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Historical baselines and the future of shell calcification for a foundation species in a changing ocean

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Seawater pH and the availability of carbonate ions are decreasing due to anthropogenic carbon dioxide emissions, posing challenges for calcifying marine species. Marine mussels are of particular concern given their role as foundation species worldwide. Here, we document shell growth and calcification patterns in *Mytilus californianus*, the California mussel, over millennial and decadal scales. By comparing shell thickness across the largest modern shells, the largest mussels collected in the 1960s–1970s and shells from two Native American midden sites (~1000–2420 years BP), we found that modern shells are thinner overall, thinner per age category and thinner per unit length. Thus, the largest individuals of this species are calcifying less now than in the past. Comparisons of shell thickness in smaller individuals over the past 10–40 years, however, do not show significant shell thinning. Given our sampling strategy, these results are unlikely to simply reflect within-site variability or preservation effects. Review of environmental and biotic drivers known to affect shell calcification suggests declining ocean pH as a likely explanation for the observed shell thinning. Further future decreases in shell thickness could have significant negative impacts on *M. californianus* survival and, in turn, negatively impact the species-rich complex that occupies mussel beds.

1. Introduction

As fossil fuel-sourced carbon dioxide continues to enter the surface ocean, ocean pH is decreasing, along with the concentration of carbonate ions available to calcifying organisms [1]. As a result, many recent studies of marine species have focused on how such changes affect the abilities of organisms to calcify (reviewed in [2,3]). Our current knowledge of how marine calcifiers are likely to respond to changing ocean pH is based, to a large extent, on laboratory experiments. An increasing number of studies have placed a variety of different organisms in seawater with pCO_2 levels ranging from approximately 380 to more than 3000 µatm and carefully monitored the responses. The majority of these experiments are short-term, ranging from multiple days to a few weeks (e.g. [2–5]). Across all studies, a complex picture emerges. Calcification in some species decreased, while those of others increased with increasing pCO_2; several showed highest rates of calcification at intermediate pCO_2 levels, and some species showed no significant change even under very high pCO_2 (e.g. [6]; see [3,7] for reviews).

In the case of shells, calcification is also affected by parameters other than seawater carbonate saturation state—notably temperature [8,9], wave exposure (e.g. [10]), food availability [11] and predation pressure [12,13], some of which can interact, either counteracting the effects of changing seawater saturation

state [11] or exacerbating them [3]. All of these factors probably contribute to how a species modulates calcification internally (e.g. [14]). In general, available data suggest that considerable variation exists within and among taxa in their ability to calcify and/or survive under changing CO_2 regimes, but the causes of that variation remain poorly understood.

Experiments have the advantage of being able to manipulate the saturation state of seawater and permit accurate measurements of the responses of the calcifiers, but the majority of the experiments so far have been short-term. Several exceptional long-term experiments show the existence of transgenerational effects on calcification [15], and the potential for adaptation [16] and evolutionary change [17,18]. However, such experiments are difficult for species with longer generation times or complex life cycles, and the responses of long-lived benthic marine species to ocean acidification (OA) remains poorly known [7]. Of particular importance is the role of intra-specific variation in calcification responses. Among population variation that is due to plasticity, standing genetic variation or local adaptation is likely to be important in determining the long-term consequences for many species [7,19,20], including macrobenthic species that have key functions in marine ecosystems. Thus, a more complete understanding of species responses to anthropogenic OA requires integrating experimental insights with information about how calcification rates vary in real populations over time, reflecting net responses to different selective forces, data that are currently scarce. Here, we quantify temporal trends in shell calcification of an intertidal mussel, *Mytilus californianus*, using field surveys in conjunction with archaeological and historical information. Specifically, we investigate how shell calcification in this species has changed over decadal to millennial scales and also discuss the implication of such changes for the future of this species.

