpacticoida; Amphipoda includes *Ampithoe rubricata, Calliopius laeviusculus, Caprella linearis, Caprella septentrionalis, Gammarellus angulosus, Gammarus oceanicus, Gammarus setosus, Ischyrocerus anguipes, Leptocheirus pinguis, Pontogeneia inermis,* and *Stenothoe brevicornis*; Polychaeta includes *Alitta virens, Autolytinae* sp., *Bylgides sarsi, Lepidonotus squamatus, Nereis pelagica, Phyllodoce mucosa,* and *Spirorbis borealis*; Isopoda includes *Idotea baltica* and *Munna* sp.

Table 3.5. Summary of two-way ANOVAs (applied to raw data) examining the effect of Seaweed (*Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum*) and Month (each of nine sampling months: February to October, 2011) on the Shannon diversity index (*H'*) and Pielou's evenness index (*J'*) of seaweed epifauna at Keys Point (see Section 3.2.3 for a description of the sampling methods).

Source of variation	DF	MS	*F*-value	*p*
Diversity (*H'*)				
Seaweed	2	0.018	0.03	0.97
Month	8	3.55	5.45	0.002
Seaweed × Month	16	0.68	6.37	<0.01
Error	182	0.11		
Total	208			
Evenness (*J'*)				
Seaweed	2	0.12	1.35	0.28
Month	8	0.95	8.71	<0.01
Seaweed × Month	16	0.11	8.15	<0.01
Error	182	0.014		
Total	208			

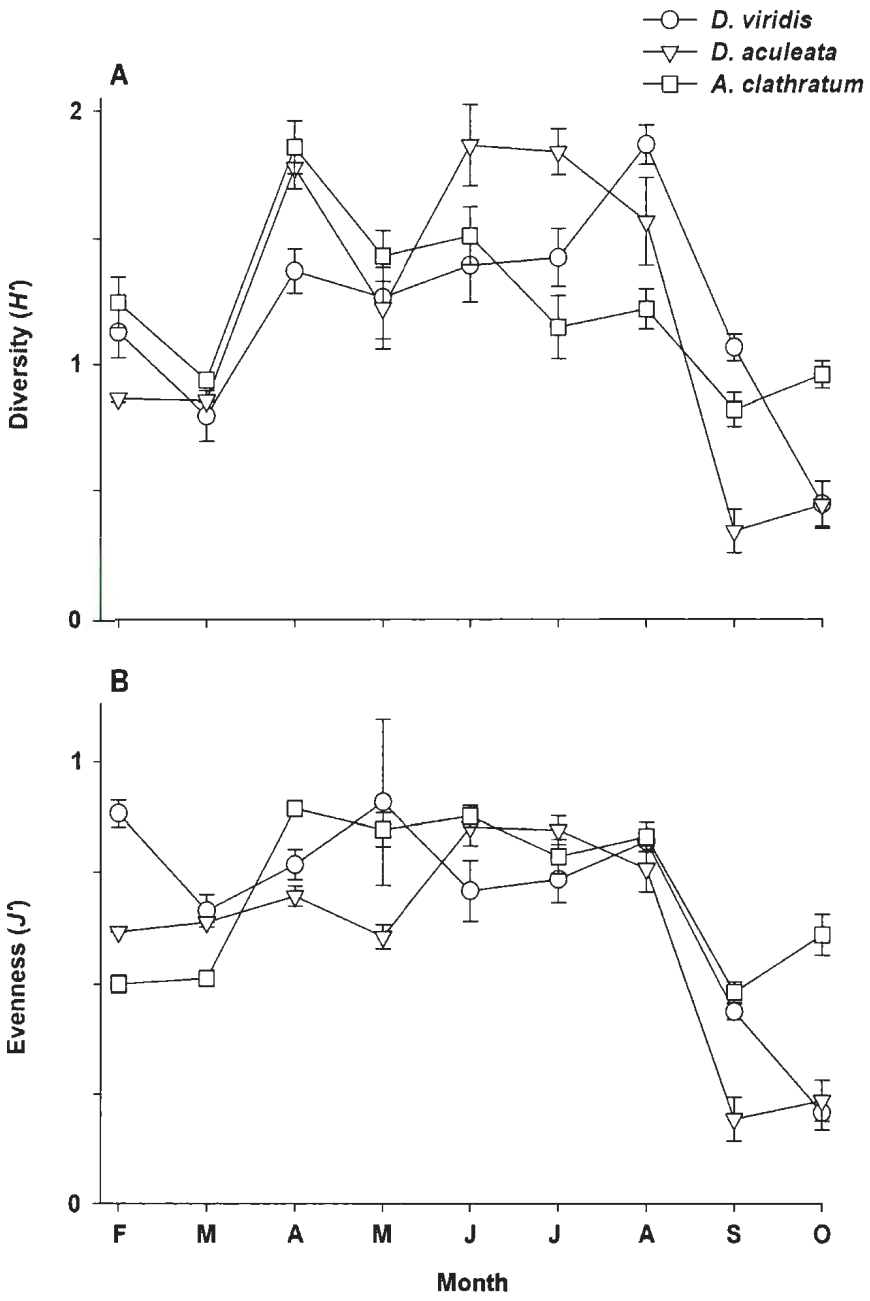


Figure 3.5. Mean (±SE) Shannon diversity index, *H'* (A) and Pielou's evenness index, *J'* (B) of epifauna on *Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum* from 18 February to 9 October, 2011 at Keys Point (n=8 to 10 for each data point, except for *A. clathratum* in February where n=3).

March to April in all seaweeds, four-fold decrease from August to October in the two Desmarestiales, and two-fold decrease from April to October in *A. clathratum* (Fig. 3.5). The Pielou's evenness index also differed significantly among seaweeds over time (Table 3.5), generally peaking between 0.7 and 0.9 from June to August in all seaweeds (Fig. 3.5). Like diversity, evenness in the two Desmarestiales decreased rapidly, by at least three times, in the last two months, while decreasing only slightly in *A. clathratum* (Fig. 3.5). Declines in diversity and evenness from August to October in *D. viridis* and *D. aculeata* were largely caused by major increases in recruits of gastropods (*L. vincta* and *Margarites helicinus*), in the former, and bivalves (*Mytilus* sp. and *Hiatella arctica*) in the latter.

3.3.3 Vulnerability to grazing

Analysis of data from Experiment 1 with *Desmarestia viridis*, *Desmarestia aculeata*, *Agarum clathratum*, and *Alaria esculenta* sporophytes exposed to 10 green sea urchins for 48 hours indicated that tissue loss to grazing among the first three species was similar and at least 36% less than in *A. esculenta* (one-way ANOVA [generalized linear model, Section 3.2.5]: Factor = Seaweed, $G_{3,53}$=9.81, p=0.020, Fig. 3.6). Analysis of data from Experiment 2 with agar-embedded extracts of the same four seaweeds exposed to the same grazing environment showed a slightly different outcome whereby only *D. viridis* and *A. clathratum* were the least grazed (one-way ANOVA [generalized linear model, Section 3.2.5]: Factor = Seaweed, $G_{4,97}$=11.15, p=0.025, Fig. 3.6). As in Experiment 1, grazing was highest on *A. esculenta*, followed by *D. aculeata* with a difference of 11.5% between the two (LS Means, p=0.021, n=19 or 20 for each seaweed

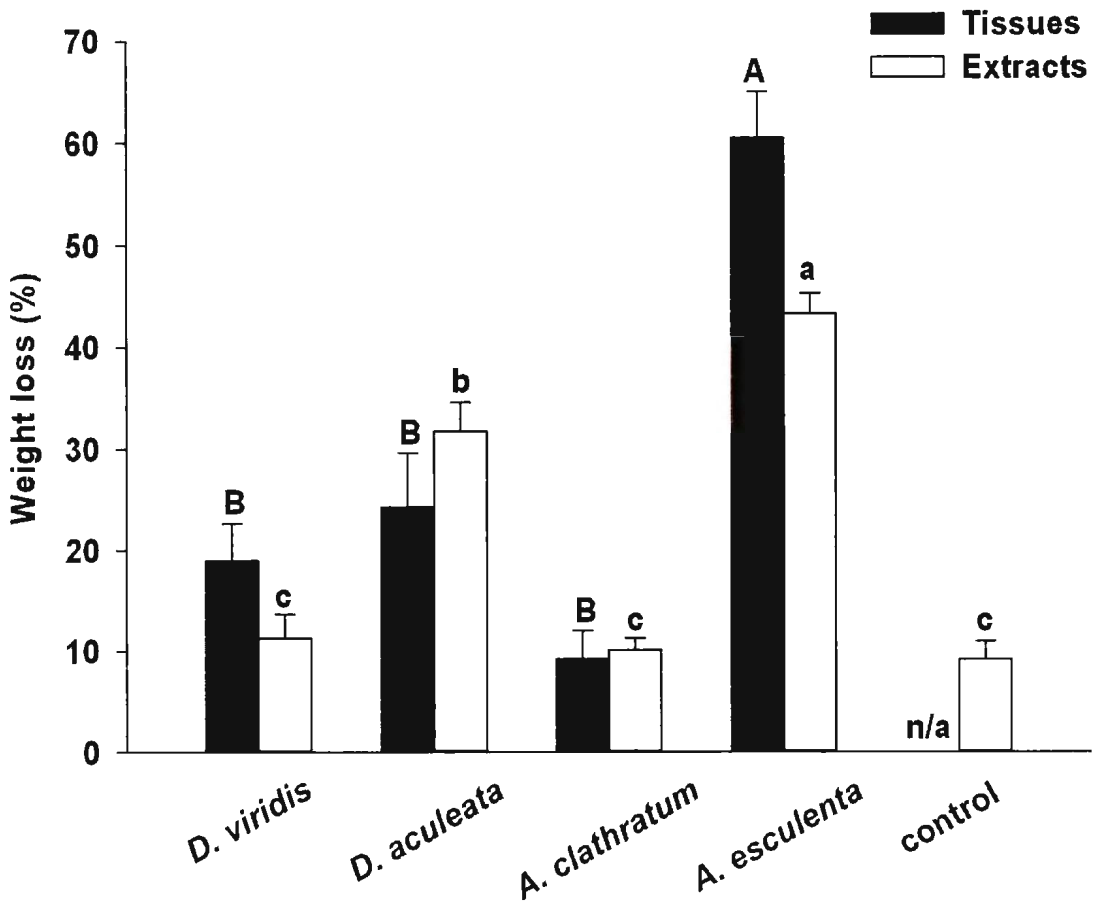


Fig. 3.6. Decrease in mean (+SE) wet weight as a percentage of initial wet weight of tissues (Experiment 1) and agar-embedded extracts (Experiment 2) of *Desmarestia viridis*, *Desmarestia aculeata*, *Agarum clathratum*, and *Alaria esculenta* sporophytes exposed 48 h to grazing by 10 green sea urchins, *Strongylocentrotus droebachiensis*. Bars not sharing the same letter are different (LS means tests, $p<0.05$; n=11 to 15 [Experiment 1] and 19 or 20 [Experiment 2] for each seaweed or control treatments) (see Section 3.2.4 for a description of the experiments and nature of the control treatment).

or control treatment). That grazing on agar-embedded extracts of *D. viridis* and *A. clathratum* was as low as in the control with no seaweed extracts (Fig. 3.6) suggests the chemical makeup of both seaweeds did not deter or attract urchins.

3.4 DISCUSSION

Several studies have helped elevate the ecological significance of unusual morphology and acid (H_2SO_4) production in the annual, brown seaweed *Desmarestia viridis* in the northwestern Atlantic (NWA): (1) the wave-induced sweeping motion of *D. viridis* sporophytes creates benthic habitat patches with higher recruitment in other seaweeds than in non-swept areas with higher urchin [*Strongylocentrotus droebachiensis*] density, i.e. a defense by association for seaweed recruits that may also represent a successional step (Gagnon et al. 2003a), (2) the chemical makeup of *D. viridis* alone is neither necessary nor sufficient to limit contacts by urchins - the wave-induced sweeping motion of sporophytes provides density-dependent, mechanical protection against urchin grazing (Gagnon et al. 2006), and (3) the acid is constitutively [regardless of grazing] and irreversibly accumulated [resulting in intracellular pH as low as 0.53] as sporophytes grow from recruits [March] to adults [June and July]. Such build-up inevitably culminates into dramatic mass releases of acid and die offs [August to October]; when mean sea temperature rises to above 10°C [the species is intolerant to temperatures above ~12°C, Gagnon et al. 2013, Chapter II]. These and the present studies provide the arguments needed to generate a comprehensive model of ecological interactions in *D. viridis* sporophytes (Fig. 3.7) and propose an important change to our

