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Temperature and salinity effects on submerged aquatic vegetation traits and susceptibility to grazing

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ABSTRACT

Submerged macrophyte vegetation provides valuable ecosystem services, but climate- and management-driven changes may alter plant traits in unexpected and interactive ways. Further, such changes in plant traits may influence herbivore response, with feedback to bed characteristics. We manipulated temperature (20, 25, 30 °C) and salinity (0, 6, 12) in mesocosms to simulate current and predicted scenarios for the San Francisco Bay area. We measured traits of *Stuckenia pectinata* (sago pondweed) and subsequent effects on invertebrate (amphipod, *Ampithoe valida*) grazing. Counter to predictions, higher temperatures tended to have positive effects on plant traits (leaf area, aboveground biomass, nitrogen [N], phosphorus [P], protein, and total phenolic content). Also, unexpectedly, the highest salinity treatment had few negative effects except when temperature was coolest; *i.e.*, 20 °C and a salinity of 12 led to decreased carbon [C], N, P, protein, phenolic concentrations, and aboveground biomass. Conversely, the highest salinity (12) at the highest temperature (30 °C) produced the highest leaf N and P, and plants from this treatment suffered most from herbivory. Consumption rates significantly increased with lower leaf fiber and higher total leaf N and protein content; *i.e.*, plants with more nutritious leaves and less structural defense were consumed most. Climate change is expected to increase both salinity and temperature, but manipulation of freshwater supply could lead to decreased salinity. The range of responses in *S. pectinata* traits and invertebrate grazing shown by our results imply that the specific combination and magnitude of human influences will differentially shape these submerged macrophyte beds and their functions.

1. Introduction

Submerged macrophyte beds are a common and often highly valued feature of estuaries and coastal lagoons worldwide. These beds help to stabilize sediments and take up excess nitrogen and phosphorus, contributing to reductions in nuisance algal blooms (Chen et al., 2012). Submerged macrophyte canopies provide shelter from predators, substrate for egg masses, and a food source for various species of invertebrates and waterfowl (Jepson, 1905; Kantrud, 1990). These beds also serve as nursery grounds for fishes, creating the foundation for ecologically and economically important ecosystems (Kantrud, 1990; Costanza et al., 1997).

The occurrence of submerged macrophytes in nearshore coastal areas makes these ecosystems and their associated services vulnerable to impacts from humans, as population centers are often concentrated along coasts (Halpern et al., 2008; Barbier, 2011). Human-mediated alterations in geomorphology and hydrology within the coastal zone affect water exchange and flow, water depth, and other factors that can influence the growth and persistence of these beds (Martins et al., 2013). Increases in salinity, *e.g.*, from diversion of fresh water for drinking or irrigation of crops, can change the distribution, vigor, and composition of submerged macrophyte species (Borgnis and Boyer, 2015). In addition, global climate change is increasing water temperatures through the greenhouse effect and salinities via sea level rise; however, there may also be localized changes in the timing of fresh water inputs from snowmelt and managed water releases, complicating the overall effect (Cayan et al., 2008). Understanding how various combinations of anthropogenic- and climate-driven environmental changes can shift macrophyte distribution and their associated functions could help inform management decisions as well as conservation and restoration actions.

In the San Francisco Bay Area, submerged macrophyte beds can be found across a wide range of temperatures and salinities. Within the San Francisco Bay Delta region (confluence of the Sacramento and San Joaquin Rivers, ~ 70 km east of the Pacific Ocean), it is projected that annual average salinities will increase by 2.2, and average water temperatures will rise from 17 °C to 20 °C by 2099, with maximum levels reaching a salinity of 20 and water temperature of 25 °C (Cloern et al.,

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2011). Further, sea level at the mouth of San Francisco Bay is expected to increase by at least 125 cm in this same timeframe, shifting saline waters further up the estuary (Cloern et al., 2011). These scenarios are not unique to this region, but rather will occur in coastal water bodies worldwide as climate change intensifies.

The focal macrophyte species for this study was *Stuckenia pectinata* (syn. *Potamogeton pectinatus* L.), commonly known as sago pondweed. It has a global distribution and can be found in freshwater lakes and ponds, coastal lagoons, and brackish (mixohaline to oligohaline) reaches of bays and estuaries. In many regions, *S. pectinata* is considered a valuable native species for restoration and conservation efforts because of its early colonization potential and role as a foundational habitat (Wersal et al., 2006; Hengst et al., 2010; Boyer and Sutula, 2015; Hilt et al., 2018). As a native species with habitat value, *S. pectinata* is now a focus of restoration in both the San Francisco Estuary and in local lagoons and lakes (Mountain Lake Adaptive Management Plan, 2014).

In general, we expected that increased water temperatures and salinities would negatively affect *S. pectinata*, based on previous studies. Water temperatures above 30 °C were shown to decrease the rate of photosynthesis, germination, shoot elongation, and leaf area of *S. pectinata* (Spencer, 1986; Madsen and Adams, 1989; Pilon and Santamaría, 2002). In addition, salinities greater than 3 decreased the total biomass of *S. pectinata* (Teeter, 1965). Other studies have found a similar benefit of low salinity, with biomass increasing at salinities of 0 and 5, doubling at 10, and remaining unchanged at 15 (Borgnis and Boyer, 2015; Aichner et al., 2017).