Marine mussels in the genus *Mytilus* are found across sharp environmental gradients in intertidal and subtidal areas. Many *Mytilus* species are locally abundant and can play a foundational role in coastal environments by filtering great amounts of seawater and creating habitats that harbour a diverse assemblage of algal and invertebrate species [21–24]. Marine mussels are also extensively harvested for human consumption and are an important component of commercial aquaculture. Because of their importance as a food item and easy accessibility, intertidal mussels have been the target of subsistence harvesting over millennia and *Mytilus* shells are often common in archaeological middens. Owing to both their global importance and findings that suggest they are vulnerable to a changing ocean carbonate system [25,26], an assessment of changes to shell calcification in marine mussels over time is needed on a time scale relevant to population and evolutionary processes as well as changes in ocean chemistry. Here, we address this issue by testing whether shell calcification in *M. californianus* has changed in the northeast Pacific Ocean over decadal and millennial time scales. Inferring the cause(s) of any observed long-term trend in shell calcification can be challenging, given the diverse set of processes that influence calcification, many of which vary with the microhabitats a species experiences. We thus consider the role of diverse environmental drivers, including testing the effect of variation along intertidal gradients. Despite the potential for differences in shell thickness through time to be muted by confounding factors, we find strong differences across decadal and millennial scales.

2. Material and methods

We compared individual measurements from living populations of *M. californianus* with those from archival shell collections and previous studies at two different sites in the northeast Pacific: Tatoosh Island, WA (48.32° N, 127.74° W) and Sand Point, WA (48.126° N, 124.702° W) in the Olympic National Park, USA (electronic supplementary material, table S1). Tatoosh Island has been used by the Makah tribe for millennia [27,28] and has been the site of extensive ecological investigation of intertidal communities for five decades [25,29–32]. Sand Point is 30 km south of Tatoosh Island and also has a documented history of human shellfish harvesting for millennia [27]. Both sites are relatively remote and free of impacts such as point-source pollution and agricultural runoff. Based on the availability of historical information, we present analyses at two different temporal scales—millennial and decadal.

(a) Millennial-scale comparisons

We used archival shell collections to test whether shell thickness of mussels (a proxy for calcification) has changed through time by measuring the thickness of the calcitic inner prismatic layer (e.g. [33]) of modern shells collected from 2009–2011 and that of shells from the archaeological middens. Measuring the thickness after sectioning the shell along the axis of maximum growth allowed us to estimate thickness in a uniform way, even for partially preserved shells. At Tatoosh Island, we collected live *M. californianus* approximately one decade old in 2009 ($n = 31$ among three sites; electronic supplementary material, table S1). We compared these large, modern shells to Native American midden shells radiocarbon dated from 1000–1340 years BP in 2010 (AD 663–1008, analysed at the National Ocean Sciences Accelerator Mass Spectrometry facility, www.whoi.edu/nosams; $n = 11$; estimated lengths 80–117 mm) provided by the Makah Cultural and Research Center in 2009. At Sand Point, we obtained midden shells that ranged from 55 to 112 mm in estimated length ($n = 18$) and subsampled 10 shells to obtain radiocarbon dates from 2150–2420 years BP in 2015. Modern shells were collected at two locales closest to the midden site at approximately 1.0 m above mean lower low water (MLLW), with six collected 1 km north of the midden site by C.A.P. (Sand Point North, 48.135° N, 124.415° W, measured lengths = 49–62 mm, July 2010) and 10 collected by JTW at an adjacent rocky point 2 km south (Yellow Banks, 48.104° N, 124.670° W, measured lengths = 98–117 mm, August 2010). After collection, we removed the soft tissue and any epibionts, dried the shells to a constant mass, and measured the length and mass of a single valve. Because of post-mortem breakage of midden shells, we measured the fragment size and then estimated the total length of the individual using extrapolations based on the living mussels. Of the 18 midden shells we obtained from Sand Point, three could not be aged due to poor preservation of annual bands.

Annual and total shell growth was estimated by measuring the calcitic inner prismatic layer of the shell in a perpendicular direction to the main anterior–posterior axis of the shell, after preparing the shells using the methodology described by Pfister *et al.* [34]. Although there are many regions in cross-section where annual growth bands can be seen, we measured them in the thickest area of calcite in the shell distal to the umbo region. Measurements were taken from digital images using ImageJ (http://imagej.nih.gov/ij). Years were matched to each shell layer by assigning the first layer near the mantle as the most recent year of growth prior to collection. Successively older annual bands were then counted outward. The year of recruitment (1999–2000) for a cohort of the modern shells from Tatoosh Island was known [35], and thus confirmed the accuracy of our identification of annual bands. Although annual banding has been confirmed in many molluscs and is often attributed to winter cessation of growth [36], the precise cause and role, if any, of dissolution remains unknown. In cases where distinct annual

bands were difficult to determine, the total thickness of the calcite was still measured, but the shell was not used when analysing thickness related to age. To control for age differences among shells, we restricted our analyses to shells of similar lifespans in each time bin and thus focused on shells greater than 5 years of age. The shell material that persisted in the middens was from larger/older mussels and thus our modern samples also focused on longer-lived shells.

