Vol. 537: 105–119, 2015
doi: 10.3354/meps11421

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 14

Kelp in hot water: II. Effects of warming seawater temperature on kelp quality as a food source and settlement substrate

E. J. Simonson[1,*], A. Metaxas[2], R. E. Scheibling[1]

[1] Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada
[2]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada

ABSTRACT: Predicting the effect of climate change on communities requires an understanding of the effects of environmental conditions on species and their interactions. We investigated the potential for warming seawater temperature to modify the interactions of the gastropod mesograzer *Lacuna vincta* and the invasive bryozoan *Membranipora membranacea* with kelps in Nova Scotia. The nutritional content (C/N) of the kelps *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum* were unaffected by temperature (11, 18 and 21°C), and chemical defenses (phlorotannins) were reduced only in *A. clathratum* after 1 wk exposure to 21°C. C/N and phlorotannin content increased over the season in *S. latissima* collected monthly in summer 2013 and 2014. The effect of temperature-induced changes in kelp on the grazing of *L. vincta* was assessed using feeding experiments with *S. latissima* pretreated at 11 or 21°C. Snails consumed more kelp pretreated at 21°C only when grazing rate was high. The quality of *S. latissima* as a food source for *L. vincta* was not affected by temperature, as diets of kelp pretreated at 11 and 21°C supported similar growth, reproduction, and survival of snails. Temperature also did not affect the quality of kelp as a substrate for *M. membranacea*, since settlement rates were not different between *S. latissima* pretreated at ambient temperature (9 to 14) and 21°C. The absence of temperature-induced changes in kelp quality suggests that the effects of *L. vincta* and *M. membranacea* will act additively with the direct effects of temperature and cause increased biomass loss from kelp beds.

KEY WORDS: Kelp · Climate change · Temperature · *Saccharina latissima* · *Lacuna vincta* · Herbivory · *Membranipora membranacea* · Bryozoans

INTRODUCTION

Effects of climate change on community structure and function will integrate environmental impacts on individual species with changes in interactions among species (Harley et al. 2006). Alteration of biotic interactions can have significant effects on community composition and ecosystem function that can enhance or diminish the effects of climate change (Zarnetske et al. 2012, HilleRisLambers et al. 2013). Climate-driven changes in biotic interactions can occur through introduction or loss of interacting species due to changes in distributional range or phenology, or through changes in the strength of interactions due to alteration of behavior or physiology (Zarneste etl al. 2012, HilleRisLambers et al. 2013, Vergés et al. 2014). Changing climate also can facilitate the invasion of non-native species or exacerbate their effects on native communities (Cockrell & Sorte 2013, Floerl et al. 2013). Alteration of biotic interactions can combine with the direct effects of climate change on a species, resulting in impacts that may not have been expected when considering the effect of climate alone (HilleRisLambers et al. 2013).

*Corresponding author: erika.simonson@dal.ca

Globally, increasing seawater temperature has been linked to range contractions and declines in kelp populations (Andersen et al. 2011, Fernández 2011, Tuya et al. 2012, Wernberg et al. 2013). Kelp (large brown algae of the order Laminariales) are important foundation species on temperate rocky reefs, supporting high community productivity and biodiversity (Dayton 1985, Steneck et al. 2002). Direct effects of increasing temperature on kelp include reduced growth (Bolton & Lüning 1982, Gerard & Du Bois 1988, Davison 1991, Andersen et al. 2013), weakening and loss of kelp tissue (Simonson et al. 2015, this volume), and mortality when physiological limits are exceeded (Wernberg et al. 2013). Temperature-mediated changes in kelp bed communities also may occur indirectly through the alteration of interspecific interactions (Harley et al. 2012, Vergés et al. 2014). The interaction between herbivores and their algal food sources can regulate kelp communities through the consumption of algal biomass (Lubchenco & Gaines 1981), and may be altered by temperature stress due to changes in either algal growth rate or consumption (O'Connor 2009), or changes in algal palatability (Harley et al. 2012). For example, warming seawater temperatures in southern Japan have both impacted kelp directly and enhanced feeding rates of herbivorous fish, triggering shifts from beds of *Ecklonia cava* to barrens (Vergés et al. 2014).

Changes in the palatability of kelp, and thus its vulnerability to herbivores, could be driven by temperature-induced changes to the morphological or chemical characteristics of the kelp tissue. Herbivores alter both rate of consumption and feeding preference based on the palatability of their food sources, selecting foods that have fewer mechanical or chemical defenses, or greater nutritional content (Steneck & Watling 1982, Duffy & Paul 1992, Kraufvelin et al. 2006, Pansch et al. 2008). Warmer temperatures decreased palatability of *Ecklonia radiata* by increasing the C/N ratio (Staehr & Wernberg 2009), as herbivores tend to select food sources enriched in nitrogen (Duffy & Paul 1992). In contrast, increases in temperature prevented the induction of anti-herbivore defenses in the brown alga *Fucus vesiculosus*, increasing consumption (Weinberger et al. 2011).

Kelp (and other brown algae) produce phlorotannins (polymers of phloroglucinol) that can act as a chemical defense against herbivory (Steinberg 1984, Targett & Arnold 1998). However, the effectiveness of phlorotannins as a deterrent may depend on their molecular size or structural characteristics (Boettcher & Targett 1993, Van Altena & Steinberg 1992), as well as herbivore tolerance (Kubanek et al. 2004). Phlorotannins likely have multiple ecological roles (Amsler & Fairhead 2006), as they also act as antioxidant compounds and can be induced by UV stress (Cruces et al. 2012). Kelp may defend against cellular damage caused by thermal stress by upregulating antioxidant compounds, such as phlorotannins, to combat the effects of reactive oxygen species. However, in the 3 species examined to date, thermal stress was not observed to induce phlorotannins, and at high levels may even inhibit phlorotannin production (Cruces et al. 2012, 2013). Phlorotannins have also been implicated as chemical defenses against epibionts (Wikström & Pavia 2004, Iken et al. 2009), suggesting that temperature-induced changes in phlorotannin content could affect the vulnerability of kelp to both grazing and fouling.

In Nova Scotia kelp beds, the gastropod mesograzer *Lacuna vincta* is a dominant herbivore that feeds by creating surface excavations and perforations on kelp blades (Johnson & Mann 1986). Grazing by *L. vincta* removes kelp tissue not only directly, but also indirectly, by increasing erosion and breakage of blades (Johnson & Mann 1986, Duggins et al. 2001, Krumhansl & Scheibling 2011, Krumhansl et al. 2011), which can result in large-scale loss of kelp biomass (Fralick et al. 1974, O'Brien et al. 2015). Although a generalist herbivore, *L. vincta* exhibits dietary selectivity both among algal species and among tissue types within species, with a strong preference for *Saccharina latissima* and avoidance of tissues high in phlorotannins (Johnson & Mann 1986, Chavanich & Harris 2002, Toth & Pavia 2002, Dubois & Iken 2012). Temperature-mediated changes in defensive chemicals, tissue toughness, or nutritional value of *S. latissima* could affect feeding rate and selectivity of *L. vincta* and the impact of these snails on kelp beds.

The invasive bryozoan *Membranipora membranacea* also causes extensive biomass loss from Nova Scotian kelp beds, by encrusting kelp blades, weakening the tissue and leading to blade breakage (Krumhansl et al. 2011). Outbreaks of *M. membranacea*, combined with large wave events, result in the large-scale defoliation of kelp beds (Saunders & Metaxas 2008, Scheibling & Gagnon 2009). Since the introduction of *M. membranacea* to the region in the early 1990s, the extent of bryozoan outbreaks and consequent damage to kelp beds have been closely tied to warming temperatures (Scheibling & Gagnon 2009, Saunders et al. 2010), which result in earlier settlement (Saunders & Metaxas 2007) and increased colony growth of the bryozoan (Saunders & Metaxas 2009). The damage inflicted on kelp beds by *M. membranacea* is an indirect effect of increasing tem-

perature, which may act synergistically with the direct effects of increased temperature on kelp. Increased temperature also may limit population growth of *M. membranacea* on kelp blades if it causes a reduction in tissue quality that limits larval settlement or the persistence of newly established colonies. In the laboratory, larvae of *M. membranacea* exhibit settlement preferences, both for algal species and for specific areas of kelp blade (Matson et al. 2010), suggesting that they can detect differences in substrate quality. Increases in temperature that degrade the structural integrity of blade tissue, or increase phlorotannin content, could prevent settling larvae from attaching, resulting in an antagonism between the direct effects of temperature on the kelp and the effects of temperature on the interaction between kelp and *M. membranacea.*

We examined the potential for synergistic or antagonistic effects of *L. vincta*, *M. membranacea* and warming seawater temperature on biomass loss from Nova Scotia kelp beds. Specifically, we investigated the impact of warming temperatures on the phlorotannnin content and C/N ratio of tissue of *Saccharina latissima*, *Laminaria digitata* and *Agarum clathratum* in a laboratory experiment, as changes in these chemical properties could potentially alter interactions of the kelp with *L. vincta* or *M. membranacea.* We used the feeding experiments to determine whether temperature-induced changes in kelp tissue quality altered the feeding rate of *L.vincta*. We predicted that changes in the kelp tissue at warmer temperatures would make kelp easier to consume or more palatable, leading to increased feeding rate. Increases in feeding rate would indicate a synergism where warming temperature both directly and indirectly (through herbivory) increases kelp tissue loss. We also examined the effect of temperature-induced changes in kelp tissue quality on settlement of *M. membranacea*, predicting that settlement would be reduced on degraded kelp exposed to warmer temperatures. Reduced settlement rate would indicate an antagonism, whereby increasing temperature directly would result in increased loss of kelp biomass, but at the same time mitigate loss caused through encrustation by the bryozoan.

