CALIFORNIA MUSSELS AS BIO-INDICATORS OF OCEAN ACIDIFICATION

A Report for:

California's Fourth Climate Change Assessment

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PREFACE

California's Climate Change Assessments provide a scientific foundation for understanding climate-related vulnerability at the local scale and informing resilience actions. These Assessments contribute to the advancement of science-based policies, plans, and programs to promote effective climate leadership in California. In 2006, California released its First Climate Change Assessment, which shed light on the impacts of climate change on specific sectors in California and was instrumental in supporting the passage of the landmark legislation Assembly Bill 32 (Núñez, Chapter 488, Statutes of 2006), California's Global Warming Solutions Act. The Second Assessment concluded that adaptation is a crucial complement to reducing greenhouse gas emissions (2009), given that some changes to the climate are ongoing and inevitable, motivating and informing California's first Climate Adaptation Strategy released the same year. In 2012, California's Third Climate Change Assessment made substantial progress in projecting local impacts of climate change, investigating consequences to human and natural systems, and exploring barriers to adaptation.

Under the leadership of Governor Edmund G. Brown, Jr., a trio of state agencies jointly managed and supported California's Fourth Climate Change Assessment: California's Natural Resources Agency (CNRA), the Governor's Office of Planning and Research (OPR), and the California Energy Commission (Energy Commission). The Climate Action Team Research Working Group, through which more than 20 state agencies coordinate climate-related research, served as the steering committee, providing input for a multisector call for proposals, participating in selection of research teams, and offering technical guidance throughout the process.

California's Fourth Climate Change Assessment (Fourth Assessment) advances actionable science that serves the growing needs of state and local-level decision-makers from a variety of sectors. It includes research to develop rigorous, comprehensive climate change scenarios at a scale suitable for illuminating regional vulnerabilities and localized adaptation strategies in California; datasets and tools that improve integration of observed and projected knowledge about climate change into decision-making; and recommendations and information to directly inform vulnerability assessments and adaptation strategies for California's energy sector, water resources and management, oceans and coasts, forests, wildfires, agriculture, biodiversity and habitat, and public health.

The Fourth Assessment includes 44 technical reports to advance the scientific foundation for understanding climate-related risks and resilience options, nine regional reports plus an oceans and coast report to outline climate risks and adaptation options, reports on tribal and indigenous issues as well as climate justice, and a comprehensive statewide summary report. All research contributing to the Fourth Assessment was peer-reviewed to ensure scientific rigor and relevance to practitioners and stakeholders.

For the full suite of Fourth Assessment research products, please visit www.climateassessment.ca.gov. This report explores the utility of employing newly settled California mussels as a bio-indicator of effects of ocean acidification.

ABSTRACT

A critical need in California is to develop robust biological indicators that can be used to understand emerging impacts to marine systems arising from human-induced global change. Among the most worrisome environmental stressors are those associated with shifts in the carbonate system of seawater, including reductions in ocean pH and decreased availability of carbonate ions (together termed 'ocean acidification'). In this study, we explored the utility of employing newly settled California mussels (*Mytilus californianus*) as a bio-indicator of effects of ocean acidification. Our approach involved a field assessment of the capacity to link patterns of mussel recruitment to climate-related oceanographic drivers, with the additional step of conducting measurements of mussel morphology and body condition to maximize the sensitivity of the bio-indicator. Our results indicate that larval shells retained in mussels that have settled on the shore are smaller in area when larval stages were likely to have been subjected to more acidic (lower-pH) seawater. Similarly, the body condition -- a measure of general health -- of newly settled juveniles subjected to lower-pH seawater was reduced in cases where those waters were also warm. These findings suggest a strong potential for newly settled California mussels to serve as informative bio-indicators of ocean acidification in California's coastal waters. Future efforts should pursue additional validation and possible expansion of this methodology, as well as the feasibility of a sustained commitment to sampling newly settled individuals of this species at multiple locations throughout the State.

Keywords: bio-indicator, climate change, mussel, ocean acidification, pH, temperature

HIGHLIGHTS

- Ocean acidification -- human-induced changes to the chemistry of seawater (including reductions in pH and decreased availability of carbonate ions) -- is a growing threat to coastal waters of California.

- Multiple lines of evidence suggest that the California mussel (*Mytilus californianus*) is in decline over large portions of the State, potentially due in part to ocean acidification.

- The current study demonstrates that the area of the larval shell of California mussels is smaller in individuals that likely experienced lower-pH waters while in the plankton. The larval shell, produced during the planktonic phase, remains visible in young juveniles that have settled into shoreline habitat.

- The 'body condition' -- a metric of overall health -- of newly settled juvenile California mussels is reduced in individuals that have been subjected to lower-pH waters, under conditions when those waters were also warm. The body condition is the ratio of dry tissue mass of individuals, divided by their total dry weight including the shell.

- Overall, the California mussel meets multiple criteria for serving as a bio-indicator of the advance of ocean acidification and its consequence for marine systems within the State and could be utilized more broadly by monitoring programs, potentially in collaboration with citizen science efforts.

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1: Introduction

1.1 Ocean Acidification in California Waters

Marine waters offshore of California are part of the California Current Large Marine Ecosystem (CCLME), one of Earth's four major upwelling systems. Such systems are unusually productive biologically and support many of the world's most valuable fisheries. In addition, upwelling systems are characterized by surface waters that can be low in pH. This latter trait arises due to coastal winds that cause deeper waters that have relatively high concentrations of carbon dioxide (CO_2) to rise to shallower depths where they bathe shoreline communities. CO_2 and pH are inversely related in seawater, and low-pH waters are often depleted in oxygen (even becoming hypoxic in some areas).

The low-pH seawater conditions that occur naturally in the CCLME are exacerbated by the absorption of human-produced CO_2 into the ocean (Chan et al. 2017). Such 'ocean acidification' occurs through a set of linked chemical reactions that increase the concentration of free hydrogen ions in seawater and decrease the availability of carbonate ions (e.g., Zeebe and Wolf-Gladrow 2001; Doney et al. 2009). Approximately a third of the CO_2 that has been emitted through human activities to date has dissolved into seawater (Sabine et al. 2004; Liu and Xie 2017), and this process is continuing.

Because of the coupling between natural (upwelling-driven) and anthropogenic (CO_2 emission-driven) processes, California waters are already experiencing declines in pH that are not expected in other areas of the world's oceans for decades (Feely et al. 2008; Chan et al. 2017). These perturbations to seawater chemistry join others associated with changing seawater temperatures (García-Reyes and Largier 2010) and reductions in ocean oxygenation (Bograd et al. 2008; Chan et al. 2008). Therefore, marine communities along the coast of California are increasingly subjected to a suite of concurrent environmental stressors. Substantial impetus exists to understand, quantify, and project biological and ecological consequences of these stressors, which current work suggests may be pervasive and diverse (Kroeker et al. 2010, 2013; Gaylord et al. 2015).

Ocean acidification is widely recognized as a challenge for calcifying species. Reductions in the availability of carbonate ions impair this process, making it more difficult for marine organisms to produce shells and skeletons. Evidence is especially widespread for tropical corals (e.g., Kleypas et al. 1999; Langdon 2000; Albright et al. 2016) and many bivalves (e.g., Talmage and Gobler 2009; Gazeau et al. 2010), including taxa that inhabit the U.S. west coast (Gaylord et al. 2011; Hettinger et al. 2012, 2013; Barton et al. 2015; Frieder et al. 2014; Waldbusser et al. 2015). Additional effects on the physiology and behavior of marine species can accrue as organisms face increased challenges in maintaining internal acid-base balance in ocean waters of reduced pH (e.g. Munday et al. 2009; Somero et al. 2016; Jellison et al. 2016). Broader ecological consequences are expected (Gaylord et al. 2015), such as those due to altered predator-prey relationships (e.g., Ferrari et al. 2011; Kroeker et al. 2014b; Sanford et al. 2014; Jellison et al. 2016) and degradation of habitat provisioning by structure-forming taxa like corals and mussels (e.g., Sunday et al. 2017).

Portions of what is now a large body of work concerning effects of ocean acidification, hypoxia, and shifts in temperature -- including implications for California species -- have been

summarized by the West Coast Ocean Acidification and Hypoxia Panel (www.westcoastoah.org). Additional reviews address effects of these factors in more detail as well as synergies among them (e.g., Hofmann and Todgham 2010; Kordas et al. 2011; Kroeker et al. 2013; Sunday et al. 2017).

1.2 Identifying Biological Indicator Species

One focus in planning for ocean acidification has been to identify sentinel organisms that might respond early or acutely to this process. Such 'bio-indicator' species allow scientists and managers to move beyond the mere assessment of physical shifts in ocean conditions to an integrated consideration of biological and ecological consequences.

In pursuing effective bio-indicator species, it is essential to recognize that even taxa with demonstrated vulnerability to acidification are unlikely to respond to only this factor. For example, all organisms possess temperature-dependent physiology (this is a basic tenet of biology; e.g., Brown et al. 2004; O'Connor et al. 2007), and connections between temperature, metabolic rate, and food availability are known to modulate the physiological consequences of environmental stress (Thomsen et al. 2013; Kroeker et al. 2016). Therefore, the key to identifying an effective bio-indicator is to find one sensitive enough to the environmental driver of interest that other abiotic and biotic processes play a secondary role.