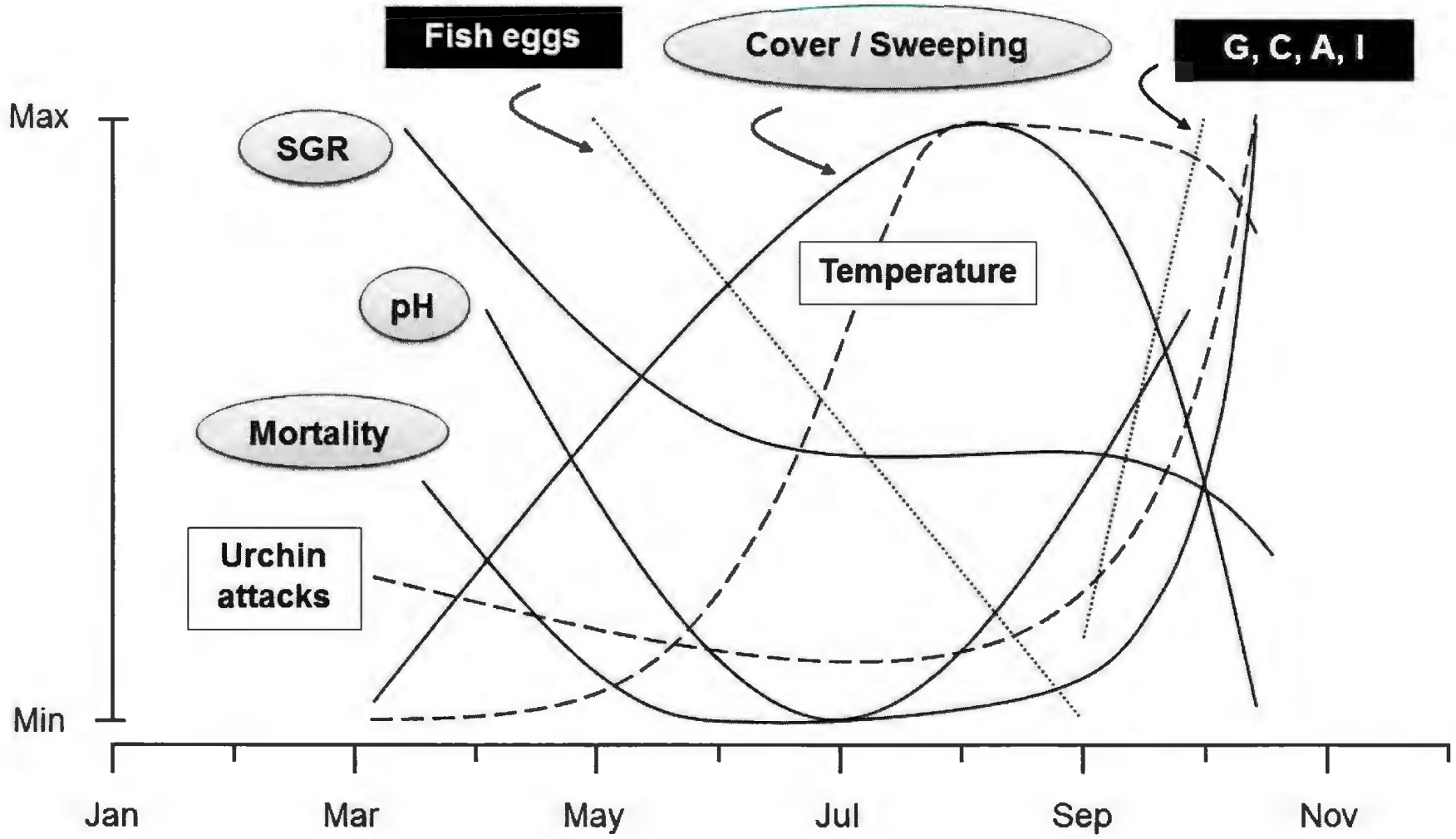

Fish eggs
Cover / Sweeping
G, C, A, I
Max
SGR
pH
Mortality
Urchin attacks
Temperature
Min
Jan
Mar
May
Jul
Sep
Nov

Figure 3.7. Empirical model of ecological interactions in sporophytes of the annual, acidic (H_2SO_4), brown seaweed *Desmarestia viridis* in urchin (*Strongylocentrotus droebachiensis*) barrens in the northwestern Atlantic (NWA) throughout an entire growth season (March to October) (main environmental controls shown as open rectangles and dashed lines; main *D. viridis* traits shown as gray ellipses and solid lines; dominant epifauna shown as solid rectangles and dotted lines). Small (~10 to 15 cm in length) *D. viridis* recruits exhibit highest (Max) arithmetic growth rates (AGR) in early March, when cover and the amplitude of the wave-induced sweeping motion are lowest (Min), and mortality and grazing by urchins are moderate. As recruits grow to adult-size (~50 to 60 cm) sporophytes in July, AGR and intracellular pH decrease (the latter being indicative of sulfuric acid production and accumulation) at a decelerating rate, whereas mortality decreases as a result of increasing cover and sweeping further reducing urchin grazing. The rapid increase in mean sea temperature in June and July to above 10°C marks the onset of mass release of acid to the environment (as shown by increasing pH) and senescence (as shown by sudden declines in cover and AGR) until all sporophytes disappear in October. The sudden increase in the frequency of urchin grazing on decaying sporophytes in August and September precipitates mortality and decline in cover. Throughout its existence as a highly branched, sweeping sporophyte, *D. viridis* provides a suitable surface for deposition of eggs by fish (May to September), as well as to distinct assemblages of mobile epifauna to recruit, cling, graze, and capture prey, as notably shown by dramatic increases of up to >150 000 individuals kg^{-1} *D. viridis* in herbivorous gastropods (G), and predatory copepods (C), amphipods (A), and isopods (I) in September (refer to the discussion for proposed cascades resulting from acid build-up and release in *D. viridis*).

perception of, and hence the way we should study, community dynamics associated with shifts between alternative stable states in shallow, rocky ecosystems in the NWA.

The need to integrate a third, transient community state: "*Desmarestia* bed"

We showed that *D. viridis* can form a transient (March to October) canopy, covering up to 25% of otherwise bare (or coralline seaweed encrusted) rocky substratum within the 2-to-8 m depth range. The initiation of the canopy in March and April occurred when significant wave height and sea temperature were nearing annual maxima and minima, respectively (see the Integrated Science Data Management [ISDM] database [www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/index-eng.html] for wave height and the Coastal Time Series [CTS] database [http://www.bio.gc.ca/science/data-donnees/base/data-donnees/cts-eng.php] for temperature), and urchin displacement and grazing were greatly limited - urchins largely clung to small topographic depressions at our two study sites, Keys Point (KP) and Bread and Cheese Cove (BCC), throughout much of the winter and early spring. These observations, together with findings that during the same period, mortality in *D. viridis* sporophytes is relatively high (up to 40% bi-weekly), arithmetic growth rate (~4 to 6% day^{-1}) peaks, and intracellular acidity increases rapidly (Gagnon et al. 2013, Chapter II), suggest that recruits largely depend on the severity of the physical environment to escape grazing while they invest resources in the production of tissues and sulfuric acid. Sporophytes attain a size refuge of ~40 cm

(stipe length) in early May, when significant wave height and sea temperature begin to decrease and increase, respectively (ISDM and CTS databases), and urchins become more active - urchins had largely left topographic depressions by the middle of June at KP and BCC and were mainly found on flat, bare rock throughout the rest of the summer and early fall, except after occasional stormy days when they had moved again to depressions.

Wave action at KP was significantly higher than at BCC during the positive (March to early July) and negative (mid-August to October) growth phases in *D. viridis*, with no difference during the transitional (early July to mid-August), null growth phase (Chapter II). These observations, together with our study of relationships between *D. viridis* cover and urchin density at 2, 3, 4, and 8 m depths, provide the first evidence from *in situ* data that wave action (water velocity and acceleration at the substratum), which normally decreases logarithmically with depth within the 0-to-10 m range (Denny and Wethey 2001), modulates interactions between *D. viridis* and urchins throughout the entire existence of *D. viridis* as a sporophyte. This was particularly well demonstrated by our findings that negative relationships between *D. viridis* cover and urchin density at 2 and 3 m were significant at BCC [explaining up to 61% of the variation] but not at KP, whereas the theoretical (regardless of wave action) vulnerability of *D. viridis* to urchin grazing (as suggested by density-to-cover ratios) at KP was higher and lower than at BCC at 2 and 3 m, respectively. Moreover, negative relationships between cover and density at 4 and 8 m were significant at KP [explaining up to 45% of the variation] but not BCC, whereas the vulnerability of *D. viridis* to grazing at these depths was higher at the latter site.

Consistent with the experimental demonstration that moderate, wave-induced sweeping motion of *D. viridis* sporophytes provides mechanical protection against urchin grazing up to a threshold value between 136 and 194 urchins m^{-2} (Gagnon et al. 2006), as well as the above confirmed between-site and postulated within-site differences in hydrodynamic conditions, we propose that: 1) high wave action at KP at 2 and 3 m largely prevented urchins from moving and grazing fast-sweeping *D. viridis* sporophytes [while possibly restricting recruitment and growth in the latter given the relatively low cover at 2 m throughout the study], 2) moderate wave action at 2 and 3 m at BCC, and 4 and 8 m at KP, provided *D. viridis* with efficient mechanical protection against urchin grazing at relatively low urchin densities, and 3) low wave action at 4 and 8 m at BCC did not induce sufficient sweeping in *D. viridis*, which was readily grazed by urchins, even at low densities. Alternatively, the lack of a significant relationship between urchin density and *D. viridis* at 2 m at KP could simply mean that the generally high urchin abundance (>150 individuals m^{-2} in >80% of the time from June to October) was sufficient to keep *D. viridis* largely in check. We witnessed, in 2012, the establishment of a thick, virtually continuous *D. viridis* canopy throughout much of the 3-to-8 m depth range (i.e. over several 1000s of m^2) at BCC (~80-90% cover) and KP (~70-80% cover), together with a substantial drop in urchin density to only a few 10s of individuals m^{-2} (C. Blain and P. Gagnon, personal observations), which is well below suggested threshold densities for destructive grazing of adjacent, shallower kelp beds (Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay and Scheibling 2007). This situation was not unique to our particular study sites. Large stands of *D. viridis* frequently develop in urchin barrens, most often

right below the lower edge of kelp beds, in the northern Gulf of St. Lawrence (Gagnon et al. 2004) and along the coasts of Newfoundland (Himmelman 1984, this study, P. Gagnon, personal observations at many other sites in the last six years), Labrador (Adey and Hayek 2011), and Nova Scotia (Moore et al. 1986, Chapman and Johnson 1990, Scheibling et al. 1999, P. Gagnon, unpublished data), as well as in the Aleutian (northern Pacific) and Svalbard (Arctic Ocean) archipelagos (Konar 2000, Molis et al. 2009).

A first important conclusion, therefore, is that *D. viridis* has the ability, at least in the NWA, to annually change large tracts of bare, rocky seabed into spatially and structurally complex habitats. It can form a third (in addition to kelp beds and urchin barrens), transient community state, hereafter termed "*Desmarestia* bed". Exceptionally high recruitment may, in some years, facilitate the transition of the system towards the *Desmarestia* bed state. A key difference among the three states is that the timing of alternations between barrens and *Desmarestia* beds is much easier to predict, because of the annual life cycle of *D. viridis*, than alternations between barrens and kelp beds, which may occur over monthly to decadal time scales depending on synergistic effects of abiotic and biotic factors on urchin and kelp populations (Scheibling 1986, Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay et al. 2009). Should *Desmarestia* beds indeed function as "giant sweepers" retarding or preventing the formation of urchin fronts at the lower edge of kelp beds, as inferred above and in studies in other regions (Himmelman 1984, Konar 2000, Gagnon et al. 2006, Molis et al. 2009), then their annual outbreak and die-off could represent a cyclical, natural disturbance disrupting urchin-kelp interactions, and ultimately allowing kelp beds to re-establish over the barrens. Longer-term studies of

relationships between the physical environment, *D. viridis* and urchin abundances, as well as the frequency and extent to which kelp beds re-establish over barrens previously colonized (or not) by *Desmarestia* beds are needed to determine the exact contribution of *D. viridis* to ecosystem resilience (Knowlton 2004, Hughes et al. 2005, Baskett and Salomon 2010, Watson and Estes 2011).

The need to elevate *D. viridis* to the rank of foundation species

We showed that the quick development of *Desmarestia* beds in urchin barrens creates biological structure for major recruitment pulses in characteristic invertebrate (including gastropods, copepods, amphipods, and isopods) and fish assemblages. Most recruitment pulses measured at KP (and observed at BCC), including increases in the herbivorous snail *Lacuna vincta* from virtual absence to >150 000 recruits kg^{-1} *D. viridis*, were restricted to only a few weeks in August and September, which is when: 1) mean sea temperature fluctuates around the lethal 12°C for *D. viridis*, 2) *D. viridis* releases sulfuric acid to the environment and undergoes senescence [which is accompanied by a change in tissue color from dark brown to light yellow], and 3) urchins start to massively graze *D. viridis* [Chapter II, this study]. These synchronous, annual events, which also characterize *Desmarestia* beds in the northern Gulf of St. Lawrence, Nova Scotia, and the rest of Newfoundland and Labrador (P Gagnon, personal observations), suggest complex, environmentally (largely temperature)-driven cascades whereby a self-defended, fast-growing seaweed suddenly turns into a highly vulnerable prey for numerically dominant benthic (e.g. urchin) and epifaunal grazers (e.g. *L. vincta*, see below). That *L. vincta* and

other invertebrates, mainly bivalves and polychaetes, deposited eggs or recruited almost exclusively on two longer-lived, perennial seaweeds in the barrens, the non-acidic sister species *Desmarestia aculeata* and kelp *Agarum clathratum*, further suggests ontogenetic partitioning in the use of habitat-forming species among invertebrates. Such partitioning may also exist in fish, as shown by large numbers of fish egg masses deposited almost exclusively on *D. viridis* from April to June, when cover, wave-induced sweeping motion, and intracellular acidity all increase rapidly (Gagnon et al. 2013).