MATERIALS AND METHODS

Chemical composition of kelp tissue

Experimental design. Mature individuals of *Saccharina latissima* (1 to 1.5 m blade length), *Laminaria digitata* and *Agarum clathratum* (0.5 to 1.0 m), were collected by SCUBA from 12 m depth at Splitnose Point (44° 28′ 38.45″ N, 63° 32′ 48.21″ W) in June/July 2013. Kelp were transported to the laboratory in coolers, and stored upon arrival in a 3000 l circular tank (1.87 m diameter, 1.08 m height) with continuously flowing ambient seawater (flow rate: 430 l h^{-1}) by fixing the kelp holdfasts with elastic bands to plastic racks suspended within the tank. Within 24 h of collection, 9 individuals of each species were suspended in experimental tanks of similar size and flow rate (total: 27 individuals per tank) and exposed to 1 of 3 temperature treatments: 11, 18 or 21°C. These temperatures represent a growth optimum for *S. latissima* and *L. digitata* (11°C), a typical maximum average temperature experienced over 1 to 2 weeks (18°C) (Scheibling et al. 2013), and an anticipated maximum temperature based on climate change predictions (21°C) (Müller et al. 2009). Any herbivores present on the surface of the kelp were removed before placement in the temperature treatments.

Tissue samples (5.5 cm diameter) were collected for chemical analysis from 3 individuals of each species within 24 h of field collection (before temperature treatment), from 3 individuals of each species after 1 wk exposure to 11 and 21°C, and from another 3 individuals of each species after 2 wk exposure to 11 and 18°C (for phlorotannin content, % dry wt) or 3 wk exposure to 11 and 18°C (for C/N ratio) (Fig. 1). Exposure time at which phlorotannins and C/N were assessed differed because low survival after 3 wk at 18°C did not provide sufficient replicates for phlorotannin quantification. Tissue samples were excised 30 cm from the blade–stipe interface in *S. latissima*, and 15 cm from the blade–stipe interface in *L. digitata* and *A. clathratum*, to control for any variation in chemical properties along the length of a blade. Three trials of the experiment were conducted in June/July 2013 and all tissue samples were stored at −80°C until further processing. Tissue samples for determination of C/N were collected only in the first trial (n = 3 individuals) and were dried at 60°C for 48 h until constant weight. Tissue samples for phlorotannin content were collected in all 3 trials (n = 9 individuals). During the trials, temperature was recorded in each tank with data loggers (Maxim Integrated Thermochron iButtons) as 11.6 ± 0.9, 18.2 ± 1.6, and 20.8 ± 1.1°C (mean ±1 SD, n = 58).

To track changes in the chemical composition of kelp blades over the summer, 10 to 15 mature *S. latissima* (1.0 to 1.5 m blade length) were collected monthly from 8 m depth at Splitnose Point, from 25 June to 27 August 2013 and from 30 June to 25 September 2014. Kelp were transported to the laboratory

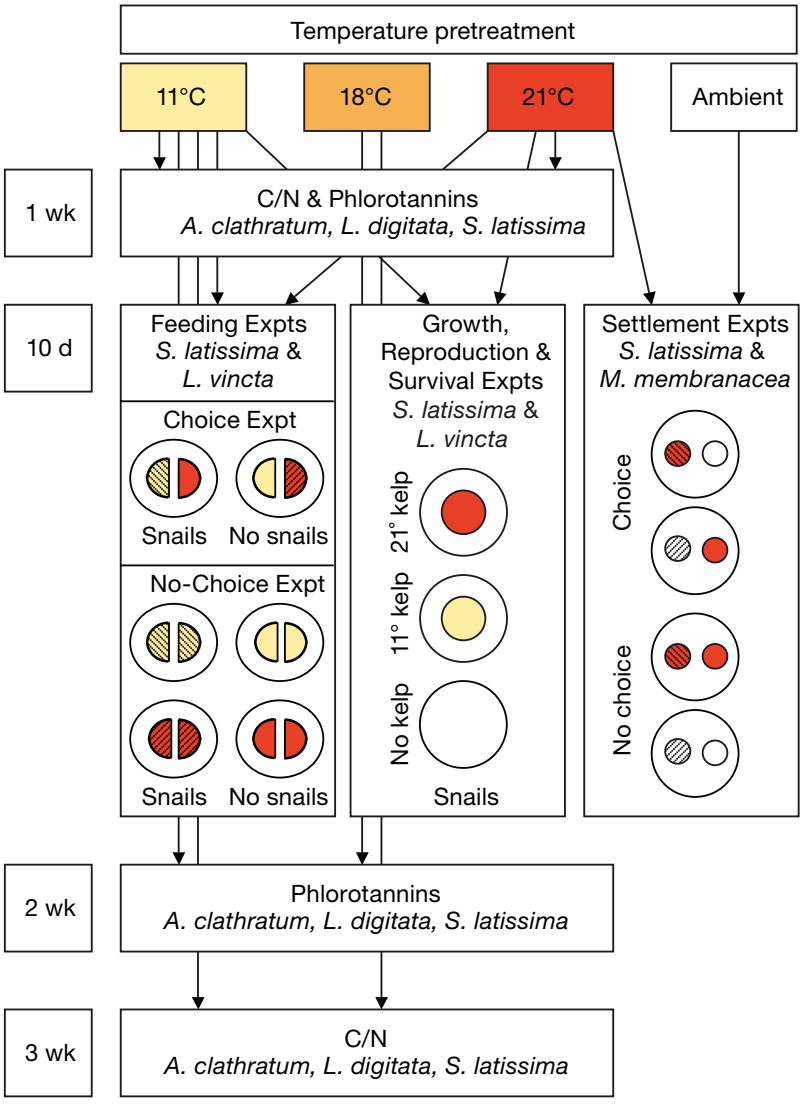

Fig. 1. Schematic of temperature pretreatments and experimental design for quantification of C/N and phlorotannin in *Agarum clathratum*, *Laminaria digitata* and *Saccharina latissima*, feeding experiments and growth, reproduction and survival experiments (*Lacuna vincta* on *Saccharina latissima*), and settlement experiments (*Membranipora membranacea* on *S. latissima*). In feeding, growth, reproduction and survival, and settlement experiments, temperature pretreatment of disks or half-disks is indicated by colour. Pairs of disks or half-disks sharing the same texture (hatched or open) come from one individual of *S. latissima*. Pairs with one hatched and one open disk or half-disk are made up of 2 individuals. See text for full description of experimental design

in coolers, and, immediately on arrival, 5.5 cm diameter tissue samples were excised 30 cm from the blade–stipe interface. Tissue samples were stored at –80°C until further processing. Temperature at the collection site was continuously monitored over the collection periods using data loggers (Onset HOBO Pendant) anchored to the seabed.

C/N analysis. Dried algal tissue was ground to a fine homogeneous powder using a mortar and pestle and packaged in known weights into tin capsules. C and N content were determined using a Costec ECS 4010 CHNSO analyzer with acetanilide as a standard (detection limit = 0.001 mg).

Phlorotannin analysis. To extract phlorotannins, tissue samples were first freeze-dried and ground to a fine powder with a mortar and pestle. For each sample, 100 mg dried tissue was placed in 5 ml 70% acetone and extracted overnight with continuous shaking (Koivikko et al. 2005). Samples were then centrifuged for 10 min at 3200 × *g* and 0.05 ml supernatant was withdrawn for phlorotannin quantification. Phlorotannin content was determined using the Folin-Ciocalteu assay (Van Alstyne 1995) with phloroglucinol (1,3,5-trihodroxybenzene, Sigma-Aldrich) as a standard. The 0.05 ml aliquot was mixed with 1.0 ml distilled water and 1.0 ml 40% Folin-Ciocalteu reagent. After standing 5 min, 1.0 ml $NaCO_3$ was added and samples were incubated for 30 min at 50°C. Absorbance was read at 765 nm using a Cary WinUV 4000 spectrophotometer (Agilent Technologies, detection limit = 0.23 mg).

Statistical analyses. The effect of temperature treatment after 1 wk (11 and 21°C) or after 3 wk (11 and 18°C) on C/N ratio was analyzed using 2-tailed independent samples *t*-tests, for each kelp species. Variance was heterogeneous for C/N ratio data for *A. clathratum* and *L. digitata* after 1 wk, so the Welch-Satterthwaite modification of degrees of freedom was used. The effect of temperature treatment (1 wk at 11 or 21°C, or 2 wk at 11 or 18°C; fixed factors) and trial (random factor) on phlorotannin content was examined using 2-way ANOVA. If the trial × treatment interaction was highly non-significant ($p > 0.20$), the interaction was removed and the main effects of temperature and trial were tested against the pooled trial × treatment MS and residual MS (Winer et al. 1991). Seasonal differences in phlorotannin content or C/N ratio among sampling dates were examined using 1-way ANOVA. Post hoc comparisons of sampling dates were performed using Tukey's HSD test.