The age of the midden shells thus did not differ significantly from modern ones (mean of 7.07 years versus 6.44, $F_{1,29} = 0.690$, $p = 0.413$), but our samples of living individuals (in 2010) from two localities surrounding Sand Point differed in the length-to-age relationship: shells from Yellow Banks were longer per year of age than those from Sand Point North. We combined the two sites in order to bracket the greatest range of relationships that we might see in the midden shells and make the most conservative comparison, but our conclusions are qualitatively the same when we use only the larger modern shells from Yellow Banks.

(b) Decadal-scale comparisons

On Tatoosh Island, we compared the thickness of shell calcite layers of the 2009 population to shells collected by T.H.S. at the Glacier (GL) site and archived ($n = 6$). Other historical measurements from the GL site (1974–1976 versus 2011), as well as the North Island (NI) site (2001 versus 2011–2012), were made for smaller individuals. For Tatoosh GL, we used the measurements reported by Paine [29], supplemented by those of Suchanek [37], because the shells were measured in the 1970s and no longer exist. In cases where the original data from the published studies were not available, we digitized the x and y coordinates of each point in the published figures using ImageJ. These 1970s mussel measurements from the GL site were the smallest measurable individuals (13–45 mm in length) and reside at the upper margin of the mussel bed. We made a corresponding collection of 130 small individuals less than 46 mm SL at GL in 2011–2012 for comparisons to these earlier studies by T.H.S. and R.T.P. The collection of the smallest GL individuals was at the upper edge of the mussel bed (approx. 2.0 m above MLLW) where R.T.P. and T.H.S. had noted that they collected. We made further comparisons at the Tatoosh NI locales of Sweet 16 and NW Point using live shells collected in 2001 and 2011–2012. We compared live-collected shells with those collected in the middle tide heights (approx. 1.0 m above MLLW) of the mussel bed by E.S. in 2001 ($n = 42$) [38]. For comparison, at the two NI sites, C.A.P. collected 111 living individuals from the mid intertidal zone in 2011 and 2012, and targeted the same range of lengths (56–72 mm). After collection, we removed the soft tissue and any epibionts, dried the shells to a constant mass, and measured length and mass.

(c) Habitat-related variation

Because intertidal areas over which many *Mytilus* species range represent a strong gradient of emersion and access to food, we tested the effect of intertidal height on mass-to-length relationships in *M. californianus*. In 2011 and 2012, C.A.P. supplemented the 111 mid intertidal mussels at the NI sites, with 45 shells in the highest part of the mussel bed (approx. 2.0 m above MLLW) and 59 in the lowest band of the mussel bed (approx. 0.0 m) for a total of 215 *M. californianus* sampled from Tatoosh Island ranging from 39.8 to 87.6 mm in length. *Mytilus californianus* spans a large vertical gradient at these locales, because they face into a prominent swell direction, allowing us to look for submersion effects over a pronounced gradient.

3. Results

At Tatoosh Island, the total thickness of midden shells of *M. californianus* older than 5 years of age and living between

