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MICROSCALE pH AND DISSOLVED OXYGEN FLUCTUATIONS WITHIN MUSSEL AGGREGATIONS AND THEIR IMPLICATIONS FOR MUSSEL ATTACHMENT AND RAFT AQUACULTURE

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ABSTRACT Mussel mariculture uses the natural attachment strategy of marine mussels by allowing them to aggregate on submerged rope lines that are then pulled to the surface and harvested. Mussels attach to ropes using a network of byssal threads, proteinaceous fibers that adhere to surfaces underwater using a powerful biological glue (adhesive plaque). Plaques use the surrounding seawater as a molecular trigger during adhesive curing, a process that requires a pH greater than 7.0 and an abundance of dissolved oxygen to progress. To ascertain whether mussels experience seawater conditions that are potentially harmful to mussel attachment, this study measured the conditions within mussel aggregations at a mussel farm in Washington state and, then, applied those conditions to plaques to determine whether such conditions are sufficient to weaken attachment. Seawater monitoring demonstrated that mussels infrequently experience acidic (pH <5.0) and hypoxic excursions (O_2 <2 mg L^{-1}) in the summer, especially near the seafloor. When reproduced in laboratory assays, the most extreme pH excursions observed delayed plaque strengthening when applied early in the plaque-curing process, whereas extreme excursions in hypoxia decreased adhesion strength after the adhesive had fully matured. In either case, adhesion strength was rescued after reimmersion in open-ocean seawater conditions, highlighting the resilience of the mussel holdfast to stresses other than mechanical strain. The window of susceptibility to changes in environmental conditions during and after curing could contribute to fall-off events at mussel farms, especially in the late summer months.

KEY WORDS: underwater adhesion, mussel foot protein (Mfp), *Mytilus trossulus*, mussel raft aquaculture

INTRODUCTION

Bivalve mariculture is a rapidly growing industry, with worldwide harvests exceeding a value of 20.6 billion USD per year (FAO 2019, Wijsman et al. 2019). As part of this global trend, suspended raft culture of marine mussels is becoming increasingly popular because of farming practices that are efficient, sustainable, and require limited investment after seed cultivation (Shumway et al. 2003, Lindahl et al. 2005, Whitmarsh et al. 2006, Troell et al. 2009, Lozano et al. 2010). Despite these advantages, mussel growers must contend with one particularly troublesome aspect of farming near coastlines that fisheries offshore do not. Unlike the open ocean, nearshore environments can experience large oscillations in seawater chemistry as a result of riverine inputs (Rysgaard et al. 2012), industrial pollution (van Dam et al. 2011, Förstner & Wittmann 2012), coastal upwelling (Wang et al. 2015), and agricultural runoff (Shaw et al. 2010) that can influence the growth and survival of marine organisms (Doney et al. 2011, Bakun et al. 2015). Given these challenges, the identification of environmental parameters that impact mussel settlement, growth, and attachment will be of paramount importance for growers in the future, especially as global ocean conditions continue to change because of human activities (Doney et al. 2009, Levin et al. 2009).

During the production process, shellfish growers commonly leverage the underwater attachment strategies of marine bivalves to promote cultivation on man-made structures that stimulate rapid production and efficient harvest. Marine mussels attach to surfaces underwater using a network of proteinaceous fibers called byssal threads. Co-opting this strategy, shellfish growers apply postlarval mussels onto rope lines made from braided plastics or natural fibers (Brenner & Buck 2010). Mussels naturally form attachment to culture lines that are hung *en masse* from floating rafts to protect mussels from predators on the seafloor and provide free access to microalgae in the water column. When cultivated with this method, mussels must remain attached to culture lines anywhere between 12 and 18 mo before reaching a marketable size (>6 cm; Ian Jefferds, personal communication). To remain attached, mussels continually make new byssal threads throughout this period as older threads decay and fall away (Moeser & Carrington 2006). In this way, mussel attachment strength plays an integral role in the survival of the organism throughout its lifecycle and can influence farm yield at the end of the growing season (Carrington et al. 2015).

In addition to their utility in mariculture, byssal threads are an important feature of the functional morphology of mussels, preventing dislodgement through the absorption of wave energy (Bell & Gosline 1996, Carrington et al. 2009). Mussels produce byssal threads by secreting protein precursors into a specialized groove that runs along the length of their foot, with each fiber imbedded in an adhesive plaque that is deposited on a surface (Tamarin et al. 1976). Plaque production begins when a small depression at the tip of the foot (the "distal depression") is pressed against a surface, and the resulting cavity is filled with adhesive proteins (mussel foot proteins, hereafter referred to as Mfp). During protein secretion, mussels control the chemistry within the distal depression, maintaining a highly acidic (Martinez Rodriguez et al. 2015), anoxic (Nicklisch et al. 2016), and ionically sparse (Yu et al. 2011, Miller et al. 2015) environment. Under these conditions, adhesive 3,4-dihydroxyphenylalanine (DOPA) residues preferentially interact with the surface rather than with each other (Anderson et al. 2010,

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Danner et al. 2012) and protein side chain oxidation is also minimized (Xu et al. 2012, Miller et al. 2015). After a few minutes, seawater infiltrates the cavity as the foot is removed and the conditions around the adhesive are changed drastically (pH *ca*. 8.0, O_2 *ca*. 8 mg L^{-1}, salinity *ca*. 31). The result is a rapid transition from a fluidic state during protein secretion to a porous solid over the course of a few seconds, with seawater acting as a molecular trigger to "cure" the newly formed adhesive (Hwang et al. 2010, Lim et al. 2010, Wei et al. 2014).

In addition to seawater facilitating this initial phase change, recent work investigating the temporal dynamics of this process demonstrates that adhesive curing continues long after the initial transition from liquid to solid. When held in open-ocean conditions (pH *ca*. 8.0, O_2 *ca*. 8.5 mg L^{-1}), the adhesion strength of the plaque doubles over the course of 8 days, while also shifting color from translucent white to dark tan (George & Carrington 2018). By contrast, holding plaques in low pH (<5.0) and hypoxic (<2 mg L^{-1}) seawater conditions halts strengthening altogether, causing the adhesive to peel from the substrate before the material could be fully loaded during tensile testing (George et al. 2018, George & Carrington 2018). These results show a direct link between the pH and oxygen environment surrounding a plaque (an external, nonliving material) and its eventual adhesion strength. By contrast, experiments that investigate the effect of environmental stressors on mussel attachment strength by using live animal exposures, for example, ocean acidification (O'Donnell et al. 2013, Zhao et al. 2017), warming (Newcomb 2015), or their interaction (Newcomb 2015, Clements et al. 2018), cannot distinguish between direct effects on the plaque material versus indirect effects on the mussel that may alter the thread manufacturing process (e.g., altered gene expression and nutritional limitation).

Although incorporating seawater into the curing process ultimately skirts the problem of water absorption that many adhesives face in wet environments (Comyn 1981), the interaction between mussel attachment and the inherent instability of seawater conditions in nearshore environments remains perplexing (Waite & Broomell 2012, Carrington et al. 2015). In estuaries, large diel fluctuations in seawater pH and dissolved oxygen saturation can be metabolically driven by the local biology (Lowe et al. 2019), with ranges over 2 pH units and 100% oxygen saturation reported at sites throughout the United States (Baumann & Smith 2017). To make matters worse, these ranges almost surely underestimate the variability seen in mussel mariculture, as the large-scale addition of biomass on culture lines has been found to drastically change the biogeochemistry of the local environment (Christensen et al. 2003, Lozano et al. 2010). In addition, the high stocking density of mussels on culture lines could lead to localized regions of hypoxia and acidification within mussel aggregations, a likely consequence of observed flow reductions of up to 70% underneath mariculture rafts (Grant & Bacher 2001, Strohmeier et al. 2005). Given that mussels cannot protect threads from environmental conditions of this kind after they are made, the sensitivity of the curing process to environmental fluctuations after adhesive formation may influence the timing and magnitude of mussel falloff.