Temperature-induced changes in kelp as a food source

Material collection and preparation. *Lacuna vincta* were collected along with associated *S. latissima* from 8 to 12 m depth at Splitnose Point in June/July 2013. Snails and kelp were transported to the laboratory in coolers. Within 24 h of collection, *L. vincta* were removed from the kelp blades and placed within fine mesh bags in a tank with flow-through ambient seawater. Once cleaned of snails, thalli of *S. latissima* were suspended in the temperature treatment tanks (as in temperature experiment) and pretreated for 10 d at either 11 or 21°C before use in feeding or survival, growth and reproduction experiments. *S. latissima* were placed in temperature pretreatments within 24 h of collection for use in feeding experiments, and within 2 wk of collection for the survival, growth and reproduction experiment. Before the start of the feeding experiments, *L. vincta* were fed kelp ad libitum for 7 d, and then starved for 3 d.

Feeding experiments. The effect of temperature-induced changes in kelp tissue quality on *L. vincta* feeding rate and preference were examined by conducting choice and no-choice feeding experiments with 2 diet treatments: *S. latissima* pretreated at 11 or 21°C. Two disks of kelp (5.5 cm diameter) were excised 30 cm from the blade–stipe interface from each of 8 individuals of *S. latissima* (maintained for 10 d in the experimental temperatures), and then cut in half. In the choice experiment, the initial blotted wet weight of each half-disk was recorded, and half-disks from the 2 diet treatments were paired by weight (Fig. 1). Each set of paired half-disks was placed in a flow-through cylindrical container (10 cm diameter, 8 cm height, with 0.2 cm holes) with a mesh top (n = 8). Four of the containers were randomly designated as autogenic controls to record changes in kelp mass in the absence of snails. Groups of 8 snails (shell length 4 to 7 mm) were individually measured (0.1 mm precision) and added to each of the other 4 containers. In the no-choice experiment, half-disks from the same individual of *S. latissima* were paired, weighed and placed together in 16 flow-through containers. Eight containers (4 with *S. latissima* pretreated at 11°C, 4 with *S. latissima* pretreated at 21°C) were randomly designated as autogenic controls and the remaining 4 containers for each diet treatment received groups of 8 snails from the same population and size range as those in the choice experiment (Fig. 1).

The containers were weighted and submerged, and randomly interspersed in the same flow-through seawater table, where they were maintained for 5 d. The mass of algal tissue grazed in each container was calculated as the change in blotted wet weight (0.001 g precision) of the half-disk(s) from the beginning to the end of the 5 d period. For both the choice and no-choice experiments, 3 trials with 3 to 4 replicate containers were conducted in June and July 2013. Water temperature during each trial was (°C, mean ± SD, n = 6): trial 1, 7.89 ± 1.27; trial 2, 10.17 ± 0.68; trial 3, 8.96 ± 1.58. Because of a recording failure within the Aquatron facility at Dalhousie University, temperature during each trial was obtained from average daily seawater temperature at 8 m depth at Splitnose Point (~15 km from the Aquatron seawater intake, and at the same depth). Temperatures in Aquatron during August 2013 were generally within 1°C of those at Splitnose Point (mean ± SD difference in temperature: 0.11 ± 0.86°C; n = 28)

Survival, growth and egg production. The effect of temperature-induced changes to kelp tissue quality on the survival, growth and reproduction of *L. vincta* was determined in an 8 wk laboratory experiment (13 July to 5 September 2013). Groups of 8 snails were placed in flow-through containers (as in feeding experiments) and randomly assigned 1 of 3 diet treatments: *S. latissima* pretreated at 11°C, *S. latissima* pretreated at 21°C, or starved controls (n = 15 containers for each treatment). All containers were randomly placed in a flow-through seawater table. Fed treatments were provided 5.5 cm diameter disks of kelp pretreated for 10 d in the 11 or 21°C temperature treatments, as in the feeding experiments. Kelp disks were replaced weekly. Snails were marked with nail polish and their individual growth rates recorded by measuring changes in shell length from digital photographs, taken initially and then at biweekly intervals for 8 wk. Shell length was measured using image analysis software (ImageJ). Initial shell lengths were 3 to 8 mm, and did not differ among treatments (1-way ANOVA: $F_{2,357} = 0.33$, p = 0.72). Growth was calculated as the change in shell length over each 2 wk sampling interval. Dead snails and egg masses were counted and removed at each measurement interval. Reproductive output per snail for each container was calculated as the number of egg masses produced divided by the number of snails. Upon termination of the experiment, a subset of surviving snails (from 30 containers) was sexed. Sex ratio in the sampled containers did not differ among treatments (1-way ANOVA, $F_{2,27} = 0.11$, p = 0.90) and the mean (± SD) proportion of females was

0.51 ± 0.29 (n = 123). The sex ratio in each container was therefore assumed to be 1:1.

Statistical analyses. Analysis of feeding experiments incorporated the controls for autogenic changes in kelp mass as described in Peterson & Renaud (1989). In the no-choice experiment, mass changes (mg d^{-1}) of half-disks were analyzed using 3-way ANOVA with diet treatment (pretreatment at 11 or 21°C) and herbivore (presence, absence) as fixed factors, and trial as a random factor. There was a significant trial × herbivore × diet interaction ($F_{2,32}$ = 11.42, p < 0.001), so trials were analyzed separately with 2-way ANOVA. Data in trial 3 were log transformed to meet the assumption of homogeneity of variance (Levene's test, p > 0.05). In the choice experiment, the difference in mass change between diet treatments was calculated for each replicate container, and the differences for containers with and without snails were compared using a 2-way ANOVA with herbivore (presence, absence) as a fixed factor and trial as a random factor. Although the interaction between trial and herbivore was not significant ($F_{2,16}$ = 13.03, p = 0.11), the trials were analyzed separately using 1-way ANOVA for consistency with the no-choice feeding experiment. Data in trial 3 did not meet the assumption of homogeneity of variance even after transformation, and results from untransformed data are presented.

Differences in growth rate of *L. vincta* between diet treatments (*S. latissima* pretreated at 11 or 21°C; fixed factor) and among weeks (random factor), with repeated measures within weeks, were examined using repeated-measures ANOVA on the average length change (mm) of snails within each container over each 2 wk interval. Similarly, differences in the effect of diet (kelp pretreated at 11 or 21°C; fixed factor) on the number of egg masses $snail^{-1}$ and proportion of snails surviving over each 2 wk interval (random factor) in each container were analyzed using repeated measures ANOVA, with repeated measures within weeks. The proportion of snails surviving violated the assumption of sphericity (Mauchly's test, p < 0.05), and the Greenhouse-Geisser adjustment was used for p-values.

Temperature-induced changes in kelp as a substrate

Material collection and preparation. The effect of the temperature-induced changes in kelp tissue on settlement of *Membranipora membranacea* was examined with a settlement choice experiment. Larvae of *M. membranacea* were isolated from plankton samples collected from St. Margaret's Bay (~35 km west of Splitnose Point) in October/November 2013 and July/August 2014. *S. latissima* was collected over the same periods from 8 to 12 m depth at Splitnose Point, and pretreated for 10 d in experimental tanks with either ambient (see below) or 21°C seawater (tank set up as in temperature experiment). Tissue samples (trials 1 and 2: 11 cm^2 half-disks; trials 3 to 5: 8 cm^2 disks) were excised from pretreated kelp, 30 cm from the blade-stipe interface, paired and placed in 250 ml beakers with filtered seawater. Pairs consisted either of 2 samples pretreated at ambient temperature, 2 samples pretreated at 21°C, or 1 sample pretreated at ambient temperature and 1 sample at 21°C (Fig. 1). Groups of 30 competent larvae of *M. membranacea* were visually identified and isolated from the plankton samples using a dissecting microscope (20× magnification), and then added to each beaker and allowed 72 h to settle on the kelp substrates. During this time, beakers were placed in a continuous-flow seawater table to maintain the beakers at ambient seawater temperature. After 72 h, the kelp was examined microscopically and the number of larvae that settled on each sample was counted. 3 trials were conducted in October to November 2013 (mean ± SD ambient water temperature: 14.4 ± 2.2°C, n = 30) and 2 trials in July to August 2014 (9.3 ± 2.1°C, n = 20). Ambient water temperature was recorded in the pretreatment tank at 1 h intervals with data loggers (Maxim Integrated Thermochron iButtons). A total of 28 larvae were scored, and all were observed to settle on one of the 2 kelp substrates. Although area of tissue varied among trials, total settlement within beakers did not differ with total area available for settlement (1-way ANOVA, $F_{1,18}$ = 0.02, p = 0.40). The number of settlers on each sample was divided by the area of the sample, giving settlers cm^{-2}.

Statistical analyses. The effect of the temperature-mediated quality of the kelp tissue on settlement of *M. membranacea* was determined by comparing the number of larvae that settled on a substrate (kelp pretreated at ambient temperature or 21°C) when larvae were given a choice of substrates to the number of larvae that settled on that substrate when there was no choice. Beakers in which no settlement occurred were not included in the analyses. For the comparisons using kelp pretreated at ambient temperature, all 5 trials were included in the analysis. However, for the comparisons using kelp pretreated at 21°C, only 3 trials (those with settlement in both choice and no-choice beakers) were included. In the no-choice

beakers, settlement did not differ between the 2 tissue samples within each beaker (paired *t*-tests, ambient beakers: $t_5 = 0.31$, p = 0.77; 21°C beakers: $t_4 = -0.93$, p = 0.41); to maintain independence of replicates, 1 sample was randomly selected from each no-choice beaker for the analysis. The effect of treatment (choice, no-choice; fixed factor) and trial (random factor) on the number of settlers cm^{-2} was examined using 2-way ANOVA for each temperature pretreatment (ambient, 21°C). Because there was no treatment by trial interaction (p > 0.20) at either temperature, the interaction was removed and treatment and trial were tested against pooled treatment × trial MS and residual MS (Winer et al. 1991).