We expected that increased temperatures and salinities would alter the nutritive content, morphometrics, and chemical traits of *S. pectinata* through the reallocation of resources (Spencer, 1986; Madsen and Adams, 1989; Gleeson and Tilman, 1992). Such changes in chemistry and morphology may influence the susceptibility of *S. pectinata* to herbivory. Further, warmer water temperatures may increase periphyton shading (Mahdy et al., 2015), potentially contributing to increased palatability (Hidding et al., 2016). Elevated temperatures can also stimulate the metabolism and abundance of grazers, which in combination with altered chemical composition of the plant (*e.g.*, increased nitrogen or decreased defensive compounds), could lead to increased herbivory (Sun et al., 2013). We expected that the highest herbivory would occur in treatments with the highest levels of salinity and temperature, as higher temperatures may decrease plant defense and degrade tissue structure, with osmotic stress potentially exacerbating these effects.

Previous work on *S. pectinata* often focused on the effects of singular abiotic stressors (Kantrud, 1990; Wersal et al., 2006; Zhang et al., 2015) or herbivory preference among multiple macrophytes (Miller and Provenza, 2007; Kapuscinski et al., 2014). It is still unclear, however, if altered environmental conditions directly or indirectly shape the consumptive behavior of invertebrate grazers. Thus our objectives are: 1) to understand the independent and interactive effects of temperature and salinity on *S. pectinata* nutritive and structural traits and 2) to assess how alterations in these traits may influence its susceptibility to invertebrate consumption.

2. Experimental

2.1. Mesocosm experiment & plant traits

Drupelets (fruits) and turions (vegetative buds) of *S. pectinata* were collected from the outer lagoon of Abbott's Lagoon in Point Reyes National Seashore, CA (38.1147°N, 122.9536°W) in September of 2014. Collected material was placed in a refrigerator for approximately six months to shed the tough exterior coat, which has been indicated as a potential barrier to germination (Kantrud, 1990; Santamaría et al., 2002). Abbott's Lagoon served as a representative collection site because it spans a wide range of temperatures (12–20 °C) and salinities (< 1 up to 8), similar to other locations within the San Francisco Bay Area where *S. pectinata* occurs (Wallitner, 2015, Point Reyes National Seashore, National Park Service, unpublished data).

In late March of 2015, collected material was planted in propagation flats (40 cm × 40 cm x 12.7 cm) and placed in 200-liter, translucent tanks at a water depth of 0.76 m in the greenhouse at the Estuary and Ocean Science Center in Tiburon, CA. Each flat was filled with sandy-loam terrestrial soil (American Stone and Soil, San Rafael, CA; 26% silt and clay, 74% sand, 4.6% organic matter, 0.11% nitrogen) and a thin layer of rocks to prevent sediment resuspension. A SeaChem Flourish tab (0.28% nitrogen and 0.17% phosphorus) was placed at mid-depth of the soil in each propagation flat to provide 8.4 mg N and 5.1 mg P for plant growth. Plants were grown at a salinity of 0, and an average water temperature of 18 to 20 °C. In June of 2015, substantial plant growth and the formation of drupelets had begun. At this point, each tank was randomly assigned to one of nine treatments, composed of three salinities (0, 6, 12) crossed with three temperatures (20, 25, 30 °C), with three replicate tanks per treatment. The factorial design of this experiment allowed for the testing of both freshening and salinization scenarios. One anomalous replicate with a treatment of 0 salinity and 25°C was removed from analyses due to excessive algae growth that was not seen in other tanks. Treatments with a salinity of 0 at 20 °C were maintained by tap water and ambient greenhouse conditions, while all other temperature and salinity treatments were set by Hydor submersible heaters and the addition of Instant Ocean sea salts. Weekly measurements using a portable YSI meter led to occasional adjustments of salinity with tap water or Instant Ocean. All tanks were acclimated to their respective treatments over the course of four weeks, with tanks then held at treatment conditions for an additional four weeks.

Temperature treatments of 20 and 25 °C and salinity treatments of 0 and 6 were based on average, present-day conditions collected by the National Park Service in Abbott's Lagoon. Temperatures within *S. pectinata* beds in Abbott's Lagoon (Wallitner, 2015, Point Reyes National Seashore, National Park Service, unpublished data), Suisun Bay, and the western Delta (Borgnis and Boyer, 2015) currently reach between the low and middle temperature treatments (˜22 °C). Thus, temperature treatments of 30 °C and salinity treatments of 12 reflected predicted climate change scenarios for these areas.

