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PRIMARY RESEARCH ARTICLE

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Coralline algal skeletal mineralogy affects grazer impacts

Sophie J. McCoy[1] | Nicholas A. Kamenos[2]

[1]Department of Biological Science, Florida State University, Tallahassee, Florida

[2]School of Geographical and Earth Science, University of Glasgow, Glasgow, UK

Correspondence
Sophie J. McCoy, Department of Biological Science, Florida State University, Tallahassee, FL 32306-4295.
Email: mccoy@bio.fsu.edu

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Abstract

In macroalgal-dominated systems, herbivory is a major driver in controlling ecosystem structure. However, the role of altered plant–herbivore interactions and effects of changes to trophic control under global change are poorly understood. This is because both macroalgae and grazers themselves may be affected by global change, making changes in plant–herbivore interactions hard to predict. Coralline algae lay down a calcium carbonate skeleton, which serves as protection from grazing and is preserved in archival samples. Here, we compare grazing damage and intensity to coralline algae in situ over 4 decades characterized by changing seawater acidity. While grazing intensity, herbivore abundance and identity remained constant over time, grazing wound width increased together with Mg content of the skeleton and variability in its mineral organization. In one species, decreases in skeletal organization were found concurrent with deeper skeletal damage by grazers over time since the 1980s. Thus, in a future characterized by acidification, we suggest coralline algae may be more prone to grazing damage, mediated by effects of variability between individuals and species.

KEYWORDS
coralline algae, herbivory, mineralogy, ocean acidification, Raman spectroscopy

1 | INTRODUCTION

Recent advances in climate change ecology have focused on traits that influence species interactions. In macroalgal-dominated systems, much of this research has centered on herbivory and the scope for altered plant–herbivore interactions and effects of changes to trophic control (Connell & Russell, 2010; Falkenberg, Russell, & Connell, 2013; Ghedini, Russell, & Connell, 2015; McCoy & Pfister, 2014; McCoy, Allesina, & Pfister, 2016; Russell et al., 2013; Vergés et al., 2014). Similarly, coralline algae stand to be affected by such community processes, yet are also vulnerable as calcifiers among macroalgae (McCoy & Kamenos, 2015). Coralline algal resistance to grazers is largely dependent on thallus thickness (Paine, 1984; Steneck, Hacker, & Dethier, 1991) and morphology (Steneck, 1982). These skeletal morphological traits may be at risk from ocean acidification (McCoy & Ragazzola, 2014), including effects on external and internal skeletal structure metrics. Further, the rate of environmental change (Kamenos et al., 2013) as well as the duration of stress (Ragazzola et al., 2012, 2013) has been shown to affect the magnitude of skeletal impacts in this group.

Previous examination of field specimens from the Northeast Pacific provides evidence for morphology-dependent responses of crustose coralline algal skeletons to ongoing in situ ocean acidification between the 1980s and 2010s. The thicker species *Pseudolithophyllum muricatum* and *Lithophyllum impressum* have thinned over time, but have preserved their internal skeletal density (McCoy, 2013; McCoy & Ragazzola, 2014). Conversely, thinner species including *Pseudolithophyllum whidbeyense* have not changed in thickness, but have reduced internal skeletal density (McCoy & Ragazzola, 2014). Together, these results suggest an energetic tradeoff under acidification stress, affecting the quantity of skeletal material laid down. It is therefore likely that changes to the quality of skeletal material, identified by changes in skeletal chemistry, have also occurred over this 30-year span. Both reduced thallus thickness and the potential for reduced quality of skeletal material will have effects

on coralline algal traits relevant to competitive or trophic interactions.

Such ecosystem studies are conducted in situ, within ecological context, and thus sublethal skeletal responses of coralline algae to ocean acidification may be mediated by grazing intensity and damage, or affect the extent to which they are damaged by grazing. Crustose coralline algae from Mediterranean CO_2 vents (*Lithophyllum*, *Titanoderma*, and *Phymatolithon* spp.) (Kamenos, Perna, Gambi, Micheli, & Kroeker, 2016) and temperate laboratory studies (Pauly, Kamenos, Donohue, & LeDrew, 2015) (*Lithothamnion glaciale*) provide evidence that they are able to buffer against changes in water pCO_2, preserving their Mg concentrations until extreme (pH <7.4) pCO_2 conditions are encountered demonstrating absence of sublethal skeletal trade-offs in low pH. This is possible because of coralline algal skeletal mineralogy, a high-Mg calcite (Kamenos, Cusack, Huthwelker, Lagarde, & Scheibling, 2009) and their ability to change its polymorph (Kamenos et al., 2016). The strength of the Mg-O bond, which is inversely proportional to positional disorder or organization within the crystal lattice, has been linked to a variety of acidification-relative metrics, including pCO_2 of ambient seawater in the cold-water coralline *Lithothamnion glaciale* (Pauly et al., 2015) and aragonite saturation of tropical coral calcifying fluid (DeCarlo et al., 2017).