At the State and Federal level, excellent progress has been made in identifying preliminary indicator species that can be employed at local and regional scales to identify, track, and respond to multiple components of global environmental change. For example, the Ocean Climate Indicators report summarizes potential indicator species for North-Central California (National Marine Sanctuaries, https://farallones.noaa.gov/manage/climate/indicators.html). Aspects of this progress at the State level have been summarized in the Indicators of Climate Change in California report produced by California's Office of Environmental Health and Hazard Assessment [OEHHA] (oehha.ca.gov/climate-change/document/indicators-climate-change-california). One key trend discussed in the 2018 report is that the majority of long-term marine biological datasets in California have been investigated for less than 10 years, with very few extending beyond 20 years. The longest biological dataset for California quantifies zooplankton volume and diversity from quarterly California Cooperative Oceanic Fisheries Investigations (CALCOFI) cruises (64 years; e.g., Bograd et al. 2003). These biological data are paired with measurements of dissolved oxygen, but few records are linked to samples of seawater carbonate chemistry (e.g., pH, pCO_2). Another available dataset is based on surveys of *Macrocystis pyrifera* (giant kelp) and several fish species conducted in the Santa Barbara Channel through the Santa Barbara Coastal Long Term Ecological Research program. However, these data are more limited in duration (12 years) and geographic extent (Southern California only). The Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO) has generated an additional 12-year record of abundance, growth, and fecundity of several invertebrate and algal species. The relatively high resolution (monthly sampling) of this latter time series increases its utility for quantifying climatic patterns (http://www.pacificrockyintertidal.org).

Here, we report on the possibility of building upon these existing monitoring programs, employing the California mussel (*Mytilus californianus*) as a bio-indicator of ocean acidification. Within this context, we consider the suitability of this species relative to several desirable traits for bio-indicators:

- Sensitive to environmental change
- Easily recognizable by people
- Simple to access
- Possess ecological and/or economic value

As we discuss in the following section, California mussels appear to meet all of these criteria. They are susceptible to changes in seawater chemistry and/or temperature. They are familiar to State residents as one of the most common intertidal organisms along the west coast of the U.S. (Morris et al. 1980). They are readily accessible, because they become emergent on the shore as tides recede. Also, they are important community members whose physiological and ecological performance may operate as an index of broader ecosystem function. *M. californianus* is a classic 'foundation species' that strongly influences community structure both through its dominant status in competing for space (Paine 1974) and because mussel beds provide habitat and protection from thermal stress for hundreds of other species that reside within them (Suchanek 1992; Lafferty and Suchanek 2016; Jurgens and Gaylord 2017). Therefore, this species has been identified previously as a potential bio-indicator by two National Marine Sanctuaries in California (Greater Farallones and Cordell Bank; https://farallones.noaa.gov/manage/climate/indicators.html). Moreover, California mussels are the foci of other long-term ecological monitoring programs for intertidal and subtidal ecosystems (e.g., LiMPETS [http://www.limpets.org/], MARINe [http://www.marine.gov/], and PISCO [http://www.piscoweb.org/]).

1.3 Background Evidence of Susceptibility of the California Mussel to Environmental Change

Prior and emerging information provide a first indication that the California mussel might exhibit susceptibility to changing environmental drivers, which is the most important criterion for a bio-indicator. Lines of evidence, including data from various locations along the west coast of the U.S., include the following:

- Percent cover of adult mussels in Southern California has decreased relative to historical levels (Smith et al. 2006). More recent reports at scientific conferences indicate that this trend extends to the present day (Robles et al. 2015, Western Society of Naturalists published abstract, November 2015 conference).
- Declines in abundance and size of intertidal mussels (*M. californianus* and *Mytilus trossulus*) have been observed in northern Washington State (Wootton et al. 2008). These declines were correlated with reductions in ocean pH documented over the same 8-year period, and modeling of the phenomenon indicates a possible causal link to ocean acidification.
- An analysis of data spanning >14 years has documented remarkably tight relationships between large-scale oceanographic indices (in particular, the North Pacific Gyre Oscillation, or NPGO) and mussel recruitment at several sites along the Oregon coast (Menge et al., 2009). These shifts, which indicate a close tie between climatic alterations in ocean circulation and larval success, have been attributed to NPGO-associated changes in phytoplankton food availability that altered survival and rates of return of

larval mussels to the shore. Subsequent work has followed up on these trends to reveal a 'filtering' effect of species interactions on later life stages of mussels (Menge et al. 2011). These latter data imply that relatively new recruits might provide a stronger link than adult mussels to environmental fluctuations.

- In an explicit test of the susceptibility of larval stages of the California mussel to ocean acidification, Gaylord et al. (2011) have shown that larval mussels exposed to elevated-CO_2 seawater grow smaller, thinner, and weaker shells and produce considerably less tissue mass for their body size. The latter trend suggests that conditions of ocean acidification impose appreciable energetic costs for larvae, a possibility also supported by additional studies (e.g., Frieder et al. 2014).

- The Bodega Ocean Acidification Research (BOAR) group, in collaboration with a consortium of west-coast marine ecologists and oceanographers (Ocean Margin Ecosystem Group for Acidification Studies; OMEGAS), led a large-scale field experiment examining how geographic variation in the strength of upwelling might influence growth of juvenile mussels and their susceptibility to predation by drilling whelks. Results indicate that decreased pH and low food, which are common during the early stages of upwelling, are associated with decreased growth, as are peak aerial temperatures during low-tide emergence (Kroeker et al. 2016). These data further support a connection between climatic factors and mussel performance throughout California and along other portions of the west coast of the U.S.

- Researchers have documented decadal-scale changes in the thickness of *M. californianus* shells (Pfister et al. 2016). These efforts focused on comparisons of the thickness of present-day mussel shells to those collected in the 1970s and those from Native American middens (~1000-2400 years before present). For large, adult mussels, present-day shells are significantly thinner than both shells from the 1970s and midden shells.

- Mussels as a general group may be experiencing declines, potentially due to climate-change drivers. Sorte et al. (2017) report that a congener, *Mytilus edulis*, on the east coast of the U.S. has decreased in abundance by more than 60% compared to the 1970s. Kroeker et al. (2014a) have demonstrated vulnerability of an additional congener, *Mytilus galloprovincialis*, to ocean acidification in the laboratory.

1.4 Goal of the Present Study

Together, the above findings point to the possible suitability of mussels to operate as early indicators of ecosystem vulnerability. We therefore undertook an exploration of the potential for newly settled California mussels to serve as a biological indicator of ocean acidification in coastal waters of the State of California.

Although ocean acidification covaries with hypoxia in many cases, intertidal organisms will not typically provide good indicators for hypoxia because breaking waves in the surf zone rapidly inject oxygen into the waters that bathe these habitats (rates of off-gassing of CO_2, by contrast, are much slower due to properties of the coupled reactions that underlie the carbonate system of seawater).

Our approach to examine the utility of *M. californianus* as a bio-indicator of ocean acidification focused on 1) quantifying the intensity and temporal pattern of mussel recruitment in the face of variable environmental conditions, and 2) quantifying the relationship between seawater properties and simple morphological and body condition characteristics of mussel recruits.

2: Methods

2.1 Mussel Recruitment

Utilizing techniques that have been employed for decades along the west coast of North America (Broitman et al. 2008), we deployed mussel recruit collectors (plastic meshes that mimic mussel byssal threads, a preferred settlement substrate) in the rocky intertidal zone of focal field sites. Collectors were positioned in the low intertidal zone along horizontal rocky benches at the lower edge of mussel beds that were 6-10 cm (2.4-3.9 in) thick and subjected to near-continuous wave action. Our primary sampling targeted mussel recruitment patterns within the Bodega Marine Reserve (BMR; 38.191, -123.440), where we conducted deployments approximately every two weeks in 2016-2017, encompassing the full reproductive season of the California mussel. For the purposes of this report, these data were also linked to additional data collected by E. Rivest during 2015, funded by other sources. This approach also built upon available preliminary data collected at BMR from 2007-2008 (Sanford and Worth 2010).

Newly settled mussels (also termed recruits in this report) were additionally collected within three other marine protected areas situated from northern to southern California (Waddell Creek: 37.108, -122.294; Asilomar State Beach: 36.622, -121.942; and Crystal Cove State Park: 33.565, -117.833) as an initial assessment of the viability of using such methods throughout the State. Mussels were collected in these marine protected areas using California Department of Parks and Recreation Permit No. 17-820-32 and California Department of Fish and Wildlife Permit No. SC-751.

For each deployment, five mussel recruit collectors were secured in the low-intertidal zone using stainless steel fasteners screwed into pre-drilled holes in the rock bench. The collectors were left in the field for two to four weeks, after which they were retrieved and returned immediately to the laboratory. The mussel recruit collectors were processed at that time or after storage at -20 °C (-4 °F) as follows. Deployment collectors were cut open, and contents of the five mussel recruit collectors from a given deployment were rinsed into a finger bowl using filtered seawater. All organisms in the finger bowl were then sorted under a dissecting microscope to find *Mytilus* individuals. Additionally, each rinsed recruitment collector was examined under the microscope to extract mussels still attached by their byssal threads. All sorted *Mytilus* individuals were counted and stored individually at -20 °C (-4 °F) until further analysis (occasionally an individual mussel was damaged during manipulation, in which case it was discarded). Since *M. californianus* recruits cannot be morphologically distinguished from the two congeners that also inhabit the U.S. west coast (*M. galloprovincialis* and *M. trossulus*), we used polymerase chain reaction (PCR) techniques to sequence the cytochrome oxidase I gene, which revealed that 97% of the recruits we sampled at our focal BMR site were *M. californianus* (E. Rivest, unpublished data).

2.2 Morphology and Body Condition of Mussel Recruits

2.2.1 Sample Processing of Mussel Recruits

Mussel recruits were dried in individual aluminum boats at 60 °C (140 °F) until they reached a constant weight. The dry weights of intact mussels were then measured using a Mettler Toledo XP2U microbalance. Next, the tissue of each mussel was removed from the shell by incubating

it in 15% H_2O_2, 0.05 M NaOH for 4-6 hours at room temperature with periodic agitation. Afterwards, the cleaned shell was rinsed in de-ionized water and dried to constant weight at 60 °C (140 °F). The dry weights of the cleaned shells were measured, and the weight of dry tissue mass was calculated by subtracting the weight of the clean, dry shell from the weight of the dry intact mussel. The two valves of the clean mussel shells were then separated and laid flat, and their exterior was photographed using a Leica MC170 HD Camera attached to a Leica M125 dissecting scope.