At the end of the growing period in August of 2015, all plant material was harvested, and ten random leaves from the terminal ends of branches were sampled from each tank to collect morphometric data. Measurements were taken with electronic calipers to the nearest millimeter. All remaining plant material was held in a −80 °C freezer until processing. Frozen tissue was lyophilized and ground to a fine powder using a mini Wiley-Mill fitted with a 40-mesh sieve. Eighteen variables were measured across reproductive (mature and immature drupelets and turions), above ground, and below ground tissues. Only biomass and carbon [mol C] : nitrogen [mol N] (C:N) were measured in below ground and reproductive tissues, while aboveground tissues were analyzed for biomass, C:N, C:P, N:P, %C, %N, and %P (dry weight), total phenolic concentrations, total soluble protein content, neutral detergent fiber content (NDF), and leaf area. C and N content was measured using a CHN Elemental Analyzer. Both P and NDF (cellulose, hemicellulose, and lignin) were measured at the UC Davis Agricultural and Natural Resources Analytical Lab using acetic acid extractions and the Reflux method, respectively. Total soluble protein content was analyzed using a modified Bradford assay with a bovine serum albumin (BSA) standard and absorbance measured at 595 nm (Kapuscinski et al., 2014). Total phenolic concentrations were analyzed following a modified protocol of the Folin-Ciocalteu method (Kapuscinski et al., 2014) using a gallic acid standard and absorbance read at 765 nm.

2.2. Feeding assays

Feeding assays were used to examine how alterations in plant tissue caused by temperature and salinity might affect invertebrate consumption rate. The amphipod, *Ampithoe valida*, one of the most

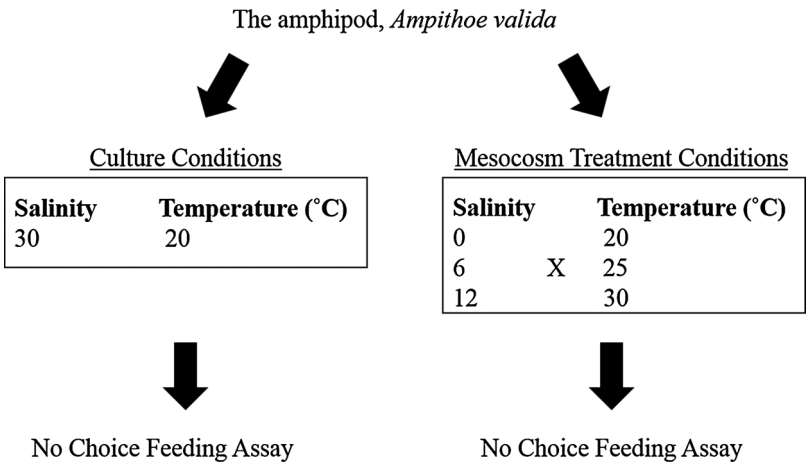

Fig. 1. Design of no-choice feeding assays utilizing two populations of invertebrates, one in culture conditions, and the other in treatment conditions.

abundant grazer species in *S. pectinata* beds in San Francisco Bay (Boyer et al., 2016), was used for these trials. *Ampithoe valida* individuals were previously collected and held in culture at the Estuary and Ocean Science Center. Individuals were removed from culture tanks and then divided into two groups, the first to remain in culture temperatures and salinities, and the second to be acclimated to the same nine treatments as the mesocosm tanks (Fig. 1). This design allowed us to differentiate between alterations in herbivory caused by organismal stress versus changes in the palatability of the plant material. Cultured individuals were kept at their original conditions with a salinity of 30 and 20 °C (Fig. 1). Acclimated individuals were divided into plastic containers and slowly acclimated to their assigned treatment values. The acclimation period lasted approximately nine days for each species with an average daily maximum change in salinity of 3 and 1 °C. Prior to each assay, individuals were held for a 24 -h starvation period.

Two no-choice feeding assays were conducted simultaneously for each species, one with cultured individuals and one with acclimated (Fig. 1). Live tissue from the terminal end of branches was randomly collected from each experimental *S. pectinata* tank and standardized by size (8–10 cm length), and by the number of leaves (4–6). Each feeding assay unit consisted of a 0.27 L Dixie clear plastic cup with a 5 cm^2 piece of fiberglass window screen, 3–4 individuals of an invertebrate species, and a pre-measured piece of *S. pectinata.* There were ten replicate units for each of the salinity and temperature treatments for both acclimated and cultured grazers. In addition, there were ten control cups per treatment that contained only plant material to account for changes occurring in the plant tissue in the absence of grazers (Peterson and Renaud, 1989). Invertebrates were fed for up to 48 h, or until at least 50% of the plant material had been visibly consumed (Sotka and Hay, 2002; Tomas et al., 2011; Goodman and Hay, 2013). The change in biomass indicative of herbivory was calculated using the equation (M_i x C_f/C_i) - M_f, where M_i and M_f were the initial and final weight of tissue used in the feeding assay, and C_f and C_i were the average final and initial mass of the control plant tissue (Tomas et al., 2011; Goodman and Hay, 2013; Lewis and Boyer, 2014).

2.3. Statistics

R software (version 3.3.0, R Core Team, 2015) was used for statistical analyses. All variables were tested for normality and homoscedasticity and those which did not meet these assumptions were transformed using Box-Cox transformations. A principal component analysis (PCA) was used to 1) identify potential correlations between continuous response variables, 2) reduce the number of variables (18 initially) to a smaller number of meaningful, uncorrelated summary components and 3) highlight the main patterns of variation in response to experimental conditions. A two-way ANOVA was run on the resulting summary components from the PCA to test for the independent and interactive effects of temperature and salinity. ANCOVA analyses identified relationships between significantly affected plant traits and herbivory rates from the three feeding assays.