By forming large aggregations relative to the size of the organisms that they facilitate, marine foundation species markedly increase environmental heterogeneity, often transforming a two-dimensional, featureless landscape into a complex, three-dimensional structure (Bruno and Bertness 2001). The notion that *D. viridis*, and perhaps *D. aculeata* and *A. clathratum*, facilitate recruitment of distinct groups of invertebrates and fish, and hence function as foundation species in urchin barrens in the NWA, is also well supported by our findings that epifaunal assemblages in the three seaweeds were: 1) composed almost exclusively [~75%] of recruits and juveniles, 2) more stable in *A. clathratum* that the two Desmarestiales from February to August, 3) more similar [diversity and evenness] from February to August between the two Desmarestiales than between any of the Desmarestiales and *A. clathratum*, and 4) markedly different in September and October than in the previous eight months in each seaweed. In a study of invertebrate assemblages associated with seaweed canopies in the northern Gulf of St. Lawrence, Bégin et al. (2004) found only trace abundance of *L. vincta* and the snail *Margarites helicinus* on fronds of *D. viridis*, as well as a higher invertebrate diversity on

A. clathratum than *D. viridis*. By sampling a broader range of invertebrate sizes (250+ μm versus 1+ mm) over a longer period (nine months versus two months) than Bégin et al. (2004), the present study draws opposite conclusions whereby *D. viridis* sporophytes can be heavily colonized by *L. vincta* and *M. helicinus* (and many other invertebrates common to both studies), while supporting a generally higher, albeit less even, epifaunal diversity than *A. clathratum*, at least in the last few months of existence of *D. viridis*. Therefore, our findings also underscore the importance of conducting studies over a range of spatial and temporal scales, while studying organisms in close connection with environmental variability, to gain a more accurate depiction of patterns and processes in marine subtidal communities.

The majority of characteristic invertebrate species on *D. viridis* and *D. aculeata* were highly mobile amphipods (e.g. *Ischyrocerus anguipes*, *Pontogeneia inermis*, *Calliopius laeviusculus*), whereas most on *A. clathratum* were encrusting or largely sedentary taxa (e.g. *Spirorbis borealis, Lichenopora* sp.). Conceivably, the highly branched and sweeping frond in the two Desmarestiales provides a suitable surface for small, mobile epifauna to cling or entwine, while reducing the risk of predation and dislodgement by waves (Carr 1994, Levin 1994, Schmidt and Scheibling 2007), whereas the relatively flat and stationary frond of *A. clathratum* provides a more stable surface to encased, self-protected, filter-feeding invertebrates as also suggested in other studies of epifaunal assemblages on seaweed canopies (Dean and Connell 1987a, c, Hacker and Steneck 1990, Taylor and Cole 1994, Chemello and Milazzo 2002). Such high host-plant specificity in many mobile and sessile invertebrates further corroborates our suggestion

that *D. viridis*, *D. aculeata*, and *A. clathratum* play an underappreciated role in the structure and function of shallow rocky subtidal ecosystems in the NWA.

We showed that urchin grazing on non-sweeping sporophytes is equally as low in the three seaweeds, while being significantly lower than in the preferred kelp *Alaria esculenta* (Himmelman 1984, Himmelman and Nédélec 1990), whereas grazing on agar-embedded extracts is lower in *D. viridis* and *A. clathratum* than in *D. aculeata*, which in turn is lower than in *A. esculenta*. These results suggest that the chemical makeup of *D. viridis* and *A. clathratum* deters urchin grazing, whereas *D. aculeata* may be even more dependent on the wave-induced sweeping motion of the frond to reduce contact with urchins, than *D. viridis* (see above). Pelletreau and Muller-Parker (2002) concluded, based on laboratory feeding preference experiments, that sulfuric acid in *Desmarestia munda* from Washington (USA) provides an important defense against grazing by green sea urchins. Sulfuric acid in *D. viridis* and phenolics in *A. clathratum* may also help retard urchin grazing, and hence be regarded as chemical defenses (Vadas 1977, Gagnon et al. 2003a, Gagnon et al. 2006, Molis et al. 2009, this study). Yet, the prospect that annual, mass releases of acid in *D. viridis* serves other biological functions for the seaweed itself (Gagnon et al. 2013), while constituting a chemical cue triggering biotic interactions, should not be overlooked. Indeed, we propose, in light of the overwhelming evidence that chemical cues regulate key aspects of the behavior of most marine organisms, including habitat selection, feeding choices, and competitive interactions (see review by Hay 2009), that planktonic larvae in herbivorous gastropods (mainly *L. vincta*) perceived the acid released to the environment in September as a chemical indicator of the existence of a readily available food item in the decaying

D. viridis sporophytes triggering dramatic recruitment pulses on the latter. Simultaneous increases in copepods, amphipods, and isopods on *D. viridis* could be due to gastropod recruits becoming an easily extractable food source attracting such highly mobile predators. Alternatively, settlement out of the water column may have occurred in a highly synchronized fashion (typical at this latitude) that happened to coincide with decay and acid release. Evidence is needed that the concomitance is due to response to acid release.

Summary and future research directions

The present study provides the first integrated model of ecological interactions in *Desmarestia viridis*, while demonstrating its ability to form extensive, relatively grazing-resistant canopies ("*Desmarestia* beds") representing a third (in addition to kelp beds and urchin barrens), transient community state in shallow rocky subtidal ecosystems in the NWA. The high foundational potential of *D. viridis*, *Desmarestia aculeata*, and *Agarum clathratum* in urchin barrens, which, as we showed, can host high abundances of recruits, juveniles, and adults in a minimum of 41 invertebrate and fish taxa at specific times of year, suggests that canopy-forming seaweeds in so-called urchin barrens, play an underappreciated role in the overall ecosystem dynamics. We conclude that the traditional view that shallow rocky subtidal ecosystems in the NWA alternate between two community states, kelp beds and urchin barrens (e.g. Scheibling et al. 1999, Gagnon et al. 2004, Lauzon-Guay and Scheibling 2010), is obsolete. At the same time we call for a comprehensive examination of the contribution of *D. viridis* and other canopy-forming seaweeds in urchin barrens to ecosystem resilience. In particular, the prospect that

Desmarestia beds disrupt cyclical alternations between kelp bed and urchin barrens states, as suggested for *A. clathratum* in the northern Gulf of St. Lawrence (Gagnon et al. 2004, Gagnon et al. 2005), should be investigated through manipulative experiments and multiyear mensurative studies. Such disruptive potential by canopy-forming seaweeds in urchin barrens could help explain the re-establishment of kelp beds over barren grounds in northern regions of the NWA, where mass mortality of urchins, as occurs in Nova Scotia, has never been reported (Gagnon et al. 2004, Scheibling and Hatcher 2007). By emphasizing the need to regard certain canopy-forming seaweeds as structuring forces interfering with top-down processes rather than simple prey for keystone grazers, our study also adds to recent calls for critical reassessments of the generality of phase-shifts and their mechanisms in iconic marine ecosystems, including kelp beds (Heck and Valentine 2007, Bruno et al. 2009, Foster and Schiel 2010, Connell et al. 2011). Given the low tolerance of *D. viridis* to variation in the thermal environment, further studies need to address direct and indirect effects that increased sea temperature predicted to occur from climate change will have on *D. viridis* populations and their interactions with dominant grazers and associated epifaunal assemblages.

CHAPTER IV

SUMMARY

4.1 Overall objective of the study

Erect fleshy seaweeds are a dominant component of shallow, marine, rocky benthic ecosystems in cold seas. Present-day models of shallow rocky community organization in the northwestern Atlantic (NWA; Nova Scotia and northwards) emphasize the ecological importance of kelps, mainly *Saccharina longicruris* and *Alaria esculenta*, in particular their vulnerability to grazing by the omnivorous green sea urchin, *Strongylocentrotus droebachiensis*, and other mortality or displacement agents such as the introduced bryozoan *Membranipora membranacea* and green seaweed *Codium fragile* ssp. *fragile*. These models may bias our perception of patterns and processes structuring these communities, for they largely overlook the possible contribution of other indigenous seaweeds to ecosystem resilience. The annual, brown seaweed *Desmarestia viridis* is one of only a few species of erect fleshy seaweeds commonly found in urchin barrens in the NWA. The exceptional ability of sporophytes (macroscopic, asexually reproducing individuals) in this highly-branched, morphologically delicate seaweed to produce and store sulfuric acid (H_2SO_4) in intracellular vacuoles as well as thrive among dense urchin populations, makes *D. viridis* a compelling model for studies of controls of acid production in seaweeds, while raising fundamental questions about its contribution to community stability.

The overall objective of this study was to determine causal relationships between abiotic and biotic factors and intracellular acidity in *D. viridis* sporophytes to gain a better understanding of the sensitivity of the species to environmental variability (Chapter II), while characterizing the persistence and functional importance of sporophytes in urchin barrens to test the overall postulate that *D. viridis* act as a foundation species facilitating

recruitment in, and supporting distinct assemblages of, invertebrates (Chapter III). Work involved laboratory experiments in aquaria and wave tanks at the Ocean Sciences Centre of Memorial University of Newfoundland, as well as surveys of individuals and populations (via SCUBA diving) throughout an entire growth season (February to October 2011) in *D. viridis* sporophytes at two subtidal sites, Bread and Cheese Cove (BCC) and Keys Point (KP), in Bay Bulls, on the southeastern tip of Newfoundland, Canada.

4.2 Controls of intracellular acidity

Chapter II presented four laboratory experiments to determine effects of temperature, light, wave action, grazing, and epibionts on intracellular acidity in *D. viridis* sporophytes, as well as measurements, from March to October 2011, of the length and survival of sporophytes, sea temperature, and wave height at BCC and KP to characterize growth and mortality patterns and their relationship with natural environmental variability. We showed that 1) light, grazing, and epibionts did not affect acidity, 2) temperature and waves had strong synergistic effects on acidity, and 3) growth and survival at both sites mirrored seasonal shifts in temperature and waves. Specifically, acid loss in the absence of waves 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 (KP) in September, when temperature was ~10°C, facilitated the dispersal of released acid, which delayed mortality by ~10 days compared to the other site (BCC). All sporophytes had disappeared at both sites in late October.

4.3 Persistence and functional importance of seaweeds in urchin barrens

Chapter III presented measurements, from February to October 2011, of *D. viridis* cover on the seabed and urchin density at multiple depths (2, 3, 4, and 8 m) at BCC and KP, as well as epifauna on *D. viridis* and two other conspicuous seaweeds in urchin barrens, the non-acidic Desmarestiales *Desmarestia aculeata* and grazing-resistant kelp *Agarum clathratum*, at KP, to assess the persistence of *D. viridis* and compare the functional importance of the three seaweeds. Two complementary laboratory experiments in which tissues and tissue extracts of the three seaweeds and the highly consumed, bed-forming kelp *Alaria esculenta* were exposed to grazing by urchins, were also used to gain insights into factors contributing to the observed variability in the distribution of *D. viridis* and epifaunal assemblages among seaweeds. We showed that 1) *D. viridis* can form a transient canopy covering up to 25% of otherwise bare [or coralline seaweed encrusted] rocky substratum within the 2-to-8 m depth range, 2) wave action [water velocity and acceleration at the substratum] modulates interactions between *D. viridis* and urchins throughout the existence of *D. viridis* as a sporophyte, 3) *D. viridis* has the ability,

at least in the NWA, to annually change large tracts of bare, rocky seabed into spatially and structurally complex habitats forming a third [in addition to kelp beds and urchin barrens], transient community state termed "*Desmarestia* bed", and 4) the quick development of *Desmarestia* beds in urchin barrens creates biological structure for major recruitment pulses in characteristic invertebrate and fish assemblages. Results also suggested that 5) the chemical makeup of *D. viridis* and *A. clathratum* deters urchin grazing, whereas *D. aculeata* may be even more dependent on the wave-induced sweeping motion of the frond to reduce contact with urchins, than *D. viridis*.

Findings in this and other studies were assimilated into a comprehensive model of ecological interactions in *D. viridis* sporophytes in urchin barrens in the NWA. Essentially, small (~10 to 15 cm in length) sporophytes in March largely depend on the severity of the physical environment, which adversely affects urchin foraging, to escape grazing while they invest resources in the production of tissues and sulfuric acid. Sporophytes attain a size refuge of ~40 cm (stipe length) in early May, which is when significant wave height and sea temperature begin to decrease and increase, respectively, and urchins become more active. As sporophytes grow to adult-size (~50 to 60 cm) in July, mortality decreases as a result of increasing cover and wave-induced sweeping further reducing urchin grazing. The rapid increase in mean sea temperature in June and July to above 10°C marks the onset of mass release of acid to the environment and senescence until all sporophytes disappear in October. The sudden increase in the frequency of urchin grazing on decaying sporophytes in August and September precipitates mortality and decline in cover. Throughout its existence as a sporophyte,

D. viridis provides a suitable surface for deposition of eggs by fish (May to September), as well as to distinct assemblages of mobile epifauna to recruit, cling, graze, and capture prey, as notably shown by dramatic increases, in September, of up to >150 000 individuals kg^{-1} *D. viridis* in herbivorous gastropods, and predatory copepods, amphipods, and isopods.

4.4 Importance of the study

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 support the notion that thermal and wave environments play a key role 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. The high foundational potential of *D. viridis*, *D. aculeata*, and *A. clathratum* in urchin barrens, which, as we found, can host high densities of recruits, juveniles, and adults in a minimum of 41 invertebrate and fish taxa at specific times of year, suggest that, in so-called urchin barrens, canopy-forming seaweeds play an underappreciated role in the overall ecosystem dynamics. In addition to providing the first model of ecological interactions for *D. viridis* sporophytes in urchin barrens in the NWA, we propose an important change to the study of community dynamics associated with shifts between alternative stable states in shallow, rocky ecosystems in the NWA. Specifically, we put forward the need to regard certain canopy-forming

seaweeds, including *D. viridis* and *A. clathratum*, as structuring forces interfering with top-down processes rather than simple prey for keystone grazers. Overall, this study enlarges the ongoing debate about the need to critically reassess the generality of phase-shifts and their mechanisms in iconic marine ecosystems such as kelp beds, coral reefs, and seagrass meadows.