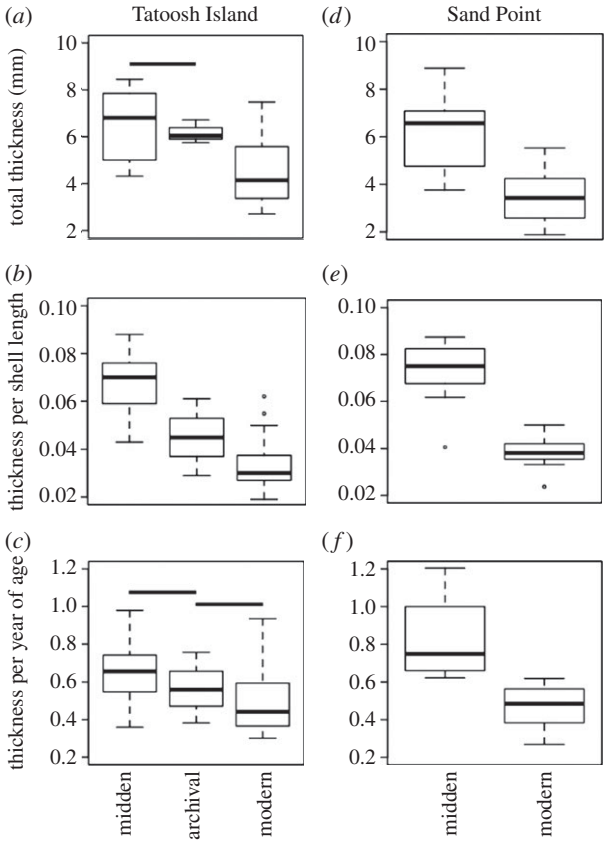

Figure 1. A comparison of the relative thickness of modern, archival and midden *Mytilus californianus* at two sites in Washington state: Tatoosh Island (*a–c*) and Sand Point (*d–f*); the total thickness (*a,d*), the thickness per shell length (*b,e*) and the thickness per year of age (*c,f*) were greater in the past at both sites. Boxplots show medians and lower and upper quantiles. ANOVA and Tukey HSD indicated significant differences among Tatoosh Island time periods, excepting those denoted with shared horizontal lines ($n = 11$ midden, $n = 5$ archival, $n = 31$ modern shells). Sand Point midden shells ($n = 12–15$) always differed significantly from their modern counterparts ($n = 11$) ((*d*) $t = 6.071$, d.f. $= 32$, $p < 0.001$; (*e*) $t = 9.896$, d.f. $= 32$, $p < 0.001$; (*f*) $t = 6.487$, d.f. $= 30$, $p < 0.001$). Only shells older than 5 years of age based on growth bands were included.

AD 663 and 1008 was on average 27.6% greater than that of a modern counterpart, while archival shells from the 1970s were similar to midden shells and 32.2% thicker than modern shells (figure 1*a*). When shell thickness was normalized to either shell length or to the age of the individual, the midden shells remained thicker per unit length (42.1%) and thicker per unit age (32.9%) compared with modern shells (figure 1*b,c*). Archival shells, when normalized to shell length, were thicker than modern shells (figure 1*b*), but significantly thinner per unit length than midden shells (figure 1*b*).

At Sand Point, WA, midden shells dated from 2150 to 2420 years BP were also thicker (93.9%) than modern shells of comparable size (figure 1*d*). The thickness per unit length (figure 1*e*) or thickness per year of age (figure 1*f*) of these midden shells was almost twice that of their modern counterparts.

Because shells from modern populations were always thinner than shells from the last century or earlier, we asked whether this thinning showed a trend with age in modern individuals. A linear mixed-effects model found no effect of year in the annual shell thickness from 1997 to 2010 at Tatoosh Island (individual shell was a random effect, year was a fixed effect; coeff. $= 0.0053$, $p = 0.313$, number of measurements $= 225$, number of shells $= 24$).

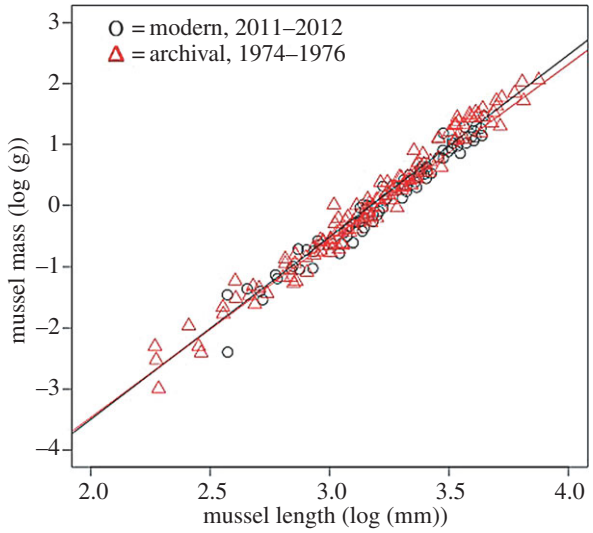

Figure 2. The mass-to-length relationship among 130 mussels on Tatoosh Island at the GL site 2011–2012, compared with shells from 1974–1976 [37] (R.T.P., unpublished; $n = 86$; 13–45 mm length) at the highest zone of the intertidal. Log mass (g) versus log length (mm) of both valves is plotted; confidence intervals for the slope and the intercept overlapped between the two groups with Model II regression. The modern mussels had a slope of 3.076 and an intercept of −9.752 ($r^2 = 0.967$), while the archival shells had a slope of 2.978 and an intercept of −9.528 ($r^2 = 0.968$).