3. Results

3.1. Plant traits

The first five components of the PCA represented 71.4% of the total variance in the data set. PC1, which accounted for 24.8% of the total variance, was positively correlated to aboveground C:N and C:P, and negatively correlated with %N, %P, and leaf area (Table 1; Fig. 2). PC2, which accounted for 16.6% of the total variance, was positively correlated with aboveground biomass, %C and total phenolic concentrations (Table 1; Fig. 2). There was a significant effect of both temperature and salinity on PC1 and PC2, and a significant effect of salinity on PC5 (ANOVA, Table 1). There was no effect of temperature or salinity on PC3 or PC4, thus PC5 was not explored further. There was no significant interactive effect of temperature and salinity on any of the five components (Table 1).

Independent of salinity, temperature elicited a response from *S. pectinata*. There was a significantly positive effect of temperature, with the highest treatments of 25 and 30 °C producing the highest values of %N, %P, total soluble protein content, total phenolic concentrations

Table 1
Results of two-factor ANOVA on new indices derived from the Principal Component Analysis. The response variables most correlated (positively, +, or negatively, -) with each principal component (PC) are listed. An * indicates significance at $p < 0.05$.

PC #	Correlated variables	Temperature	Salinity	Interaction
1	+: aboveground C:N, C:P, mature drupelet C:N, belowground C:N, belowground turion biomass -: %N, %P, leaf area	p = 0.0002*	p = 0.003*	p = 0.778
2	+: aboveground biomass, %C, phenolics, mature drupelet biomass, immature drupelet biomass	p = 0.0002*	p = 0.002*	p = 0.071
3	+: immature drupelet C:N, seed area -: aboveground N:P	p = 0.505	p = 0.128	p = 0.178
4	+: belowground biomass -: protein, belowground turion C:N	p = 0.973	p = 0.930	p = 0.903
5	+: fiber (NDF)	p = 0.471	p = 0.004*	p = 0.474

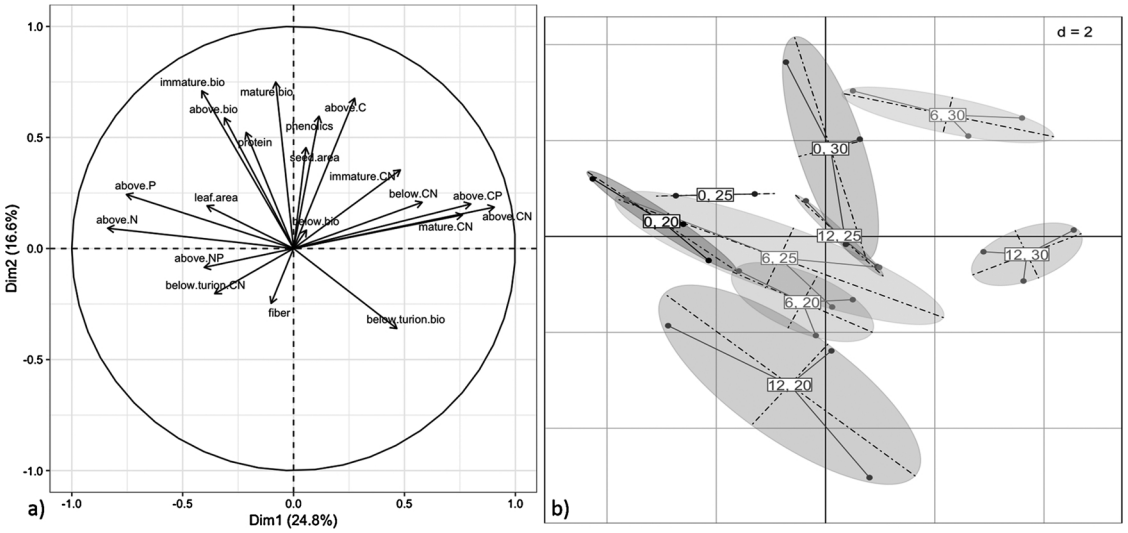

Fig. 2. (a) Correlation circle displaying PC1 and PC2 (Dimension [Dim] 1 and 2) from Principal Component Analysis (PCA). The length of the arrows indicate the strength of representation and contribution of each variable to PC1 or PC2. (b) Grouping of salinity (0, 6, 12) and temperature (20, 25, 30°C) treatments from PC1 and PC2 generated from the PCA.