Since the larval shell of *M. californianus* is retained after settlement and remains visually distinct from the recruit shell, individual parameters could be calculated for both the larval and recruit period (Fig. 1). To image the larval shell, which is located at the anterior end of the recruit shell, the two valves of the mussel shell were mounted on a modified microscope slide so that the larval shell was visible and positioned in a level plane in the photographs.

2.2.2 Morphological Parameters

Several morphological metrics were determined from the above weight and shell measurements. The following metrics were quantified using ImageJ v.1.50 (Schneider et al. 2012).

1) Larval shell area, assessed from a tracing of the perimeter of the larval shell.
2) Larval shell length, determined as the longest diameter of the larval portion of the shell.
3) Recruit shell area, quantified from a tracing of the perimeter of the overall valve.
4) Recruit shell length, measured as the total length from the umbo (including the larval shell) to the posterior growing edge of the shell.

Each of these measurements was made for the right and left valves of each individual, and average values of the two valves were calculated for analysis.

5) A simple metric related to shell thickness was also quantified by dividing the total dry weight of both clean mussel valves by the total projected area of both valves. Although this metric does not equal thickness per se, it tends to co-vary with thickness. Similar metrics have been employed in other studies due to their ease of use (e.g., Reimer and Tedengren 1996; Freeman and Byers 2006).

Figure 1. Example of a newly settled mussel (*Mytilus californianus*) individual extracted from a recruitment collector for processing. The larval portion of the shell is visible as the slightly darker (yellow or tan-hued) prominence at the apex of the umbo, visible at the anterior end of each valve, located here at the bottom of the image.

2.2.3 Body Condition Parameter

Finally, a metric of generalized health or body condition of mussel recruits was assessed.

6) 'Body condition' was calculated as: (Weight of the dry tissue mass)/(Weight of dry intact mussel) * 100 (see, e.g., Mann 1978).

2.3 Environmental Data Collection

Field conditions of seawater pH and temperature during the mussel collector deployments were determined using both an autonomously recording sensor that quantified conditions every ten minutes and discrete water samples collected weekly to monthly.

2.3.1 Continuous pH and Temperature Measurements

Autonomous recordings relied on a TidalFET instrument, which employs a Honeywell DuraFET sensor to measure pH (total scale; Zeebe and Wolf-Gladrow 2001) along with temperature (Martz et al. 2010; Hofmann et al. 2011; Chan et al. 2017). It was deployed in the

low intertidal zone and recorded every 10 minutes between February 19, 2016 - April 5, 2017. Due to instrument failures, TidalFET data were not collected during other parts of the project period. The instrument was affixed to the rocky substrate using a protective polyvinyl-chloride housing at +0 m Mean Lower Low Water (MLLW), and the active element of the pH sensor was encased by a copper cage to prevent biofouling. The instrument was recovered every four to six weeks for data acquisition and re-calibration. The DuraFET-based pH sensor was calibrated using a Tris buffer (2 amino-2-hydroxymethyl 1,3-propanediol in synthetic seawater; Certified Reference Material, A. Dickson, Scripps Institution of Oceanography) while immersed in a 25 °C (77 °F) water bath for at least six hours. *In situ* water temperature was calculated using thermistor calibration methods from Steinhart & Hart (1968) and pH values were then calculated using predetermined offsets between the Dickson TRIS buffer, a reference electrode (Chan et al. 2017), and the electromotive force (EMF) of the DuraFET pH sensor.

2.3.2 Discrete Samples

Bottle samples were collected near the TidalFET one to two times per month and analyzed for seawater pH and total alkalinity. Temperature and salinity data were collected alongside these samples in the field using a YSI Professional Plus handheld multi-parameter meter, since this information is required during analysis of the bottle samples once they are returned to the laboratory. Samples for total alkalinity were frozen immediately following collection, and pH samples that could not be run immediately were preserved with $4 \times 10^{-4}\%$ $HgCl_2$ fixative. Seawater samples were collected and processed following best practices (Dickson et al. 2007). Seawater pH was measured (total scale; Zeebe and Wolf-Gladrow 2001) via spectrophotometric analysis using a pH-sensitive dye (m-cresol purple). Total alkalinity was determined using potentiometric acid titration. Carbonate chemistry parameters were calculated using the carbonate system computer software CO2SYS v2.1 (http://cdiac.ess-dive.lbl.gov/ftp/co2sys/), with K_1 and K_2 dissociation constants based on Mehrbach et al. (1973) as refit by Dickson and Millero (1987), and $KHSO_4$ based on Dickson et al. (2007).

2.3.3 Seawater Conditions during each Mussel Recruitment Interval

Average seawater pH and temperature conditions relevant to mussels that settled during each collector deployment were determined. Conditions pertinent to larvae were distinguished from those pertinent to recruits using the following methodology.

Seawater conditions experienced by larvae were assumed to span the two weeks preceding the mid-point of each collector deployment. Statistical analyses of larval characteristics (larval shell area, larval shell length) therefore relied on averages of pH and temperature over this duration. This approach takes into account the fact that the larval shell forms prior to settlement on the shore. Although the larval duration of *M. californianus* is known with relatively low precision, two weeks is a reasonable estimate. For instance, Strathmann (1987) lists the larval duration for this species as 10 days, while Trevelyan and Chang (1983) found in laboratory experiments that some *M. californianus* larvae could stay alive in the water column for as long as 45 days if never provided a settlement cue.

For statistical analyses of recruit characteristics (recruit shell area, recruit shell length, recruit shell thickness, and recruit body condition), average seawater pH and temperature were calculated for the duration of the deployment of each collector.

When analyzing TidalFET records, data quality was maximized by eliminating time periods when tidal height was less than +0.3 m (+1 ft) above MLLW -- that is, when the TidalFET might have been partially or fully exposed.

In the case of the discrete water samples, because rough weather often precluded collection of these samples at BMR, additional samples from a more accessible site a few hundred meters away (Horseshoe Cove: 38.316, -123.072) were employed in calculations of average seawater pH and temperature during poor-weather deployments. When this data replacement approach was used, estimated pH and temperature values for BMR were generated by applying an offset to the Horseshoe Cove data that was determined previously by comparing pairs of BMR and Horseshoe Cove data from periods when both records were available.

2.4 Statistical Analyses

All data were analyzed using R v.3.4.3 (R Core Team 2017). Statistical assumptions of normality and heteroscedasticity were met based on Q-Q plots and score tests for non-constant error variance, sometimes following a natural log or inverse transformation.

Linear mixed effects models (lmertest package in R; Kuznetsova et al, 2017) were used to estimate effects on larval shell area and length, recruit shell area and length, recruit shell thickness, and recruit condition index, with seawater pH and seawater temperature as continuous fixed factors and collector deployment as a random effect. For shell thickness, residuals of the relationship between shell thickness and shell length were evaluated in the model, to address the possibility that thickness varied with size. For all analyses, model selection was performed incrementally, following Burnham and Anderson (2002). Additionally, in models where the random effects did not add sufficient explanatory capability, the random effects were dropped before proceeding with model selection. Likelihood ratio tests (type III sum of squares) were conducted on selected models.

Statistical analyses examining relationships between seawater properties (pH and temperature) and mussel morphology and/or body condition were conducted primarily on the higher-quality datasets derived from the continuous, autonomously recording TidalFET sensors. However, to explore the possibility of extending the dataset to periods where high-resolution TidalFET data were unavailable, additional statistical tests were run using a combination of TidalFET data and discrete water sample (bottle) data to characterize pH and temperature. Using the latter data required linking morphological and/or body condition data to estimates of deployment-average pH that were based on only one or two instantaneous measurements (each bottle sample was acquired at a particular point in time). As a consequence, statistical models involving discrete water samples innately led to more tenuous inferences. Such lower-confidence results therefore appear exclusively in the Appendix.

3: Results and Discussion

3.1 Rates of Mussel Recruitment

Mussel recruits were collected in the low-intertidal zone at BMR from March 2015 to September 2017. Recruitment was detected throughout this two-year period. Although recruitment rates

were more variable during the summer, recruitment occurred year-round, with at least one mussel individual recovered for each collector deployment. Daily recruitment rates calculated from each deployment ranged from 0.04 to 2.21 mussel recruits per day per set of five collectors (Fig. 2). Recruitment in 2015 was higher than in the other two years. In 2016 and 2017, recruitment was lower during the summer than in the spring and fall, on average.