Here, we investigated the role of acidification-induced changes to the coralline algal skeleton in determining their susceptibility to grazing damage left on the coralline algal surface by limpets and chitons and determine (a) whether grazers preferentially target a particular species, (b) whether species differ in their skeletal chemistry and (c) if so, whether grazing intensity and degree of damage has a greater impact on coralline algae with different skeletal chemistries. We also investigated relationships within sample groups to (d) determine the degree of variability across individuals. Last, we asked (v) if changes in those relationships have occurred under in situ ocean acidification over the past 30 years.

2 | MATERIALS AND METHODS

2.1 | Specimens

Coralline algal specimens were sampled from Hedophyllum Cove on the NE coast of Tatoosh Island, WA (48.32°N, 127.74°W) during summer months. All sampled specimens were adults of at least 10 years of age, estimated based on specimen size and growth rates (McCoy & Pfister, 2014). Prior to collection, all specimens were transplanted in situ onto epoxy putty discs (Sea Goin' Poxy Putty, Permalite Corp.) in areas with natural grazer abundance for >2 years. Collections were made by hammer and chisel, and epoxy discs bearing coralline algal samples were left to dry in the shade and stored dry prior to analysis. Archival *Lithophyllum impressum*, *Pseudolithophyllum muricatum*, and *Pseudolithophyllum whidbeyense* samples of different individuals from 1981, 1982, 1994, and 1997 were obtained from R.T. Paine and those from 2013 were sampled following Paine's methods.

2.2 | A note on taxonomy

Pseudolithophyllum muricatum, as previously studied on Tatoosh Island, WA (McCoy, 2013; McCoy & Pfister, 2014; McCoy & Ragazzola, 2014; McCoy et al., 2016; Paine, 1980, 1984 ; Steneck & Paine, 1986), has since been regrouped into the *Crusticorallina* species complex (Hind, Gabrielson, Jensen, & Martone, 2016). A genetic re-characterization of *Pseudolithophyllum whidbeyense* is currently underway (P. Gabrielson, personal communication). We thus refer to these species as *P. muricatum* and *P. whidbeyense* herein to provide consistency with the previous work at this site and to avoid confusion while larger scale taxonomic relationships are still being clarified. However, we note that *P. muricatum* and *P. whidbeyense* should no longer be interpreted as congeners. We did not study specimens of *Lithothamnion phymatodeum* or articulated *Bossiella* and *Corallina* spp. as those species use physical topography as a grazer defense mechanism.

2.3 | 3D SEM

Uncoated, dry samples were imaged under SEM (FEI Quanta 200F) in low vacuum at both a 0° and 10° stage tilt, using the same physical focal point at the center of each image. These image stereopairs were converted to a 3D image using Alicona MeX 6.1 software (Alicona Imaging). Though samples varied in size, each sample was imaged at ~7 locations to cover its full surface area. Using each composite 3D SEM image, transects were drawn perpendicular to grazing scars to generate a topographical profile of each scar in Alicona MeX. These topographical profiles were saved as image files and using ImageJ (imagej.nih.gov) the width and depth of each grazer mark (identified as a "valley" feature) were measured and analyzed with reference to the original 3D SEM image to ensure that only true grazer marks were quantified.

Grazing scars are typically made in pairs due to the morphology of molluskan radula. Grazing pressure (defined here as the intensity of grazing) was thus calculated by tallying the number of grazing marks on each SEM micrograph, divided by two and normalized by the area of each imaged area. All SEM micrographs were taken at either 150× or 300× magnification, representing areas of 828,750 and 207,187.5 μm^2, respectively. Grazing intensity was calculated separately for each 3D *SEM* image and averaged across the individual specimen.

2.4 | Raman spectroscopy

Raman spectroscopy identifies structural molecular fingerprints of materials through a series of vibrational peaks. Raman spectroscopy was conducted using a Renishaw inVia Raman microscope equipped with a LeicaDM2500M (Leica Microsystems GmbH, Wetzlar, Germany) microscope. Coralline algae were analyzed in epoxy blocks and were excited using a 785 nm laser with a 1,200 mm^{-1} grating via a 20× objective. Raman spectra were taken from individual coralline algae adjacent to the areas where grazing was present (identified

by 3D SEM). The calcium mineral, full width at half peak maximum (FWHM) and frequency of the peak at ~1,089 cm^{-1} (v_1) were determined using Renishaw Wire v.3.2 software.