4.5 Future directions

This study provides a novel framework to the study of shallow, rocky subtidal ecosystems in the NWA by proposing alternative, seaweed-driven, community phase shifts. Further studies with *D. viridis* and other members of the intriguing taxonomic group Desmarestiales are required to determine the tradeoffs that exist among 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. We propose that the traditional view that shallow rocky subtidal ecosystems in the NWA alternate between two community states, kelp beds and urchin barrens, is obsolete. At the same time, we call for a comprehensive examination of the exact contribution of *D. viridis* and other canopy-forming seaweeds in urchin barrens to ecosystem resilience. In particular, the prospect that *Desmarestia* beds disrupt cyclical alternations between kelp bed and urchin barrens states, as suggested for *A. clathratum* in the northern Gulf of St. Lawrence, should be investigated through

manipulative experiments combined with multiyear mensurative studies. Such disruptive potential by canopy-forming seaweeds in urchin barrens could help explain the re-establishment of kelp beds over barren grounds in northern regions of the NWA, where mass mortality of urchins, as occurs in Nova Scotia, has never been reported. Given the low tolerance of *D. viridis* to variation in the thermal environment, further studies should also attempt to address direct and indirect effects that increased sea temperature predicted to occur from climate change will have on *D. viridis* populations and their interactions with dominant grazers and associated epifaunal assemblages.

LITERATURE CITED

Ackerman, J. D. and A. Okubo. 1993. Reduced mixing in a marine macrophyte canopy. Funct Ecol **7**:305-309.

Adey, W. H. and L.-A. C. Hayek. 2011. Elucidating marine biogeography with macrophytes: quantitative analysis of the North Atlantic supports the thermographic model and demonstrates a distinct subarctic region in the northwestern Atlantic. Northeast Nat **18**:1-128.

Agrawal, A. A. 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology **81**:1804-1813.

Agrawal, A. A. 2005. Future directions in the study of induced plant responses to herbivory. Entomol Exp Appl **115**:97-105.

Amsler, C. D., M. O. Amsler, J. B. McClintock, and B. J. Baker. 2009a. Filamentous algal endophytes in macrophytic Antarctic algae: prevalence in hosts and palatability to mesoherbivores. Phycologia **48**:324-334.

Amsler, C. D. and V. A. Fairhead. 2006. Defensive and sensory chemical ecology of brown algae. Adv Bot Res **43**:1-91.

Amsler, C. D., K. Iken, J. B. McClintock, M. O. Amsler, K. J. Peters, J. M. Hubbard, F. B. Furrow, and B. J. Baker. 2005. Comprehensive evaluation of the palatability and chemical defenses of subtidal macroalgae from the Antarctic Peninsula. Mar Ecol Prog Ser **294**:141-159.

Amsler, C. D., K. Iken, J. B. McClintock, and B. J. Baker. 2009b. Defenses of polar macroalgae against herbivores and biofoulers. Bot Mar **52**:535-545.

Amsler, C. D., J. B. McClintock, and B. J. Baker. 2008. Macroalgal chemical defenses in polar marine communities. Pages 91-103 *in* C. D. Amsler, editor. Algal chemical ecology. Springer-Verlag, Berlin/Heidelberg.

Amsler, C. D., R. J. Rowley, D. R. Laur, L. B. Quetin, and R. M. Ross. 1995. Vertical distribution of Antarctic peninsular macroalgae: cover, biomass and species composition. Phycologia **34**:424-430.

Anderson, R. J. and B. Velimirov. 1982. An experimental investigation of the palatability of kelp bed algae to the sea urchin *Parechinus angulosus* Leske. Mar Ecol **3**:357-373.

Ankisetty, S., S. Nandiraju, H. Win, Y. C. Park, C. D. Amsler, J. B. McClintock, J. A. Baker, T. K. Diyabalanage, A. Pasaribu, M. P. Singh, W. M. Maiese, R. D. Walsh, M. J. Zaworotko, and B. J. Baker. 2004. Chemical investigation of predator-deterred macroalgae from the Antarctic Peninsula. J Nat Prod **67**:1295-1302.

Aumack, C. F., C. D. Amsler, J. B. McClintock, and B. J. Baker. 2011. Impacts of mesograzers on epiphyte and endophyte growth associated with chemically defended macroalgae from the western Antarctic Peninsula: a mesocosm experiment. J Phycol **47**:36-41.

Barko, J. W., D. G. Hardin, and M. S. Matthews. 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. Can J Bot **60**:877-887.

Baskett, M. L. and A. K. Salomon. 2010. Recruitment facilitation can drive alternative states on temperate reefs. Ecology **91**:1763-1773.

Bax, N., A. Williamson, M. Aguero, E. Gonzalez, and W. Geeves. 2003. Marine invasive alien species: a threat to global biodiversity. Mar Policy **27**:313-323.

Bégin, C., L. E. Johnson, and J. H. Himmelman. 2004. Macroalgal canopies: distribution and diversity of associated invertebrates and effects on the recruitment and growth of mussels. Mar Ecol Prog Ser **271**:121-132.

Blanchette, C. A. 1997. Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. Ecology **78**:1563-1578.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. Withe. 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol **24**:127-135.

Bolser, R. C. and M. E. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology **77**:2269-2286.

Brey, T. and A. Clarke. 1993. Population dynamics of marine benthic invertebrates in Antarctic and subantarctic environments: are there unique adaptations. Antarct Sci **5**:253-266.

Brown, T. E. and R. L. Richardson. 1968. The effect of growth environment on the physiology of algae: light intensity. J Phycol **4**:38-54.

Bruno, J. F. and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. Pages 119-125 *in* M. D. Bertness, editor. Marine community ecology. Sinauer Associates Inc., Sunderland.

Bruno, J. F., H. Sweatman, W. F. Precht, E. R. Selig, and V. G. W. Schutte. 2009. Assessing evidence of phase shifts from coral to macrroalgal dominance on coral reefs. Ecology **90**:1478-1148.

Carpenter, R. C. 1986. Partitioning herbivory and its effects on coral reef algal communities. Ecol Monogr **56**:345-363.

Carr, M. H. 1994. Effects of macroalgal dynamics on recruitment of a temperate reef fish. Ecology **75**:1320-1333.

Ceh, J., M. Molis, T. M. Dzeha, and M. Wahl. 2005. Induction and reduction of anti-herbivore defenses in brown and red macroalgae off the Kenyan coast. J Phycol **41**:726-731.

Chapman, A. R. O. 1972a. Morphological variation and its taxonomic implications in the ligulate members of the genus *Desmarestia* occuring on the west coast of North America. Syesis **5**:1-20.

Chapman, A. R. O. 1972b. Species delimitation in the filiform, oppositely branched members of the genus *Desmarestia* Lamour. (Phaeophyceae, Desmarestiales) in the northern hemisphere. Phycologia **11**:225-231.

Chapman, A. R. O. and E. M. Burrows. 1970. Experimental investigations into the controlling effects of light conditions on the development and growth of *Desmarestia aculeata* (L.) Lamour. Phycologia **9**:103-108.

Chapman, A. R. O. and E. M. Burrows. 1971. Field and culture studies of *Desmarestia aculeata* (L.) Lamour. Phycologia **10**:63-76.

Chapman, A. R. O. and J. S. Craigie. 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar Biol **40**:197-205.

Chapman, A. R. O. and C. R. Johnson. 1990. Disturbance and organization of macroalgal assemblages in the Northwest Atlantic. Hydrobiologia **192**:77-121.

Chemello, R. and M. Milazzo. 2002. Effect of algal architecture on associated fauna: some evidence from phytal molluscs. Mar Biol **140**:981-990.

Clark, R. P., M. S. Edwards, and M. S. Foster. 2004. Effects of shade from multiple kelp canopies on an understory algal assemblage. Mar Ecol Prog Ser **267**:107-119.

Clarke, K. R. and R. N. Gorley. 2006. PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth.

Coleman, F. C. and S. L. Williams. 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. Trends Ecol Evol **17**:40-44.

Connell, S. D., B. D. Russell, and A. D. Irving. 2011. Can strong consumer and producer effects be reconciled to better forecast 'catastrophic' phase-shifts in marine ecosystems? J Exp Mar Biol Ecol **400**:296-301.

Conover, W. J. 1980. Practical nonparametric statistics. John Wiley & Sons, New York.

Cronin, G. and M. E. Hay. 1996. Effects of light and nutrient availability on the growth, secondary chemistry, and resistance to herbivory of two brown seaweeds. Oikos **77**:93-106.

Crowder, M. J. and D. J. Hand. 1990. Analysis of repeated measures. Chapman & Hall, London.

Cyr, H. and M. Pace. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature **361**:148-150.

Dawson, R., W. Schramm, and M. Bolter. 1985. Factors influencing the production, decomposition and distribution of organic matter in Admiralty Bay, King George Island. Pages 109-114 *in* W. R. Siegfried, P. R. Condy, and R. M. Laws, editors. Antarctic nutrient cycles and food webs. Springer, Berlin.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecol Monogr **41**:351-389.

Dayton, P. K. 1985a. Ecology of kelp communities. Annu Rev Ecol Syst **16**:215-245.

Dayton, P. K. 1985b. The structure and regulation of some South American kelp communities. Ecol Monogr **55**:447-468.

Dean, R. L. and J. H. Connell. 1987a. Marine invertebrates in an algal succession III. Mechanisms linking habitat complexity with diversity. J Exp Mar Biol Ecol **109**:249-274.

Dean, R. L. and J. H. Connell. 1987b. Marine invertebrates in an algal succession. I. Variations in abundance and diversity with succession. J Exp Mar Biol Ecol **109**:195-215.

Dean, R. L. and J. H. Connell. 1987c. Marine invertebrates in an algal succession. II. Tests of hypotheses to explain changes in diversity with succession. J Exp Mar Biol Ecol **109**:217-247.

DeLaca, T. E. and J. H. Lipps. 1976. Shallow-water marine associations, Antarctic Peninsula. Antarct J **11**:12-20.

Denny, M. and D. Wethey. 2001. Physical processes that generate patterns in marine communities. Pages 3-37 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine community ecology. Sinauer Associates, Sunderland.

Dieckmann, G., W. Reichardt, and D. Zielinski. 1985. Growth and production of the seaweed, *Himantothallus grandifolius*, at King George Island. Pages 104-108 *in*

W. R. Siegfried, P. Condy, and R. M. Laws, editors. Antarctic nutrient cycles and food webs. Springer, Berlin.

Dorenbosch, M. and E. S. Bakker. 2011. Herbivory in omnivorous fishes: effect of plant secondary metabolites and prey stoichiometry. Freshwater Biol **56**:1783-1797.

Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. Oikos **99**:201-219.

Duffy, J. E. 2006. Biodiversity and the functioning of seagrass ecosystems. Mar Ecol Prog Ser **311**:233-250.

Duffy, J. E. and M. E. Hay. 1990. Seaweed adaptations to herbivory. Bioscience **40**:368-375.

Duffy, J. E. and M. E. Hay. 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. Ecology **72**:1286-1298.

Duffy, J. E. and M. E. Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. Ecol Monogr **70**:237-263.

Duggins, D. O., J. E. Eckman, and A. T. Sewell. 1990. Ecology of understory kelp environments II. Effects of kelps on recruitment of benthic invertebrates. J Exp Mar Biol Ecol **143**:27-46.

Duggins, D. O., C. A. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science **245**:170.

Dunton, K. 2001. $d^{15}N$ and $d^{13}C$ measurements of Antarctic Peninsula fauna: trophic relationships and assimilation of benthic seaweeds. Am Zool **41**:99-112.

Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of understory kelp environments I. Effects of kelps on flow and particle transport near the bottom. J Exp Mar Biol Ecol **129**:173-188.

Fairhead, V. A., C. D. Amsler, J. B. McClintock, and B. J. Baker. 2005. Variation in phlorotannin content within two species of brown macroalgae (*Desmarestia anceps* and *D. menziesii*) from the Western Antarctic Peninsula. Polar Biol **28**:680-686.

Fischer, G. and C. Wiencke. 1992. Stable carbon isotope composition, depth distribution and fate of macroalgae from the Antarctic Peninsula region. Polar Biol **12**:341-348.

Fletcher, W. J. 1987. Interactions among subtidal Australian sea urchins, gastopods, and algae: effects of experimental removals. Ecol Monogr **57**:89-109.

Fortes, M. D. and K. Lüning. 1980. Growth rate of North Sea macroalgae in relation to temperature, irradiance and photoperiod. Helgol Meeresunters **34**:15-29.

Foster, M. S. and D. R. Schiel. 2010. Loss of predators and the collapse of southern California kelp forests (?): alternatives, eplainations and generalizations. J Exp Mar Biol Ecol **393**:59-70.

Gagné, J. A., K. H. Mann, and A. R. O. Chapman. 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., C. Blain, and J. Vad. 2013. Living within constraints: irreversible chemical buildup and seasonal temperature-mediated die-off in a highly acidic (H_2SO_4) annual seaweed (*Desmarestia viridis*). Mar Biol **160**:439-451.

Gagnon, P., J. H. Himmelman, and L. E. Johnson. 2003a. Algal colonization in urchin barrens: defense by association during recruitment of the brown alga *Agarum cribrosum*. J Exp Mar Biol Ecol **290**:179-196.