RESULTS

Chemical composition of kelp tissue

There was no effect of temperature on C/N ratio for any of the 3 kelp species after 1 wk exposure between 11 and 21°C, or after 3 wk exposure between 11 and 18°C (Fig. 2, Table 1). There was a trend of increasing C/N ratio over the summer in both 2013 and 2014 (Fig. 3), although differences among sampling dates were not significant (Table 2).

Phlorotannin content of *Agarum clathratum* was significantly lower after 1 wk exposure to 21°C compared to 11°C (Fig. 2, Table 3), but there was no difference after 2 wk exposure between 11 and 18°C (Fig. 2, Table 3). Phlorotannin content in *Laminaria digitata* and *Saccharina latissima* was approximately 10% and 25% that of *A. clathratum*, respectively (Fig. 2). Phlorotannin content of *L. digitata* was slightly lower (by ~0.2% dry wt) at 18°C than 11°C, after 2 wk exposure. There was no difference in phlorotannin content for *S. latissima* species after 1 wk exposure between 11 and 21°C, or after 2 wk exposure between 11 and 18°C (Fig. 2, Table 3). Phlorotannin content of *S. latissima* collected from Split-nose Point was higher in 2013 than in 2014 across all sampling dates (Fig. 3). During 2013, phlorotannin content increased throughout the summer (Fig. 3), although there were no significant differences among sampling dates (Table 2). In 2014, phlorotannin content of *S. latissima* increased slightly from June to August and then decreased significantly by 0.6% dry wt in September (Fig. 3, Table 2).

Temperature-induced changes in kelp as a food source

In the no-choice feeding experiment, kelp loss was greater in the presence of *Lacuna vincta*, and auto-

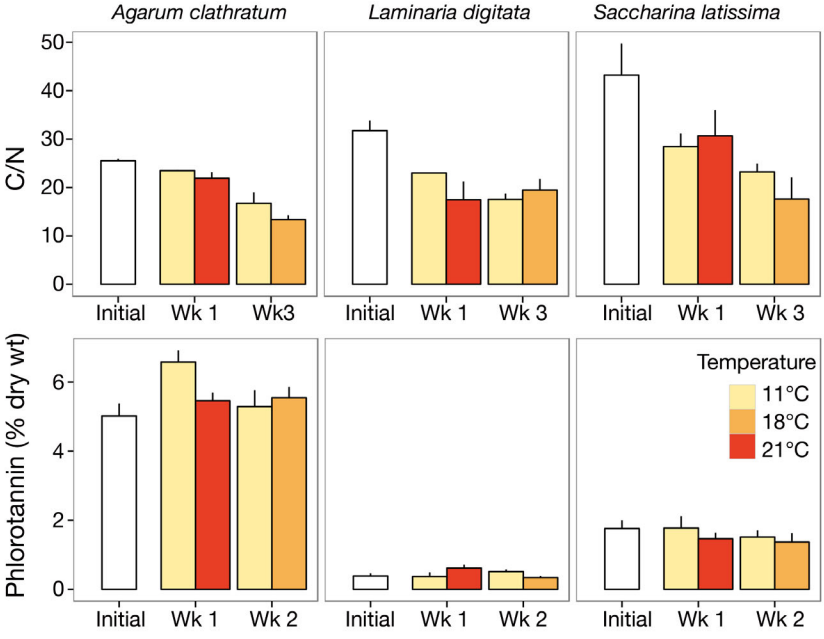

Fig. 2. Mean (+ 1 SE) C/N ratio (n = 3) and phlorotannin content (% dry wt; n = 9) of *Agarum clathratum*, *Laminaria digitata* and *Saccharina latissima* immediately after collection from the field (initial) and after 1 wk exposure to 11 and 21°C, and 2 wk or 3 wk exposure to 11 and 18°C in the laboratory

Table 1. Results of independent samples *t*-test to examine differences in C/N ratio between 11 and 21°C temperature treatments after 1 wk exposure, or between 11 and 18°C treatments after 3 wk exposure, for each kelp species

Species	Exposure (wk)	Temperature (°C)	*t*	df	p
Agarum clathratum	1	11 vs. 21	1.24	2.1[a]	0.34
	3	11 vs. 18	1.37	4	0.24
Laminaria digitata	1	11 vs. 21	1.45	2.0[a]	0.28
	3	11 vs. 18	−0.73	4	0.51
Saccharina latissima	1	11 vs. 21	−3.76	4	0.73
	3	11 vs. 18	1.17	4	0.30

[a]Welch-Satterthwaite adjustment due to unequal variance (*F*-test, p < 0.05)

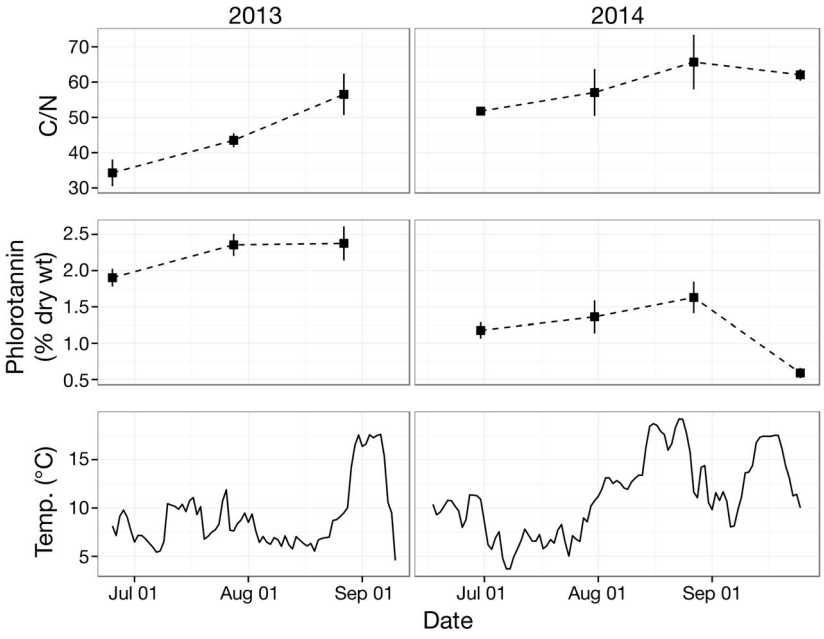

Fig. 3. Mean (± 1 SE) C/N ratio (n = 3) and phlorotannin content (% dry wt; n = 10) of blade tissue of *Saccharina latissima* collected monthly in summer 2013 and 2014 from 8 m depth at Splitnose Point (black squares), and mean daily temperature at the collection site

Table 2. Results of ANOVA comparing phlorotannin content (Phl) or C/N ratio of blade tissue of *Saccharina latissima* in the field, among sampling dates (2013: 18 and 25 June, 15 July (Phl only), 28 July, 27 August; 2014: 30 June, 31 July, 27 August, 25 September). Significant results are shown in **bold**

Year	Variable	*F*	df	p	Tukey HSD
2013	C/N	3.70	3, 8	0.06	
	Phl	2.61	4, 31	0.09	
2014	C/N	1.46	3, 8	0.30	
	Phl	4.08	3, 24	**0.001**	Sep < Aug = Jul

genic loss of kelp pretreated at 21°C was greater than for kelp pretreated at 11°C (Fig. 4, Table 4). In trial 1, a significant diet by herbivore interaction indicated that grazing rates of *L. vincta* were greater on kelp pretreated at 21°C than at 11°C (Fig. 4, Table 4). There was no difference in grazing rates between diets in trials 2 and 3, as indicated by the non-significant interaction term (Fig. 4, Table 4). In the choice experiment, the difference in mass change between half-disks of kelp pretreated at 11 and 21°C was significantly greater in treatments with *L. vincta* than without *L. vincta* in trial 1, indicating that snails fed preferentially on kelp pretreated at 21°C (Fig. 4, Table 5). This was not the case in trials 2 and 3, although the direction of differences was consistent (Fig. 4, Table 5). As in the no-choice experiment, autogenic mass loss was greater for kelp pretreated at 21°C in the choice experiment (Fig. 4).

At the end of the 8 wk growth extperiment, mean growth (change in shell length) in containers of snails fed either of the 2 kelp diets was an order of magnitude higher than the mean growth of starved controls (Fig. 5a). There was no effect of kelp diet (*S. latissima* pretreated at 11 or 21°C) on the growth of snails over the 8 wk experiment, and no interaction between kelp diet and sampling week, although growth did differ among sampling weeks (Table 6). There was no production of egg masses in the starved controls after week 2, and egg production snail^{-1} increased with sampling week in containers fed either kelp diet (Fig. 5b). There was no difference in the number of egg masses produced snail^{-1} between containers fed kelp pretreated at 11°C and those fed kelp pretreated at 21°C (Table 6). Egg production did differ among weeks, but there was no interaction between kelp diet and sampling week (Table 6). Mean survival of snails in the experiment was high, exceeding 90% over the first 6 wk in all diet treatments (Fig. 5c). After 8 wk, mean survival of the starved controls declined to 75% (Fig. 5c). There was no difference in the survival between the 2 kelp diets, and no interaction between diet and sampling week, but there was a difference in survival among weeks (Table 6).