The microenvironment mussels experience on rope lines during suspended raft culture has the potential to dramatically affect attachment strength, through direct means, by (1) preventing plaque-curing or (2) damaging mature threads, or indirect means, by (3) decreasing thread production or (4) altering the quality of threads. Despite these potential interactions, the magnitude and duration of pH and dissolved oxygen excursions within mussel aggregations remain unknown. In this study, water quality measurements were taken underneath a suspended culture raft deployed within the Puget Sound of Washington state to quantify the spatial (depth) and temporal (season) variation in water conditions that mussels experience throughout the year. In addition, microscale modifications to seawater pH and dissolved oxygen at the site of plaque adhesion were measured by imbedding sensors directly in mussel aggregations. The effect of temporal dynamics on adhesive curing was additionally investigated in the laboratory, replicating extreme excursions in pH and oxygen either early or late in the curing process. Data from these experiments aim to answer whether extreme excursions in pH and dissolved oxygen, within mussel aggregations, are capable of interrupting plaque-curing and/or decreasing thread production, a result that could contribute to holdfast weakening and mussel falloff.

MATERIALS AND METHODS

Seawater Monitoring

Seawater Conditions Under a Mussel Aquaculture Raft

Water conditions were monitored underneath a mussel raft over the course of 3 y (April 1, 2015 to March 3, 2018) at the Penn Cove Shellfish hatchery, located in Quilcene Bay, Quilcene, WA (47° 47′48.0″ N, 122° 51′16.6″ W). The mussel raft chosen was closest to the inlet and was approximately 15 × 18 m, supported *ca*. 1,500 mussel lines, with a typical yield of *ca*. 20 kg mussels per line (Dominic Pangelinan, personal communication). Although the environmental characteristics and hydrodynamics of this particular site have yet to be described, the Salish Sea has been shown to experience tidally driven bouts of hypoxia and acidification, largely driven by riverine inputs that surge with snowmelt in spring and summer (Moore et al. 2008, Feely et al. 2010). Water quality sondes (YSI EXO2 #599502-00; Yellow Springs, OH), hereafter referred to as "raft sensors," were suspended from ropes in the center of the raft, deployed at 1 and 7 m below the surface (Fig. 1), approximately 0.5 km from the inlet to the bay.

Each sonde was equipped with four EXO sensors: conductivity and temperature (accuracy ±0.5%; YSI #599870), pH (accuracy ±0.1 pH units; YSI #599701), optical dissolved oxygen (accuracy ±1%; YSI #599100-01), and total algae PE (precision 0–100 μg L^{-1}; YSI #599103-01). Water temperature (°C), salinity, pH, dissolved oxygen concentration (mg L^{-1}), and chlorophyll concentration (μg L^{-1}) were recorded as the average of 10-min samples, taken every hour, and radio transmitted to a database. Sensors were calibrated monthly against a 50,000-μS cm^{-1} conductivity standard (YSI #3169), NBS pH standards (YSI #3822), and air-saturated DI water. Total algae sensors were calibrated against a 0.625-mg L^{-1} rhodamine FWT red dye solution (Kingscote Chemicals, Miamisburg, OH; #106023).

The effect of seasonality and depth on each measured seawater parameter was investigated using a two-way ANOVA. Seasons were defined by the spring equinox, summer solstice, autumn equinox, and winter solstice of each year. Each parameter was rank transformed to achieve normality with the

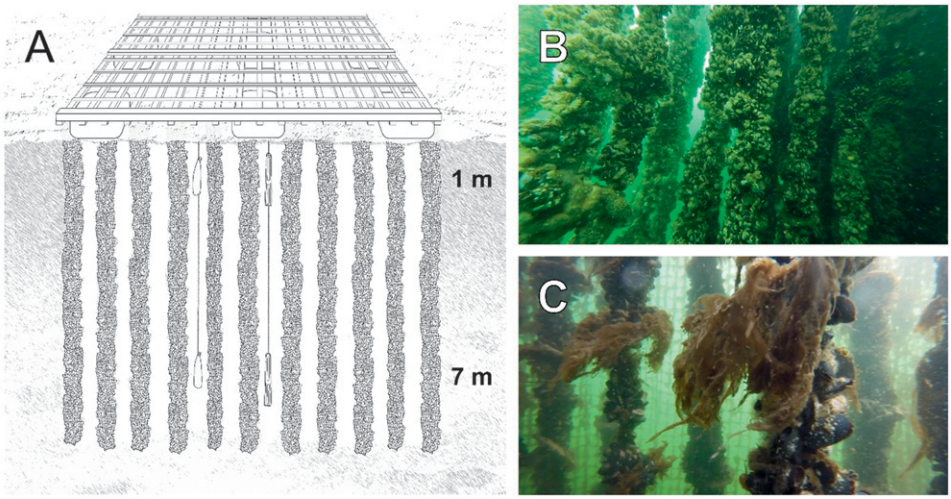

Figure 1. Simplified diagram of a mussel aquaculture raft with hanging rope lines (A), describing the position of hanging bags ("aggregation sensors," left) and YSI water quality sondes ("raft sensors," right). Rope lines were approximately 0.3 m apart. Hanging bags were filled with mussels (*ca.* 5 kg), with a pH and oxygen sensor embedded in the center of each bag. Aggregation and raft sensors recorded conditions every hour at two depths (1 and 7 m below the surface). Mussel density on rope lines varied seasonally, with noticeable differences observed between spring (B) and late autumn (C), presumably because of fall-off events.

normal quantile transformation (Ryan & Ulrich 2017). The Pearson correlation test was used to determine whether seawater parameters covaried. All statistical analyses were performed in R (Version 3.4.1; http://www.r-project.org/) with RStudio IDE (Version 1.0.153; http://www.rstudio.com/).

Seawater Conditions in Mussel Aggregations

The pH and oxygen conditions mussels experience within aggregations were measured and compared across depth and season. Nylon mesh bags filled with mussels (*ca.* 5 kg total weight) were hung from rope lines and positioned adjacent to the 1- and 7-m sondes (Fig. 1). An "aggregation sensor" was placed in the center of each bag, comprised of a Durafet III pH electrode (Honeywell, Fort Washington, PA; accuracy ±0.01; Martz et al. 2010) and a Honeywell DirectLine DL5000 equilibrium probe (accuracy ±0.1 mg L^{-1}). Aggregation sensors recorded 10 min of measurements every hour that were then averaged to report a single hourly measurement. Measurements of pH (NBS) and oxygen concentration (mg L^{-1}) were monitored using a Honeywell UDA2182 analyzer, powered by a 12-V AGM battery (Universal Power Group, Coppell, TX; #UB12900), and logged using a 4–20 mA data logger (Lascar Electronics, Erie, PA; #EL-USB-4). Aggregation sensors and sondes were calibrated at the same time and were temporally synchronized to record at the top of every hour. Bags were deployed four times during both the summer (June–September) and winter (December–March) of 2015 to 2016, for up to 3 wk at a time, and sensors were cleaned between deployments to limit any effects of fouling.