The v_1 Raman peak (at ~1,085–1,089 cm^{-1}) identifies as the Mg-O bond from trace Mg present in all natural marine carbonates (Bischoff, Sharma, & MacKenzie, 1985). The shape of the spectrum is used to differentiate calcite and aragonite (Bischoff et al., 1985), and the Raman shift of the ~1,089 cm^{-1} peak is a proxy of Mg content of high Mg calcite (Bischoff et al., 1985; Pauly et al., 2015). Positional disorder of the mineral lattice is caused by shorter and stronger Mg-O bonds, which correspond to smaller FWHM of the ~1,089 cm^{-1} peak (Bischoff et al., 1985; Perrin et al., 2016). While Mg concentrations affect FWHM in synthetic carbonates, no consistent relationship has been found in biogenic minerals (Bischoff et al., 1985). To account for this potential effect, we normalized our FWHM data for Mg concentrations by dividing by Raman peak center of the ~v_1 peak (sensu Pauly et al., 2015). We thus use normalized FWHM to refer to relative mineral organization indicative of changes in positional disorder; higher relative mineral organization (lower positional disorder) is representative of a more robust skeleton (Pauly et al., 2015).Raw FWHM data are reported in the Supporting information Appendix S1. We note there can be a difference in FWHM between biogenic and abiotic carbonates due to difference in the organic load causing difference in symmetrical stretching of C-O bonding. However, this does not apply in our case as we (a) only measured biogenic carbonates (Von Euw et al., 2017), (b) photo-bleached our samples (laser) to remove organic interference, and (c) are using a 785 nm laser, which has little sensitivity to organic load.

2.5 | Environmental datasets

Sea surface temperature (SST) data measured in situ every 30 min at Hedophyllum Cove on Tatoosh Island, WA are available since 2000. Nearby NOAA buoys measuring SST are located at Neah Bay (NB, No. 46087) and Cape Elizabeth (CE, No. 46041). The NB buoy has been in operation since 2004, and the CE buoy since 1987. While the CE buoy shows a close relationship in SST between Cape Elizabeth and Tatoosh Island (Pfister, Wootton, & Neufeld, 2007), it does not extend to 1980 to match our archival sampling. As coastal datasets have been found to be more closely predictive of Tatoosh Island seawater than offshore buoys and satellite data (Pfister et al., 2007), we have used monthly averages of the long-term in situ temperature record from Race Rocks, BC (48° 17′ N, 123° 31′ W), which extends back to 1921 (https://www.racerocks.com/racerock/abiotic/temperature/seatemperature.htm; Supporting information Figure S1). As Tatoosh Island SST data did not extend back to the 1980s, a linear regression between SST from Race Rocks, BC and SST from Tatoosh Island, WA was used to determine suitability of using the extended Race Rocks SST record for comparison to skeletal chemistry data from Tatoosh Island. Race Rocks, BC SST was closely correlated to Tatoosh Island, WA, SST (linear regression, $r^2 = 0.297$, $F_{1,47} = 21.3$, $p < 0.001$; Supporting information Figure S2).

The Pacific Decadal Oscillation (PDO) index was obtained from NOAA (https://www.esrl.noaa.gov/psd/gcos_wgsp/Timeseries/PDO/; Supporting information Figures S3 and S4). We expect SST and PDO to be highly correlated with each other and other environmental metrics, yet it is unclear which is the most informative (Pfister et al., 2007). We calculated linear relationships between PDO calculated monthly, yearly, monthly minimum, maximum, and *SD* by year, and summer (April–July) as well as yearly June–May (the preceding year of growth) and determined that yearly mean PDO was most representative of other metrics, including mean annual SST, except for *SD* (sensu Kroeker et al., 2016; Supporting information Figures S5 and S6). Thus, to rule out the hypothesis of changes in skeletal chemistry resulting from response to SST or PDO, we tested for relationships between Raman metrics of skeletal chemistry and yearly mean and *SD* of the PDO Index and Raman metrics.

Carbonate chemistry data are available from Tatoosh Island only since 2000 (Wootton & Pfister, 2012; Wootton, Pfister, & Forester, 2008), thus we do not present correlations between our skeletal chemistry data and carbonate chemistry data. However, isotopic and trace mineral analyses from Tatoosh Island mussel shells reveal abrupt and ongoing changes to the inorganic carbon cycle at this site since the 1970s (Bian, 2013; Pfister et al., 2011). These changes are of a similar magnitude to observed changes in pH in the 2000–2009 period (Pfister et al., 2011), providing evidence for recent changes to the inorganic carbon cycle over the span of our study specimens from 1981–2013.

2.6 | Statistical analysis

To determine if grazers target a particular species, we tested grazer mark shape metrics (width, depth) and total grazing pressure by algal species, using a mixed effects model with sample as an interactive random effect (slope and intercept, R package nlme v. 3.1-131) to account for multiple measurements per sample. Within *P. muricatum*, trends in grazer wounds over time were assessed using a 1-way ANOVA, as only one sample was available from each of 1981, 1982, 1994, and 1997, conflating year and individual identity; accordingly, we treat this analysis qualitatively.

To test whether coralline algal species differ in their skeletal chemistry and if chemistry changed over time, we ran a mixed effects model on Raman metrics indicative of Mg content (Raman shift) and relative mineral organization (normalized FWHM) by species and decade. To account for multiple measurements per sample, this was used as an interactive random effect (slope and intercept, R package nlme v. 3.1-131). As both species and decade were significant factors, we tested for effects of decade within each species, again using a mixed effects model with sample as an interactive random effect to better understand these differences.