Mussel recruitment rates at the BMR site during 2015-2017 were depressed compared to recruitment levels observed in 2007-2008 (Fig. 3; see also Sanford and Worth 2010). We note, however, that due to slight differences in methodology, the two datasets may not be exactly comparable (e.g., mussels during 2007-2008 were collected from locations slightly higher on the shore).
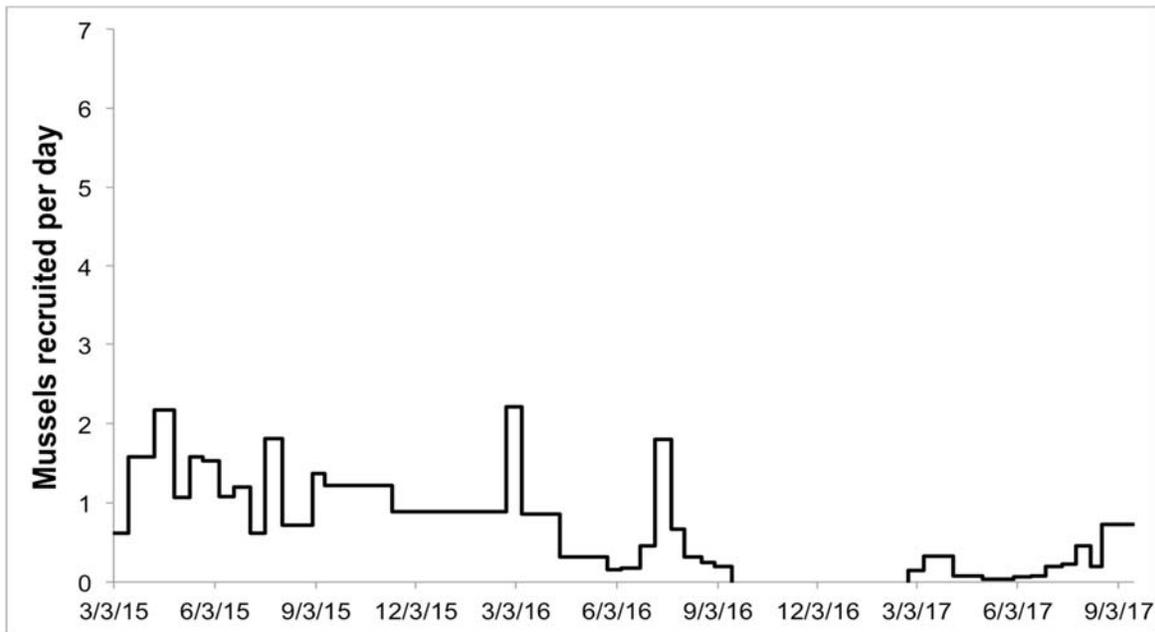

Figure 2. Recruitment rates (number of mussels per day per set of five collectors) of the California mussel, *Mytilus californianus*, at Bodega Marine Reserve (BMR) from 2015-2017. No data were collected from 9/2016 to 3/2017 due to chronic large waves that made the site inaccessible during periods of low tide when sampling would have otherwise occurred.
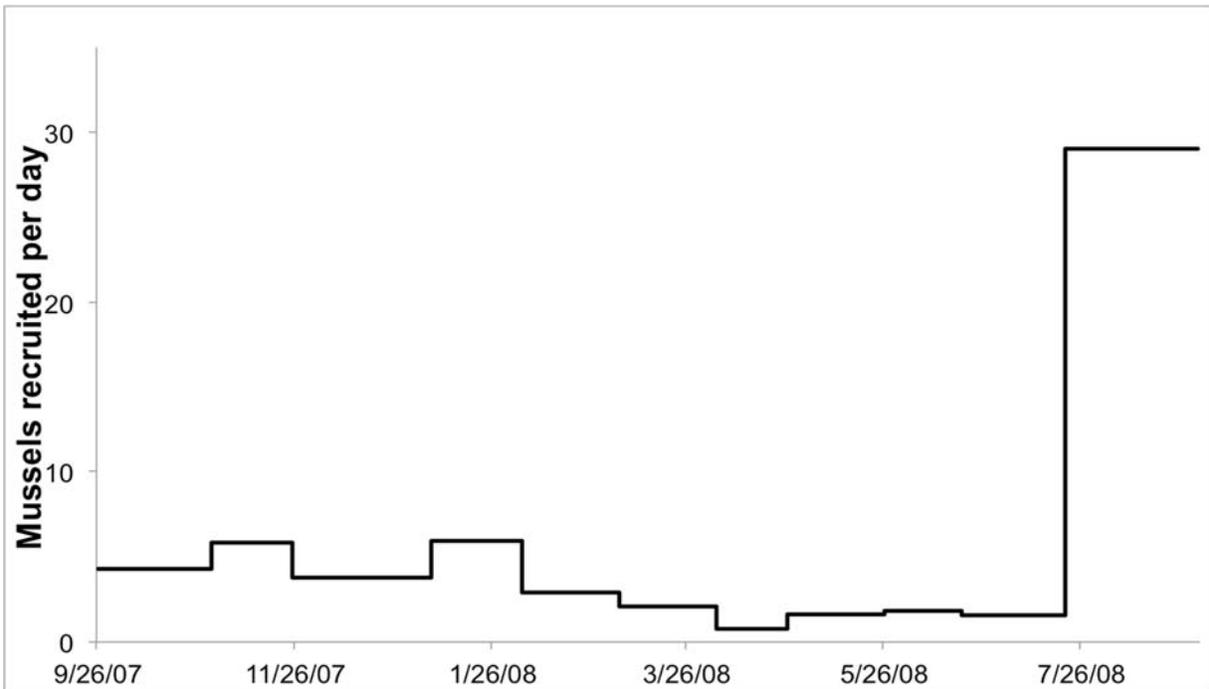
Figure 3. Recruitment rates (number of mussels per day per set of five collectors) of *M. californianus* at Bodega Marine Reserve in 2007-2008. These rates (typically 5/day, maximum of 30/day) were substantially higher than what was observed in 2015-2017. Note that the scale on the ordinate axis differs from that in Fig. 2. Redrawn from Sanford and Worth (2010).

M. californianus recruits were also successfully collected at additional California marine reserves besides BMR (August-September 2017) as part of the current study (Table 1). These results suggest that techniques applied at BMR for this species and these life stages could be used more broadly at additional California sites if there were interest in expanding efforts to employ *M. californianus* as a bio-indicator.

Table 1. Number of *Mytilus* mussel recruits per day per set of five collectors, sampled during one-month collector deployments at additional intertidal sites along the coast of California. Note the suggestion of a potential latitudinal trend with lower recruitment in the south, consistent with the findings of Broitman et al. (2008).

Intertidal Site	Marine Protected Area	Number of Recruits Per Set of Five Collectors
Bodega Marine Reserve	Bodega Head State Marine Reserve	21
Waddell Creek	Año Nuevo State Marine Reserve	35
Asilomar State Beach	Asilomar State Marine Reserve	2
Crystal Cove State Park	Crystal Cove State Marine Conservation Area	8

3.2 Environmental Data

Seawater pH (total scale) varied between extremes of 7.6 and 8.4, with an average seawater pH of ~7.9 between 2015 and 2017 (Fig. 4). For continuous pH measurements collected by TidalFETs in 2016, seawater pH reached lower values in spring and summer due to seasonal upwelling, and average seawater pH was higher in the fall. Values of pH in early spring 2017 were higher than in the previous year. The large fluctuations visible especially in spring and summer 2016 occurred on a weekly to biweekly time scale in association with transitions between oceanographic upwelling and relaxation processes that can dominate in these seasons.
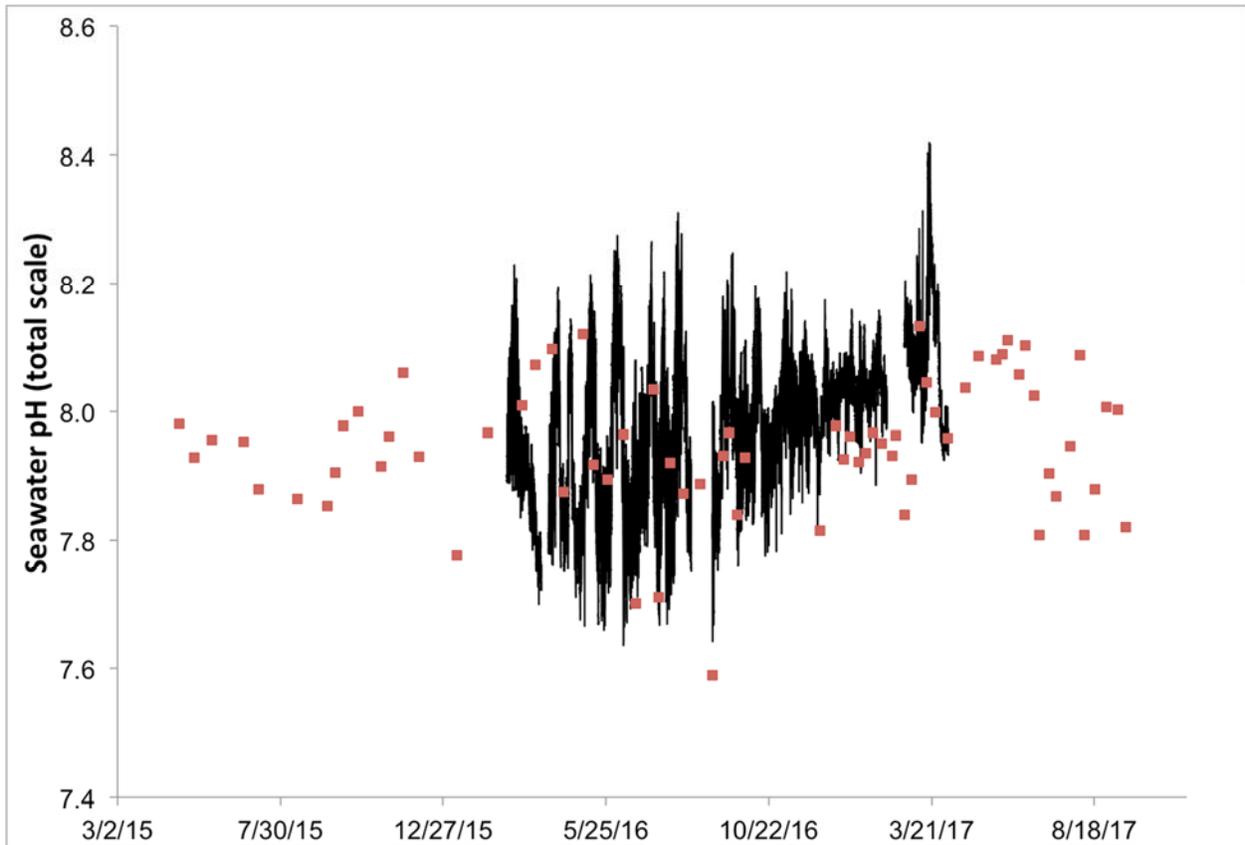
Figure 4. Seawater pH (total scale) measured in the low intertidal zone at Bodega Marine Reserve during 2015-2017. Continuous measurements from autonomously recording TidalFET instruments are shown in black. Discrete measurements from bottles samples are shown in red.