Gagnon, P., J. H. Himmelman, and L. E. Johnson. 2004. Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. Mar Biol **144**:1191-1203.

Gagnon, P., L. E. Johnson, and J. H. Himmelman. 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**:498-505.

Gagnon, P., L. V. St-Hilaire-Gravel, J. H. Himmelman, and L. E. Johnson. 2006. Organismal defenses versus environmentally mediated protection from herbivores: unraveling the puzzling case of *Desmarestia viridis* (Phaeophyta). J Exp Mar Biol Ecol **334**:10-19.

Gagnon, P., G. Wagner, and J. H. Himmelman. 2003b. 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.

Gilinsky, E. 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology **65**:455-468.

Hacker, S. D. and R. S. Steneck. 1990. Habitat architecture and the abundance and body-size-dependent habitat selection of a phytal amphipod. Ecology **71**:2269-2285.

Hagen, N. T. 1995. Recurrent destructive grazing of successionally immature kelp forests by green sea urchins in Vestfjorden, Northern Norway. Mar Ecol Prog Ser **123**:95-106.

Hand, D. J. and C. C. Taylor. 1987. Multivariate analysis of variance and repeated measures. Chapman & Hall, London.

Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 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**: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. and W. Fenical. 1988. Marine plant-herbivore interactions: the ecology of chemical defense. Annu Rev Ecol Syst **19**:111-145.

Hay, M. E. and P. D. Steinberg. 1992. The chemical ecology of plant-herbivore interactions in marine vs. terrestrial communities. Pages 371-413 *in* G. Rosenthal and M. Berenbaum, editors. Herbivores: their interaction with secondary plant metabolites. Evolutionary and ecological processes. Academic Press, San Diego, California, USA.

Heck, K. L. and J. F. Valentine. 2007. The primacy of top-down effects in shallow benthic ecosystems. Estuar and Coast **30**:371-381.

Himmelman, J. H. 1984. Sea urchin feeding and macroalgal distribution in Newfoundland and eastern Canada. Nat Can **111**:337-348.

Himmelman, J. H. and H. Nédélec. 1990. Urchin foraging and algal survival strategies in intensely grazed communities in eastern Canada. Can J Fish Aquat Sci **47**:1011-1026.

Himmelman, J. H. and D. H. Steele. 1971. Foods and predators of the green sea urchin *Strongylocentrotus droebachiensis* in Newfoundland waters. Mar Biol **9**:315-322.

Howe, G. A. and G. Jander. 2008. Plant immunity to insect herbivores. Annu Rev Plant Biol **59**:41-66.

Huang, Y. M., M. O. Amsler, J. B. McClintock, C. D. Amsler, and B. J. Baker. 2007. Patterns of gammaridean amphipod abundance and species composition associated with dominant subtidal macroalgae from the western Antarctic Peninsula. Polar Biol **30**:1417-1430.

Huang, Y. M., J. B. McClintock, C. D. Amsler, K. J. Peters, and B. J. Baker. 2006. Feeding rates of common Antarctic gammarid amphipods on ecologically important sympatric macroalgae. J Exp Mar Biol Ecol **329**:55-65.

Hughes, T. P., D. R. Bellwood, C. Folke, R. S. Steneck, and J. Wilson. 2005. New paradigms for supporting the resilience of marine ecosystems. Trends Ecol Evol **20**:380-386.

Iken, K. 1999. Feeding ecology of the Antarctic herbivorous gastropod *Laevilacunaria antarctica* Martens. J Exp Mar Biol Ecol **236**:133-148.

Iken, K., E. R. Barrera-Oro, M. L. Quartino, R. J. Casaux, and T. Brey. 1997. Grazing by the Antarctic fish *Notothenia coriiceps*: evidence for selective feeding on macroalgae. Antarct Sci **9**:386-391.

Irlandi, E. A. and C. H. Peterson. 1991. Modification of animal habitat by large plants: mechanisms by which seagrasses influence clam growth. Oecologia **87**:307-318.

Jormalainen, V. and T. Honkanen. 2008. Macroalgal chemical defenses and their roles in structuring temperate marine communities. Pages 57-89 *in* C. D. Amsler, editor. Algal Chemical Ecology. Springer-Verlag, Berlin.

Karban, R., A. A. Agrawal, J. S. Thaler, and L. S. Adler. 1999. Induced plant responses and information content about risk of herbivory. Trends Ecol Evol **14**:443-447.

Karban, R. and I. T. Baldwin. 1997. Induced responses to herbivory. The University of Chicago Press, Chicago.

Karban, R. and J. H. Myers. 1989. Induced plant responses to herbivory. Annu Rev Ecol Syst **20**:331-348.

Keats, D. W., G. R. South, and D. H. Steele. 1990. Effects of an experimental reduction in grazing by green sea urchins on a benthic macroalgal community in eastern Newfoundland. Mar Ecol Prog Ser **68**:181-193.

Kelly, J. R., R. E. Scheibling, and T. Balch. 2011. Invasion-mediated shifts in the macrobenthic assemblage of a rocky subtidal ecosystem. Mar Ecol Prog Ser **437**:69-78.

Kennelly, S. J. 1989. Effects of kelp canopies on understory species due to shade and scour Mar Ecol Prog Ser **50**:215-224.

Knowlton, N. 2004. Multiple 'stable' states and the conservation of marine ecosystems. Prog Oceanogr **60**:387-396.

Konar, B. 2000. Seasonal inhibitory effects of marine plants on sea urchins: structuring communities the algal way. Oecologia **125**:208-217.

Konar, B. and J. A. Estes. 2003. The stability of boundary regions between kelp beds and deforested areas. Ecology **84**:174-185.

Kübler, J. E. and I. R. Davison. 1993. High-temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. Mar Biol **117**:327-335.

Lauzon-Guay, J.-S. and R. E. Scheibling. 2007. Behaviour of sea urchin *Strongylocentrotus droebachiensis* grazing fronts: food-mediated aggregation and density-dependent facilitation. Mar Ecol Prog Ser **329**:191-204.

Lauzon-Guay, J.-S. and R. E. Scheibling. 2010. Spatial dynamics, ecological thresholds and phase shifts: modelling grazer aggregation and gap formation in kelp beds. Mar Ecol Prog Ser **403**:29-41.

Lauzon-Guay, J.-S., R. E. Scheibling, and M. A. Barbeau. 2009. Modelling phase shifts in a rocky subtidal ecosystem. Mar Ecol Prog Ser **375**:25-39.

Lawrence, J. M. 1975. On the relationships between marine plants and sea urchins. Oceanogr Mar Biol Annu Rev **13**:213-286.

Lee, K. S., S. R. Park, and Y. K. Kim. 2007. Effects of irrodiance, temperature, and nutrients on growth dynamics of seagrasses: a review. J Exp Mar Biol Ecol **350**:144-175.

Levin, P. S. 1994. Small-scale recruitment variation in a temperate fish: the roles of macrophytes and food supply. Environ Biol Fish **40**:271-281.

Lobban, C. S. and P. J. Harrison. 1994. Seaweed ecology and physiology. Cambridge University Press, Cambridge.

Long, J. D. and G. C. Trussell. 2007. Geographic variation in seaweed induced responses to herbivory. Mar Ecol Prog Ser **333**:75-80.

Lubchenco, J. and J. Cubit. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. Ecology **61**:676-687.

Lubchenco, J. and S. D. Gaines. 1981. A unified approach to marine plant-herbivore interactions. I. Populations and communities. Annu Rev Ecol Syst **12**:405-437.

Lüning, K. 1990. Seaweeds: their environment, biogeography and ecophysiology. Wiley, New York.

Lüning, K. and M. J. Dring. 1985. Action spectra and spectral quantum yield of photosynthesis in marine macroalgae with thin and thick thalli. Mar Biol **87**:119-130.

Lyons, D. A., K. L. Van Alstyne, and R. E. Scheibling. 2007. Anti-grazing activity and seasonal variation of dimethylsulfoniopropionate-associated compounds in the invasive alga *Codium fragile* ssp. *tomentosoides*. Mar Biol **153**:179-188.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science **182**:975-981.

Mann, K. H. 1977. Destruction of kelp-beds by sea-urchins: a cyclical phenomenon or irreversible degradation. Helgol Wiss Meeresunters **30**:455-467.

McClintock, M., N. Higinbotham, E. G. Uribe, and R. E. Cleland. 1982. Active, irreversible accumulation of extreme levels of H_2SO_4 in the brown alga, *Desmarestia*. Plant Physiol **70**:771-774.

McCullagh, P. and J. A. Nelder, editors. 1989. Generalized linear models. Chapman & Hall, New York.

Menge, B. A. 1976. Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. Ecol Monogr **46**:355-393.

Menge, B. A. 1978. Predation intensity in a rocky intertidal community: effect of an algal canopy, wave action and desiccation on predator feeding rates. Oecologia **34**:17-36.

Menge, B. A., J. Lubchenco, and L. R. Ashkenas. 1985. Diversity, heterogeneity and consumer pressure in a tropical rocky intertidal community. Oecologia **65**:394-405.

Milchunas, D. G. and I. Noy-Meir. 2002. Grazing refuges, external avoidance of herbivory and plant diversity. Oikos **99**:113-130.

Moe, R. L. and P. C. Silva. 1977. The Antarctic marine flora: uniquely devoid of kelps. Science **196**:1206-1208.

Molis, M., H. Wessels, W. Hagen, U. Karsten, and C. Wiencke. 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**:71-82.

Moore, D. S., R. J. Miller, and L. D. Meade. 1986. Survey of shallow benthic habitat: eastern Shore and Cape Breton, Nova Scotia. Report No. 1546. Canadian Technical Report of Fisheries and Aquatic Sciences.

Orth, R. J., K. L. Heck, Jr. , and J. van Montfrans. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries **7**:12.

Pakker, H. and A. M. Breeman. 1996. Temperature responses of tropical to warm-temperature Atlantic seaweeds. II. Evidence for ecotypic differentiation in amphi-Atlantic tropical-Mediterranean species. Eur J Phycol **31**:133-141.

Pelletreau, K. N. and G. Muller-Parker. 2002. Sulfuric acid in the phaeophyte alga *Desmarestia munda* deters feeding by the sea urchin *Strongylocentrotus droebachiensis*. Mar Biol **141**:1-9.

Peters, A. F., M. J. H. Van Oppen, C. Wiencke, W. T. Stam, and J. L. Olsen. 1997. Phylogeny and historical ecology of the Desmarestiaceae (Phaeophyceae) support a southern hemisphere origin. J Phycol **33**:294-309.

Peterson, C. H. 1982. Clam predation by whelks (*Busycon* spp.): experimental tests of the importance of prey size, prey density, and seagrass cover. Mar Biol **66**:159-170.

Pinet, P. R. 2012. Invitation to oceanography. Sixth edition edition. Jones & Bartlett Learning, Burlington.

Reichardt, W. 1987. Burial of antarctic macroalgal debris in bioturbated deep-sea sediments. Deep-Sea Res **34**:1761-1770.

Ruitton, S., P. Francour, and C. F. Boudouresque. 2000. Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). Estuar Coast Shelf S **50**:217-230.

Ruiz, G. M., J. T. Carlton, E. Grosholz, and A. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Amer Zool **37**:621-632.

Sasaki, H., H. Kataoka, M. Kamiya, and H. Kawai. 1999. Accumulation of sulfuric acid in *Dictyotales* (Phaeophyceae): taxonomic distribution and ion chromatography of cell extracts. J Phycol **35**:732-739.

Sasaki, H., H. Kataoka, A. Murakami, and H. Kawai. 2004. Inorganic ion compositions in brown algae, with special reference to sulfuric acid ion accumulations. Hydrobiologia **512**:255-262.

Sasaki, H., A. Murakami, and H. Kawai. 2005. Seasonal stability of sulfuric acid accumulation in the Dictyotales (Phaeophyceae). Phycol Res **53**:134-137.

Scheibling, R. 1986. Increased macroalgal abundance following mass mortalities of sea urchins (*Strongylocentrotus droebachiensis*) along the Atlantic coast of Nova Scotia. Oecologia **68**:186-198.

Scheibling, R. and P. Gagnon. 2006. Competitive interatction between the invasive green alga *Codium fragile* ssp. *tomentosoides* and native canopy-forming seaweeds in Nova Scotia (Canada). Mar Ecol Prog Ser **325**:1-14.

Scheibling, R. and B. G. Hatcher. 2007. Ecology of *Strongylocentrotus droebachiensis*. Pages 353-392 *in* J. M. Lamwrence, editor. Edible sea urchins: biology and ecology. Elsevier, Amsterdam.

Scheibling, R. E., A. W. Hennigar, and T. Balch. 1999. Destructive grazing, epiphytism, and disease: the dynamics of sea urchin - kelp interactions in Nova Scotia. Can J Fish Aquat Sci **56**:2300-2314.

Schiel, D. R. and M. J. H. Hickford. 2001. Biological structure of nearshore rocky subtidal habitats in southern New Zealand. Sci Conserv **182**:54.

Schmidt, A. L. and R. E. Scheibling. 2007. Effects of native and invasive macroalgal canopies on composition and abundance of mobile benthic macrofauna and turf-forming algae. J Exp Mar Biol Ecol **341**:110-130.

Shears, N. T. and R. C. Babcock. 2002. Marine reserves demonstrate top-down control of community structure on temperate reefs. Oecologia **132**:131-142.

Snedecor, G. W. and W. G. Cochran. 1989. Statistical methods. Iowa State University Press, Ames.

Sokal, R. R. and F. J. Rohlf, editors. 2012. Biometry: the principles and practice of statistics in biological research. W. H. Freeman, New York.