Relationships between grazer mark shape (depth and width) and skeletal chemistry were assessed using linear regressions. Due to cost and time of SEM analysis, we were not able to study the morphology of grazer marks on historical specimens of *L. impressum* and *P. whidbeyense*. To account for differences in specimen sampling

methodology between each analytical technique, we were unable to pair measurement points at the subsample level between grazer mark shape and skeletal chemistry. Thus, our smallest unit of variability is the specimen, and we took means and standard deviations by species and decade.

3 | RESULTS

3.1 | Grazing pressure

Among our samples, all grazer marks were visually identified as molluskan by the parallel lines generated by their radula (Steneck, 1982). No urchin grazing scars were present, which would be shaped in a star pattern generated by the Aristotle's lantern (Steneck, Bellwood, & Hay, 2017). Within modern 2010s samples, we found no variability in grazing pressure by species ($F_{2,3}$ = 0.62, p = 0.595). In *P. muricatum*, there was no variation in grazing pressure by decade ($F_{1,2}$ = 0.13, p = 0.759) (Figure 1).

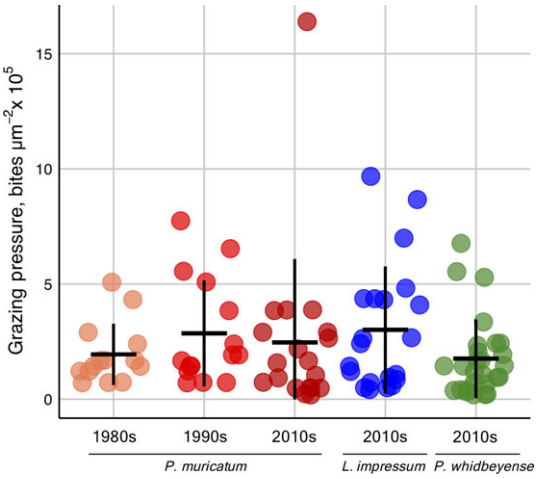

FIGURE 1 Grazing pressure in bites $\mu m^{-2} \times 10^5$ per sample group. *P. muricatum* from the 1980s in light red, 1990s in bright red, and 2010s in maroon. *L. impressum* from the 2010s in blue, and *P. whidbeyense* from the 2010s in green. Species and decade are also labeled on the *x*-axis. Each point represents grazing pressure observed on an SEM micrograph. Points are jittered for data visualization. Horizontal black lines represent group means and vertical black lines show standard deviation

3.2 | Grazing wound shape

Grazer mark depth and width varied significantly between algal species in modern 2010s samples (depth, $F_{2,116}$ = 25.5, $p < 0.001$; width, $F_{2,848}$ = 77.47, $p < 0.001$). Among these modern samples, *P. muricatum* exhibited the smallest grazer marks and least variation in grazer mark shape by species (Supporting information Table S2). *P. whidbeyense* exhibited the largest grazer marks both in width and depth. Overall, the ratio of grazing wound depth and width is consistent across all specimen types, all marks being much wider than they were deep (Figure 2c,d). Within modern samples, grazer marks on *P. whidbeyense* were deeper per unit width than those on *L. impressum* and *P. muricatum* (Figure 2d).

Due to historical sample availability, we analyzed historical specimens for only one individual per calendar year in 1981, 1982, 1994, and 1997. We thus pooled samples by decade (1980s, 1990s, 2010s) to better assess the effect of individual on grazer mark shape trends over time. We analyzed variation in grazer mark depth and width over time on *P. muricatum*, which varied between decades (depth, $F_{1,441}$ = 38.3, $p < 0.001$; width, $F_{1,441}$ = 52.3, $p < 0.001$) (Figure 2a,b). Interestingly, the ratio of width to depth did not differ from historical specimens, though the grazer marks were shallower and less wide on 2010 s samples compared to pooled 1980s and 1990s individuals (1-way ANOVA, $F_{1,441}$ = 56.79, $p < 0.001$).

3.3 | Skeletal mineralogy

Relative mineral organization (positional disorder), indicated by normalized FWHM, differed by species ($F_{2,51}$ = 29.9, $p < 0.001$) and decade ($F_{2,8}$ = 10.8, p = 0.006), as did Mg concentrations (species, $F_{2,52}$ = 14.7, $p < 0.001$; decade, $F_{2,8}$ = 7.95, p = 0.013; Figure 3). Specifically, each species exhibited a different trend over time, with only *P. whidbeyense* showing a decrease in relative mineral organization over time ($F_{1,19}$ = 17.7, $p < 0.001$) and no longitudinal trend in either *P. muricatum* ($F_{1,19}$ = 0.008, p = 0.93) or *L. impressum* ($F_{1,16}$ = 0.02, p = 0.88; Figure 3b). The same pattern was observed in each species with regard to Mg concentrations over time, with *P. whidbeyense* showing a decline in Raman peak shift ($F_{1,19}$ = 9.7, p = 0.006), and no trend over time in *P. muricatum* ($F_{1,19}$ = 2.3, p = 0.14) or *L. impressum* ($F_{1,16}$ = 1.8, p = 0.20; Figure 3a). There were no strong relationships between relative mineral organization or Mg concentration and PDO Index (Supporting information Figure S8), nor between grazer mark width or depth and PDO Index (Supporting information Figure S9). All Raman spectra were indicative of high-Mg calcite mineral.