The records of intertidal pH collected with the autonomously recording TidalFET sensors and those collected via discrete bottle samples exhibited similar ranges through the year (Fig. 4). The pH ranges of both the bottle samples and TidalFET records also resembled those quantified at a mooring 1.5 km offshore (Chan et al. 2017). The latter correspondence suggests that the pH of pelagic water masses experienced by larvae resembles shoreline conditions experienced by recruits. However, a regression between point-by-point data from the TidalFET and shoreline bottle samples exhibited no significant correlation (p=0.492), a likely consequence of the substantial spatio-temporal variation in pH that characterizes coastal environments, coupled with the difficulty of ensuring that the discrete bottle collections and the TidalFET sampled the identical water masses at the same time. This issue is a clear limitation in intertidal environments that are strongly dynamic and variable.

Average seawater temperature was 11.9 °C (53 °F) between 2015 and 2017, with values varying between extremes of 7 °C (45 °F) and 18°C (64 °F) (Fig. 5). For the year 2016, when continuous temperature data were available from TidalFET deployments, lower temperature values were observed during spring and summer when seasonal upwelling was occurring, but temperatures remained strongly variable throughout the year. Temperatures recorded via the TidalFET were correlated with bottle sample temperatures (p=0.049, R^2=0.309).
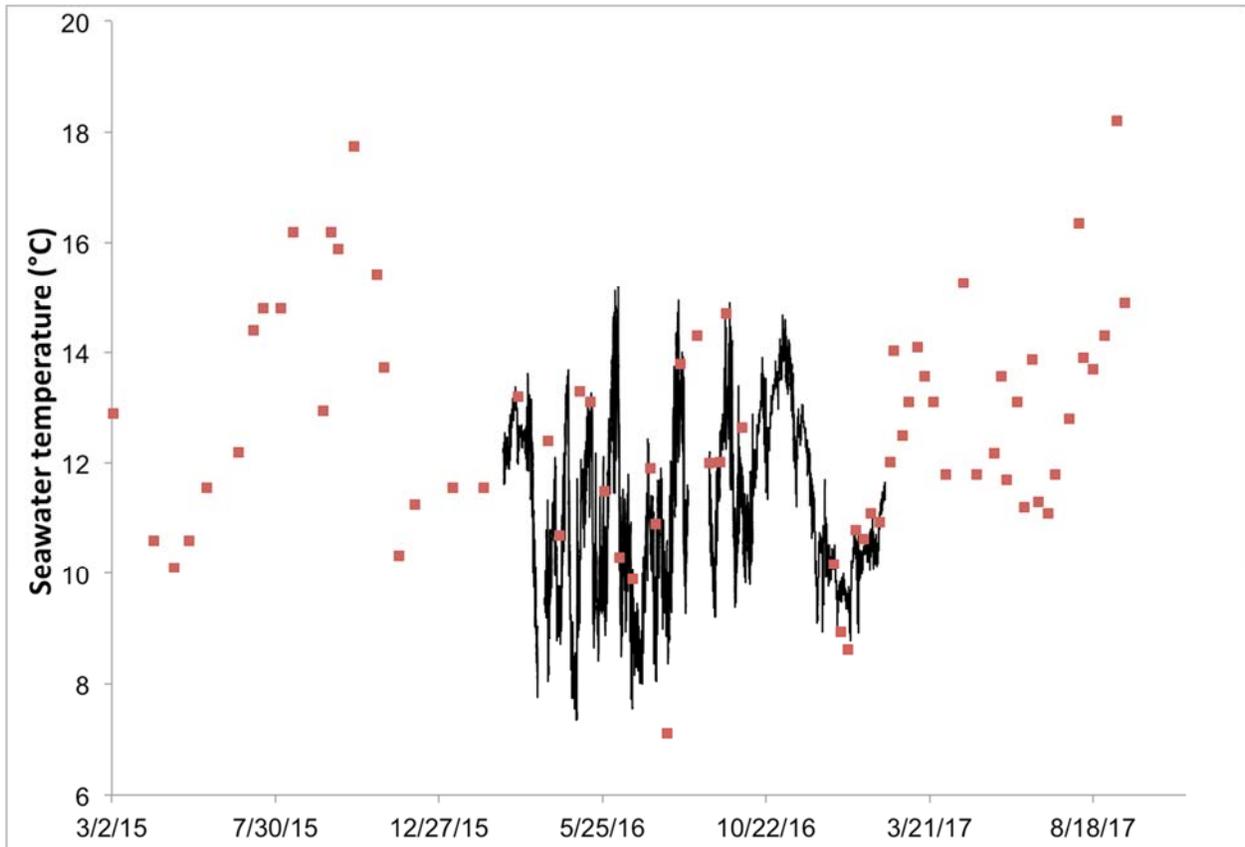
Figure 5. Seawater temperature measured in the low intertidal zone at Bodega Marine Reserve during 2015-2017. Continuous measurements from TidalFET instruments are shown in black. Discrete measurements from a handheld instrument are shown in red.

3.3 Mussel Morphology

3.3.1 Larval Mussels

For mussels collected during time periods with continuous pH and temperature data, the area of the mussel's larval shell correlated significantly with seawater pH (Table 2). As seawater pH declined, larval shell area decreased by 7.5% for every 0.1 pH units (Figs. 6 and 7). Larval shell area was not affected by temperature alone or by the interaction of pH and temperature. The length of the larval shell was not significantly affected by pH, temperature, or their interaction (Table 2).

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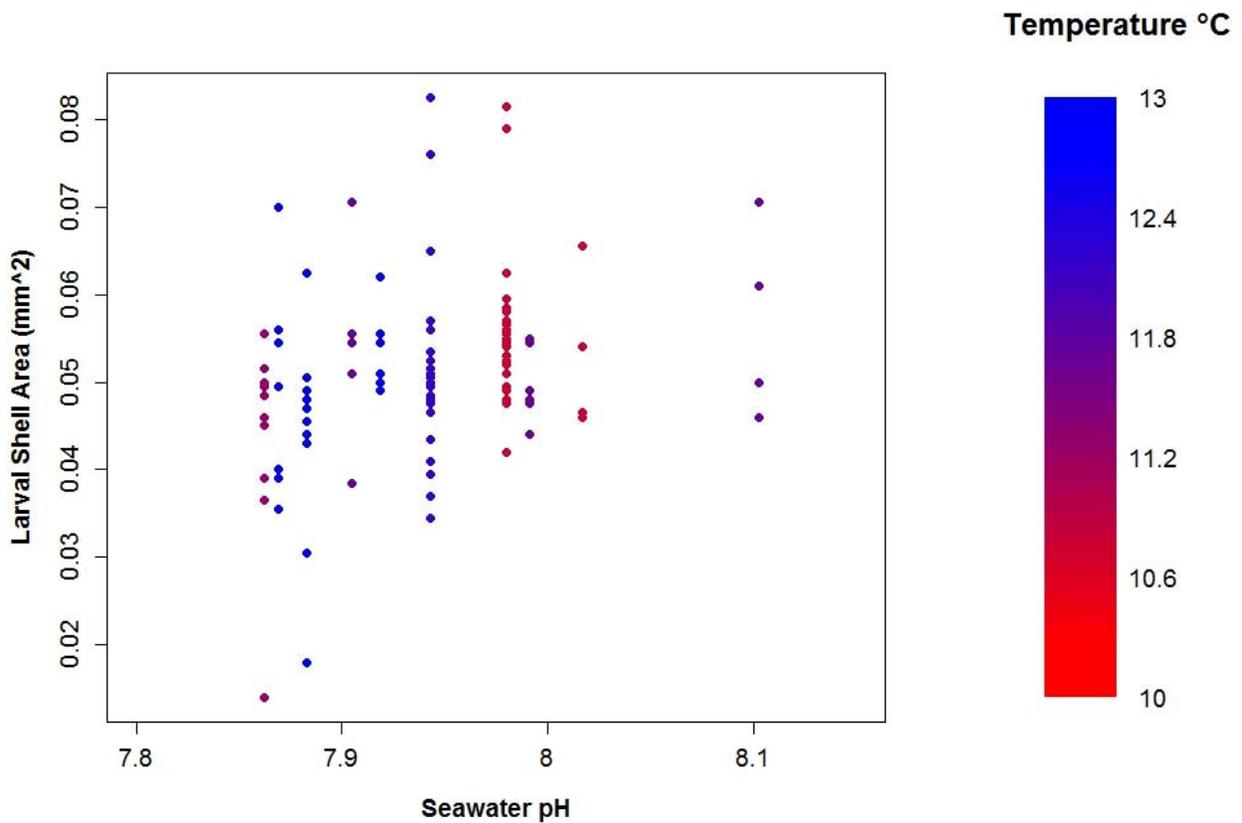
Figure 6. Larval shell area decreases with declining seawater pH. The data shown include periods when high resolution environmental data were available from autonomously recording TidalFET instruments. Colors indicate accompanying temperatures (red = warmer, blue = colder), but no effect of temperature was detected here. As is explained in the text, values of pH and temperature were calculated as the means over the two weeks preceding the mid-point of each collector deployment, consistent with the best estimate of when larvae were in the water column.
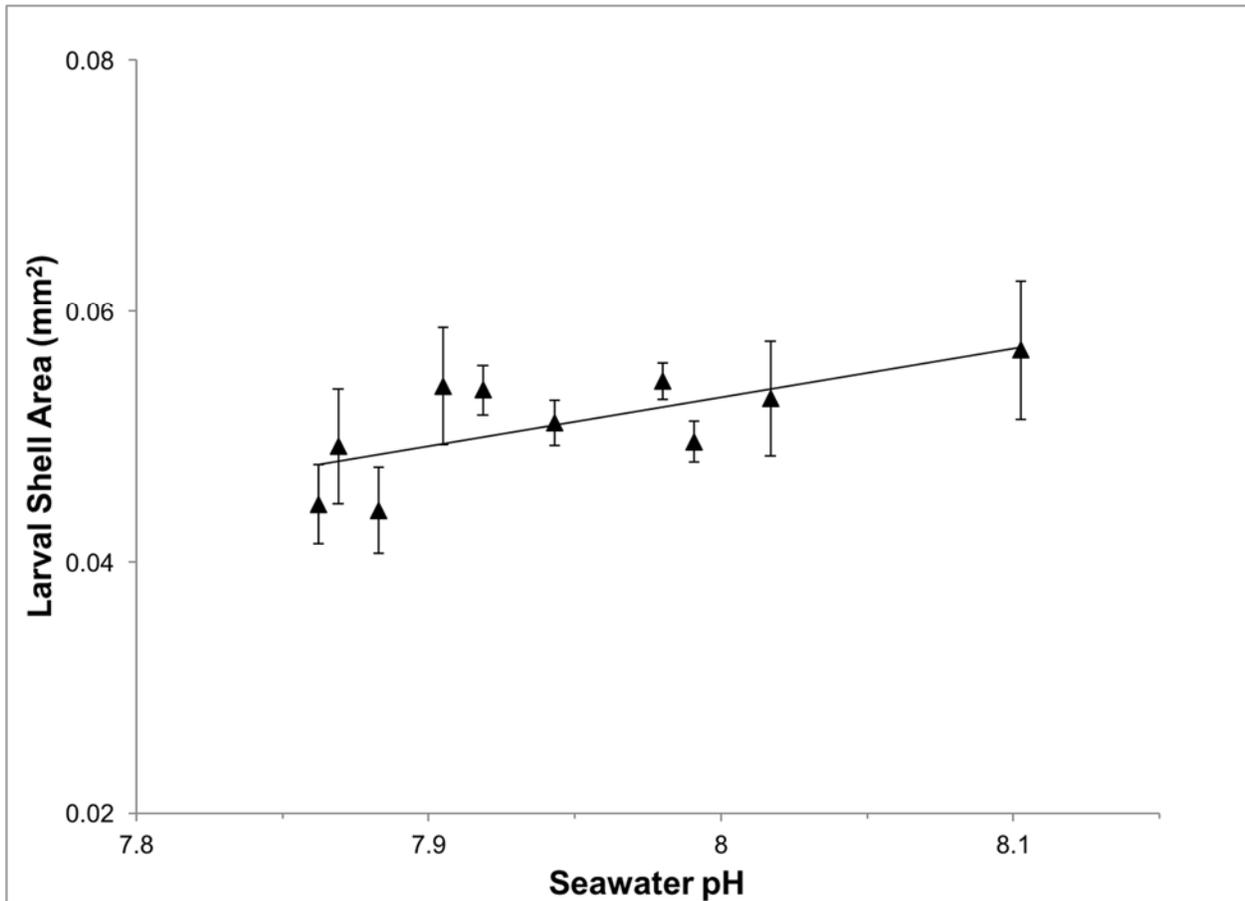
Figure 7. Larval shell area as a function of pH as in Fig. 6, except that deployment-averaged values of larval shell area are shown (with standard errors) and temperature is ignored. This replotting highlights the minor deviation of individual deployments from the overall trend, even for this set of results where the random effect of deployment contributed meaningfully to the final reduced statistical model (Table 2).