To characterize the occurrence of rare, extreme, sustained reductions in seawater pH or dissolved oxygen within mussel aggregations, a threshold analysis was performed on measurements collected from aggregation sensors. Excursions were defined as two consecutive measurements from a single sensor (2 h) reporting a pH of less than 7.0 or a dissolved oxygen of less than 5.0 mg L^{-1}. The duration of each excursion and their frequency were then recorded and compared across the season and depth using the Kolmogorov–Smirnov test.

Laboratory Experiments

Six cohorts of adult mussels *Mytilus trossulus* (Gould, 1850; *ca.* 50 mussels per collection) were gathered from the top of aquaculture rope lines during the winter of 2015 (November 2015 to February 2016), transported on ice to the laboratory, and kept in 50-L aquaria. Aquaria typically contained 20–30 mussels and were filled with 0.2 µm filtered, UV-sterilized seawater, with constant aeration. Mussels were in the laboratory for no longer than 3 wk and were fed Shellfish Diet 1800 (Reed Mariculture, Campbell, CA) up to 5% of their wet tissue mass day^{-1}, dispensed at a concentration of 2,000 algal cells mL^{-1}, a diet that has been shown to maintain body weight for up to 1 mo (M. George & E. Carrington, unpublished data). After a week of acclimation, mussels either produced threads that were included in plaque-curing experiments or the animal itself that was included in a thread production assay.

Mussel shell length (±0.1 cm), reproductive condition (Gonad Index, GI), and physiological condition (Condition Index, CI) were measured in accordance with the methods outlined in Baird (1958). In brief, gonadal tissue was excised from each animal by prying apart the valves of the shell, peeling back the gill flaps, and removing the underlying tissue with a scalpel. The remaining somatic tissue was then removed and dried separately from the gonads at 60°C until a constant dry weight was achieved (*ca.* 3 days). Gonad Index was calculated as the ratio of dried gonadal tissue to total tissue mass for each individual (Carrington 2002) and CI was calculated as the total dry tissue mass normalized to the shell length cubed (Moeser & Carrington 2006). Gonad and Condition Index were measured for all mussels that contributed threads to an experimental treatment, for mussels that produced threads under an experimental condition, and for a nonexperimental laboratory control group that was maintained in the laboratory for 20 days. Mussel condition was compared across all groups to screen for the possible influence of captivity and cohort, with no significant effect found (data not shown).

Byssal threads were collected in the laboratory by securing mussels to mica plates with rubber bands, orienting the valve opening toward the substrate, and allowing them to attach under seawater conditions that mimicked those found in the open ocean (pH *ca.* 8, O_2 *ca.* 8.5 mg L^{-1}, Sal *ca.* 30, T *ca.* 9°C). After 4 h, threads were separated from each animal at the shell margin by cutting the proximal region of each thread, preserving the attachment with each plate. Plates with attached threads were then incubated in seawater treatments, using only plates from mussels that made three or more attachments. After incubation, plates were removed from seawater, dried, and stored for up to 2 wk before mechanical testing was performed. Storage in air for up to 2 wk arrests the curing process, which can then be resumed on resubmersion in seawater, effectively preventing adhesive plaque-curing during storage without adversely affecting adhesion strength (George & Carrington 2018).

For each experiment described in the following, linear mixed-effects models were constructed to investigate whether plaque adhesive strength was impacted by either a pH (fixed effect with two levels) or dissolved oxygen (fixed effect with two levels) excursion (nmle package; Pinheiro et al. 2017). Each model also tested for the independent and interactive effects of adhesive age (fixed effect with six levels) while each mussel was incorporated as a random effect, nested within tank ID. Before inclusion in a model, the assumptions of normality and homoscedasticity for each variable were confirmed using Shapiro test in combination with the visual assessment of Q-Q and residual-fitted plots. When normality was violated, variables were transformed using Johnson transformation (Fernandez 2014). To control for the possibility of temporal autocorrelation, a first-order autoregressive correlation structure was also imposed on each model, using AIC/log likelihood comparisons to choose the model with the lowest value. Linear mixed effects without an imposed correlation structure always displayed the lowest AIC values (data not shown). For each significant effect, the multcomp package was used to perform pairwise comparisons of groups using Tukey HSD *post hoc* tests (Hothorn et al. 2008).

The Effect of Experimental Seawater Excursions on Adhesive Plaque-Curing

To determine whether rare, extreme excursions in pH and dissolved oxygen can directly affect the plaque-curing process, plaques were aged to maturity in fluctuating seawater treatments that mimicked the magnitude and duration of the "worst-case" scenario, as defined by the most extreme excursion observed in field measurements (pH <5.0 or O_2 <2 mg L^{-1}, for 5 days). Mica plates with freshly attached (*ca.* 4 h after deposition) threads were haphazardly assigned to one of five experimental treatments, controlled using the pH and oxygen-stat system previously described. Threads aged in the first two experiments experienced constant dissolved oxygen (*ca.* 8 mg L^{-1}), temperature (*ca.* 9°C), and salinity conditions (*ca.* 29) while also being subjected to an excursion in seawater pH (pH *ca.* 5.0) after either 1 (Exp. 1) or 8 (Exp. 2) days. The second two experiments mimicked the conditions of the first, except that seawater pH was maintained at *ca.* 8.0 throughout and threads were exposed to hypoxia excursions (O_2 <2 mg L^{-1}) either at 1 (Exp. 3) or 8 (Exp. 4) days into the experiment. pH and oxygen excursions were maintained for 5 days, after which conditions returned to a baseline that represented open-ocean conditions (pH *ca.* 8.0, O_2 *ca.* 8.5, T *ca.* 9°C, Sal *ca.* 29). A subset of plates was removed within each experiment after 3, 5, 8, 12, or 20 days and stored dry for up to 2 wk before mechanical testing was performed. A control treatment wherein open-ocean conditions were maintained for 20 days was also performed with the same sampling regime.

Byssal Thread Production During Experimental Seawater Excursions

Byssal thread production during acidification and hypoxia excursions was investigated by placing mussels secured to mica plates in one of five pH treatments (pH target = 5.0, 6.0, 7.0, 7.5, or 8.0) or one of two dissolved oxygen treatments (O_2 target = <2.0 or >8.0 mg L^{-1}) for 7 days. pH treatments were maintained using a pH-stat system similar to the one described in O'Donnell et al. (2013). In brief, seawater pH (NBS) and temperature (°C) were measured with a Honeywell Durafet III pH electrode and monitored with a Honeywell UDA2182 analyzer that controlled the operation of a solenoid valve. The solenoid value regulated the flow of CO_2 into the aerator of each tank. Using a PID loop, the analyzer tailored a CO_2:air mixture by controlling the proportional operation of the valve, using pH as the response variable. Dissolved oxygen treatments were accomplished in a similar way by equipping the analyzer with a Honeywell DL5000 equilibrium oxygen probe (accuracy ±0.1) and replacing the CO_2 cylinder with N_2 gas. The salinity in each treatment was monitored with a Honeywell DL4000 conductivity cell (accuracy ±1), which was also monitored by the analyzer. pH, oxygen, temperature, and salinity were logged every 10 min using a 4–20 mA data logger. Any pre-existing byssal threads were removed from each mussel, by cutting threads in the proximal region at the shell margin, before being placed in a treatment. Once in a treatment, a subset of mussels (*ca.* 20) was removed at 1, 3, 5, and 7 days, counting the number of new threads each mussel produced before determining the CI and GI for each animal. Linear mixed-effects models were constructed to investigate the effect of pH or dissolved oxygen on the number of threads produced, including the exposure time as a cofactor.