3.4 | Relationships between grazing and skeletal chemistry

Mean grazer mark width increased with Mg concentration (slope = 7.58, r^2 = 0.823, p = 0.021; Figure 4a). There were no significant relationships between grazer mark morphometrics and Raman metrics of skeletal chemistry on a per specimen basis (Supporting information Figure S10a,d,g,j). We measured variability within a sample group (grouped by species and decade) by its standard deviation (*SD*). Variability in the grazing mark width was strongly correlated with increased Mg concentrations, indicated by increased Raman shift (slope = 7.85, r^2 = 0.987, $p < 0.001$), and with increased mineral organization (slope = 1911.8, r^2 = 0.755, p = 0.04; Figure 4b,c). No additional relationships were revealed by other pairwise comparisons between individual specimens, sample group means, and sample group SDs (Supporting information Figure S10).

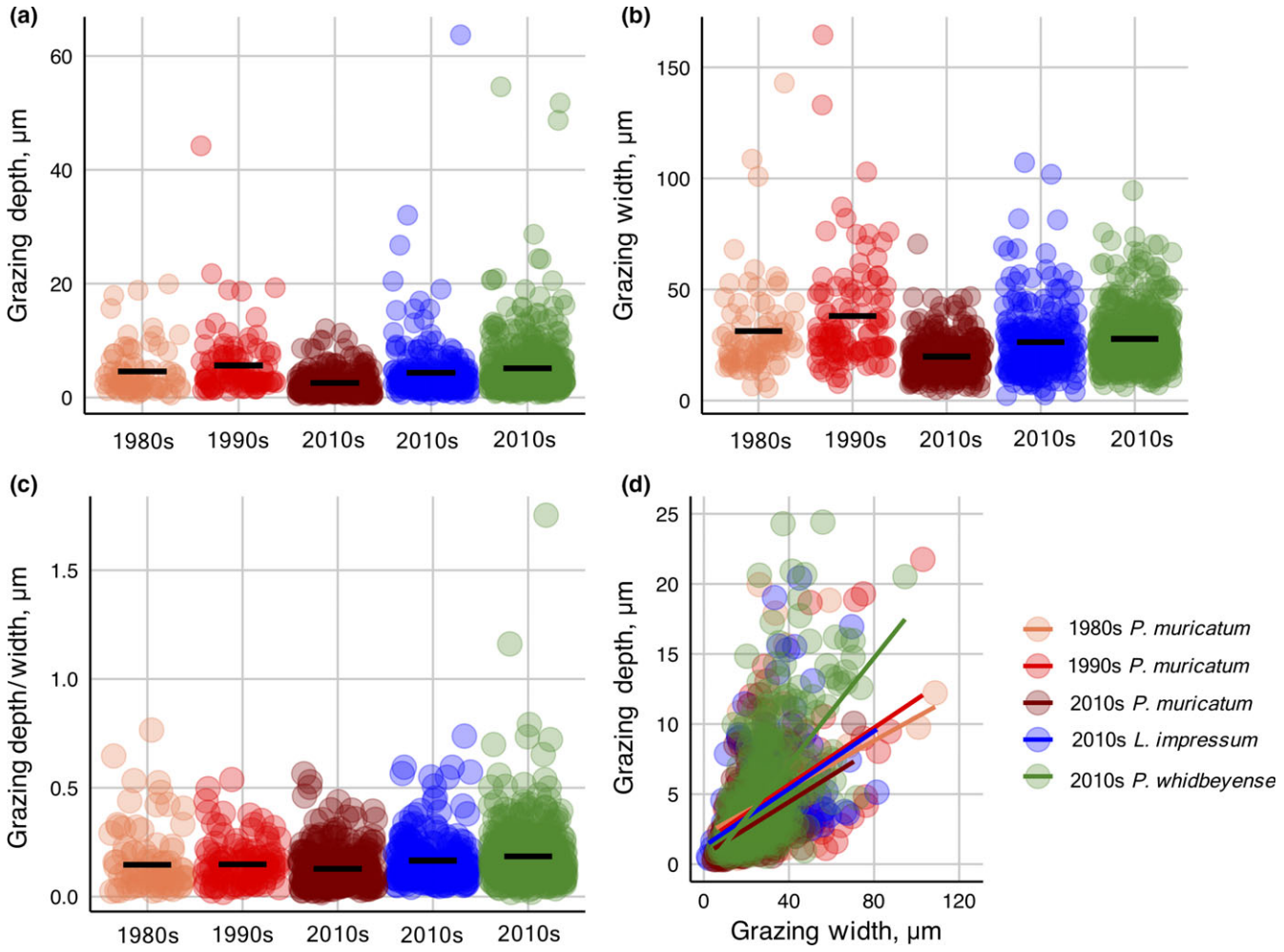

FIGURE 2 (a) Grazing mark depth and (b) width plotted by specimen type. (c) Grazing mark depth over width plotted by specimen type. (d) Grazing mark depth plotted versus width (µm), with outliers removed for better visualization. Outliers are included in panels a–c. *P. muricatum* from the 1980s in light red, 1990s in bright red, and 2010s in maroon. *L. impressum* from the 2010s in blue, and *P. whidbeyense* from the 2010s in green. Each point represents an individual grazing mark. Points are jittered for data visualization, and statistics are given in text

4 | DISCUSSION

Grazer intensity remained constant over time, and radula marks were proportionally smaller in modern samples (Figure 2). Grazer intensity was calculated by density of grazing scars on our specimens (Figure 1) and inferred from field abundance measurements (McCoy & Pfister, 2014). Morphological characteristics of radula marks were used to infer the consistency of grazer identity (Figure 2). Together, we