The observed effect of pH on larval shell area is consistent with results from previous, controlled laboratory experiments on *M. californianus* (Gaylord et al. 2011; Frieder et al. 2014), where larvae also produced smaller shells under elevated CO_2 (or lower pH) conditions. Thus, although the findings reported here are correlative and cannot directly address cause, it is reasonable to interpret the smaller sizes of larval shells sampled in the current field study as arising due to negative effects of pH on growth during the planktonic larval phase. Because it can be more costly to maintain correct acid-base balance and other biochemical functions (e.g., ion transport, protein synthesis; Pan et al. 2015) in seawater of increased acidity, physiological studies often emphasize the potential for degraded energy balances in marine organisms subjected to ocean acidification (reviewed in Widdicombe and Spicer 2008; Hofmann and Todgham 2010, Gazeau et al. 2013).

3.3.2 Mussel Recruits

For mussels collected during time periods with continuous pH and temperature data, the morphology of the recruit shell, both area and length, was not significantly affected by seawater pH, temperature, or their interaction (Table 2). The shell thickness metric for newly settled juveniles was also not significantly affected by seawater pH, temperature, or their interaction (Table 2).

The absence of detectable effects of ocean acidification on the morphology of newly settled juveniles could derive from multiple reasons. First, settled juveniles may be less sensitive than larvae to changes in the carbonate system of seawater. Other work has suggested that larvae may be especially vulnerable to environmental stressors (Pechenik 2006). In addition, studies on other species of bivalves have revealed that effects of low-pH exposure during the juvenile stage can be swamped by those originating during the larval period (termed a 'carryover effect;' Hettinger et al. 2012, 2013). To the extent that such trends hold for *M. californianus*, the larval metrics explored here may be more effective than the recruit metrics when using this species as a bio-indicator of ocean acidification.

The lack of correlation between recruit morphologies and pH in the present study might also arise because of the much greater capacity for growth of mussels after settlement. The amount of shell precipitated in two weeks during the early benthic phase is orders of magnitude greater than that produced during the larval period (see Fig. 1, where the shell formed following settlement is dramatically larger than that associated with the larval period). This substantially faster growth may introduce more variability into estimates of recruit shell area compared to larval shell area because some recruits will arrive into collectors at the beginning of a given deployment compared to others settling just before the collectors are retrieved. As a consequence, it may be more difficult to detect relationships between seawater properties (pH and temperature) and morphological or body condition parameters in juveniles. In contrast, larval shell features may be more constrained, dictated by a largely prescribed developmental trajectory of larvae, which could cause them to exit the water column at a common age and within a similar size range.

We also note that previous field growth experiments conducted using somewhat older juveniles showed significant effects of pH and temperature (Kroeker et al. 2016). Thus, it is possible that the absence of detectable relationships between recruit morphology and pH in the current data indeed reflects issues of differences in when mussels settled into collectors and how long they grew as recruits prior to retrieval and processing.

3.4 Body Condition of Recruits

For mussels collected during time periods with continuous pH and temperature data, lower body condition indices of recruits were associated with reductions in pH when temperatures were warm, but not when temperatures were cool. This temperature-dependent outcome appears in the statistical analyses as a significant interaction between seawater pH and temperature (Fig. 8; Table 2).
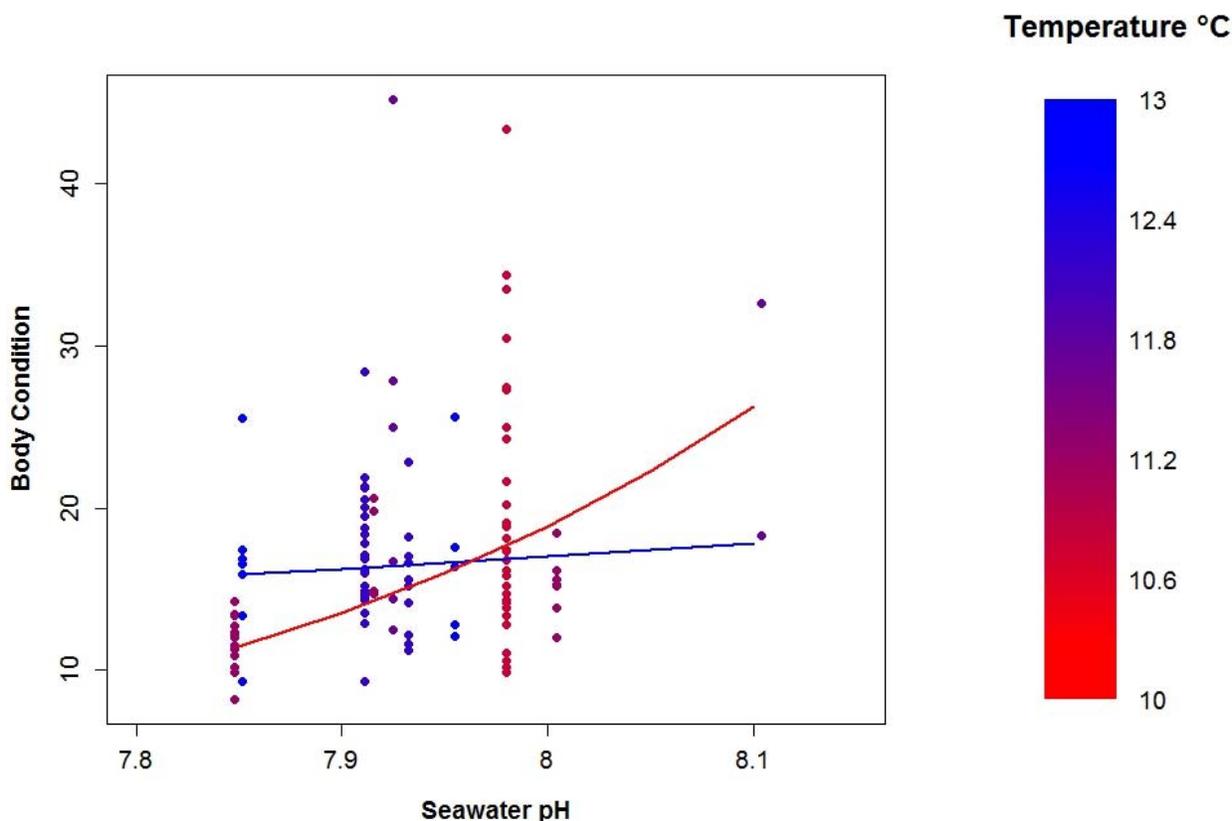
Figure 8. The relationship between seawater pH and body condition index of newly settled juvenile _M. californianus_ mussels depends on the temperature of the seawater. The data shown include periods when high resolution environmental data were available from autonomously recording TidalFET instruments. Trend lines are depicted to aid visual comparison between collector deployments characterized by warmer versus cooler waters; the red curve represents the best fit through data points where temperatures exceeded 11.5 °C (53 °F), and the blue curve corresponds to the best fit through data points where temperatures were less than 10.5 °C (51 °F). Note that the prediction lines are curved as they are derived from a model containing a log-transformed response that has been subsequently untransformed. Values of pH and temperature are the means recorded during each collector deployment.