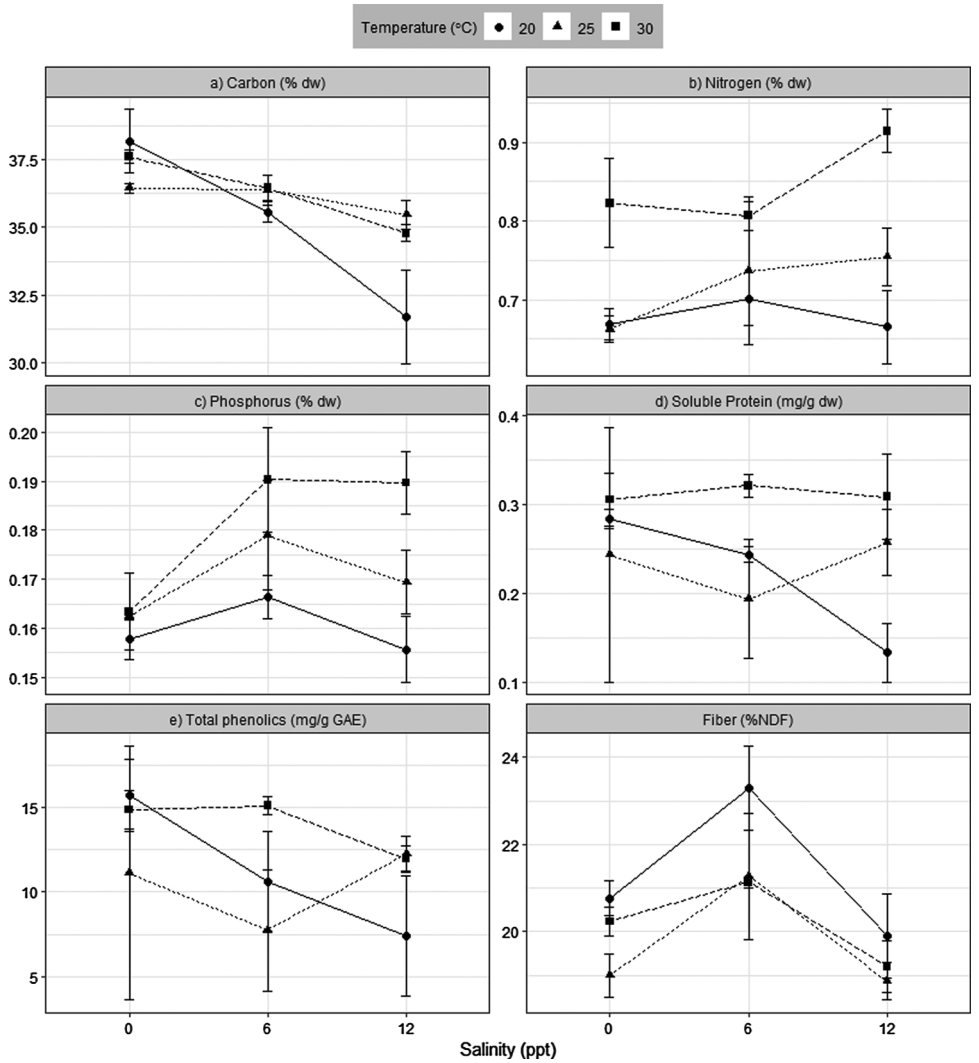

Fig. 3. Mean values of a) carbon (% dry weight), b) nitrogen (% dry weight), c) phosphorus (% dry weight), d) total soluble protein (mg/g dry weight), e) total phenolic concentrations (mg/g Gallic Acid Equivalent (GAE)), and f) neutral detergent fiber (NDF) measured in S. pectinata tissues subjected to salinity (0, 6, 12) and temperature (20, 25, 30°C) treatments. Error bars represent standard error.

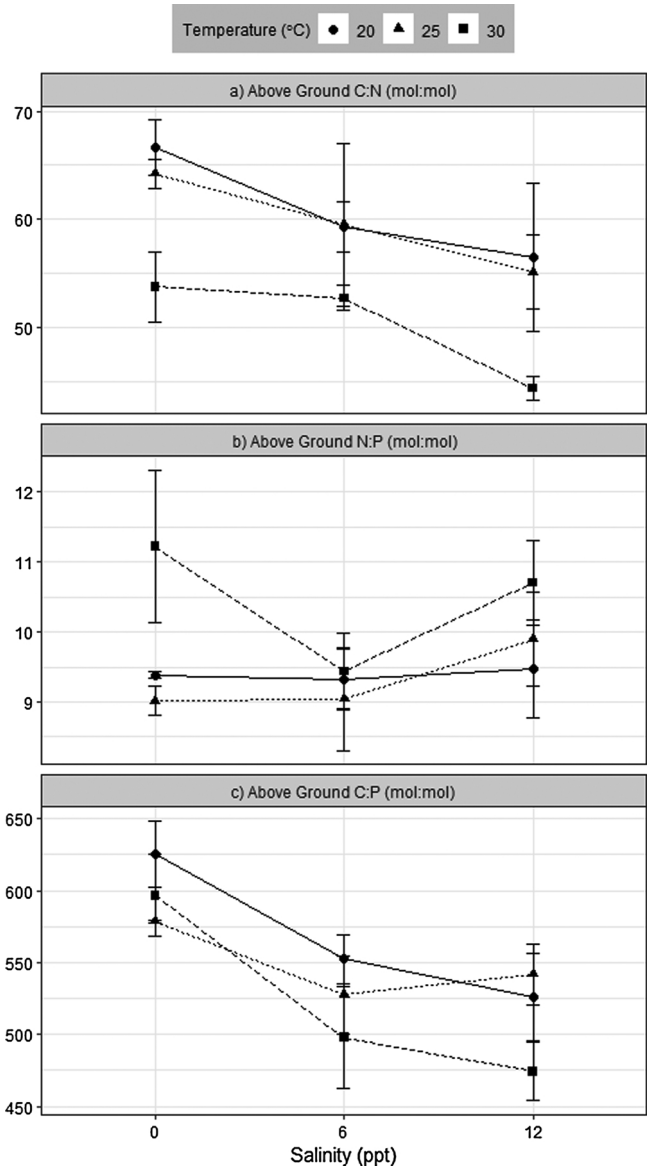

Fig. 4. Mean a) C:N (mol:mol), b) N:P (mol:mol) and c) C:P (mol:mol) of above ground S. pectinata tissues subjected to salinity (0, 6, 12) and temperature (20, 25, 30°C) treatments. Error bars represent standard error.