Mechanical Testing

Plaque attachment strength was determined by gripping the distal region of each byssal thread and pulling perpendicular (90°) to the substrate until failure, following the protocol of George and Carrington (2018). This testing angle was chosen for its reproducibility; it should be noted that the contact angle of the thread with the plaque varies and threads are rarely brought into tension fully perpendicular to the substrate (Desmond et al. 2015). Plaques were rehydrated in their respective seawater treatments before mechanical testing for more than 5 min. The thread distal region was gripped with a hemostat *ca.* 1 mm above the plaque-thread junction, and force was recorded using a 10 N digital force gauge (OMEGA, Stamford, CT; accuracy ±0.01 N) attached to a motor-driven testing frame. Threads were pulled at an extension of 10 mm min^{-1} until plaque failure (the distal region is much stronger than the plaque; Bell & Gosline 1996) and force (N) were recorded at 20 Hz. The adhesion strength (kPa) of each plaque was determined by normalizing the maximum force required to dislodge each plaque by the attachment planform area (mm^2),

TABLE 1.

Water conditions (temperature, salinity, dissolved oxygen, pH, and chlorophyll concentration, measured hourly) recorded by raft sensors in Quilcene Bay, from April 2015 to March 2018.

Season	Depth (m)	*n*	T (°C)	*n*	Salinity	*n*	O_2 (mg L^{-1})	*n*	pH (NBS)	*n*	Chlorophyll (μg L^{-1})
Spring	1	4,897	13.2 ± 2.6 (8.1–20.7)	4,731	25.7 ± 2.1 (13.7–30.0)	4,871	11.0 ± 2.1 (3.1–18.9)	4,342	8.15 ± 0.21 (7.26–8.73)	4,871	9 ± 16 (0–342)
	7	4,895	11.0 ± 1.2 (7.8–18.6)	3,864	27.8 ± 1.7 (20.3–36.2)	4,895	8.5 ± 2.9 (0.4–18.5)	4,885	7.89 ± 0.29 (7.15–8.58)	4,843	7 ± 18 (0–435)
Summer	1	4,355	18.0 ± 2.3 (11.7–24.1)	1,246	26.6 ± 3.3 (13.5–33.0)	4,833	8.3 ± 4.1 (0.3–19.1)	3,108	8.08 ± 0.18 (7.35–8.67)	4,861	6 ± 10 (0–321)
	7	4,435	13.2 ± 1.6 (10.3–19.3)	3,354	29.4 ± 1.6 (25.1–35.4)	4,435	7.7 ± 3.4 (0.1–18.1)	4,435	7.77 ± 0.28 (7.14–8.56)	4,457	10 ± 18 (0–238)
Autumn	1	2,641	10.9 ± 2.5 (6.4–15.7)	2,642	26.9 ± 5.4 (2.1–31.4)	3,783	9.2 ± 1.2 (5.0–12.6)	3,783	7.80 ± 0.14 (7.32–8.28)	3,783	27 ± 56 (0–433)
	7	4,049	11.4 ± 4.3 (7.3–15.0)	4,049	29.7 ± 2.5 (21.9–34.5)	4,049	7.2 ± 1.7 (2.6–11.3)	4,049	7.66 ± 0.15 (7.32–8.07)	4,029	2 ± 6 (0–237)
Winter	1	3,514	7.9 ± 1.0 (5.3–10.1)	2,639	23.8 ± 4.1 (2.6–33.0)	3,514	10.1 ± 1.8 (5.4–17.5)	3,514	7.85 ± 0.19 (7.43–8.54)	3,513	6 ± 10 (0–215)
	7	2,712	9.4 ± 2.5 (6.9–11.2)	1,767	28.4 ± 2.4 (21.2–33.7)	2,712	8.2 ± 1.9 (4.5–14.5)	2,712	7.77 ± 0.19 (7.40–8.34)	2,691	3 ± 18 (0–486)

Reported values are aggregated season means ± SD, recorded at 1 and 7 m below the surface. The minimum and maximum values observed for each seawater parameter, at a given depth and season, are listed in parentheses.

TABLE 2.

Results of two-way ANOVA testing for independent and interactive effects of season and depth on seawater temperature (°C), salinity, dissolved oxygen concentration (mg L^{-1}), pH (NBS), and chlorophyll concentration (μg L^{-1}) from April 2015 to March 2018.

Source of error				
Temperature	**df**	**SS**	***F***	***P* value**
Season	3	112,617	33,378	<0.001*
Depth	1	31,128	9,226	<0.004*
Season × depth	3	68,282	6,746	<0.001*
Salinity				
Season	3	3,585	1,377	<0.001*
Depth	1	1,293	1,490	<0.001*
Season × depth	3	1,664	639	<0.001*
Dissolved oxygen				
Season	3	4,469	1,749	<0.001*
Depth	1	1,639	1,924	<0.001*
Season × depth	3	1,249	489	<0.001*
pH				
Season	3	4,825	2,130	<0.001*
Depth	1	2,208	2,924	<0.001*
Season × depth	3	5,113	2,257	<0.001*
Chlorophyll				
Season	3	232	85	<0.001*
Depth	1	769	849	<0.001*
Season × depth	3	1,232	453	<0.001*

* Statistically significant (alpha = 0.05).

measured by tracing the outline of each plaque from above using a dissection scope with an accompanying AmScope MU1000 camera (Irvine, CA) and AmScope X imaging software before testing (Burkett et al. 2009). The mean adhesion strength of 3–5 plaques is reported for each mussel.

In an effort to link observed differences in plaque adhesion with the failure mechanics of the adhesive, the failure mode of each plaque was also scored visually during mechanical testing following Young and Crisp (1982) as outlined in George and Carrington (2018). In brief, plaques were binned within three failure types: adhesive, peeling, or tearing. In the case of adhesive failure, plaques detached from the substrate in a single, swift, plunger-like motion. Peeling failure was characterized by a detachment beginning at a location along the perimeter of the plaque, propagating from one side of the structure to the other. Tearing failure was evident when a portion of the plaque remained attached to the substrate after the test was completed, or the thread became dislodged from the attachment plaque at the thread–plaque junction. The failure mode of plaques at each time point was pooled and compared with an open-ocean control treatment (expected values) using a chi-square test.

RESULTS

Seawater Conditions Under a Mussel Aquaculture Raft

Raft sensor measurements of seawater temperature, salinity, dissolved oxygen concentration, pH, and chlorophyll concentration varied because of the interaction between seasonality and depth (Tables 1 and 2, Fig. 2). Over the 3 y measured, seawater