Temperature-induced changes in kelp as a substrate

There was no difference in the number of larvae of *Membranipora membranacea* cm^{-2} that settled on *S. latissima* pretreated at 21°C or at ambient temperature when it was offered as a choice, compared to when it was offered without a choice (Fig. 6, Table 7). The lack of a difference between choice and no-choice treatments indicates that larvae of *M. membranacea* have no preference for, or avoidance of, kelp pretreated at 21°C compared to kelp pretreated at ambient temperature.

Table 3. Results of 2-way ANOVA to examine differences in phlorotannin content (% dry wt) between 11 and 21°C temperature treatments (fixed factor) and trials (random factor) after 1 wk exposure, or between 11 and 18°C treatments after 2 wk exposure, for each kelp species. Temperature and Trial were tested against pooled Temp. × Trial MS and residual MS. Significant results are shown in **bold**

Exposure (wk)	Source of variation	df	MS	*F*	p
Agarum clathratum					
1	Temp. (11 vs. 21)	1	4.27	6.37	**0.03**
	Trial	2	0.43	0.64	0.54
	Temp. × Trial	2	0.38	0.51	0.61
	Residual	10	0.73		
2	Temp. (11 vs. 18)	1	0.30	0.22	0.64
	Trial	2	2.16	1.56	0.24
	Temp. × Trial	2	0.47	0.31	0.74
	Residual	12	1.53		
Laminaria digitata					
1	Temp. (11 vs. 21)	1	0.27	2.64	0.13
	Trial	2	0.14	1.38	0.28
	Temp. × Trial	2	0.05	0.50	0.62
	Residual	12	0.11		
2	Temp. (11 vs. 18)	1	0.15	5.28	**0.04**
	Trial	2	0.06	2.20	0.15
	Temp. × Trial	2	0.04	1.65	0.23
	Residual	11	0.03		
Saccharina latissima					
1	Temp. (11 vs. 21)	1	0.43	0.69	0.42
	Trial	2	1.02	1.65	0.23
	Temp. × Trial	2	0.46	0.71	0.51
	Residual	12	0.65		
2	Temp. (11 vs. 18)	1	0.09	0.21	0.65
	Trial	2	0.67	1.51	0.25
	Temp. × Trial	2	0.31	0.66	0.54
	Residual	12	0.46		

DISCUSSION

Effects of temperature on chemical composition of kelp tissue

The increase in C/N ratio observed in *Saccharina latissima* over the summer follows the expected pattern of seasonal variation in this species (Gevaert et al. 2001, Nielsen et al. 2014). This pattern results from both a decrease in N and an increase in C over the summer (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m537p105_supp.pdf, Gevaert et al. 2001, Nielsen et al. 2014). Over winter, when growth is light-limited, N is stored and then used to support growth in spring and summer (Nielsen et al. 2014). During N-limited growth in

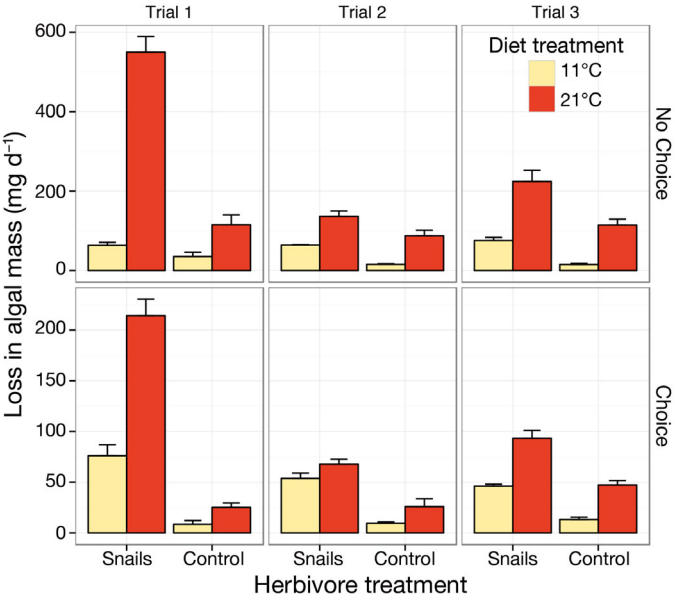

Fig. 4. Mean (+ 1 SE) rates of mass loss of *Saccharina latissima* pretreated for 10 d at 11°C or 21°C in the presence of *Lacuna vincta*, or in controls without *L. vincta* in 3 trials of choice and no-choice feeding experiments (trial 1: n = 3; trials 2 & 3: n = 4)

Table 4. Results of 2-way ANOVA examining effects of diet (*Saccharina latissima* pretreated for 10 d at 11 or 21°C) and herbivore (presence or absence of *Lacuna vincta*) on rate of kelp mass loss (mg d^{-1}) in 3 trials of the no-choice feeding experiment. Significant results are given in **bold**

Trial	Source	df	*F*	p
1	Diet	1, 8	45.81	**<0.001**
	Herbivore	1, 8	30.55	**<0.001**
	Diet × Herbivore	1, 8	23.53	**0.001**
2	Diet	1, 12	13.25	**0.003**
	Herbivore	1, 12	6.08	**0.03**
	Diet × Herbivore	1, 12	0.00	0.99
3[a]	Diet	1, 12	33.89	**<0.001**
	Herbivore	1, 12	18.91	**<0.001**
	Diet × Herbivore	1, 12	3.87	0.073

[a]Mass change log-transformed to meet assumption of homoscedasticity

Table 5. Results of 1-way ANOVA comparing difference in rate of mass change (mg d^{-1}) of *Saccharina latissima* pretreated for 10 d at 11 vs. 21°C between herbivore treatments (presence or absence of *Lacuna vincta*) in 3 trials of the choice feeding experiment. Significant results are given in **bold**

Trial	df	F	p
1	1, 4	102.6	**<0.001**
2	1, 6	0.016	0.91
3[a]	1, 6	0.605	0.47

[a]Data heteroscedastic even after transformation. Untransformed data are presented

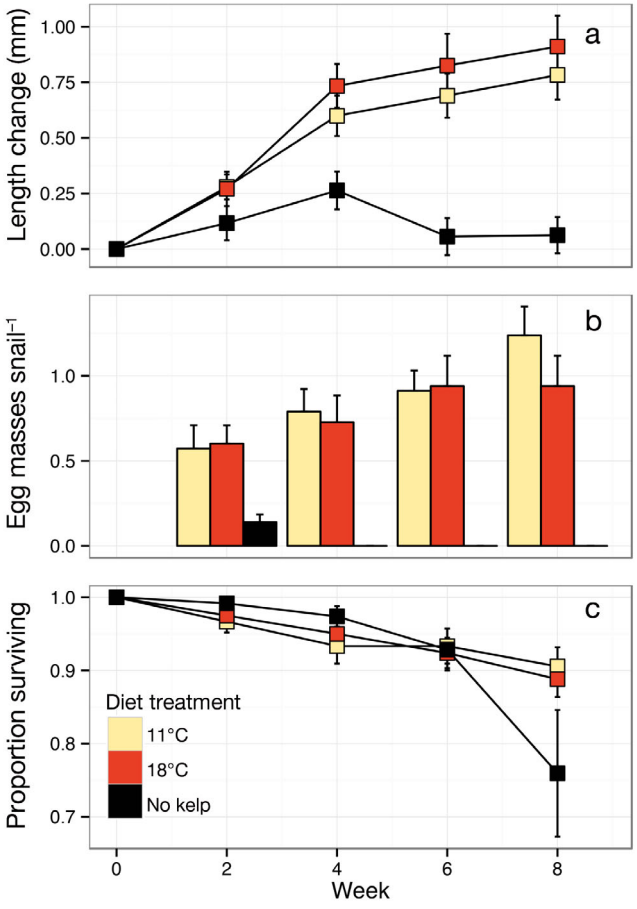

Fig. 5. (a) Mean (± 1 SE) growth (length change) of *Lacuna vincta* relative to initial shell length when fed 3 diets: *Saccharina latissima* pretreated 10 d at 11°C, *S. latissima* pretreated 10 d at 21°C, or no kelp. SE represents the variation among 15 containers. (b) Mean (+ 1 SE) egg masses snail^{-1} produced by *L. vincta* when fed 3 diets (as above; n = 15 containers). (c) Mean (± 1 SE) proportion of *L. vincta* surviving (relative to initial number of individuals) when fed 3 diets (as above; n = 15 containers)

summer, C is stored as carbohydrates to allow for continued growth through the winter (Nielsen et al. 2014). It has been suggested that changes in temperature also may contribute to this pattern. A decrease in N content with increasing temperature has been observed for *S. latissima* from Helgoland (Olischläger et al. 2014) as well as in the kelps *Eklonia radiata* (Staehr & Wernberg 2009) and *Eisenia arborea* (Matson & Edwards 2007). We found that neither 1 wk exposure to 21°C nor 3 wk exposure to 18°C changed the C/N ratio in any of the species we examined, relative to 11°C. N content (% dry wt) of field-collected kelps at the start our experiment (mean ± SE: *Agarum clathratum*, 1.29 ± 0.02; *Laminaria digitata*, 0.92 ± 0.05; *S. latissima*, 0.67 ± 0.08) was less than the critical value of 1.8%, below which the growth of *S. latissima* is N-limited (Chapman et al. 1978). This suggests that upon collection any stores of N had already been consumed and the kelps were N limited throughout the experiment.