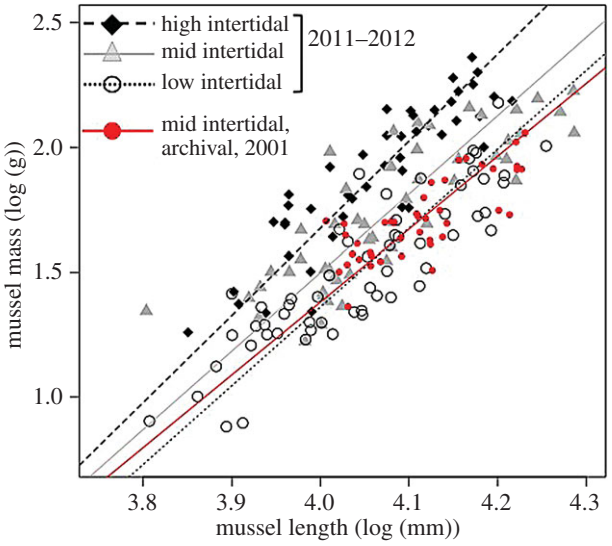

Figure 3. The mass-to-length relationship among 215 mussels at the Sweet 16 and NW Point sites on Tatoosh Island in 2011–2012, demonstrating that at the same locale, mussels that spend more time emergent are thicker per unit size. There was no statistical interaction between tide height and shell length as a predictor of shell mass (ANOVA, $F_{3,207} = 0.892$, $p = 0.446$), indicating the slopes did not differ for high = 3.488 (dashed line), mid = 3.142 (grey line) and low = 3.156 (dotted line). However, the investment of shell mass : length did differ and shells in the high zone were thicker than those in either the mid or low zones (ANCOVA, $F_{3,207} = 37.713$, $p < 0.001$, high = −12.277, mid = −11.067, low = −11.263). The red points are 42 *Mytilus californianus* collected at the same site in 2001, at the mid intertidal height. These shells showed a statistically similar mass-to-length relationship as the mid intertidal mussels from 2011–2012 (slope = 2.917, intercept = −10.287, $r^2 = 0.612$). Best-fit lines from a Model II regression (major axis) are shown. Log mass (g) versus log length (mm) of a single valve is plotted.

When we tested whether the annual thickness as a function of age differed between modern and midden shells at Tatoosh Island, we found no difference in the intercept ($p = 0.209$), slope ($p = 0.137$) or interaction term ($p = 0.608$, number of observations = 328, $n = 34$). Thus, there was no systematic change in the thickness of the annual bands of Tatoosh Island mussels during the lifespan of each individual, regardless of whether they lived approximately 1000 years ago or in the past two decades. Similarly, the Sand Point *M. californianus* shells showed no age-related trend, regardless of whether they were midden or modern shells (year coeff. = −0.016, $p = 0.211$; interaction coeff. = 0.022, $p = 0.238$). The intercept for midden shells, however, was significantly greater than that of modern shells (0.892 versus 0.431, $p = 0.0002$, number of measurements = 175, number of shells = 23). Thus, although overall shell thickness of *M. californianus* has decreased significantly over time at both of our sites, the width of annual increments of shell growth has not changed systematically over the past decades.

The shell mass per unit length of the smallest *M. californianus* showed no significant change from the 1970s compared with 2011–2012 on Tatoosh Island (figure 2). Model II regression (R package 'lmodel2'; www.r-project.org) indicated that the confidence intervals for both the intercepts and the slopes overlapped for both archival and modern shells at the GL site. Similarly, at the NI site, shell thickness per length also did not differ in 2011–2012 versus 2001 for mussels occupying the middle of the mussel bed and ranging in length from 56 mm to 72 mm (figure 3).