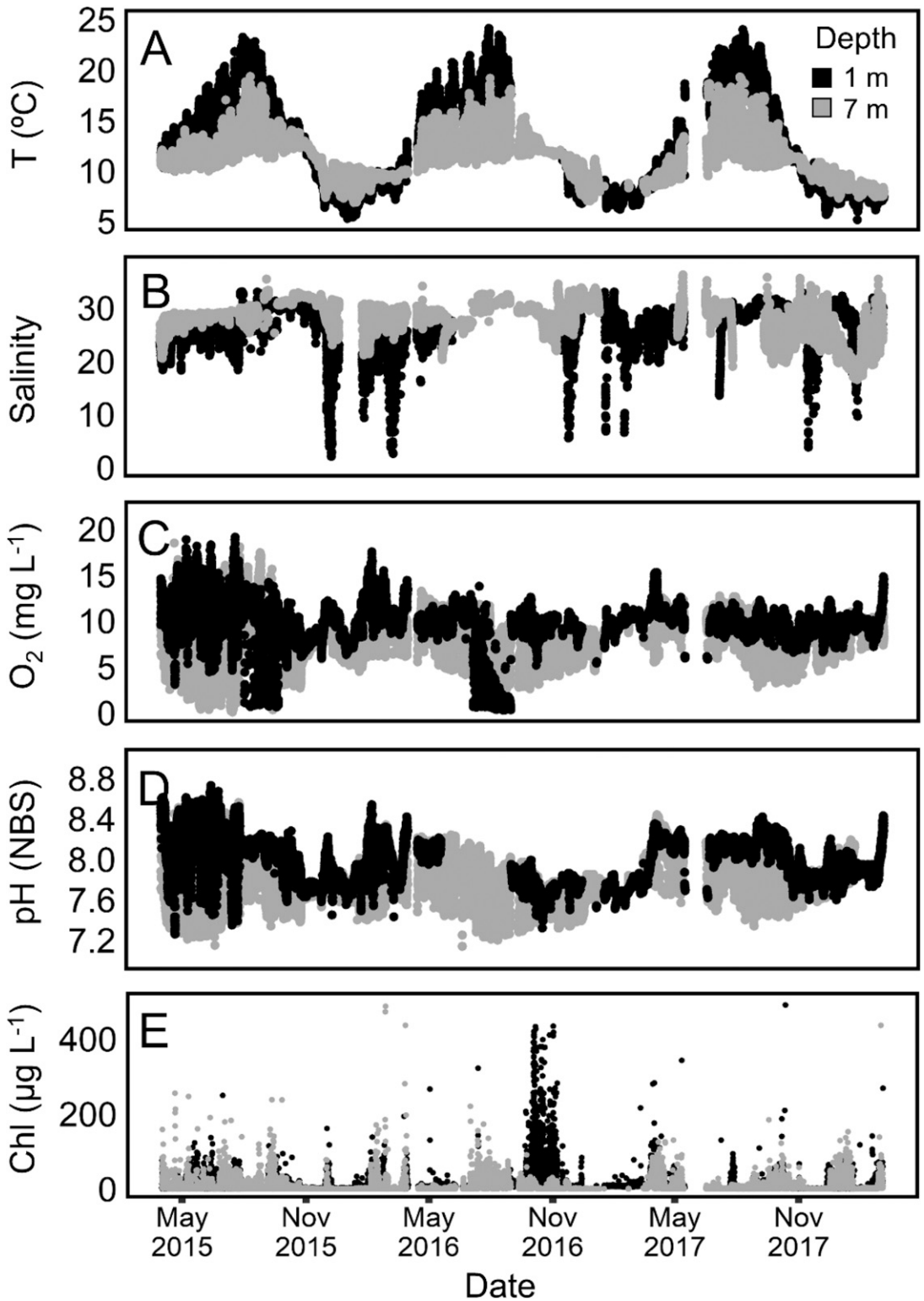

Figure 2. Seawater conditions underneath a mussel aquaculture raft in Quilcene Bay, Quilcene, WA, from March 2015 through March 2018. Seawater temperature (A, °C), salinity (B), dissolved oxygen concentration (C, mg L^{-1}), pH (D, NBS scale), and chlorophyll concentration (E, µg L^{-1}) were measured at two depths (1 and 7 m) below the surface hanging two raft sensors between mussel lines. A summary of conditions by season is presented in Table 1.

temperature was typically higher at the surface (1 m) in the spring and summer than at depth (7 m), with the trend reversing in the autumn and winter (Fig. 2A). Low salinity events were observed exclusively at the surface and were isolated to the autumn and winter months (Fig. 2B), whereas dissolved oxygen and seawater pH remained higher at the surface than at depth, regardless of season (Fig. 2C, D). Chlorophyll concentration varied widely between seasons and years, with spikes frequently observed in the autumn and winter at the surface (Fig. 2E). Of the five parameters measured, seawater pH and dissolved oxygen concentration were most strongly correlated (1 m: slope = 5.13, $R = 0.68$; 7 m: slope = 9.31, $R = 0.82$; Fig. 3A), with reductions in pH and dissolved oxygen commonly co-occurring in the summer and spring at both depths (Fig. 3B, C).

pH and Oxygen Excursions in Mussel Aggregations

Measurements of pH and dissolved oxygen from within mussel aggregations were tightly correlated with raft sensor recordings between mussel lines during the winter months, at both the surface (pH: slope = 1.15, $R = 0.77$; O_2: slope = 0.88, $R = 0.94$; Fig. 4A, C) and at depth (pH: slope = 0.90, $R = 0.64$; O_2: slope = 0.85, $R = 0.92$; Fig. 4A, C). The same was true for pH during the summer at the surface (slope = 0.80, $R = 0.82$), but not at depth (slope = 1.71, $R = 0.52$), where measurements from within aggregations often fell well below values recorded by raft sensors hanging less than a meter away (Fig. 4B). Similarly, "excursions" of low dissolved oxygen concentration commonly accompanied declines in seawater pH within aggregations (slope = 1.6, $R = 0.46$, $P < 0.001$), particularly in the summer at depth (slope = 0.52, $R = 0.65$, $P < 0.001$; Fig. 4D). An example of such an excursion of low pH and dissolved oxygen concentration from July 2015 is illustrated in E and F of Figure 4.

Using data collected from aggregation sensors deployed in the winter and fall, threshold analysis indicated that extreme excursions in seawater pH (<7.0) and dissolved oxygen (<5.0 mg L^{-1}) were exceedingly rare and short lived, with 75% of all pH excursions and 52% of all dissolved oxygen excursions lasting less than 10 h, with the longest pH and oxygen excursion lasting just over 5 days (Fig. 4G, H). Excursions were less frequent and of shorter duration in the winter with the longest dissolved oxygen excursion lasting 29 h and no excursions in seawater pH during the sampling period. Kolmogorov–Smirnov tests comparing excursion durations and frequency across seasons indicated that the frequency distribution of excursions was significantly different across seasons for both pH ($D = 1$, $P < 0.001$) and dissolved oxygen ($D = 0.5$, $P = 0.007$).

Adhesive Plaque-Curing During Experimental Seawater Excursions

Plaque adhesion strength increased overtime, more than doubling in strength after 8 days in the control condition (+117%, Fig. 5). When pH was held below 5.0 during days 1–6 of the curing process, plaque strengthening was delayed, leading to weaker attachments at 5 and 8 days after deposition (time × pH, $P = 0.017$, Table 3, Fig. 5E). During this period, plaques also peeled more frequently from the substrate during tensile tests, failing before the thread could be loaded (age = 8 days, $P = 0.006$, Table 4, Fig. 5I). After the pH excursion ended and conditions returned to the baseline, plaques resumed strengthening and were not significantly different than the control at day 12 (Fig. 5I). By contrast, a low pH excursion (pH <5) after 8 days of maturation did not significantly affect adhesion strength ($P = 0.114$, Table 3, Fig. 5F) nor the way that plaques failed (Table 4) when compared with the control.