Sousa, W. P. 1979. Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. Ecol Monogr **49**:227-254.

Steinberg, P. D. and R. de Nys. 2002. Chemical mediation of colonization of seaweed surfaces. J Phycol **38**:621-629.

Steneck, R. S. 1983. Escalating herbivory and resulting adaptive trends in calcareous algal crusts. Paleobiology **9**:44-61.

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., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: Biodiversity, stability, resilience and future. Environ Conserv **29**:436-459.

Steneck, R. S., J. Vavrinec, and A. V. Leland. 2004. Accelerating trophic-level dysfunction in kelp forest ecosytems of the western North Atlantic. Ecosystems **7**:323-332.

Steneck, R. S. and L. Watling. 1982. Feed capabilities and limitation of herbivorous molluscs: a functional group approach. Mar Biol **68**:299-319.

Stoner, A. W. 1980. The role of seagrass biomass in the organization of benthic macrofaunal assemblages. B Mar Sci **30**:537-551.

Strand, J. A. and S. E. B. Weisner. 2001. Morphological plastic responses to water depth and wave exposure in an aquatic plant (*Myriophyllum spicatum*). J Ecol **89**.

Strauss, S. Y. and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol Evol **14**:179-185.

Taylor, R. B. and R. G. Cole. 1994. Mobile epifauna on subtidal brown seaweeds in northeastern New Zealand. Mar Ecol Prog Ser **115**:271-282.

Twilley, R. R. and J. W. Barko. 1990. The growth of submersed macrophytes under experimental salinity and light conditions. Estuaries **13**:311-321.

Vadas, R. 1968. The ecology of *Agarum* and the kelp bed community. University of Washington.

Vadas, R. L. 1977. Preferential feeding: an optimization strategy in sea urchins. Ecol Monogr **47**:337-371.

Van Alstyne, K. L., J. J. McCarthy III, C. L. Hustead, and D. O. Duggins. 1999. Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. Mar Biol **133**:371-379.

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. Helgol Meeresunters **35**:153-214.

Watanabe, J. M. and C. Harrold. 1991. Destructive grazing by sea urchins *Strongylocentrotus* spp. in a central California kelp forest: potential roles of recruitment, depth, and predation. Mar Ecol Prog Ser **71**:125-141.

Watson, J. and J. E. Estes. 2011. Stability, resilience and phase shifts in rocky subtidal communities along the west coast of Vancouver Island, Canada. Ecol Monogr **81**:215-239.

Wessels, H., W. Hagen, M. Molis, C. Wiencke, and U. Karsten. 2006. Intra- and interspecific differences in palatability of Arctic macroalgae from Kongsfjorden (Spitsbergen) for two benthic sympatric invertebrates. J Exp Mar Biol Ecol **329**:20-33.

Wiencke, C. and C. D. Amsler. 2012. Seaweeds and their communities in polar regions. Pages 265-294 *in* C. Wiencke and K. Bischof, editors. Seaweed Biology: Novel insights into ecophysiology, ecology and utilization. Springer-Verlag, Berlin.

Wiencke, C., I. Bartsch, B. Bischoff, A. F. Peters, and A. M. Breeman. 1994. Temperature requirements and biogeography of Antarctic, Arctic and Amphiequatorial seaweeds. Bot Mar **37**:247-259.

Wiencke, C. and M. Clayton. 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., M. Clayton, and C. Langreder. 1996. Life history and seasonal morphogenesis of the endemic antarctic brown alga *Desmarestia anceps* Montagne. Bot Mar **39**:435-444.

Wiencke, C., M. N. Clayton, I. Gómez, K. Iken, U. H. Lüder, C. D. Amsler, U. Karsten, D. Hanelt, K. Bischof, and K. Dunton. 2007. Life strategy, ecophysiology and ecology of seaweeds in polar waters. Rev Environ Sci Biotechnol **6**:95-126.

Wiencke, C., M. N. Clayton, and D. Schulz. 1995. Life history, reproductive morphology and development of the Antarctic brown alga *Desmarestia menziesii* J. Agardh. Bot Acta **108**:201-208.

Wiencke, C. and I. tom Dieck. 1989. Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. Mar Ecol Prog Ser **54**:189-197.

Worm, B. and J. E. Duffy. 2003. Biodiversity, productivity and stability in real food webs. Trends Ecol Evol **18**:628-632.

Yñiguez, A. T., J. W. McManus, and L. Collado-Vides. 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., J. A. Hodar, and J. M. Gomez. 1999. Plant-herbivore interaction: beyond a binary vision. Pages 677-718 *in* F. I. Pugnaire and F. Valladares, editors. Handbook of functional plant ecology. Marcel Dekker, Inc., New York.

Zamzow, J. P., C. F. Aumack, C. D. Amsler, J. McClintock, M. O. Amsler, and B. J. Baker. 2011. Gut contents and stable isotope analyses of the Antarctic fish, *Notothenia coriiceps* Richardson, from two macroalgal communities. Antarctic Sci **23**:107-116.

Zar. 2010. Biostatistical analysis. Prentice-Hall, Upper Saddle River, NJ.

APPENDIX A

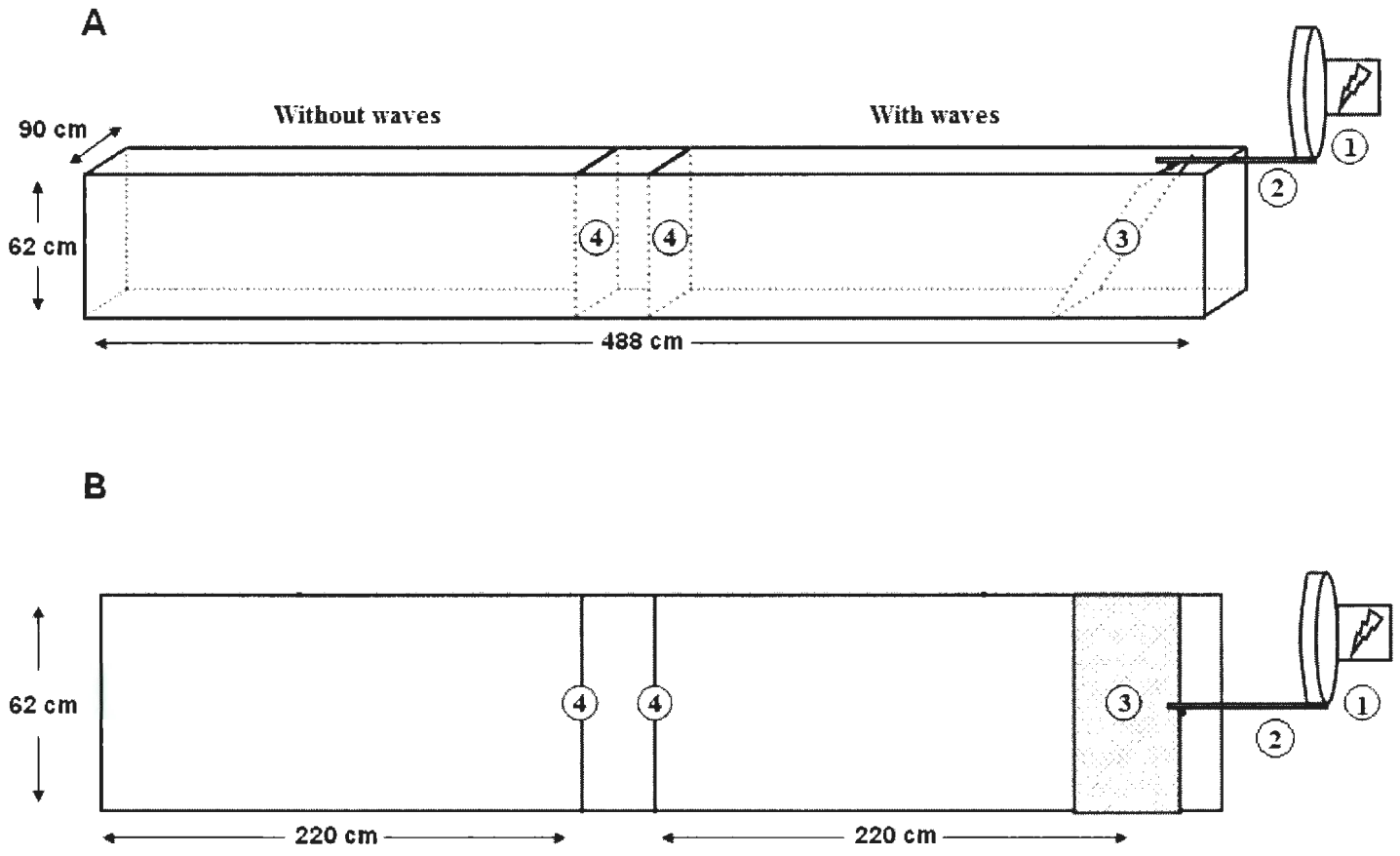

A
90 cm
Without waves
With waves
62 cm
4
4
3
1
2
488 cm
B
62 cm
4
4
3
1
2
220 cm
220 cm

Figure A1. Side (A) and top (B) views of the oscillatory wave tank used in Experiment 2 of Chapter II (see Section 2.2.3). The tank system consisted of a 2 HP electric motor (1) with adjustable rotation speed (2000 rpm maximum; TB Woods Incorporated, X4C1S010C) connected with a perforated metal bar (2) to the top of a ply-wood panel (3) hinged to the bottom of the tank (488[L]×90[W]×62[H]). The rotational force of the motor was converted into a bi-directional force that moved the plywood panel to produce waves. A 1-cm gap between the panel and the sides and bottom of the tank allowed the water to circulate from one side of the panel to the other. The amplitude of the waves created in the tank could be varied with the depth of water in the tank (30 cm in this experiment), the speed of rotation of the motor, and the position on the perforated bar to which the bar connecting the panel was attached. Two unmovable panels (4) were inserted in the centre of the tank to create two working sections (220[L]×90[W]×62[H] cm each), one with and one without waves (20 wave cycles min^{-1}, 0.2 m s^{-1}). In each trial, three *D. viridis* sporophytes (~50 cm in length) were attached to the bottom of the tank in each working section. Sporophytes were positioned so that fronds did not come in contact with one another or with the sides of the tank.

APPENDIX B

Analysis of residuals and data transformation (ANOVAs)

All the statistical analyses in this thesis were conducted in conformity with widely used techniques in the area of marine benthic ecology. Although these analyses reflect accurate usage of techniques presented in some of the most respected and highly cited textbooks in statistics (Hand and Taylor 1987, McCullagh and Nelder 1989, Snedecor and Cochran 1989, Crowder and Hand 1990, Zar 2010, Sokal and Rohlf 2012), they may not necessarily reflect the most recent paradigms taught in university classrooms or published in the statistical literature. Efforts were made to employ more recent techniques (Bolker et al. 2008, Zar 2010, Sokal and Rohlf 2012) where deemed relevant by the author (C. Blain) and supervisor (P. Gagnon) of the thesis.

All of the ANOVAs in this thesis were first run on raw data. Homogeneity of the variance was verified by examining the distribution of the resulting residuals in plots of residuals versus predicted values. The distribution was considered homogeneous if residuals were equally spread across the full spectrum of predicted values (i.e. no bowl- or arch-shaped pattern). Visual inspections of the plots were complemented with Levene tests in those cases where no definitive statement could be made from the plots. Normality in the distribution of the residuals was verified by visually inspecting the normal probability plot of the residuals (fractiles of error distribution versus fractiles of a normal distribution having the same mean and variance). The distribution was considered normal when points on the probability plot followed a diagonal line with no excessive skewness (bow-shaped pattern) or kurtosis (S-shaped pattern). Visual inspections of the

plots were complemented by considering the Shapiro-Wilk's statistics in those cases where no definitive statement could be made from the plots. Results of ANOVAs on raw data were presented when the associated residuals showed both homogeneous and normally distributed. Else, non-linear data transformation appropriate for the type of independent variable modeled was applied to the raw data. Associated residuals were inspected for normality and homogeneity as described above. Results of ANOVAs on transformed data were presented when the associated residuals showed both homogeneous and normally distributed in relevant plots. If non-linear data transformations failed to correct problems of normality and homogeneity, the raw data were rank-transformed and the ANOVA was applied to the latter. The p-value of each factor in the ANOVA on raw data was compared with that of each corresponding factor in the ANOVA on rank-transformed data. Results of ANOVAs on raw data were presented when the significance of p-values was the same between the two ANOVAs (which was always the case). In a few cases, generalized linear models were employed to accommodate the particular nature of independent variables (e.g. a proportion, which is bound to lie between a minimum [0] and maximum [100] value, and hence not entirely amenable to analysis with standard ANOVAs assuming normally distributed residuals). Randomization tests were not used due to difficulties in applying them uniformly across those cases where ANOVA assumptions were systematically violated or sample size was small.

APPENDIX C

Table C.1. Mean acidity (expressed in pH units) and concentration of sulfuric acid (H_2SO_4, expressed in molarity [M]) of *Desmarestia viridis* sporophytes at the beginning and end of Experiment 1 (temperature and light), Experiment 2 (wave action), and Experiment 3 (grazing and epibionts) in Chapter II, as well as corresponding difference (see Sections 2.2.2, 2.2.3, and 2.2.4 for an explanation of each experiment and section 2.2.6 for calculation of intracellular acidity).