Our collection of individuals across three specific tidal heights (highest zone, mid zone and lower zone) showed no statistical interaction between tide height and shell length as a predictor of shell mass (ANOVA, $F_{3,207} = 0.892$, $p = 0.446$), indicating the slopes did not differ (figure 3). However, the investment of shell mass per unit length did differ and shells in the high zone were significantly thicker than those in either the mid or low zones ($F_{3,207} = 37.713$, $p < 0.001$), highlighting plasticity in shell thickness.

4. Discussion

Our results show that shells of *M. californianus* in Washington State are significantly thinner today compared with conspecific individuals in middens dating from 1000 to 2500 years BP. Although our extrapolation of shell dimensions in midden shells assumed that shell shape did not change, we note that this would not have changed our finding that these midden shells surpass in thickness or in thickness per unit age any modern shell we have ever observed in the vicinity of these midden locales. When we compared mussels in the largest sizes on Tatoosh Island over recent decades, total thickness and thickness per shell length were significantly lower in modern shells compared with archived shells from the 1970s; however, thickness per shell age did not exhibit a significant relationship. For mussels in the smallest sizes, we found no significant difference in the mass per unit size of mussels from the 1970s until the present (figure 2) or over the past decade (figure 3). For both sets of shells, the mass per unit shell length was unchanged through time.

The relationship of shell mass to length was a function of tidal height, with the most calcium carbonate per unit length seen in the highest part of the tidal range of *M. californianus* (figure 3). These tidal height patterns highlight the importance of comparing shells through time from areas of

similar intertidal height. Owing to the dispersal distance of California mussel larvae, their propensity to dislodge and move as adults, and the lack of substantial genetic differentiation across the geographical range of *M. californianus* [39], could be the result of strong local selection or a phenotypic response to local conditions. Although increased submergence time and access to food is known to increase linear growth rates in *M. californianus* [40,41], it may also result in increased allocation to the thickness of the shell material with increasing tidal height, as demonstrated at other locales for *Mytilus* species. For example, *M. edulis* in north Wales showed increased shell mass to area at greater intertidal heights, a pattern attributed to increased protection from predators [42], but may also be due to decreased food [22]. Regardless of the cause, shell thickness differences across the intertidal zone provide a cautionary tale about latitudinal or temporal comparisons among archival shells and motivated our careful comparison among studies. For the midden versus modern comparisons, there is no possibility that intertidal height plays a role in explaining the differences, as we never found modern mussels on Tatoosh Island that rivalled those of the midden shell in thickness. For the NI shells collection in figure 3, we compared the shell at the middle level based on collection notes (by E.S.).

The discrepancy between a decline in our measure of shell calcite thickness (figure 1) while shell mass-to-length ratio was unaffected (figures 2 and 3) is somewhat at odds with the result that the two variables were positively related among 48 live-collected individuals of *M. californianus* (correlation coefficient = 0.764), though there was considerable scatter around the relationship ($r^2 = 0.58$). The decrease through time in shell calcite thickness may indicate that the thickness of the shell calcite layer is more sensitive to changes in environmental conditions than total shell mass. A second reason that shell calcite thickness showed a decline when shell mass : length did not may be related to the observation that the thickness of the calcite layer (per unit length) is a less variable metric by half than the total shell mass (per unit length) (CV = 28.2 versus 56.4), perhaps indicating that detecting relatively small population-level changes in shell mass per unit length would require sample sizes larger than the ones used here or necessitate more precise metrics of size than only length. However, because the proximal and thickest region of the shell is generally preserved in archival specimens, calcite thickness may serve as an accurate metric of shell growth through time.

The long-term decline in shell thickness over the past millennia (figure 1) has several potential explanations, including a decrease in food availability, changes in seawater temperature, changes in predator-induced mortality and reduced calcification due to increasing pCO_2 of the coastal ocean (figure 4). Shell calcification is affected by a multitude of biotic and abiotic factors. Because many of these parameters are changing in response to anthropogenic activities, understanding how calcifiers will respond to changing ocean carbon chemistry requires models that account for all direct and indirect influences on calcification and energetic trade-offs within the animal.