Plaques exposed to hypoxia during the beginning of the curing window (day 1–5) showed no sign of delayed strengthening ($P = 0.078$, Table 4, Fig. 5G), although the frequency of failure by tearing increased slightly on day 3 (Fig. 5K, Table 4). Alternatively, when plaques were exposed to hypoxia after the

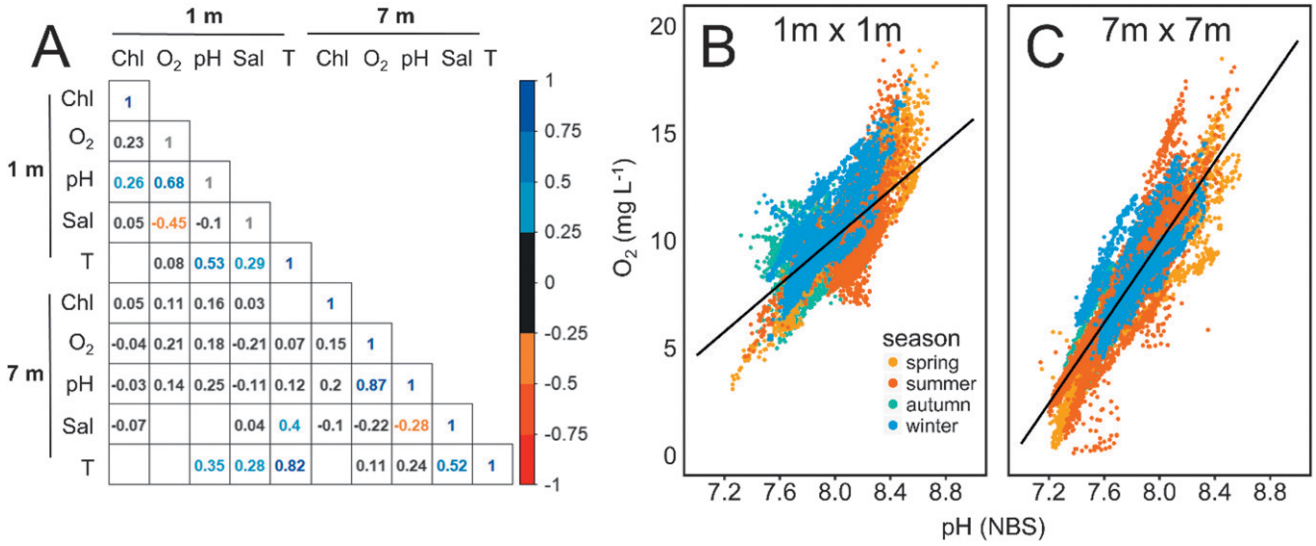

Figure 3. Seawater conditions recorded by raft sensors, at two depths below the surface (1 and 7 m), were measured from March 2015 through March 2018. The pairwise Pearson correlation test was used to generate a matrix of correlation coefficients for all parameters measured (A, alpha = 0.001). Dissolved oxygen (mg L^{-1}) was positively correlated with pH at 1 (B; slope = 5.13, $R = 0.68$) and 7 m (C; slope = 9.31, $R = 0.87$). Datasets were color coded by season (spring = orange, summer = red, autumn = teal, and winter = blue). A summary of all conditions measured, grouped by season, is presented in Table 1.

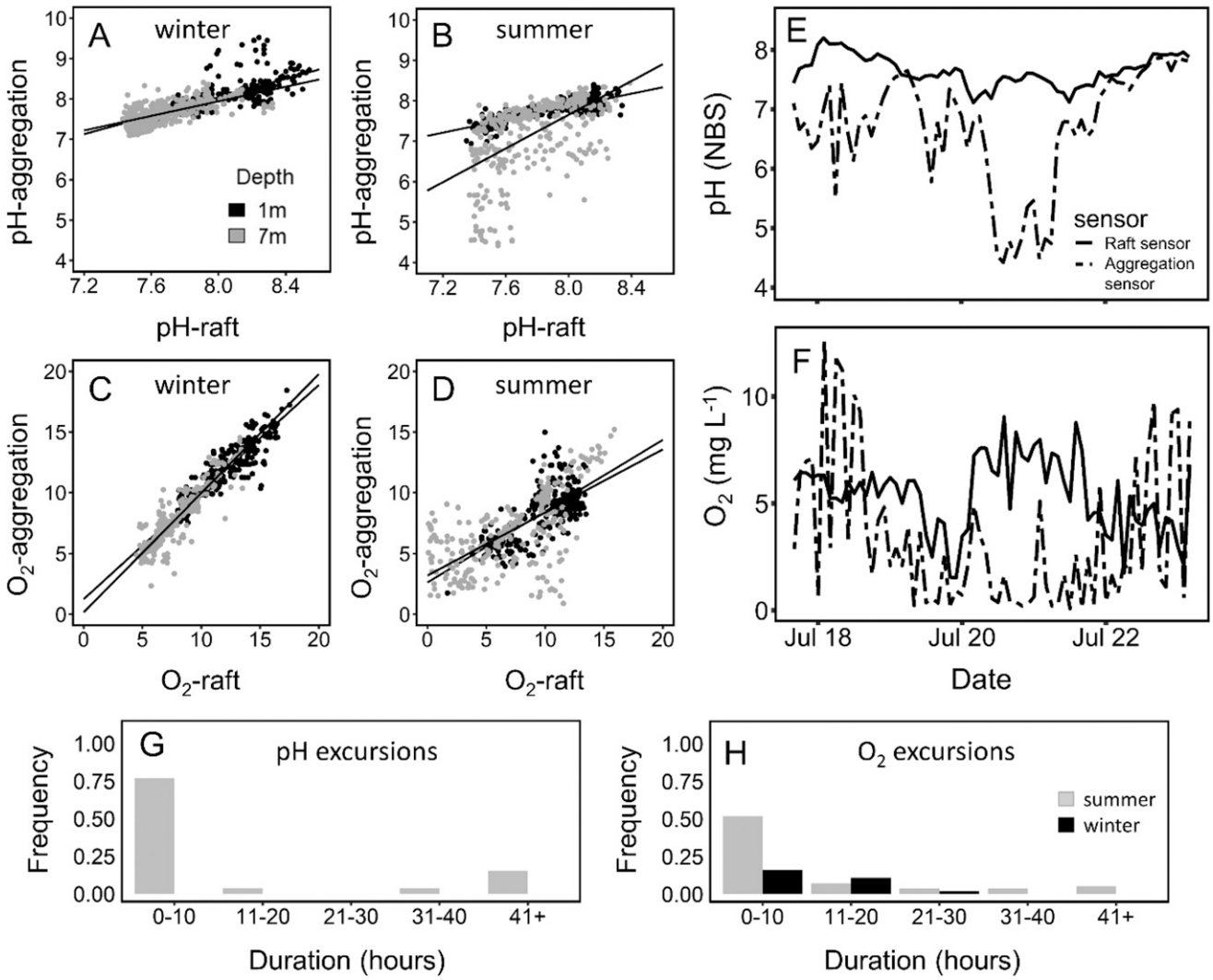

Figure 4. A comparison of seawater conditions (pH and dissolved oxygen concentration) found concurrently in mussel aggregation sensors and raft sensors. Measurements were taken during summer and winter months of 2015, at two depths below the surface (1 m, black; 7 m, gray circles). Seawater pH (NBS) within mussel aggregations was tightly correlated with raft sensor measurements in winter at both depths (A, C). In summer, low pH and oxygen excursions were recorded at depth (B, D). A typical pH/oxygen excursion (7 m, summer) that resulted in a mismatch between mussel aggregation and raft sensors is depicted in (E) and (F). The frequency and duration of pH and oxygen excursions (pH <7.0 and O_2 <5.0 mg L^{-1}) that occurred in either summer (gray) or winter (black) are presented as histograms in (G) and (H), respectively.

curing process was complete (day 8–13), plaques were significantly weaker than the control treatment ($P = 0.026$, Table 3, Fig. 5H). Plaque adhesion strength then recovered after oxygen returned to baseline levels, and plaques were tested at day 20 (Fig. 5H). While weakened, the proportion of plaques that failed by peeling from the substrate drastically increased to 61% of those tested, leading to a significantly different failure distribution than the control ($P < 0.001$, Table 4, Fig. 5L).