		Beginning		End		Difference	
		pH	**M**	**pH**	**M**	**pH**	**M**
Experiment 1 (temperature and light)							
Cold water	Low light intensity [C/L]	0.78	0.17	0.91	0.12	(+) 0.13	(-) 0.043
	High light intensity [C/H]	0.83	0.15	0.96	0.11	(+) 0.13	(-) 0.038
Warm water	Low light intensity [W/L]	0.80	0.16	1.16	0.069	(+) 0.36	(-) 0.89
	High light intensity [W/H]	0.83	0.15	1.23	0.059	(+) 0.40	(-) 0.089
Experiment 2 (wave action)							
Waves	Late spring	0.75	0.18	0.66	0.22	(-) 0.09	(+) 0.04
	Early summer	0.76	0.17	0.68	0.21	(-) 0.08	(+) 0.04
	Late summer	0.80	0.16	0.85	0.14	(+) 0.05	(-) 0.02

Table C.1. (continued)

		Beginning		End		Difference	
		pH	**M**	**pH**	**M**	**pH**	**M**
Experiment 2 (wave action)							
No waves	Late spring	0.81	0.16	0.73	0.19	(-) 0.08	(+) 0.03
	Early summer	0.74	0.18	0.64	0.23	(-) 0.10	(+) 0.05
	Late summer	0.76	0.17	0.94	0.11	(+) 0.18	(-) 0.06
Experiment 3 (grazing and epibionts)							
Grazers	Epibionts [G+E+]	0.62	0.24	0.64	0.23	(+) 0.02	(-) 0.01
	No epibionts [G+E-]	0.54	0.29	0.55	0.29	(+) 0.01	(-) 0.00
No grazers	Epibionts [G-E+]	0.58	0.26	0.63	0.24	(+) 0.05	(-) 0.03
	No epibionts [G-E-]	0.62	0.24	0.65	0.22	(+) 0.03	(-) 0.02

APPENDIX D

Taxonomic keys and guides used to identify epifauna (Chapter III)

Abbott, R. T. 1976. Seashells. Bantam, Toronto, Canada.

Abbott, R. T. and P. A. Morris. 1995. Shells of the Atlantic and Gulf Coasts and the West Indies. 4 edition. Houghton Mifflin Company, New York, USA.

Abbott, R. T. and G. F. Sandstrom. 1996. A guide to field identification: seashells of North America. St. Martin's Press, New York, USA.

Barnard, J. L. 1969. The families and genera of marine gammaridean Amphipoda. Smithsonian Institution, United States National Museum, Washington DC, USA.

Bousfield, E. L. 1972. Shallow-water gammaridean Amphipoda of New England. Comstock Publishing Associates, Cornell University Press, Ithaca & London.

Brinkhurst, R. O., L. E. Linkletter, E. I. Lord, S. A. Connors, and M. J. Dadswell. 1976. A preliminary guide to the littoral and sublittoral marine invertebrates of Passamaquoddy Bay. Department of Environment Fisheries and Marine Service, New Brunswick, Canada.

Fenwick, G. D. and D. H. Steele. 1983. Amphipods of Placentia Bay, Newfoundland. Memorial University of Newfoundland, St. John's, Canada.

Gosner, K. L. 1978. Atlantic seashore: a field guide to sponges, jellyfish, sea urchins, and more. Houghton Mifflin Company, New York, USA.

Korczynski, R. E. 1985. A taxanomic key to the benthic amphipods (Crustacea, Amphipoda) inhabiting Tuktoyaktuk Harbor, Northwest Territories. Department of Fisheries and Oceans Canada.

Pettibone, M. H. 1963. Marine Polycheata worms of the New England region. 1. Families Apthroditidae through Trochochaetidae. Smithsonian Institute, USA.

Pocklington. 1989. Polychaetes of eastern Canada: an illustrated key to polychaetes of eastern Canada including the eastern Arctic. Department of Fisheries and Oceans Canada.

Quijon, P. 2004. Polychaetes of Bonne Bay, NL: an introductory manual for the identification of the most common species. Memorial University of Newfoundland, St. John's, Canada.

Ramey, P. A. 2001. Factors influencing patterns in distribution, abundance, and diversity of sedimentary macrofauna in deep, muddy sediments of Placentia Bay, Newfoundland and the adjacent shelf. Memorial University of Newfoundland, St. John's, Canada.

Sars, G. O. 1895. An account of the Crustacea of Norway, with short descriptions and figures of all the species. Christiania and Copenhagen, A. Cammermeyer, Norway.

Stephensen, K. 1944. The zoology of east Greenland: Amphipoda. C. A. Reitzels Forlag, Kobenhavn.

APPENDIX E

Table E.1. Mean density (individuals [or egg masses] kg^{-1} seaweed) of invertebrates and fish on *Desmarestia viridis*, *Desmarestia aculeata*, and *Agarum clathratum* sporophytes sampled monthly from 18 February to 9 October, 2011 (see Section 3.2.3 for details of sampling). Standard error is given below each mean in *italics*.

Desmarestia viridis

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Annelida									
Polychaeta									
Alitta virens M Sars	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Autolytinae sp.	0	0	0	189.9	211.2	140.7	19.8	44.5	0
	0.0	*0.0*	*0.0*	*22.1*	*34.5*	*65.4*	*11.0*	*15.5*	*0.0*
Bylgides sarsi Kinberg in Malmgren	0	0	0	87.4	131.7	90.0	69.6	15.6	0
	0.0	*0.0*	*0.0*	*22.8*	*16.8*	*45.1*	*23.7*	*3.4*	*0.0*
Lepidonotus squamatus Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Nereis pelagica Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Phyllodoce mucosa Örsted	0	0	0	121.4	69.5	0	0	0	0
	0.0	*0.0*	*0.0*	*22.4*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Spirorbis borealis Daudin	0	0	0	0	0	0	0	821.8	332.8
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*119.2*	*56.7*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Arthropoda									
Arachnida									
Halacaridae	0	132.3	16.7	18.0	17.8	23.0	138.4	69.8	0
	0.0	*5.6*	*2.9*	*3.8*	*3.8*	*5.8*	*25.8*	*14.1*	*0.0*
Crustacea									
Ampithoe rubricata Montagu	0	0	162.1	81.1	0.0	0.0	39.9	13.5	95.1
	0.0	*0.0*	*16.7*	*21.3*	*0.0*	*0.0*	*18.4*	*6.4*	*31.1*
Calliopius laeviusculus Kroyer	26.4	15.0	1232.8	527.4	378.4	142.1	1102.3	122.0	314.8
	7.7	*4.5*	*46.9*	*32.1*	*33.5*	*21.0*	*66.7*	*23.9*	*29.4*
Caprella linearis Linnaeus	866.5	621.0	224.9	38.9	0	0	0	137.8	227.7
	56.4	*87.7*	*43.5*	*18.0*	*0.0*	*0.0*	*0.0*	*25.2*	*42.2*
Caprella septentrionalis Kroyer	81.6	199.5	0	0	0	0	0	10.3	0
	10.9	*14.7*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*3.9*	*0.0*
Copepoda*	4185.5	41644.1	53571.4	9945.5	17533.0	21435.6	31042.2	11772.8	45469.4
	1087.7	*2184.8*	*2544.4*	*2138.9*	*4887.8*	*8997.2*	*9925.5*	*3589.4*	*9844.8*
Gammarellus angulosus Rathke	0	0	33.1	0	0	0	3.8	0	0
	0.0	*0.0*	*12.0*	*0.0*	*0.0*	*0.0*	*2.6*	*0.0*	*0.0*
Gammarus oceanicus Segerstråle	107.5	522.9	34.7	5.0	0	0	17.4	0	49.5
	33.3	*48.1*	*16.5*	*3.5*	*0.0*	*0.0*	*9.1*	*0.0*	*22.0*
Gammarus setosus Dementieva	0	0	0	0	0	0	0	3.2	0.0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*2.0*	*0.0*
Hyas sp.*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Idotea baltica Pallas	0	0	0	0	0	0	40.2	188.5	875.5
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*9.8*	*29.3*	*69.1*
Ischyrocerus anguipes Kroyer	1165.3	3701.5	2023.2	3573.4	3264.0	2932.4	316.3	454.2	133.6
	169.5	*284.1*	*155.2*	*281.0*	*139.9*	*157.3*	*134.5*	*99.7*	*87.0*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Leptocheirus pinguis Stimpson	0	0	0	0	316.5	370.6	251.4	182.8	118.1
	0.0	*0.0*	*0.0*	*0.0*	*68.5*	*46.0*	*55.5*	*31.7*	*28.9*
Munna sp.	0	0	0	0	0	0	31.8	51.7	65.8
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*8.8*	*11.7*	*15.9*
Pontogeneia inermis Kroyer	72.0	547.8	570.9	343.6	590.7	288.7	222.6	430.0	2143.3
	22.4	*101.8*	*83.6*	*75.8*	*88.0*	*57.4*	*44.0*	*43.8*	*101.4*
Stenothoe brevicornis G.O. Sars	0	522.6	663.7	0	74.2	19.0	229.7	374.9	1318.0
	0.0	*101.5*	*165.2*	*0.0*	*19.7*	*11.5*	*77.3*	*75.1*	*185.2*
Pycnogonida									
Phoxichilidium femoratum Rathke	0	0	0	0	0	0	0	10.7	77.7
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*4.5*	*12.2*
Chordata									
Actinopterygii									
Cyclopterus lumpus Linnaeus*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Liparis inquilinus Able*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Pholis gunnellus Linnaeus*	0	0	0	0	2.9	1.8	3.2	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.2*	*1.9*	*2.8*	*0.0*	*0.0*
Egg masses (unknown species)	0	10.0	20.1	477.4	389.5	145.0	72.5	5.3	0
	0.0	*3.1*	*11.8*	*79.8*	*66.7*	*68.4*	*30.8*	*2.9*	*0.0*
Cnidaria									
Hydrozoa									
Obelia geniculata Linnaeus**	0	0	0	0	0	0	2.8	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.4*	*0.0*	*0.0*
Scyphozoa									
Haliclystus salpinx James-Clark	0	0	0	0	0	0	39.4	20.1	167.4
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*15.2*	*5.2*	*43.1*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Echinodermata									
Asteroidea									
Asterias sp.	0	0	0	0	0	0	0	37.0	58.1
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*13.0*	*12.4*
Ophiuroidea									
Ophiopholis aculeata Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Ectoprocta									
Gymnolaemata									
Electra pilosa Linnaeus**	0	0	0	0	0	0	0	0	37.8
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*14.7*
Stenolaemata									
Lichenopora sp.**	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Mollusca									
Bivalvia									
Hiatella arctica Linnaeus	0	0	0	0	0	0	0	11838.7	1454.3
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*1534.1*	*299.0*
Modiolus modiolus Linnaeus	0	0	0	187.0	101.7	155.7	492.0	0	0
	0.0	*0.0*	*0.0*	*45.8*	*33.4*	*21.0*	*85.6*	*0.0*	*0.0*
Mytilus sp.	40.5	13.0	16.7	163.7	262.1	292.7	133.6	7931.4	26539.1
	6.8	*1.5*	*1.6*	*24.5*	*22.7*	*23.9*	*11.0*	*156.5*	*5897.5*
Gastropoda									
Dendronotus frondosus Ascanius	0	0	0	0	0	0	31.1	145.0	261.3
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*4.8*	*232.8*	*99.6*
Lacuna vincta Montagu	481.1	164.7	344.1	308.8	696.7	1035.5	1041.9	44885.5	154770.4
	45.5	*21.7*	*40.2*	*19.9*	*101.8*	*245.7*	*321.8*	*983.3*	*5994.5*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Margarites helicinus Phipps	0	0	0	0	0	196.6	111.5	7921.7	3986.6
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*22.8*	*13.4*	*389.5*	*677.0*
Egg masses (*L. vincta*)	0	0	0	0	0	0	0	5.5	9.1
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*1.2*	*1.3*
Polyplacophora									
Tonicella marmorea Fabricius	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Nemertea									
Enopla									
Tetrastemma sp.*	0	5.2	19.8	0	0	20.8	53.4	0	0
	0.0	*1.0*	*2.9*	*0.0*	*0.0*	*3.1*	*8.8*	*0.0*	*0.0*