Phlorotannin content in *Agarum clathratum* and *L. digitata* was reduced after 1 wk exposure to 21°C and 2 wk exposure to 18°C, respectively. Phlorotannins are a known antioxidant defense in brown algae, induced by exposure to ultraviolet radiation (Gómez & Huovinen 2010, Cruces et al. 2012, 2013). In these studies, phlorotannins were not induced under thermal stress for periods of up to 72 h, despite increased lipid peroxidation indicative of a rise in activity of reactive oxygen species (Cruces et al. 2012, 2013). Furthermore, temperature stress (20 and 28°C) was found to inhibit induction of phlorotannin by ultraviolet radiation (Cruces et al. 2012, 2013), suggesting that high temperatures may hinder the ability of kelp to produce phlorotannins, possibly by damaging the membranes of the Golgi-ER complex, where phlorotannins are produced (Schoenwaelder & Clayton 2000).

Temperature did not effect phlorotannin content of *S. latissima* and had only a small effect on phlorotannin content of *L. digitata*. The observed ~0.2% dry wt decrease in phlorotannin content in *L. digitata,* is unlikely to be ecologically significant, as concentrations of phlorotannins below 1% dry wt have not been reported to deter herbivores (Targett & Arnold 1998). Phlorotannin content of *S. lattisima* and *L. digitata* were low, although similar to previously reported levels in these species: 0.9 – 2.5% dry wt and 0.15 – 0.3% dry wt, respectively (Johnson & Mann 1986, Connan et al. 2006, Dubois & Iken 2012), reducing our ability to detect any effect of temperature. Measurement of non-phlorotannin compounds may have further hindered our ability to detect any effect of temperature (Van Alstyne 1995).

In the field, there was a pattern of increasing phlorotannin content over the summer in *Saccharina latissima*, followed by a decrease in September in 2014. Although phlorotannin content was similar in summer and winter in *S. latissima*, and unrelated to irradiance or nutrient availability (Dubois & Iken 2012), in other brown algae (primarily the order Fucales) phlorotannin content peaks in the spring or summer (Steinberg 1995, Stiger et al. 2004, Kamiya et al. 2010). Higher phlorotannin content in summer has been attributed to increased grazer density, which can induce phlorotannin production (Van Alstyne 1988), or greater energy availability for phlorotannin production during periods of growth limitation (Steinberg 1995, Stiger et al. 2004). Phlorotannin

Table 6. Results of repeated-measures ANOVA comparing proportion of *Lacuna vincta* surviving, average growth (length change) and egg production (egg masses snail^{-1}) between 2 diets (*Saccharina latissima* pretreated at 11 or 21°C; fixed factor). Repeated measures were taken at 2 wk intervals (2, 4, 6 and 8 wk). Significant results are given in **bold**

Variable	Source	df	*F*	p
Proportion surviving[a]	Between Subjects			
	Diet	1	0.0003	0.98
	Container(Diet)	28		
	Within Subjects			
	Week	3	13.73	**<0.001**
	Week × Diet	3	0.90	0.42
	Week × Container(Diet)	84		
Growth	Between Subjects			
	Diet	1	0.52	0.48
	Container(Diet)	28		
	Within Subjects			
	Week	3	11.28	**<0.001**
	Week × Diet	3	0.71	0.55
	Week × Container(Diet)	84		
Egg production	Between Subjects			
	Diet	1	0.21	0.65
	Container(Diet)	28		
	Within Subjects			
	Week	3	8.25	**<0.001**
	Week × Diet	3	1.05	0.37
	Week × Container(Diet)	84		

[a]Assumption of sphericity violated (Mauchly's test p < 0.001), and Greenhouse-Geisser adjustment applied

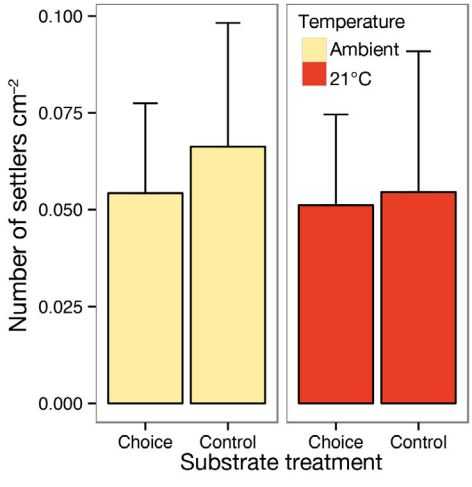

Fig. 6. Mean (+ 1 SE) number of settlers of *Membranipora membranacea* cm^{-2} on *Saccharina latissima* pretreated for 10 d at ambient temperature, or *S. latissima* pretreated for 10 d at 21°C, when a choice of substrates was offered and in a control with no choice of substrates (data from 5 trials pooled, n = 5 to 9). Ambient temperature (mean ± SD): 14.4 ± 2.2°C for 3 trials in Oct/Nov 2013; 9.3 ± 2.1°C for 2 trials in Jul/Aug 2014

content of *S. latissima* was higher in 2013 than in 2014, by ~1% dry wt. Interannual variation in phlorotannin content can reflect changes in environmental conditions, such as grazer densities, irradiance or nutrient availability (Van Alstyne 1988, Pavia & Toth 2000).

Effects of temperature on kelp as a food source

Lacuna vincta consumed more kelp pretreated at 21 than at 11°C in trial 1 of both the choice and no-choice feeding experiments, but not in the other 2 trials. The rate of consumption of kelp in trial 1 was more than twice that in trial 2 or 3 in the choice experiment. The rate of consumption of kelp pretreated at 21°C in trial 1 was at least 2.5-fold greater than that in trials 2 or 3 in the no-choice experiment. Differences in ambient temperature among trials could have caused these differences in feeding rate in response to metabolic demand; however, temperature in trial 1 was lower than that in trial 2 or 3.

The greater feeding rate of *L. vincta* on kelp pretreated at 21°C in trial 1 could reflect a temperature-induced change in the palatability of kelp tissue that is apparent only at high feeding rates. Given that the chemical quality of *S. latissima* was unaffected by temperature, temperature-induced changes to the mechanical properties of kelp may account for the observed increased feed-

Table 7. Results of 2-way ANOVA examining the effect of treatment (choice of substrates or no-choice; fixed factor) and trial (random factor) on the settlement of larvae of *Membranipora membranacea* in 2 temperature treatments, ambient (9.3–14.4°C) and 21°C. Treatment and Trial were tested against pooled Treatment × Trial MS and residual MS.

Temperature	Source of variation	df	MS	*F*	p
Ambient	Treatment	1	0.0019	0.31	0.59
	Trial	4	0.0048	0.76	0.57
	Treatment × Trial	4	0.0088	2.05	0.23
	Residual	5	0.0043		
21°C	Treatment	1	0.0006	0.12	0.74
	Trial	2	0.0031	0.60	0.57
	Treatment × Trial	2	0.0054	1.04	0.42
	Residual	5	0.0052		

ing rate. Higher grazing rates on kelp pretreated at 21°C in both the choice and no-choice experiments indicate that *L. vincta* did not have an active preference for this tissue, but rather that kelp pretreated at 21°C is easier to consume. *L. vincta* has a taenioglossan radula that is more efficient on softer tissues (Steneck & Watling 1982), and prefers young tissue, even in the absence of any difference in C/N ratio or phlorotannin content, likely due to differences in tissue toughness (Toth & Pavia 2002, Chenelot & Konar 2007). Increasing temperature damages and weakens kelp tissue (Table 8), and this weaker tissue may be easier for *L. vincta* to consume.

The greater consumption of kelp pretreated at 21°C in trial 1 indicates that the effect of temperature on the palatability of kelp tissue may depend on the feeding rate of *L. vincta*, and that the direct effects of temperature on kelp tissue loss and temperature-induced changes in herbivory of *L. vincta* can be synergistic when feeding rates are high. However, when feeding rates are lower (as in trials 2 and 3) there is no evidence for temperature-induced changes in palatability of *S. latissima*, and the impacts of temperature and *L.vincta* on kelp tissue loss will likely be additive.

S. latissima is a nutritious food source for *L. vincta* that supports greater growth rates than other algal diets (Chavanich & Harris 2002). In our study, survival, growth and egg production of snails were unaffected by temperature-induced changes in kelp tissue, indicating that the nutritional quality of kelp did not change, as evidenced by the lack of variation in C/N between temperature pretreatments. Mean growth rate of fed snails in our experiment (0.04 to 0.23 mm wk^{-1}) were similar to those previously recorded in the laboratory on a diet of *S. latissima* (0.04 to 0.12 mm wk^{-1}; Chavanich & Harris 2002). The similarity in the success of *L. vincta* (similar growth rates, survival, and reproductive output) fed kelp diets pretreated at 11 and 21°C suggests that populations of *L. vincta* will likely remain stable with increasing temperature, as long as kelp is available: i.e. there are no indirect effects of temperature that affect growth, survival or reproduction of snails.