interpreted these to imply a decline in individual grazer body size, with maintenance of overall grazing rates by the same grazer type. Indeed, this is corroborated by field abundance measurements of common grazers at the collection site, which showed no trend in grazer abundances over this time period (Figure 5). *P. whidbeyense* had larger overall grazing scars and had marks with greater depth per unit width than did *L. impressum* and *P. muricatum* (Figure 2c, Supporting information Table S2). Such differences could be explained by either species-specific grazer preferences (targeting of *P. whidbeyense* by species with differently shaped teeth) or a difference in skeletal mineralogy that affects grazer wound damage.

Generally across species and decades, we found that grazing wound width was greater at higher skeletal Mg concentration, and more variable either with increasing Mg concentration and relative mineral organization (Figure 4). Grazing mark size was not related to relative mineral organization, which implies that coralline algae have not become more vulnerable to grazing. This is not what we expected. Decreased mineral organization and an increase in its variability have been observed as a response to high seawater pCO_2 and ocean acidification (DeCarlo et al., 2017; Fitzer, Cusack, Phoenix, & Kamenos, 2014; Kamenos et al., 2013; McCoy, Kamenos, Chung, Wootton, & Pfister, 2018; Pauly et al., 2015). Given the time series of increasing acidification at our sample site (Wootton & Pfister, 2012; Wootton et al., 2008), we expected a decline in coralline algal skeletal mineral organization or an increase in the variability of mineral organization over time, indicative of poorer control over skeletal deposition. Increased variability of grazer wound width with higher mineral organization can therefore be interpreted as revealing variability of grazing damage based on grazer size and pressure on a