In considering an integrated picture of animal investment to shell growth (figure 4), we placed shell area and shell thickness on different response axes to illustrate that environmental factors can have independent effects on each. We recognize, however, that environmental factors and shell responses can

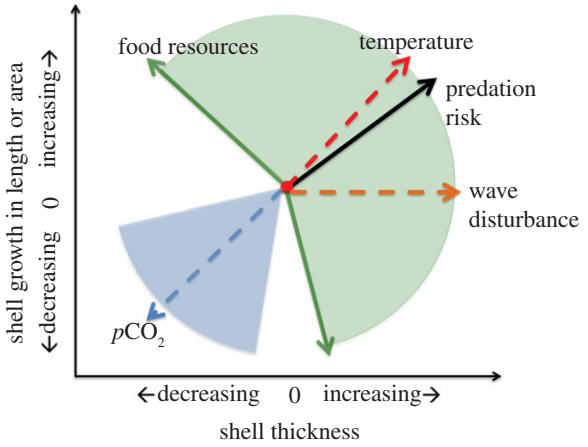

Figure 4. Possible shell length (or area) and thickness responses of a mussel shell to various biotic (solid) and abiotic factors (dashed) represented as vectors. The figure highlights the range of possible shell growth responses to environmental variables, and we suggest that coincident, opposing and interacting [43] environmental variables are possible. The green area designates the range of possible responses to increased food resources, including increases in both length and thickness, while the blue area shows possible negative responses to ocean acidification (e.g. [44]).

interact. Some molluscs also invest in increasing size for a period of time prior to investment in thickness (e.g. limpets in the genus *Patella* [45]). Thus, studies need to be attentive to the natural ontogenetic changes in shell growth to identify the appropriate response variable; short-term responses may not indicate the organism's response over its lifespan. Because we consider that museum specimens and the fossil record provide excellent archival material over the lifespan of the individual, we must look critically at which environmental drivers affect shell material investment in overall dimensions of area (size) versus thickness per unit area of shell. Further, investment in shell dimensions related to overall size versus investment to thickness can have distinct ecological outcomes for shells in their ecosystems and can imply different constraints [39].

The direct effect of increasing pCO_2 has been shown to produce a range of calcification and minerology responses in molluscs, both in the laboratory and in nature. Decreased size and thickness of mussels has resulted from elevated pCO_2, including during larval stages [26]. There are also an increasing number of studies that show decreased growth in shell area with decreasing pH (see review in [15]), sometimes as a result of net dissolution [46] and reduced shell density [47]. Melzner *et al.* [48] recorded dissolution internally in a congener (*M. edulis*) of our focal species, though we note that *M. edulis* has an aragonitic inner layer that is more susceptible to dissolution. Dissolution may also contribute to the annual banding pattern, as shown for other bivalves where winter conditions and low oxygen conditions promote dissolution and the formation of a darker organic-rich band [36]. Because the potential role of dissolution is untested here, we assumed that shell thickness reflected total calcification. Some species have been shown to maintain overall size dimensions while reducing shell thickness, as in the juveniles of *M. galloprovincialis* [49]. Shell composition can also change with pH. The mussel *M. galloprovincialis* showed a decrease in the amount of aragonitic nacreous material in the shell as pH decreased in proximity to CO_2-emitting vents [50]. Further, material properties of shells can be affected, with increased

pCO_2 increasing the stiffness of the outer shell material while increasing the softness of the interior shell in *M. edulis* [9].

While food availability can potentially affect shell calcification [11,51] and long-term global declines in phytoplankton have recently been reported [52], measurements of open ocean chlorophyll closest to our sites show no such decline over this period [34]. Nonetheless, in the absence of a millennial-scale database on phytoplankton abundance, changing food availability remains a possible explanation for the changes seen here. Though shell calcification rates in *Mytilus* correlate positively with temperature and salinity [53,54], analyses of the stable isotopes of oxygen in *M. californianus* through time provided no evidence of significant changes in seawater temperature over the past millennium in our focal region [34] and thus provide little support for warmer sea surface temperatures favouring thicker shells during the period 1000–2500 years BP.