Across all plaque-curing experiments, mussel size (shell length), reproductive condition (GI), physiological condition (CI), and plaque attachment area (mm^2) were consistent across treatments and time points (data not shown). Mussels included in experimental treatments did not have a significantly different condition ($F_{5,423} = 1.956$, $P = 0.084$) or GI ($F_{5,432} = 2.335$, $P = 0.102$) when compared with those that were freshly collected or kept in laboratory conditions during experimental manipulations. A summary of seawater conditions for each treatment is presented in Table 5.

Byssal Thread Production During Experimental Seawater Excursions

The effect of seawater pH and dissolved oxygen interacted significantly with the effect of excursion length on the number of byssal threads produced during laboratory-produced excursions ($P < 0.001$ for both pH and dissolved oxygen concentration; Fig. 6A, B, Table 6). Mussels held at a pH less than 7.0 or dissolved oxygen concentration less than 2 mg L^{-1} neglected to produce threads for up to 3 days and produced an average of less than 10 threads per individual thereafter. A summary of seawater conditions for each treatment is presented in Table 5. Mussel shell length, GI, and CI were consistent across treatments (data not shown).

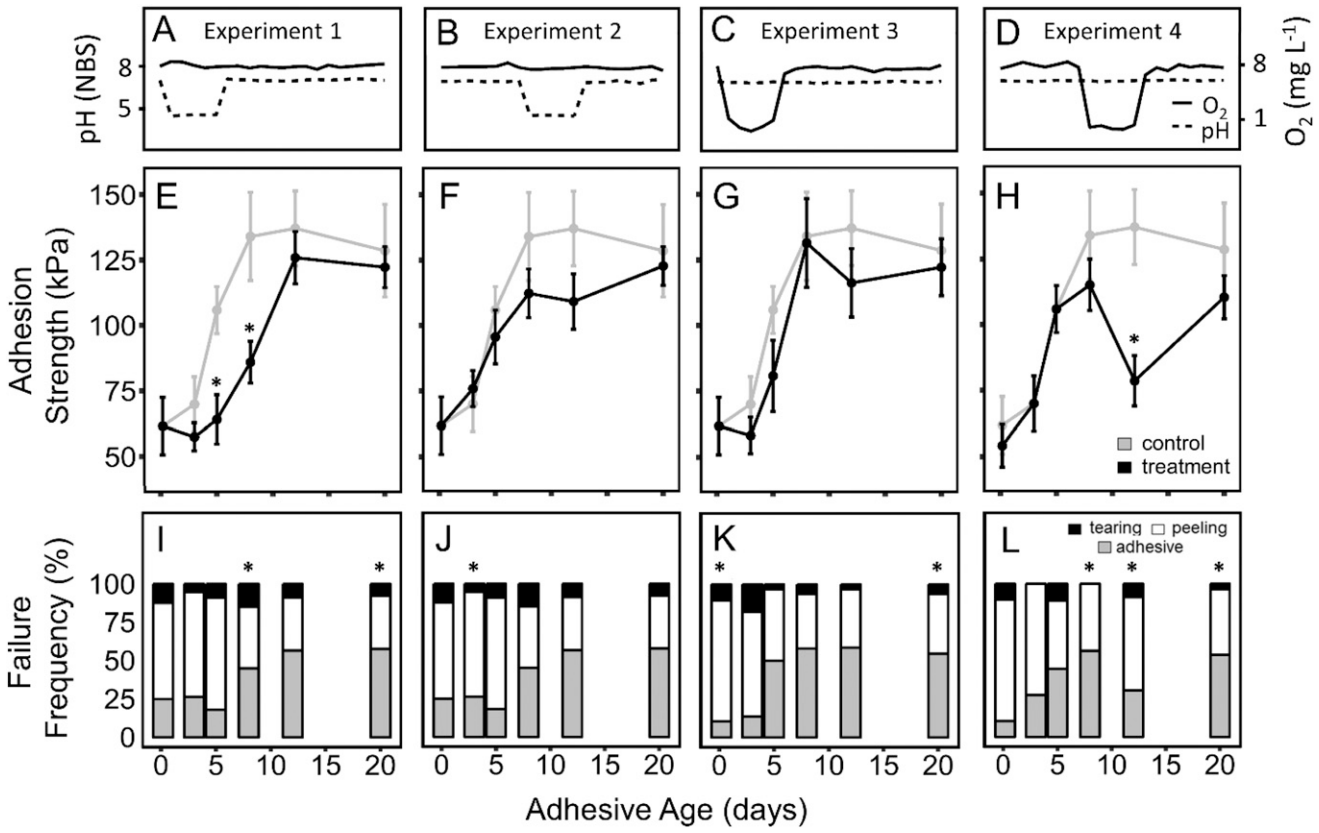

Figure 5. The effect of low pH and hypoxia excursions on adhesive plaque-curing. (A–D) pH and oxygen conditions within each experiment. (E–H) Adhesion strength (kPa) of plaques matured in each experiment over the course of 20 days, with the open-ocean control plotted in gray. (I–L) Frequency of different plaque failure modes (tearing, peeling, or adhesive failure) observed in the treatment group overtime. Error bars represent the SE about the mean for each time point. Asterisks indicate that either the mean adhesion strength of plaques were significantly different than the control at a given time point (E–H) or the failure mode distribution at a given time point was significantly different than the control (I–L), as determined by a chi-square test.

DISCUSSION

The environmental monitoring used in this study shows that mussels grown in suspended raft culture experience a wide variety of seawater conditions that vary with season. On top of this variability, the stressful temperatures, hyposalinity, acidification, and hypoxia that mussels experience are dependent on their location on a growing line. The most extreme conditions were observed at 7-m depth in the summer, within mussel aggregations, where mussels experienced a pH and dissolved oxygen minima of 4.42 and 0.89 mg L^{-1}, respectively. Although these extreme excursions were rare and typically only lasted for a few hours, decreases in pH and oxygen concentration to this degree represent a several orders of magnitude increase in the hydrogen ion concentration on surfaces where byssus attachment occurs. Interestingly, when exposed to these extremes in the laboratory, the maturation state of the byssus plaque determined whether adhesion strength was negatively affected. An acidic excursion (pH 5.0) early in the curing process (day 1–6) caused a delay in plaque strengthening, whereas a sustained hypoxic excursion (*ca.* 1 mg L^{-1}) after the curing window had passed (day 8–13) caused plaque weakening. Despite this effect, any negative consequence of acidification or hypoxia appears to be reversible, given that the material could recover under favorable conditions for a sufficient period of time. These results suggest that whereas environmental variability in the nearshore can influence plaque adhesion, the timing and duration of unfavorable conditions may determine any impact on mussel attachment.

Hypoxic and acidic conditions have been shown previously to arrest the curing process of byssus plaques, causing the material to peel from surfaces at lower forces than when fully matured (George et al. 2018, George & Carrington 2018). Both a basic pH and high oxygen availability are needed for the conversion of DOPA residues to DOPA–quinone (Haemers et al. 2002, 2003), a posttranslationally modified amino acid that is responsible for the formation of covalent cross-links between proteins in the adhesive (McDowell et al. 1999, Zhao & Waite 2006, Miserez et al. 2010). Whereas the cross-linking behavior of specific proteins is well understood (Fant et al. 2000, 2002), the cross-linking kinetics of the entire network of mussel foot proteins present within the plaque is not, particularly in the context of variability in the surrounding seawater environment (Waite & Broomell 2012). In nearshore environments, plaques are constantly inundated with unfavorable conditions during the curing process. It remains unclear from the molecular mechanics of Mfp cross-linking whether plaque strengthening is irrevocably damaged by environmental conditions or just delayed, two possibilities that could have very different outcomes for a mussel that depends on strong attachment for survival.