Desmarestia aculeata

Table E.1 (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Annelida									
Polychaeta									
Alitta virens M Sars	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Autolytinae sp.	0	0	0	45.5	45.2	0	0	0	0
	0.0	*0.0*	*0.0*	*12.3*	*14.2*	*0.0*	*0.0*	*0.0*	*0.0*
Bylgides sarsi Kinberg in	0	0	0	0	64.7	73.0	33.2	10.0	0
	0.0	*0.0*	*0.0*	*0.0*	*13.2*	*15.7*	*11.0*	*2.5*	*0.0*
Lepidonotus squamatus Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Nereis pelagica Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Phyllodoce mucosa Örsted	0	0	7.6	56.4	70.1	89.1	68.4	16.0	0
	0.0	*0.0*	*2.2*	*23.4*	*19.8*	*19.1*	*22.0*	*9.4*	*0.0*
Spirorbis borealis Daudin	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Arthropoda									
Arachnida									
Halacaridae	0	0	20.9	0	0	132.3	254.5	321.7	151.7
	0.0	*0.0*	*6.9*	*0.0*	*0.0*	*22.1*	*23.4*	*22.8*	*19.7*
Crustacea									
Ampithoe rubricata Montagu	0	0	111.3	9.0	0	0	0	0	56.8
	0.0	*0.0*	*22.8*	*2.7*	*0.0*	*0.0*	*0.0*	*0.0*	*18.6*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Calliopius laeviusculus Kroyer	113.2	115.7	640.1	131.4	251.4	1110.8	283.1	608.7	335.0
	35.8	*13.8*	*29.9*	*16.0*	*26.7*	*71.7*	*66.2*	*68.5*	*44.2*
Caprella linearis Linnaeus	121.2	120.8	133.7	95.5	18.7	14.0	11.9	120.7	1109.9
	34.5	*19.9*	*20.0*	*20.6*	*9.1*	*9.0*	*6.1*	*22.3*	*112.8*
Caprella septentrionalis Kroyer	135.7	251.1	49.9	45.7	0	0	0	11.1	101.6
	53.6	*56.6*	*21.7*	*19.3*	*0.0*	*0.0*	*0.0*	*6.2*	*28.2*
Copepoda*	5092.2	20359.1	27264.4	34738.8	22021.1	28721.0	36720.5	3507.2	22992.0
	1015.5	*4568.8*	*7411.4*	*7865.6*	*3566.3*	*15899.9*	*9448.7*	*1122.2*	*7899.5*
Gammarellus angulosus Rathke	0	55.2	33.0	20.9	11.2	67.4	6.9	0	0
	0.0	*25.5*	*18.7*	*11.1*	*4.6*	*28.8*	*4.0*	*0.0*	*0.0*
Gammarus oceanicus Segerstråle	0	0	40.7	5.1	14.9	21.5	68.0	12.7	401.8
	0.0	*0.0*	*17.3*	*3.9*	*10.0*	*12.5*	*22.8*	*5.5*	*64.4*
Gammarus setosus Dementieva	0	0	9.9	0	0	0	0	10.7	0
	0.0	*0.0*	*2.2*	*0.0*	*0.0*	*0.0*	*0.0*	*3.6*	*0.0*
Hyas sp.*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Idotea baltica Pallas	0	10.0	13.7	1.4	115.0	129.6	66.9	67.8	201.4
	0.0	*3.4*	*4.7*	*0.8*	*22.0*	*17.8*	*19.6*	*19.5*	*22.7*
Ischyrocerus anguipes Kroyer	1254.4	246.0	749.8	957.2	169.7	491.2	144.4	231.4	899.0
	160.4	*68.4*	*211.3*	*345.7*	*40.9*	*33.5*	*26.5*	*27.4*	*123.8*
Leptocheirus pinguis Stimpson	0	0	0	0	110.2	251.5	13.4	85.7	353.3
	0.0	*0.0*	*0.0*	*0.0*	*20.7*	*23.6*	*9.0*	*21.4*	*35.5*
Munna sp.	0	0	0	0	0	0	59.3	48.9	270.5
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*22.8*	*23.1*
Pontogeneia inermis Kroyer	0	0	428.7	194.5	460.0	202.8	4.1	0	199.7
	0.0	*0.0*	*59.8*	*60.1*	*66.5*	*45.8*	*2.0*	*0.0*	*45.7*
Stenothoe brevicornis G.O. Sars	0	0	151.0	151.3	301.1	51.2	20.7	9.8	85.4
	0.0	*0.0*	*34.7*	*32.6*	*29.6*	*14.0*	*10.3*	*2.7*	*25.6*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Pycnogonida									
Phoxichilidium femoratum Rathke	0	0	0	0	0	0	0	0	1704.6
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*523.5*
Chordata									
Actinopterygii									
Cyclopterus lumpus Linnaeus*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Liparis inquilinus Able*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Pholis gunnellus Linnaeus*	0	0	0	0	0	0	1.8	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.2*	*0.0*	*0.0*
Egg masses (unknown species)	0	5.1	11.9	21.7	51.6	0	0	0	0
	0.0	*2.2*	*3.4*	*17.8*	*16.5*	*0.0*	*0.0*	*0.0*	*0.0*
Cnidaria									
Hydrozoa									
Obelia geniculata Linnaeus**	0	0	0	0	0	0	10.9	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*3.3*	*0.0*	*0.0*
Scyphozoa									
Haliclystus salpinx James-Clark	0	0	0	0	0	0	43.8	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*15.4*	*0.0*	*0.0*
Echinodermata									
Asteroidea									
Asterias sp.	0	10.1	0	0	0	0	0	0	59.6
	0.0	*2.8*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*16.6*
Ophiuroidea									
Ophiopholis aculeata Linnaeus	0	0	0	5.0	32.5	0	0	0	0
	0.0	*0.0*	*0.0*	*2.4*	*12.7*	*0.0*	*0.0*	*0.0*	*0.0*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Ectoprocta									
Gymnolaemata									
Electra pilosa Linnaeus**	22.5	46.5	33.5	46.2	54.1	66.8	70.1	46.3	77.8
	7.6	*10.0*	*6.7*	*10.1*	*13.8*	*12.7*	*13.9*	*19.0*	*21.1*
Stenolaemata									
Lichenopora sp.**	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Mollusca									
Bivalvia									
Hiatella arctica Linnaeus	0	0	0	0	0	0	0	0	61.3
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*7.2*
Modiolus modiolus Linnaeus	0	0	50.8	26.3	32.5	12.6	58.0	0	0
	0.0	*0.0*	*13.8*	*15.2*	*8.7*	*6.3*	*7.9*	*0.0*	*0.0*
Mytilus sp.	178.9	257.2	346.0	165.7	205.7	122.1	74.3	204694.4	238465.9
	28.8	*49.7*	*101.2*	*133.8*	*97.5*	*77.3*	*25.4*	*1685.5*	*7985.6*
Gastropoda									
Dendronotus frondosus Ascanius	0	134.9	0	0	0	0	0	0	0
	0.0	*55.5*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Lacuna vincta Montagu	2267.5	1287.3	1454.2	1072.5	436.9	127.5	1128.2	11401.4	24002.9
	986.6	*774.2*	*452.1*	*200.8*	*98.7*	*45.2*	*106.4*	*3011.8*	*4561.2*
Margarites helicinus Phipps	0	77.8	25.9	58.3	97.0	69.1	78.4	2091.8	259.7
	0.0	*21.1*	*13.2*	*11.5*	*95.5*	*46.4*	*66.2*	*106.9*	*49.9*
Egg masses (*L. vincta*)	0	0	13.9	18.9	9.7	73.7	51.9	0	0
	0.0	*0.0*	*2.2*	*1.6*	*2.6*	*46.6*	*16.8*	*0.0*	*0.0*
Polyplacophora									
Tonicella marmorea Fabricius	0	31.8	0	0	0	0	0	0	0
	0.0	*22.8*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Nemertea									
Enopla									
Tetrastemma sp.*	0	8.0	19.8	72.3	48.0	11.6	2.7	0	0
	0.0	*0.1*	*2.7*	*9.3*	*10.7*	*0.5*	*0.1*	*0.0*	*0.0*

Agarum clathratum

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Annelida									
Polychaeta									
Alitta virens M Sars	39.6	4.2	0	0	0	0	0	0	0
	8.2	*0.9*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Autolytinae sp.	0	0	0	0	0	0	6.4	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*2.2*	*0.0*	*0.0*
Bylgides sarsi Kinberg in	0	0	0	0	0	0	6.4	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*5.1*	*0.0*	*0.0*
Lepidonotus squamatus Linnaeus	44.7	22.3	2.0	0	0	0	0	0	0
	16.1	*6.0*	*0.7*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Nereis pelagica Linnaeus	50.8	91.1	0	0	0	0	0	0	0
	9.7	*10.3*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Phyllodoce mucosa Örsted	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Spirorbis borealis Daudin	40.5	95.8	69.9	165.3	251.1	233.7	194.5	3073.6	2751.1
	8.8	*16.4*	*20.1*	*21.3*	*44.7*	*39.8*	*66.2*	*468.4*	*455.0*
Arthropoda									
Arachnida									
Halacaridae	0	16.7	0	0	0	0	0	0	0
	0.0	*2.3*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Crustacea									
Ampithoe rubricata Montagu	0	0	0	0	0	0	0	20.2	22.8
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*9.1*	*9.0*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Calliopius laeviusculus Kroyer	3.2	3.2	29.7	11.7	19.1	69.4	0	0	0
	0.5	*2.4*	*11.8*	*7.3*	*8.0*	*22.7*	*0.0*	*0.0*	*0.0*
Caprella linearis Linnaeus	0	0	0	4.1	0	0	0	6.8	0
	0.0	*0.0*	*0.0*	*2.0*	*0.0*	*0.0*	*0.0*	*3.1*	*0.0*
Caprella septentrionalis Kroyer	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Copepoda*	1098.4	975.2	1210.8	11859.4	19319.9	4157.7	17118.2	3923.0	4044.2
	163.2	*222.7*	*298.4*	*1855.2*	*5781.1*	*465.9*	*5987.5*	*1654.1*	*990.0*
Gammarellus angulosus Rathke	336.4	24.3	9.8	0	0	0	0	0	0
	55.7	*20.1*	*7.4*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Gammarus oceanicus Segerstråle	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Gammarus setosus Dementieva	2.8	4.9	1.2	0	0	0	0	0	0
	0.9	*0.7*	*0.6*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Hyas sp.*	1.0	3.9	0	0	0	0	0	0	0
	2.2	*0.9*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Idotea baltica Pallas	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Ischyrocerus anguipes Kroyer	0	241.0	200.2	161.7	159.6	625.1	128.7	201.0	135.1
	0.0	*48.5*	*36.9*	*45.8*	*63.8*	*71.1*	*52.0*	*52.4*	*43.0*
Leptocheirus pinguis Stimpson	0	0	0	0	0	0	0	51.3	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*13.5*	*0.0*
Munna sp.	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Pontogeneia inermis Kroyer	0	54.2	92.9	31.0	100.7	143.4	0	0	0
	0.0	*14.1*	*23.6*	*19.2*	*12.8*	*16.7*	*0.0*	*0.0*	*0.0*
Stenothoe brevicornis G.O. Sars	0.0	0	9.9	0.0	131.7	14.3	55.9	27.7	124.9
	0.0	*0.0*	*2.8*	*0.0*	*33.0*	*5.4*	*19.8*	*12.4*	*12.6*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Pycnogonida									
Phoxichilidium femoratum Rathke	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Chordata									
Actinopterygii									
Cyclopterus lumpus Linnaeus*	0	2.4	5.6	9.8	0	0	0	0	0
	0.0	*0.8*	*2.1*	*2.3*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Liparis inquilinus Able*	6.3	2.1	3.4	0	0	0	0	0	0
	2.2	*0.7*	*0.8*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Pholis gunnellus Linnaeus*	0	2.5	0	0	0	2.1	2.8	0	0
	0.0	*1.2*	*0.0*	*0.0*	*0.0*	*0.9*	*2.2*	*0.0*	*0.0*
Egg masses (unknown species)	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Cnidaria									
Hydrozoa									
Obelia geniculata Linnaeus**	0	0	0	0	0	2.6	2.8	15.8	40.9
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*1.1*	*0.9*	*2.8*	*13.5*
Scyphozoa									
Haliclystus salpinx James-Clark	0	0	4.6	0	16.0	5.9	37.7	29.8	22.1
	0.0	*0.0*	*0.3*	*0.0*	*2.0*	*0.9*	*4.6*	*7.1*	*6.9*
Echinodermata									
Asteroidea									
Asterias sp.	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Ophiuroidea									
Ophiopholis aculeata Linnaeus	0	0	0	0	0	0	21.5	0	9.2
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.9*	*0.0*	*2.3*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Ectoprocta									
Gymnolaemata									
Electra pilosa Linnaeus**	0	9.8	0	0	0	53.8	101.9	298.3	238.1
	0.0	*2.2*	*0.0*	*0.0*	*0.0*	*11.5*	*20.7*	*46.5*	*40.7*
Stenolaemata									
Lichenopora sp.**	63.5	86.8	76.3	50.9	12.4	113.5	277.8	191.9	4088.9
	13.8	*22.2*	*13.9*	*10.1*	*0.9*	*41.8*	*33.7*	*34.6*	*108.8*
Mollusca									
Bivalvia									
Hiatella arctica Linnaeus	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Modiolus modiolus Linnaeus	29.9	43.2	17.6	67.3	27.4	28.8	137.5	8.1	0
	2.8	*2.8*	*3.7*	*16.9*	*13.4*	*16.8*	*46.5*	*0.2*	*0.0*
Mytilus sp.	102.0	143.4	7.8	45.8	50.4	68.7	16.7	381.2	454.2
	12.9	*14.1*	*0.4*	*10.8*	*8.7*	*8.3*	*2.4*	*51.6*	*49.8*
Gastropoda									
Dendronotus frondosus Ascanius	0	0	0	0	0	0	0	0	75.0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*13.2*
Lacuna vincta Montagu	54.8	1104.0	260.2	178.0	444.2	109.2	435.4	7246.1	10496.6
	12.8	*765.1*	*46.8*	*66.0*	*169.2*	*59.6*	*44.7*	*1010.5*	*2337.4*
Margarites helicinus Phipps	33.4	45.5	35.4	28.1	39.7	19.6	9.4	281.6	30.7
	8.1	*12.3*	*13.4*	*9.5*	*10.5*	*4.2*	*1.6*	*44.8*	*13.2*
Egg masses (*L. vincta*)	5.0	0	0	70.6	76.9	43.8	28.3	10.8	247.2
	2.2	*0.0*	*0.0*	*21.0*	*21.3*	*19.1*	*5.8*	*2.5*	*46.7*
Polyplacophora									
Tonicella marmorea Fabricius	0	13.1	0	0	0	0	0	0	0
	0.0	*2.2*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

Table E.1. (continued)

Taxon	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Nemertea									
Enopla									
Tetrastemma sp.*	0	0	0	0	0	0	0	0	0
	0.0	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

* Denotes taxon excluded from MDS, ANOSIM, and SIMPER analyses.

** Denotes colonial taxon in which each colony was counted as one individual.