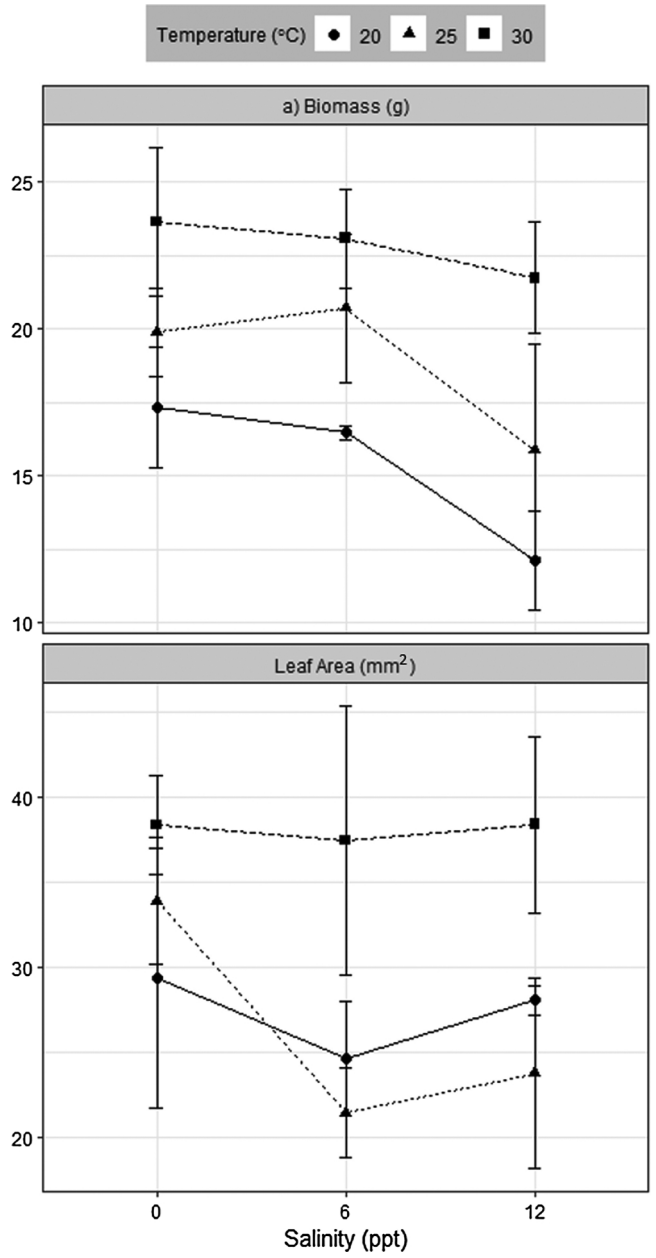

Fig. 5. Mean values of a) above ground biomass (grams) and b) leaf area (mm2) of S. pectinata tissues subjected to salinity (0, 6, 12) and temperature (20, 25, 30°C) treatments. Error bars represent standard error.

(Fig. 3b–e), N:P (Fig. 4b), and leaf area (Fig. 5b). In addition, the highest aboveground biomass was in treatments at 30 °C with an average of 22.83 g per 30 cm^2 flat (Fig. 5a). Further, there was a significant negative effect of the highest temperature treatment (30 °C) on aboveground C:N (Fig. 4a). Independent of temperature, salinity treatments of 6 and 12 had a significantly negative effect on aboveground %C (Fig. 3a), C:N, C:P (Fig. 4a and c), and aboveground biomass (Fig. 5a).

Although there were no significant interactive effects of temperature and salinity, it is interesting to note that there was a pronounced decline at the highest salinity (12) and lowest temperature (20°C) for several variables, including aboveground biomass (Fig. 5a), %C, %N, %P, protein, and phenolics (Fig. 3a–e). However, in treatments of high temperature (30°C) paired with high salinity (6 and 12), the highest %N, %P (Fig. 3b and c), and lowest C:N and C:P (Fig. 4a and c) were produced.

3.2. Feeding assays

There were six variables that were significantly affected by temperature and/or salinity that could affect the appearance and palatability of plant tissue, potentially influencing herbivory rates: total phenolic concentrations, leaf area, C:N, C:P, %N, and %P (Table 1). Although there was no effect of temperature nor salinity on N:P, soluble protein content or neutral detergent fiber (NDF) content, these variables have been shown to influence palatability (Martinez-Crego et al., 2016), so they were included as additional variables in the analysis of herbivory data.