Predators are another factor that influence the thickness of many marine molluscs [13], with a reduction in shell thickness reflecting a decline in predation pressure. Predation is a process that may have changed through time to change the selection pressures on mussel shell construction. Sea otter (*Enhydra lutris*) abundance in the Pacific Northwest is known to have decreased markedly over time [55], and there is evidence that they were used and hunted by Native Americans in this region, although their remains are nearly absent in the Sand Point midden [56]. Shell thickness may offer little protection against predation by sea otters, which prey on an entire patch of mussels at once [57]. More importantly, sea otters were successfully reintroduced south of Sand Point in 1970, and have experienced rates of population growth between 10% and 20% during the 1980s and 1990s, respectively [58]. This predicts that if shell thickness confers any protection against sea otters, it should be increasing at both Tatoosh Island and Sand Point, opposite of the trend we document. The black oystercatcher (*Haematopus bachmani*) is another predator of *M. californianus*, but oystercatcher abundance has been nearly constant for the past two decades at Tatoosh Island (J.T.W. 1974, unpublished data). Although their numbers prior to that are unknown, they are not crushing predators and are thus also unlikely to exert strong selection on shell thickness. The ability of sea stars to consume *M. californianus* is likely to be more dependent on the strength of the adductor muscle, not shell thickness, and this predator was common at all of our sites until 2015 [59]. Drilling predators such as whelks, on the other hand, are likely to select for increased shell thickness because a thicker, larger shell offers protection from such predation. Whelks were abundant in Native American middens in Washington state [27], and one common species (*Nucella canaliculata*) attains densities of 67.95 per m^2 at Tatoosh Island sites where our modern mussels were collected [32], suggesting that whelks have been a persistent selection pressure. The interaction between *M. californianus* and *N. canaliculata* varies geographically as a function of alternative prey [60,61]; the interaction is strong where alternative prey are rare (southern part of the range of *N. canaliculata* in central and northern California) and the snail drills individuals in excess of 100 mm, and weak where alternative prey such as barnacles are common (Oregon and Washington), where *M. californianus* is rarely drilled above a size of 30 mm [62,63]. In sum, the available information about spatial and temporal changes in predator communities does not provide any compelling evidence that predation pressure has had any significant influence on the concordant long-term declines in shell calcification documented here. Indeed, if decreased shell mass is occurring even as predator numbers are relatively high, as is suggested by our study, then modern mussels may be poorly matched with the predator mortality they will experience.

A difference in thickness between the midden shells and modern populations could also emerge if Native Americans were consistently harvesting shells larger than those in our modern samples. A bias towards large size is a common characteristic of marine invertebrates in archaeological middens because selective harvesting of larger individuals for human consumption is a common practice [64]. However, our thickness estimates for modern shells from Tatoosh Island (figure 1) are also biased towards large sizes because we specifically collected the largest/oldest shells for comparison with midden specimens. Additionally, we controlled for shell size and age in our analyses. Thus, it is highly unlikely that the observed trend of reduced thickness over time reflects a size-related sampling bias.

Finally, shell thickness of mussels may also be affected by wave exposure, as demonstrated in other molluscs [65], but available evidence suggests an increase in wave height over past decades [66], not the decrease expected to produce the observed decrease in shell mass shown here.

Available evidence suggests that the long-term decline in shell calcification in *M. californianus* may represent a response to OA. The decreases in pH documented at Tatoosh Island since 2000 [25,67] and the low pH and aragonite saturation states seen in California [68,69] suggest that carbon cycle changes in the ocean are producing an environment where aragonite and calcite are more difficult for organisms to accrete.

More importantly, regardless of the exact cause(s), the fact that shells of *M. californianus* are substantially thinner now compared with decades or centuries ago raises concerns about whether this species can retain its current role as a foundation species if shell thickness further declines and these thickness decreases lead to increased vulnerability to predation and disturbance (e.g. [13,43,70]). Similarly, if mussel byssal thread function is weakened with OA [71], and the viability of *M. californianus* at its upper intertidal limit is compromised by increased ambient temperatures [72], the net effect may be a decreased niche and a smaller climate envelope within which the California mussel can persist, altering the species interactions under which it evolved. If continued reductions in shell thickness significantly increase mortality risk for *M. californianus*, the integrity of their structurally complex mussel beds that support over 300 species of associated organisms [21,24] could be compromised, potentially reducing associated intertidal species throughout the range of the California mussel.

There is a clear need for careful monitoring of seawater chemistry, for further studies of shell calcification of this foundation species, and for increased understanding of the physiological, mineralogical and genetic aspects of shell calcification to aid in the development of predictive models of species response to OA. Mussels not only provide an opportunity to understand the link between seawater chemistry and calcification, but their ecosystem importance and strong links to the carbon cycle make this imperative [73].

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provided samples. C.A.P., K.R. and S.J.M. performed analyses. All authors contributed to data interpretation and to writing the manuscript. All authors gave final approval for publication.

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