The trend of decreased body condition under low pH conditions is consistent with prior measurements conducted on larvae (Gaylord et al. 2011). Although this prior result was acquired for larvae instead of newly settled juveniles, the rearing temperature used was 15 °C (59 °F), well within the range for which recruits of this study exhibited a relationship between body condition and pH.

The temperature-dependent relationship between body condition and pH in the newly settled juveniles collected is expected. As is revealed by other field experiments, rates of growth and tissue content of juvenile _M. californianus_ mussels can depend on a complicated set of relationships among pH, temperature, and food availability (Kroeker et al. 2016). Indeed, in some species of mussels, negative effects of ocean acidification on growth can be offset

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substantially, and even eliminated completely, if sufficient food is available (Thomsen et al. 2013; Kroeker et al. 2016). Upwelling processes often influence food availability.

In sum, and as shown quantitatively in Table 2, larval shell area and recruit body condition exhibit significant relationships with seawater properties associated with ocean acidification. These relationships are congruent with prior laboratory and field studies showing degraded growth of *M. californianus* under conditions of low chlorophyll-*a* abundance. They may therefore reflect underlying mechanistic causes tied to increased costs of calcification or tradeoffs with other metabolic demands in the face of altered seawater chemistry.

Finally, although we focus on univariate analyses of environmental effects on mussel condition for clarity and accessibility, we also conducted multivariate analyses based on a structural equation model framework. In particular, we developed two piecewise structural equation models on the univariate regressions to determine if the entire sets of regressions are a good fit of the data. For both the juvenile data and the larval data, we obtained Fisher C's scores that were not significant (Fisher's C = 11.755, p = 0.302 and Fisher's C = 0.127, p=0.938, respectively) when the response variables (e.g., thickness, area, length, and body condition) were allowed to covary. This result suggests that no significant pathway was omitted from the models and that the set of regressions are a good fit of the data. In other words, results from the piecewise structural equation models are qualitatively consistent with those of the univariate approach. The only change occurred with the interaction term of pH and temperature on body condition becoming just nonsignificant (p = 0.087) due to a small loss of sample size (n=6) when structuring the data sets for the structural equation model analysis.

Table 2. Analysis of statistical relationship between mussel characteristics and continuous environmental data. Comparisons were made using type III sum of squares with pH and temperature (T) as fixed effects and collector deployment as a random effect. Significant effects are shown in bold. The sum of squares, degrees of freedom (df), p-values (*p*), and intercept and slope values are shown for the final reduced model. Interaction terms removed from the model are not shown. The random effect of collector deployment did not significantly explain variation in recruit shell area, recruit shell length, recruit shell thickness, and recruit body condition, and was therefore excluded from the final reduced models in these cases. A natural log transformation was performed on body condition and recruit shell area data prior to statistical analysis.

Larval Shell Area

Effect	SS	df	*p*	Coefficients	Estimate
pH	<0.001	1	**0.017**	Intercept	-0.356
T	<0.001	1	0.925	pH	0.051
				T	<0.001

Larval Shell Length

Effect	SS	df	*p*	Coefficients	Estimate
pH	0.002	1	0.117	Intercept	-14.236
T	0.002	1	0.147	pH	1.834
pHxT	0.002	1	0.146	T	1.191
				pHxT	-0.151

Recruit Shell Area

Effect	SS	df	*p*	Coefficients	Estimate
pH	0.110	1	0.687	Intercept	4.277
T	0.003	1	0.948	pH	-0.511
				T	-0.005

Recruit Shell Length

Effect	SS	df	*p*	Coefficients	Estimate
pH	0.002	1	0.307	Intercept	6.267
T	<0.001	1	0.935	pH	-0.595
				T	0.009

Recruit Shell Thickness

Effect	SS	df	*p*	Coefficients	Estimate

Effect	SS	df	p	Coefficients	Estimate
pH	0.002	1	0.307	Intercept	0.684
T	<0.001	1	0.935	pH	-0.087
				T	<0.001

Recruit Body Condition					
Effect	SS	df	*p*	Coefficients	Estimate
pH	1.250	1	**<0.001**	Intercept	119.304
T	0.114	1	0.261	pH	--14.628
pHxT	0.352	1	**0.050**	T	-11.801
				pHxT	1.482

4: Conclusions and Future Directions

Ocean acidification is a growing threat to marine life in California waters and around the world. Therefore, an important goal is to identify viable bio-indicators that will assist State managers and policymakers in tracking biological and ecological consequences of this crucial perturbation to the chemistry of seawater.

Although a number of bio-indicator species have been proposed and even pursued (e.g., pteropods; Bednaršek et al. 2014; see also Somero et al. 2016), the ideal candidate will possess several features: sensitivity to ocean acidification, familiarity to the public, easy accessibility to monitoring efforts, and economic and/or ecological importance. The California mussel (*Mytilus californianus*) meets all of these criteria.

With regards to the issue of sensitivity in particular, multiple lines of evidence from previous research imply that mussels in general, and *M. californianus* specifically, are vulnerable to, and likely already experiencing, negative population-level consequences from large-scale environmental changes including ocean acidification. This species is well-known to people recreating on rocky shores, can be readily accessed during low tides, and plays a recognized and defining role as a crucial member of marine intertidal communities along much of the open coast of California.

We therefore examined and documented in this study the feasibility and potential value of using the California mussel as an ocean acidification bio-indicator. The approach we employed focuses on quantifying two readily measured morphological and body condition metrics in *M. californianus*. We show both to be traits that are readily quantifiable and have the capacity to inform the progression of ocean acidification regionally. In particular:

1) The area of the larval component of the shell of newly settled mussels decreases with reductions in ocean pH (such changes in pH also provide a proxy for accompanying

decreases in the availability of carbonate ions, important for calcification by marine taxa).

2) The 'body condition' (a ratio of dry tissue mass to total dry mass including the shell) in newly settled juvenile mussels declines with decreases in ocean pH under conditions when ocean temperatures are warm. This index is often interpreted as representing the overall, general health of an organism.

An important additional consideration is the ease of implementation of any bio-indicator monitoring program. The California mussel is widespread along the State's coastline, and new recruits can be collected from the shore at low tide with relatively modest effort. These features suggest that a monitoring program using *M. californianus* as a focal bio-indicator could be established and run without unreasonable effort or exorbitant financial expense. In particular, reliance on field-deployable pH sensors may become less crucial over time if future experiments validate the robustness of the relationships identified in the current study. In such a circumstance, monitoring efforts might advantageously focus on collection and analysis of recruits rather than on quantifying intertidal pH, since the former task requires only personnel and standard apparatus available in an abundance of university laboratories. It would be imperative, however, to maintain periodic checks concerning the applicability of the identified relationships between pH and mussel morphology or body condition.

For future expansion of a mussel-based bio-indicators approach, initial efforts should focus on analogous studies that parallel this one and expand the number of geographic sites in California. The relationships documented in the current report between seawater properties and the morphology and body condition of mussels will almost certainly depend on regional oceanographic processes, as well as the mussel species examined. In particular, although *M. californianus* is found along the full coast of California, *M. galloprovincialis* increases in relative abundance in Southern California. Therefore, trends in any mussel bio-indicator assay could vary geographically across the State. In such efforts to determine bio-indicator relationships at additional locations, autonomously recording pH sensors should be employed preferentially over discrete water samples. The latter are unlikely to provide adequate measures of aggregate seawater properties. That said, once high-intensity correlations are developed, recruitment collections could likely be conducted as stand-alone efforts for much of the time, accompanied by periodic checks with autonomous instruments. Such efforts could elucidate changes over time in morphological or body condition metrics of newly recruited mussels at given locations.

Mussel recruitment collections themselves have the potential to be conducted within a citizen-science framework. Assuming that links between organism responses and seawater properties have otherwise been defined (e.g., through measurements like those of the current study), then public efforts could focus only on the collection of mussels. This approach could be successful as long as the accompanying morphological and body condition measurements were made through other efforts. The latter must be done using quality microscopes and with calibrated microbalances and would be best accomplished by a finite number of trained personnel to reduce measurement error. It would also be important to undertake periodic sensor deployments to verify the continued applicability of the established statistical relationships. One strategy to accomplish this suite of tasks therefore might be to create partnerships between interested citizens and appropriate university or agency laboratories.

Regardless of the precise methodology that might be used, newly settled California mussels could serve as a valuable tool in documenting and understanding changes associated with ocean acidification and in planning for future ocean conditions.

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APPENDIX A: Statistical Relationships Based on the Combination of Both Continuous Environmental Data and Discrete Bottle Samples

As is discussed in the main text of this report, developing statistical relationships between seawater properties (pH and temperature) and the morphology and body condition of mussel recruits is best accomplished using high-resolution, continuous measurements of environmental parameters, relying on devices such as TidalFETs. However, such continuous data were missing for a subset of the collector deployments of this study. Therefore, we explored the feasibility of also using discrete water samples to extend the number of deployments that might be incorporated into the statistical analyses. Importantly, doing so relied on estimating average pH conditions associated with a given collector deployment from only a limited (usually two) bottle samples. This limitation introduced substantial uncertainty into the estimates of average pH for such deployments, leaving us cautious as to inferences that might be possible. We report below the results of such analyses for completeness, and because they might provide hints for how to target future studies, but do not consider them to be strongly credible in and of themselves.