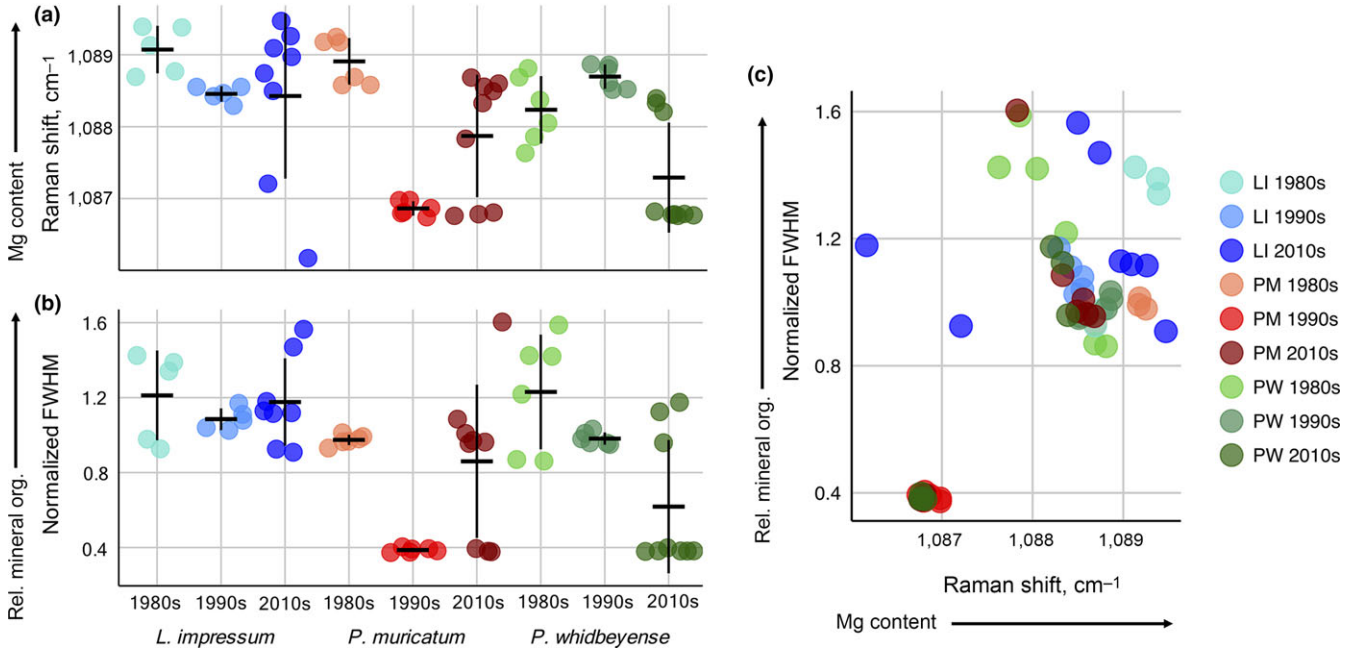

FIGURE 3 (a) Raman shift (cm^{-1}) and (b) relative mineral organization, or full width at half maximum (FWHM) normalized to Raman peak center, plotted per species over each sampling time, 1980s, 1990s, and 2010s. (c) Normalized FWHM, plotted by Raman shift for each data point. FWHM is plotted ×100 and all points are jittered for data visualization. Horizontal black lines represent group means and vertical black lines show standard deviation

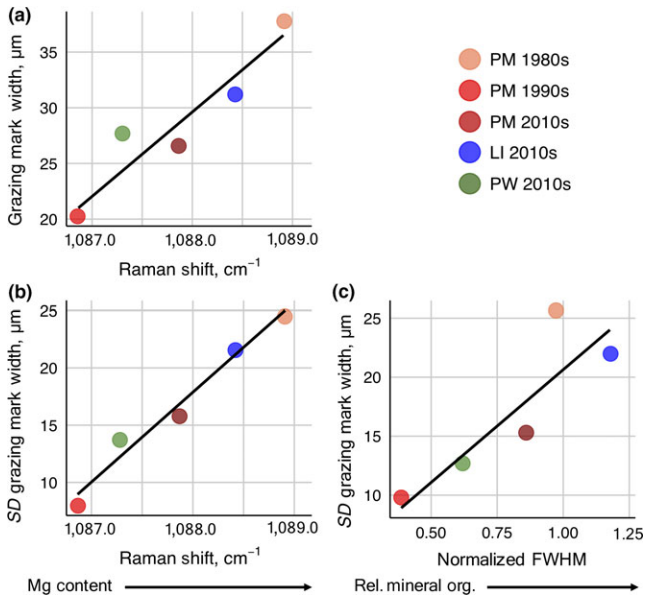

FIGURE 4 Significant relationships between (a) Raman shift (related to Mg concentration) and grazing mark width and (b) *SD* of grazing mark width, and between (c) relative mineral organization and *SD* of grazing mark width. Arrows show direction of increase and interpretation of Raman metrics along each *x*-axis. *P. muricatum* from the 1980s in light red, 1990s in bright red, and 2010s in maroon. *L. impressum* from the 2010s in blue, and *P. whidbeyense* from the 2010s in green. Linear regressions and statistics given in text. Refer to Supporting information Figure S10 for plots and statistics of all relationships between specimen mean, sample group mean, and sample group *SD*

structurally sound skeleton or possibly as grazers becoming impacted by ocean acidification before coralline algae.

We note that we did not find a strong correlation between Mg content and mineral organization across any of our samples (Figure 3c), which corresponds to the results of Bischoff et al. (1985), who found an inconsistent relationship between Mg concentration and mineral organization (positional disorder) in biogenic carbonates. While Pauly et al. (2015) noted seasonal fluctuations in Mg-O bond strength due to seasonal variation in temperature-linked Mg concentrations, we discount seasonal temperature as a potential driver in this study as all of our samples were collected in early summer across years. Similarly, we did not see a large variation in temperature or PDO across the timescale of our study (Supporting information Figures S1, S3–S6), nor did we find any significant relationships between PDO and our data (Supporting information Figures S8 and S9). Relative mineral organization (FWHM) and its variability has been found to respond to seawater carbonate saturation states more than to seawater trace element concentrations in aragonite (DeCarlo et al., 2017), potentially offering explanation for the partial decoupling of Mg content and mineral organization in our samples and showing a strong link between ocean acidification and mineralogical structure.