It is important to note that *A. valida* was unable to acclimate to a salinity of 0, so there is no herbivory data for acclimated individuals in these treatments (Fig. 6b). Therefore, ANCOVA only tested the effects of acclimation (*i.e.*, acclimated vs. cultured herbivory) and plant traits on trials held in salinities of 6 and 12. There was a significant effect of %N,

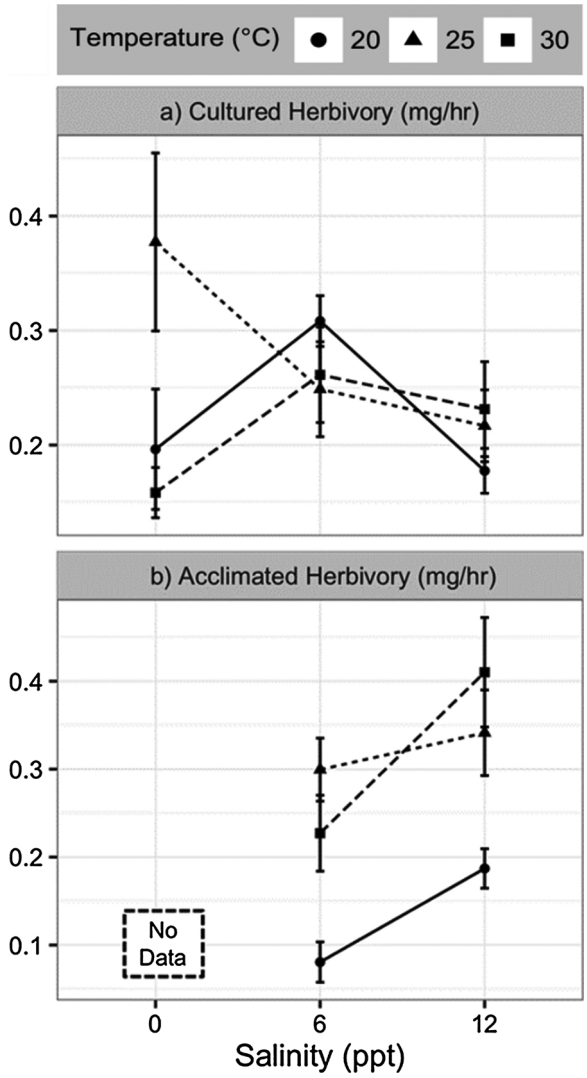

Fig. 6. The mean herbivory rates of both acclimated and cultured Ampithoe valida on S. pectinata tissues subjected to salinity (0, 6, 12) and temperature (20, 25, 30°C) treatments. Error bars represent standard error. We were unable to acclimate A. valida to a salinity of 0, so no data is presented for that salinity treatment.

Table 2
Results of ANCOVA for covariate effects on the rates of herbivory by *A. valida*. An * indicates $p < 0.05$.

Covariate	F value	P value
Acclimation	1.37	0.244
Phenolics	0.03	0.868
C:N	1.78	0.185
C:P	0.14	0.704
N:P	1.96	0.164
% N	5.72	0.018*
% P	1.22	0.270
Protein	4.94	0.028*
Leaf Area	0.27	0.607
Fiber	15.20	0.0002*

protein, and fiber content on the herbivory rate of *A. valida* (Table 2). Plants in the highest temperature treatments of 25 and 30 °C incurred the highest herbivory by both cultured and acclimated *A. valida* (Fig. 6), corresponding with low fiber, high %N and protein content. However, cultured individuals consumed the most plant tissue in salinities of 0, whereas acclimated *A. valida* consumed the most in salinities of 12 (Fig. 6). These patterns in both acclimated and cultured herbivory correspond with high %N and low fiber content (Fig. 3b and f).

4. Discussion

Submerged macrophyte beds spanning fresh to saline waters have been shown to provide a suite of valuable ecosystem services, including excess nutrient uptake, sediment stabilization, and habitat provision for invertebrates and fishes (Kantrud, 1990; Chen et al., 2012). Increased herbivory pressure and altered water temperature and salinity are cited as some of the most serious threats to submerged macrophyte ecosystems (Orth et al., 2006). In this study, high temperatures of 25°C and 30°C and salinities of 6 and 12 affected the nutritive (N, P, N:P, proteins, phenolics) and structural composition (C, C:N, C:P, fiber, leaf area) of *S. pectinata*, which in turn influenced herbivory by the amphipod, *Ampithoe valida*.

4.1. Nitrogen, phenolics, & protein

Stuckenia pectinata tissues across all treatments had much lower N than what is typically found in the field. Kantrud (1990) reported a range of 1.24–6.01 %N in *S. pectinata* collected from the Netherlands and Scotland, and Boyer et al. (2016) reported 2.4–3.2 %N in plants collected from Suisun Bay in San Francisco Estuary; whereas values from this study ranged from 0.65 – 0.95%N dry weight, falling below the 1.3%N threshold (Gerloff and Krombholz, 1966), suggesting that our tanks were nitrogen-limited. Our results for C:N (45–67 mol:mol) and N:P (8.5–12.3 mol:mol) further support this, as they are higher and lower respectively than what has previously been reported for this species, presumably a function of low N content (Xia et al., 2014). However, high temperature treatments of 30°C produced the largest leaf areas and the greatest biomass for *S. pectinata*, leading us to expect the greatest nitrogen limitation in this treatment. This suggests that in comparison to nitrogen-replete conditions, the magnitude of our reported differences may be conservative.