Table 8. Effects of temperature treatments (11, 14, 18 and 21°C) on chemical and mechanical metrics of kelp tissue quality (C/N, phlorotannin content [% dry wt], blade tissue strength [MPa] and blade tissue extensibility [% length change]) for *Agarum clathratum*, *Laminaria digitata* and *Saccharina latissima*. Quality metrics were significantly decreased by exposure to the listed temperatures

Property	Species	Temperature	Reference
C/N	*A. clathratum*	No effect	This study
	L. digitata		
	S. latissima		
Phlorotannin	*A. clathratum*	21°C	This study
	L. digitata	No effect	
	S. latissima		
Strength	*A. clathratum*	No effect	Simonson et al. (2015)
	L. digitata	18, 21°C	
	S. latissima	14, 18, 21°C	
Extensibility	*A. clathratum*	No effect	Simonson et al. (2015)
	L. digitata	21°C	
	S. latissima	18, 21°C	

Effects of temperature on kelp as a substrate

Temperature pretreatment of kelp tissue did not affect larval settlement of *Membranipora membranacea*. Abundance of settlers observed on kelp pretreated at ambient temperatures and that pretreated at 21°C (mean: 0.05 to 0.07 settlers cm^{-2}) were comparable to the abundance of settlers on *S. latissima* in the field during peak settlement (mean: 0.03 to 0.19 settlers cm^{-2}; Saunders & Metaxas 2007).

Larvae of *M. membranacea* demonstrate settlement preferences both among kelp species and among locations on the kelp thallus (Brumbaugh et al. 1994, Matson et al. 2010). They also exhibit fine-scale searching behaviour along the kelp substrate, which suggests they are able to detect differences in substrate quality (Matson et al. 2010). Across kelp species, larvae of *M. membranacea* show a preference for settlement on young tissue proximal to the blade meristem (Brumbaugh et al. 1994, Denley et al. 2014). The cues attracting larvae to young tissue are unknown, but the persistence of the preference when flow is reversed suggests that larvae use a physical or chemical characteristic of the substrate to cue settlement (Brumbaugh et al. 1994). Phlorotannins have been suggested as a chemical cue preventing the settlement of fouling organisms (Wikström & Pavia 2004). Because the phlorotannin content of *S. latissima* was unaffected by temperature, the kelp substrates in our experiment (pretreated at 21°C and pretreated at ambient temperature, 9.3–14.4°C) had similar levels of chemical deterrents. Brumbaugh et al. (1994) found that damage to the blade reduced settlement and postulated that lar-

vae may avoid older sections of the blade due to greater levels of physical damage there. Temperature stress of 21°C also damages and weakens kelp tissue (Table 8), however we saw no effect of this damage on settlement preference.

The lack of any temperature-induced changes in kelp quality as a substrate for settlement of *M. membranacea* suggests that increased population growth rate due to warming seawater temperatures (Saunders & Metaxas 2007, 2009) will not be retarded by lower settlement rates. The direct effects of temperature on kelp combined with temperature-mediated increases in *M. membranacea* populations are likely to cause large-scale biomass loss from kelp beds in Nova Scotia. However, this biomass loss could be mitigated if temperature-induced changes in substrate quality affect post-settlement mortality. Temperature-induced damage to the meristoderm could cause it break and peel away with the associated bryozoan colonies. Peeling of the outer layer of cells has been documented as a mechanical defense against fouling in several algal species (Sieburth & Tootle 1981, Nylund & Pavia 2005). Even in the absence of changes to post-settlement mortality, the loss of kelp biomass may in turn limit further outbreaks of *M. membranacea* by limiting the availability of preferred substrate.

CONCLUSIONS

An understanding of the simple and cumulative effects of environmental conditions on species interactions is imperative for predicting the effects of climate change on community function. We expected that warming seawater temperature would alter kelp tissue quality, which would in turn affect interactions between kelp and the mesograzer *L. vincta* and encrusting bryozoan *M. membranacea*. However, higher temperature did not affect the quality of *S. latissima* as a substrate for *M. membranacea,* and we observed temperature-induced changes in the palatability of kelp only when grazing rates of *L. vincta* were high. Nutrient content and chemical defense of both *S. latissima* and *Laminaria digitata* were not strongly affected by temperature, suggesting that the quality of these species as a food and a substrate would be similarly unaffected by increases in temperature. A temperature-induced reduction in the chemical defenses of *Agarum clathratum* at 21°C suggests that this species might become more vulnerable to damage by *L. vincta* or *M. membranacea* at high temperatures.

We predict that the direct effects of temperature on kelp tissue, herbivory by *L. vincta* and encrustation by *M. membranacea* will act additively to increase biomass lost from kelp beds as seawater temperature increases. Warmer temperatures are expected to increase both outbreaks of *M. membranacea* (Scheibling & Gagnon 2009, Saunders & Metaxas 2007, 2009) and metabolic rates, and therefore feeding, of herbivores (O'Connor 2009). Encrustation by bryozoans, herbivory by *L. vincta,* and temperature-induced damage all weakened kelp tissue and increased vulnerability to wave forces (Krumhansl et al. 2001, Table 8). No antagonistic effects were manifested through the inhibition of feeding by snails or settlement by *M. membranacea* that would mitigate other temperature effects on kelp. Using future climatic conditions (warming temperatures and larger waves), models of kelp detrital production have also predicted a loss of kelp biomass in Nova Scotia (Krumhansl et al. 2014). Reductions in standing kelp biomass could impact both habitat availability and community productivity (Dayton 1985, Steneck et al. 2002), while changes in the production and export of kelp detritus will impact adjacent coastal and deep-water ecosystems (Krumhansl & Scheibling 2012).

Acknowledgements. We thank J. Lindley, K. Filbee-Dexter, C. Feehan, J. O'Brien, D. Denley, and F. Francis for assistance with field work, and M. Harris, D. Durette Morin and J. O'Brien for their help in the laboratory. This research was funded by Discovery Grants to A.M. and R.E.S from the Natural Sciences and Engineering Research Council (NSERC) of Canada. E.J.S. was supported by an NSERC Canada Graduate Scholarship and a Dalhousie University Scholarship.

LITERATURE CITED

Amsler CD, Fairhead VA (2006) Defensive and sensory chemical ecology of brown algae. Adv Bot Res 43:1–91

Andersen GS, Steen H, Christie H, Fredriksen S, Moy FE (2011) Seasonal patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima* in Skagerrak, Norway: implications for forest recovery. J Mar Biol 2011:690375

Andersen GS, Pedersen MF, Nielsen SL (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). J Phycol 49:689–700

Boettcher AA, Targett NM (1993) Role of polyphenolic molecular size in reduction of assimilation efficiency in *Xiphister mocusus*. Ecology 74:891–903

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar Biol 66:89–94

Brumbaugh DR, West JM, Hintz JL, Anderson FE (1994) Determinants of recruitment by an epiphytic marine bryozoan: field manipulations of flow and host quality.

In: Wilson WH, Stricker SA, Shinn GL (eds) Reproduction and development of marine invertebrates. Johns Hopkins University Press, Baltimore, p 287–301

Chapman ARO, Markham JW, Lüning K (1978) Effect of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. J Phycol 14:195–198

Chavanich S, Harris LG (2002) The influence of macroalgae on seasonal abundance and feeding preference of a subtidal snail, *Lacuna vincta* (Montagu) (Littorinidae) in the Gulf of Maine. J Mollusc Stud 68:73–78

Chenelot H, Konar B (2007) *Lacuna vincta* (Mollusca, Neotaenioglossa) herbivory on juvenile and adult *Nereocystic luetkeana* (Heterokontophyta, Laminariales). Hydrobiologia 583:107–118

Cockrell ML, Sorte CJB (2013) Predicting climate-induced changes in population dynamics of invasive species in a marine epibenthic community. J Exp Mar Biol Ecol 440: 42–48

Connan S, Delisle F, Deslandes E, Gall EA (2006) Intrathallus phlorotannin content and antioxident activity in Phaeophycea of temperate waters. Bot Mar 49:39–46

Cruces E, Huovinen P, Gómez I (2012) Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three South Pacific kelps. Photochem Photobiol 88:58–66

Cruces E, Huovinen P, Gómez I (2013) Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxident activities of two sub-Antarctic brown algae. Mar Biol 160:1–13

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. J Phycol 27:2–8

Dayton PK (1985) Ecology of kelp communities. Annu Rev Ecol Syst 16:215–245

Denley D, Metaxas A, Short J (2014) Selective settlement by larvae of *Membranipora membranacea* and *Electra pilosa* (Ectocarpa) along kelp blades in Nova Scotia, Canada. Aquat Biol 21:47–56

Dubois A, Iken K (2012) Seasonal variation in kelp phlorotannins in relation to grazer abundance and environmental variables in the Alaskan sublittoral zone. Algae 27: 9–19

Duffy JE, Paul VJ (1992) Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. Oecologia 90:333–339

Duggins D, Eckman JE, Siddon CE, Klinger T (2001) Interactive roles of mesograzers and current flow in survival of kelps. Mar Ecol Prog Ser 223:143–155

Fernández C (2011) The retreat of large brown seaweeds on the north coast of Spain: the case of *Saccorhiza polyschides.* Eur J Phycol 46:352–360

Floerl O, Rickard G, Inglis G, Roulston H (2013) Predicted effects of climate change on potential sources of non-indigenous marine species. Divers Distrib 19:257–267