P. whidbeyense was the only species in which skeletal mineralogy changed systematically over time. Unlike our results from pooled specimens, Mg content declined and relative crystal organization decreased in *P. whidbeyense* between the 1980s and 2010s (Figure 3a,b). The changes observed over time in *P. whidbeyense* skeletal mineralogy are particularly interesting considering recent changes in

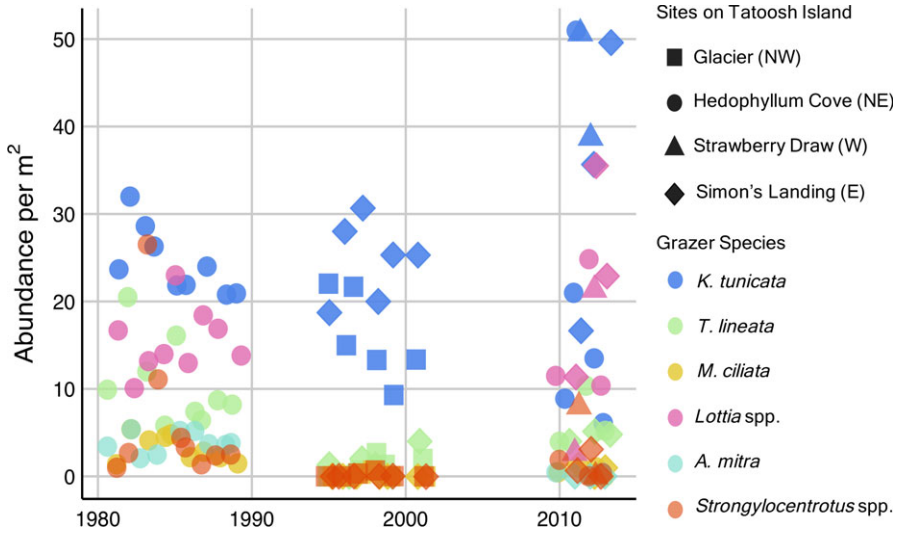

FIGURE 5 Grazer abundance over time at Tatoosh Island, WA. Shape indicates different sites around Tatoosh Island, while color denotes grazer species: blue, *Katharina tunicata*; green, *Tonicella lineata*; yellow, *Mopalia ciliata*; pink, *Lottia* spp.; light blue, *Acmea mitra*; and orange, *Strongylocentrotus* spp.
Adapted from McCoy and Pfister (2014)

the ecophysiology of this species. *P. whidbeyense* was historically thinner than both *L. impressum* and *P. muricatum*; however, recent acidification stress has reduced all three of these species to that of *P. whidbeyense*'s original thickness (McCoy & Ragazzola, 2014; Steneck & Paine, 1986). Conversely, calcified skeletal interfilament walls, providing the horizontal partition between cells, have thinned in *P. whidbeyense*, and not in *L. impressum* or *P. muricatum* (McCoy & Ragazzola, 2014). These morphology-dependent trade-offs in reducing skeletal volume point to a possible ecophysiological limitation to further thinning (McCoy & Ragazzola, 2014). The ecological niche of *P. whidbeyense* is traditionally that of a relatively poor competitor but fast grower (Paine, 1984), and the reduction of interfilament wall calcification has been interpreted as a trade-off that preserves its growth rate under acidified conditions (McCoy & Ragazzola, 2014). It seems likely that *P. whidbeyense* may then be more stressed by current seawater conditions than the other species studied here and pressured ecologically to maintain rates of growth and skeletal accretion.

We suggest that some coralline algae may be increasingly prone to grazing damage as acidification continues in the future. Acidification is also likely to affect grazers of coralline algae concurrent with algal skeletons becoming less resistant to grazing damage. Evidence from other systems suggests increased dissolution of grazing mollusk shells (Hall-Spencer et al., 2008; Rodolfo-Metalpa et al., 2011), effects on radula, and changes in feeding and metabolic rates (Leung, Russell, & Connell, 2017; Russell et al., 2013), all of which will affect grazing pressure through effects on grazer abundance, age-structure, and feeding rates. Acidification effects on noncalcified macroalgae are difficult to generalize across species and environments, but are likely to affect coralline-algal grazers as noncalcified macroalgae compete with coralline algae directly and indirectly through grazer-mediated apparent competition (Celis-Plá et al., 2015; Cornwall et al., 2011, 2017 ; Hepburn et al., 2011; Nunes et al., 2015). Overall, there is mounting evidence that trophic control in marine systems is changing and may potentially play a stabilizing role in the resonance of climate change responses across marine ecosystems (Falkenberg et al., 2013; Ghedini et al., 2015; Kroeker, Kordas, & Harley, 2017; McCoy & Pfister, 2014; McCoy et al., 2016).

Finally, we note that we observed strong relationships between sample means and standard deviations but not within individual samples. This emphasizes the importance of individual responses to stress and the variety of individual responses within a population (McCoy et al., 2018). Due to the time and cost of analysis, many geochemical studies limit sample replication. We note that in doing so, they may overlook variability due to differential responses between individuals, and may be excluding explanatory mechanisms from their findings that are important to ecophysiological response and interpretation.

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ORCID

Sophie J. McCoy http://orcid.org/0000-0003-1324-1578
Nicholas A.Kamenos http://orcid.org/0000-0003-3434-0807

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