When all deployments were considered, larval shell area was not significantly affected by pH, temperature, or their interaction (Table A1). The length of the larval shell was significantly affected by the interaction of seawater pH and temperature, indicating that the effect of pH on larval shell length depended on the temperature of the associated water (Fig. A1; Table A1).
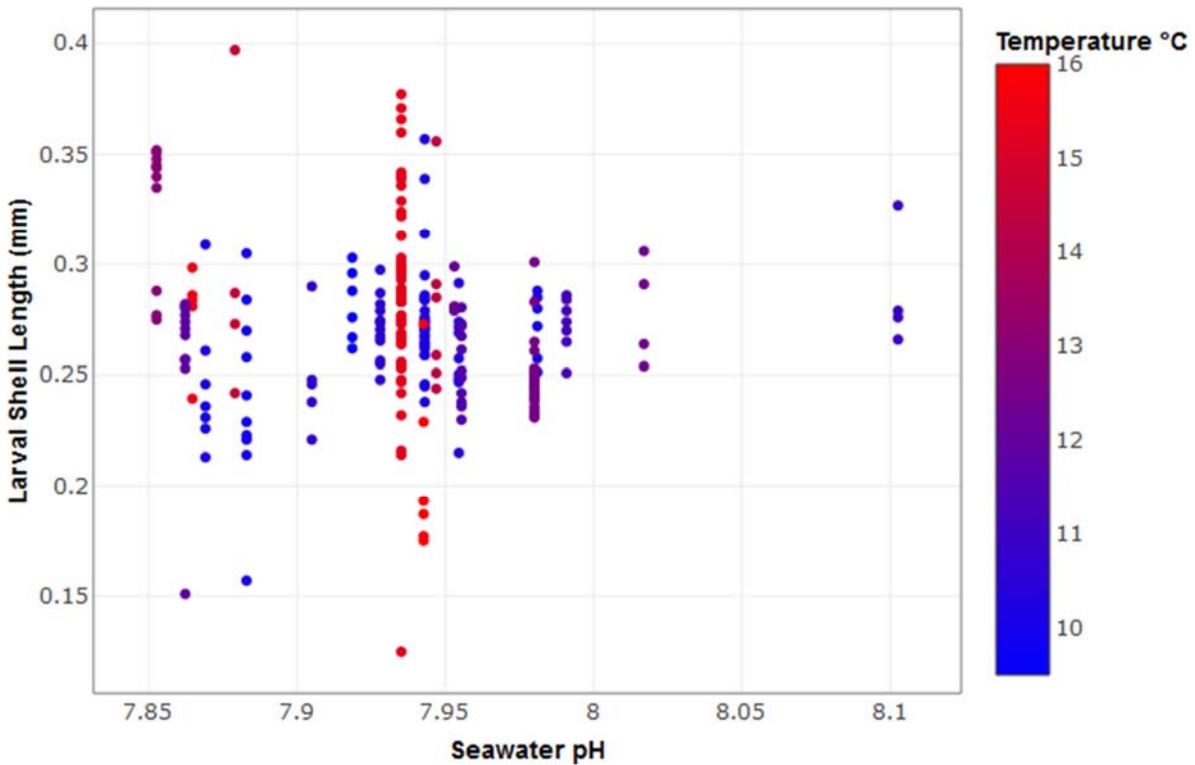
Figure A1. When including discrete bottles samples of pH in correlations between mussel morphology and seawater properties, the effect of pH on larval shell length depended on water temperature. For example, under warmer temperatures, larval shells were shorter when pH was higher. Under cooler temperatures, larval shells tended to be longer when pH was higher. Average seawater pH and temperature were calculated from the two weeks preceding the mid-point of each collector deployment.

Consistent with results reported in the main text that were based on high-resolution, continuous TidalFET records, when all collector deployments and environmental data (including discrete water samples) were considered, effects of pH, temperature, and their interaction were not significant for both recruit shell area and length (Table A1).

In contrast, the metric of recruit shell thickness was significantly affected by the interaction of seawater pH and temperature when all data and deployments were considered. In this case, the analysis also accounted for the relationship between shell thickness and shell length, and so focused on the residual trends after the effect of length was removed (Fig. A2; Table A1).
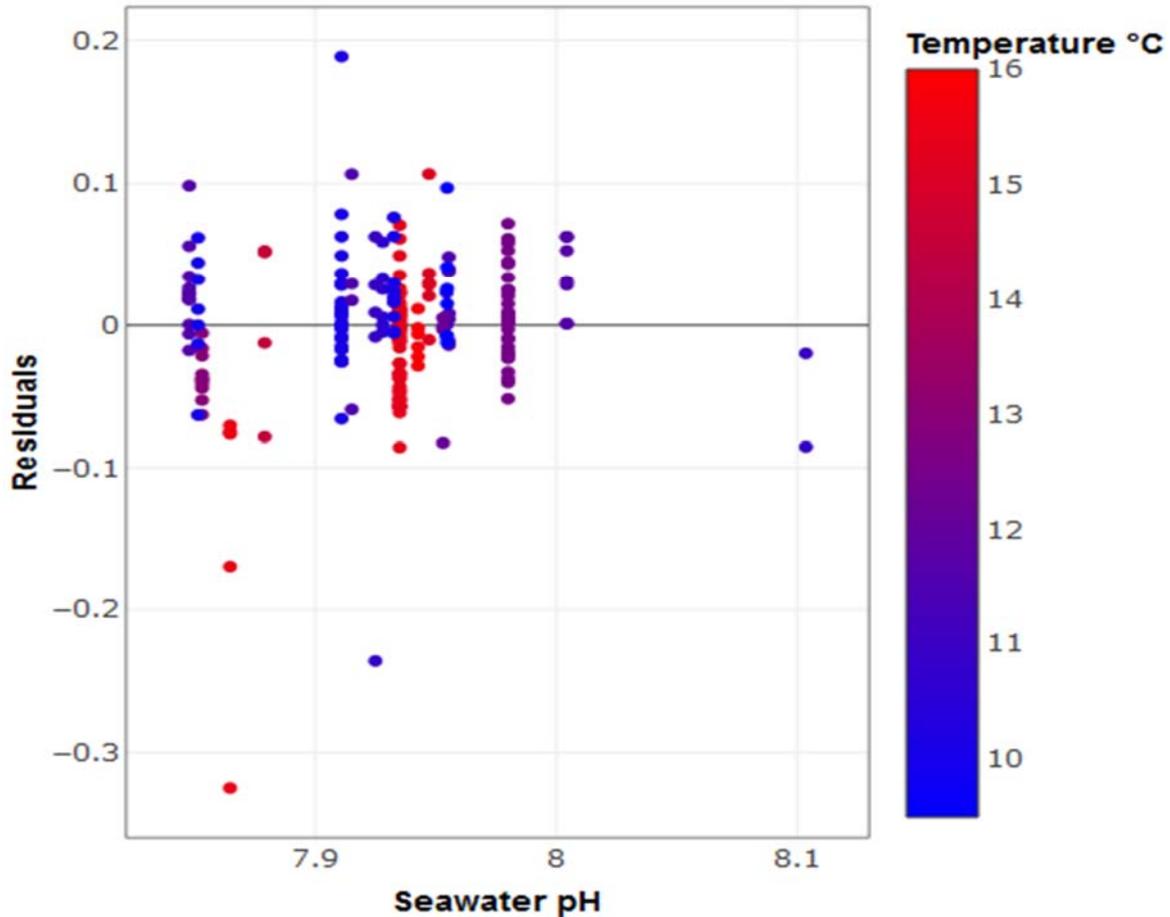
Figure A2. Residuals of the relationship between the shell thickness metric and seawater pH in newly settled juvenile *M. californianus* mussels, when assessed using both discrete bottle samples as well as higher-resolution, continuous pH recordings. The relationship with seawater pH depended on the temperature of the seawater. For example, under warmer temperatures, residuals of shell thickness increased with pH at a faster rate than when temperatures were cooler. Average seawater pH and temperature were calculated as the means of each collector deployment.

Also, in contrast to results discussed in the main text that were based only on continuous pH and temperature data, when all deployments and environmental data (including discrete water samples) were considered, body condition did not vary with seawater pH, temperature, or their interaction (Table A1).

Table A1. Analysis of statistical relationship between mussel characteristics and the combination of both continuous environmental and discrete bottle data. Comparisons were made using type III sum of squares with pH and temperature (T) as fixed effects and collector deployment as a random effect. The final reduced model is shown, with test statistic sum of squares, degrees of freedom (df), p-values (*p*), and intercept and slope values reported. Interaction terms removed from the model are not shown here. The random effect of collector deployment significantly contributed to all models in the table. An inverse transformation was performed on larval shell length and area data before statistical analyses were run. A natural log transformation was performed on recruit shell area data.

Larval Shell Area

Effect	SS	df	*p*	Coefficients	Estimate
pH	3.601	1	0.761	Intercept	65.422
T	40.290	1	0.316	pH	-6.647
				T	0.673

Larval Shell Length

Effect	SS	df	*p*	Coefficients	Estimate
pH	1.787	1	**0.018**	Intercept	230.069
T	1.751	1	**0.019**	pH	-28.550
pHxT	1.751	1	**0.019**	T	-19.142
				pHxT	2.415

Recruit Shell Area

Effect	SS	df	*p*	Coefficients	Estimate
pH	0.346	1	0.443	Intercept	13.359
T	1.726	1	0.101	pH	-1.546
				T	-0.096

Recruit Shell Length

Effect	SS	df	*p*	Coefficients	Estimate
pH	0.257	1	0.456	Intercept	10.664
T	0.937	1	0.170	pH	-1.075
				T	-0.054

Recruit Shell Thickness

Effect	SS	df	*p*	Coefficients	Estimate

pH	0.022	1	**0.002**	Intercept	26.958
T	0.024	1	**0.001**	pH	-3.391
pHxT	0.024	1	**0.001**	T	-2.351
				pHxT	0.296

Recruit Body Condition					
Effect	SS	df	*p*	Coefficients	Estimate
pH	60.314	1	0.209	Intercept	-142.584
T	5.427	1	0.703	pH	20.389
				T	-0.148