Fralick RA, Turgeon KW, Mathieson AC (1974) Destruction of kelp populations by *Lacuna vincta* (Montagu). Nautilus 88:112–114

Gerard VA, Du Bois KR (1988) Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina.* Mar Biol 97:575–580

Gevaert F, Davoult D, Creach A, Kling R, Janquin MA, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: biometrics and seasonal variations. J Mar Biol Assoc UK 81:727–734

Gómez I, Huovinen P (2010) Induction of phlorotannins during UV exposure mitigates inhibition of photosynthesis and DNA damage in the kelp *Lessonia nigrescens.* Photochem Photobiol 86:1056–1063

Harley CDG, Hughes AR, Hultgren KM, Miner BG and others (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9:228–241

Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH (2012) Effects of climate change on global seaweed communities. J Phycol 48: 1064–1078

Hille Ris Lambers J, Harsch MA, Ettinger AK, Ford KR, Theobald EJ (2013) How will biotis interactions influence climate change-induced range shifts? Ann N Y Acad Sci 1297:112–125

Iken K, Amsler CD, Amsler MO, McClintock JB, Baker BJ (2009) Field studies on deterrent properties of phlorotannins in Antarctic brown algae. Bot Mar 52:547–557

Johnson CR, Mann KH (1986) The importance of plant defense abilities to the structure of subtidal seaweed communities: the kelp *Laminaria longicruris* de la Pylaie survives grazing by the snail *Lacuna vincta* (Montagu) at high population densities. J Exp Mar Biol Ecol 97:231–267

Kamiya M, Nishio T, Yokoyama A, Yatsuya K, Nishigaki T, Yoshikawa S, Ohki K (2010) Seasonal variation of phlorotannin in sargassacean species from the coast of the Sea of Japan. Phycol Res 58:53–61

Koivikko R, Loponen J, Honkanen T, Jormalainen V (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J Chem Ecol 31: 195–212

Kraufvelin P, Salovius S, Christie H, Moy FE, Karez R, Pedersen MF (2006) Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the marine amphipod *Gammarus locusta.* Aquat Bot 84:199–209

Krumhansl KA, Scheibling RE (2011) Detrital production in Nova Scotian kelp beds: patterns and processes. Mar Ecol Prog Ser 421:67–82

Krumhansl KA, Scheibling RE (2012) Production and fate of kelp detritus. Mar Ecol Prog Ser 467:281–302

Krumhansl KA, Lee MJ, Scheibling RE (2011) Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. J Exp Mar Biol Ecol 407:12–18

Krumhansl KA, Lauzon-Guay JS, Scheibling RE (2014) Modeling effect of climate change and phase shifts on detrital production of a kelp bed. Ecology 95:763–774

Kubanek J, Lester SE, Fenical W, Hay ME (2004) Ambiguous role of phlorotannins as chemical defenses in the brown alga *Fucus vesiculosus.* Mar Ecol Prog Ser 277:79–93

Lubchenco J, Gaines SD (1981) A unified approach to marine plant–herbivore interactions. I. Populations and communities. Annu Rev Ecol Syst 12:405–437

Matson PG, Edwards MS (2007) Effects of ocean temperature on the southern range limits of two understory kelps, *Pterygophora californica* and *Eisenia arborea*, at multiple life-stages. Mar Biol 151:1941–1949

Matson PG, Steffen BT, Allen RM (2010) Settlement behavior of cyphonautes larvae of the bryozoan *Membranipora membranacea* in response to two algal substrata. Invertebr Biol 129:277–283

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. Bot Mar 52:617–638

Nielsen MM, Krause-Jensen D, Olesen B, Thinggaard R, Christensen PB, Bruhn A (2014) Growth dynamics of *Saccharina latissima* (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the species' distribution range. Mar Biol 161:2011–2022

Nylund GM, Pavia H (2005) Chemical versus mechanical inhibition of fouling in the red alga *Dilsea carnosa.* Mar Ecol Prog Ser 299:111–121

O'Brien JM, Scheibling RE, Krumhansl KA (2015) Positive feedback between large-scale disturbance and density-dependent grazing decreases resilience of a kelp bed ecosystem. Mar Ecol Prog Ser 522:1–13

O'Connor MI (2009) Warming strengthens an herbivore–plant interaction. Ecology 90:388–398

Olischläger M, Iñiguez C, Gordillo FJL, Wiencke C (2014) Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased pCO_2 and temperature reveals ecotypic variation. Planta 240:1213–1224

Pansch C, Gómez I, Rothäusler E, Veliz K, Thiel M (2008) Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelps *Lessonia nigrescens* and *Macrocystis integrifolia.* Mar Biol 155:51–62

Pavia H, Toth GB (2000) Influence of light and nitrogen on the phlorotannin content of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus.* Hydrobiologia 440:299–305

Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. Oecologia 80:82–86

Saunders M, Metaxas A (2007) Temperature explains settlement patterns of the introduced bryozoan *Membranipora membranacea* in Nova Scotia, Canada. Mar Ecol Prog Ser 344:95–106

Saunders M, Metaxas A (2008) High recruitment of the introduced bryozoan *Membranipora membranacea* is associated with kelp bed defoliation in Nova Scotia, Canada. Mar Ecol Prog Ser 369:139–151

Saunders M, Metaxas A (2009) Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. Mar Biol 156:2267–2276

Saunders M, Metaxas A, Filgueira R (2010) Implication of warming temperatures for population outbreaks of a nonindigenous species (*Membranipora membranacea*, Bryozoa) in rocky subtidal ecosystems. Limnol Oceanogr 55:1627–1642

Scheibling RE, Gagnon P (2009) Temperature-mediated outbreak dynamics of the invasive bryozoan *Membranipora membranacea* in Nova Scotian kelp beds. Mar Ecol Prog Ser 390:1–13

Scheibling RE, Feehan CJ, Lauzon-Guay JS (2013) Climate change, disease and the dynamics of a kep-bed ecosystem in Nova Scotia. In: Fernández-Palacios JM, de Nascimento L, Hernández JC, Clemente S, González A, Díaz-González JP (eds) Climate change perspectives from the Atlantic: past, present and future. Servicio de Publicaciones, Universidad de La Laguna, p 361–387

Schoenwaelder MEA, Clayton MN (2000) Physode formation in embryos of *Phyllospora comosa* and *Hormosira banksii* (Phaeophyceae). Phycologia 39:1–9

Sieburth J, Tootle JL (1981) Seasonality of microbial fouling on *Ascophyllum nodosum* (L.) LeJol., *Fucus vesiculosus* L., *Polysiphonia lanosa* (L.) Tandy and *Chondrus crispus* Stackh. J Phycol 17:57–64

Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. Mar Ecol Prog Ser 537: 89–104

Staehr PA, Wernberg T (2009) Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature. J Phycol 45:91–99

Steinberg PD (1984) Algal chemical defense against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata.* Science 223:405–407

Steinberg PD (1995) Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata.* Oecologia 102:169–173

Steneck RS, Watling L (1982) Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. Mar Biol 68:299–319

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv 29:436–459

Stiger V, Deslandes E, Payri CE (2004) Phenolic contents of two brown algae, *Turbinaria ornata* and *Sargassum mangarevenses* on Tahiti (French Polynesia): interspecific, ontogenic and spatio–temporal variations. Bot Mar 47: 402–409

Targett NM, Arnold TM (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. J Phycol 34:195–205

Toth G, Pavia H (2002) Intraplant habitat and feeding preference of two gastropod herbivores inhabiting the kelp *Laminaria hyperborea.* J Mar Biol Assoc UK 82:243–247

Tuya F, Cacabelos E, Duarte P, Jacinto D and others (2012) Patterns of landscape and assemblage structure along a latitudinal gradient in ocean climate. Mar Ecol Prog Ser 466:9–19

Van Alstyne KL (1988) Herbivore grazing increases polyphenolic defenses in the intertidal brown algae *Fucus distichus*. Ecology 69:655–663

Van Alstyne KL (1995) Comparison of three methods for quantifying brown algal polyphenolic compounds. J Chem Ecol 21:45–58

Van Altena IA, Steinberg PD (1992) Are differences in the response between North American and Australasian marine herbivore to phlorotannins due to differences in phlorotannin structure? Biochem Syst Ecol 20:493–499

Vergés A, Steinberg PD, Hay ME, Poore AGB and others (2014) The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. Proc R Soc B 281:20140846

Weinberger F, Rohde S, Oschmann Y, Shahnaz L, Dobretsov S, Wahl M (2011) Effects of limitation stress and of disruptive stress on induced antigrazing defense in the bladder wrack *Fucus vesiculosus.* Mar Ecol Prog Ser 427: 83–94

Wernberg T, Smale DA, Tuya F, Thomsen MS and others (2013) An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nat Clim Change 3:78–82

Wikström SA, Pavia H (2004) Chemical settlement inhibition versus post-settlement mortality as an explanation for differential fouling of two congeneric seaweeds. Oecologia 138:223–230

Winer BJ, Brown DR, Michels KM (1991) Statistical principles in experimental design, 3rd edn. McGraw-Hill, New York, NY

Zarnetske PL, Skelly DK, Urban MC (2012) Biotic multipliers of climate change. Science 336:1516–1518

Editorial responsibility: Morten Pedersen, Roskilde, Denmark

Submitted: March 20, 2015; Accepted: July 10, 2015
Proofs received from author(s): September 8, 2015