The concentration of total soluble protein was several orders of magnitude greater (0.1 – 0.39 mg/g dry weight) than the values reported for *S. pectinata* in previous studies (Miller and Provenza, 2007; Kapuscinski et al., 2014). In addition, phenolic concentrations of *S. pectinata* in our study were comparable to those found in other studies (Miller and Provenza, 2007; Kapuscinksi et al. 2014) and follow the Carbon Nutrient Balance Hypothesis (CNBH) which indicates that low nitrogen conditions often lead to high phenolic concentrations (Koricheva, 2002). However, it is important to note that this study examined total phenolic concentrations, as they are thought to serve in chemical defense against herbivory (Fraenkel, 1959; Hartley and Jones, 1996). Phenolics can serve a variety of other functions though, including the synthesis of structural compounds such as lignin (Jones and Hartley, 1999), albeit low in *S. pectinata* tissues, and UV protection (Close and McArthur, 2002). Because this study did not examine the concentrations of specific phenolic compounds, it is unclear whether the concentrations measured are of phenols that are used for cellular processes and protection, defense from herbivores, or more likely, a combination of both.

4.2. Plant traits and herbivory

We expected that alterations in the traits and thus palatability of *S. pectinata* would influence the herbivory rates of the amphipod, *A. valida*. Similar feeding assay studies have attempted to link food quality variables to herbivory patterns, often resulting in mixed findings (Peterson and Renaud, 1989; Sieg and Kubanek, 2013). There are four variables cited that can affect the forage quality of a food source: neutral detergent fiber (NDF), comprised of lignin, cellulose, and hemicellulose; tissue stoichiometry (%N, %P, C:N, C:P, N:P); total soluble proteins; and total phenolic concentrations, as a proxy for

chemical defensive compounds (Prusak et al., 2005; Cebrian et al., 2009). We found a significant effect of %N, protein content, and neutral detergent fiber (NDF) content on the herbivory rates of *A. valida*, which was expected, as invertebrate herbivores often forage for nitrogen-based proteins and tissue that is low in fiber content. Plant tissue grown in the highest salinity and temperature treatments (12 and 30 °C) incurred the highest herbivory from acclimated *A. valida*. This is likely because of a significant increase in N, thus increasing the forage quality of *S. pectinata*. Although there were no significant effects of temperature and salinity on fiber content, the lowest fiber content values for *S. pectinata* occurred in treatments with salinities of 0 at 25°C, aligning with the highest herbivory by cultured individuals. Conversely, the highest fiber content occurred in salinities of 6 at 20°C, corresponding with the lowest herbivory by acclimated individuals.

4.3. Climate change and management implications

Global climate change is expected to lead to increases in the sea surface temperature and the salinity of estuaries and coastal lagoons (Cloern et al., 2011). These shifts in environmental conditions can alter the habitat availability and suitability for submerged macrophyte beds. Our results indicate that *S. pectinata* can withstand and even thrive in temperatures of 25°C and 30°C, as these treatments generally increased %N, %P, total soluble protein content, and total phenolic concentrations. Higher values of aboveground biomass and leaf area also corresponded with higher temperature treatments (25 and 30°C), although these results could potentially be a function of self-shading in experimental tanks. When higher temperatures were combined with salinities of 12, *S. pectinata* tissue C:N and C:P significantly declined, similar to other studies (Ventura et al., 2008), while %N and %P increased. These changes in tissue stoichiometry are partially responsible for the increases in herbivory by acclimated *A. valida*. *Stuckenia pectinata* grown in salinities of 12 and 20 °C were the most negatively affected, resulting in the lowest aboveground biomass, %C, %N, %P, protein, and phenolic content. This indicates that increased temperatures at or above 25 °C may in effect buffer *S. pectinata* from the effects of salinities greater than 6, however, increased vulnerability to herbivory could counter this ameliorating effect.

Although it is expected that both temperature and salinity will increase under future climate change, management intervention and decisions regarding freshwater diversion may lead to an alternative scenario in which salinities decrease, while temperatures continue to increase. The results of this research indicate that across the three temperature treatments, low salinity treatments (0) led to increased %C, C:N, and C:P, which can promote structural complexity and biomass production, perhaps leading to an overall increase in *S. pectinata* bed acreage. However, competition between *S. pectinata* and non-native submerged macrophyte species increases at low salinities (Borgnis and Boyer, 2015), which may offset these benefits.

This study contributes to our understanding of how submerged macrophyte ecosystems will respond under changing environmental conditions. Further, our results can be used to inform site selection for present and future restoration efforts by providing insight into the optimal temperature and salinity conditions for plant proliferation and growth. However, further research on the specific role of phenolic compounds in *S. pectinata* tissues is needed to better determine how a changing climate and future management decisions will influence the characteristics of *S. pectinata* beds, associated invertebrate communities, and their interactions.

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CRediT authorship contribution statement

Serina Sebilian Wittyngham: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Julien Moderan:** Formal analysis, Writing - review & editing, Visualization. **Katharyn E. Boyer:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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