TABLE 3.

Results of linear mixed-effects models investigating the effect of adhesive age (days), during either a pH or O_2 excursion, and their interaction with adhesive plaque strength.

Source of error	DF_{num}	DF_{den}	*F*	*P* value
Experiment 1: pH excursion on days 1–6				
Intercept	1	91	698.51	<0.001*
Time	5	42	16.90	<0.001*
pH	1	42	14.23	<0.001*
Time × pH	5	42	3.13	0.017*
Experiment 2: pH excursion on days 8–13				
Intercept	1	77	649.55	<0.001*
Time	5	59	10.22	<0.001*
pH	1	59	2.57	0.114
Time × pH	5	59	1.12	0.360
Experiment 3: O_2 excursion on days 1–6				
Intercept	1	69	465.53	<0.001*
Time	5	57	9.49	<0.001*
Oxygen	1	57	3.21	0.078
Time × Oxygen	5	57	0.44	0.817
Experiment 4: O_2 excursion on days 8–13				
Intercept	1	69	928.38	<0.001*
Time	5	57	15.28	<0.001*
Oxygen	1	57	5.25	0.026*
Time × Oxygen	5	57	1.49	0.206

Results are presented for four laboratory experiments wherein plaques were incubated in fluctuating pH (Experiment 1, early; Experiment 2, late exposure) and dissolved oxygen (Experiment 3, early; Experiment 4, late exposure) conditions.

* Significant factors (alpha = 0.05).

TABLE 4.

Results of chi-square tests comparing the failure mode of plaques at each time point during laboratory excursion experiments.

Age (days)	χ^2	df	*n*	*P* value
Experiment 1: pH excursion on days 1–6				
3	0.94	2	16	0.626
5	3.60	2	19	0.165
8	10.14	2	11	0.006*
12	4.55	2	20	0.103
20	6.59	2	23	0.037*
Experiment 2: pH excursion on days 8–13				
3	10.01	2	26	0.007*
5	0.57	2	19	0.753
8	2.84	2	33	0.242
12	1.41	2	33	0.494
20	2.10	2	13	0.350
Experiment 3: O_2 excursion on days 1–6				
3	12.79	2	22	0.002*
5	1.24	2	30	0.538
8	3.81	2	31	0.149
12	5.83	2	29	0.054
20	11.18	2	31	0.004*
Experiment 4: O_2 excursion on days 8–13				
3	1.62	2	11	0.444
5	0.04	2	9	0.978
8	9.47	2	32	<0.001*
12	26.34	2	23	<0.001*
20	13.83	2	28	<0.001*

* Significant results (alpha = 0.05).

To test these two hypotheses, plaques in this study were exposed to hypoxic and acidic excursions either early in the curing process (day 1–5) or after they were fully mature (day 8–13). Matching the worst-case scenario from field surveys, plaques were exposed to excursions for 5 days, after which conditions returned to that of the open ocean and plaques continued to age until they were 20 days old. Early exposure to acidic conditions (pH = 5) during plaque-curing effectively delayed strengthening, causing the material to be weaker at 5 and 8 days, whereas exposure following the curing period did not cause significant weakening. The opposite was true for hypoxia (O_2 <2 mg L^{-1}), with plaque-curing proceeding normally when exposure occurred during the curing window, and plaque adhesion strength decreasing only when the material was deprived of oxygen after maturation. Nevertheless, in all cases where plaque weakening was observed, adhesion strength increased when affected plaques could continue curing in open-ocean conditions, effectively recovering from any negative impact of the local seawater environment.

The dynamic response of the plaque to pH and oxygen excursions in this study confirm that although basic pH and dissolved oxygen are required for plaque strengthening, the curing process can start and stop based on the presence or absence of these conditions. In addition, there was no evidence that plaques were permanently damaged by unfavorable conditions; mature plaques weakened by hypoxia in this study were able to recover when incubated in sufficiently high oxygen concentrations for 8 days. Placed in the context of DOPA–quinone cross-linking, these results mimic those found in the distal region of the thread, a material that is known for reforming sacrificial His–metal coordinate cross-links after they are broken during the extension of the thread (Harrington & Waite 2007, Harrington et al. 2009). Although the role of metal coordination within the protein network of the plaque remains to be explored, the plaque cuticle (Mfp-1) is known to form increasingly stable mono-, bis-, and tris-(DOPA)Fe^{3+} complexes as pH increases above a pH of 5.5, increasing the cohesive strength of the structure (Taylor et al. 1996, Xu 2013, Yang et al. 2016).

Over 3 y of measurements from underneath a mussel raft indicate that the vertical position in the water column can influence the type of environmental variation that mussels experience. For mussels at the surface, the temperature (5.3°C–24.1°C) and salinity (2.1–33.0) of seawater flowing through culture lines were the most variable, whereas mussels living just 6 m deeper experienced a broader range of pH (7.14–8.58) and dissolved oxygen concentrations (0.1–18.5 mg L^{-1}). Even so, a positive correlation of pH and dissolved oxygen was universal, leading to the co-occurrence of hypoxia and acidification at both depths, particularly during the summer months. The timing of these events implies that rather than being driven by the upwelling of oxygen-depleted waters from offshore that typically occurs in the winter months (Peterson et al. 1988), the net effect of biological processes (respiration, photosynthesis, etc.) could be responsible for moderating the carbonate chemistry in this nearshore habitat (Feely et al. 2010, Baumann & Smith 2017). This is highly likely within mussel aquaculture rafts because of the large biomass of mussels that

TABLE 5.

Seawater conditions (mean ± SD) during thread maturation and thread production laboratory experiments.

Thread maturation experiments (without mussel)							
Exp.	Excursion timing (day)	pH (NBS)		O_2 (mg L^{-1})		Sal	T (°C)
	Start–end	Baseline	Excursion	Baseline	Excursion		
Control	–	8.03 ± 0.04	–	8.54 ± 0.25	–	29 ± 1	8.8 ± 0.4
Experiment 1	1–6	8.01 ± 0.09	4.96 ± 0.03	8.33 ± 0.19	–	28 ± 2	9.1 ± 0.3
Experiment 2	8–13	8.04 ± 0.06	4.94 ± 0.06	8.36 ± 0.22	–	29 ± 1	9.1 ± 0.4
Experiment 3	1–6	7.98 ± 0.04	–	8.31 ± 0.37	1.13 ± 0.32	28 ± 2	8.8 ± 0.3
Experiment 4	8–13	7.98 ± 0.06	–	8.28 ± 0.26	1.59 ± 0.63	29 ± 2	9.2 ± 0.4
Thread production assays (with mussel)							
Experiment	**Target value**	**pH (NBS)**		**O_2 (mg L^{-1})**		**Sal**	**T (°C)**
pH	8.0	8.15 ± 0.06		8.44 ± 0.88		30 ± 2	8.9 ± 1.4
	7.5	7.48 ± 0.09		8.48 ± 1.22		29 ± 2	8.8 ± 0.9
	7.0	6.83 ± 0.06		8.83 ± 0.81		28 ± 1	9.2 ± 1.1
	6.0	6.01 ± 0.02		8.70 ± 0.50		29 ± 2	9.3 ± 0.6
O_2	>8.0	8.10 ± 0.04		8.87 ± 0.45		28 ± 1	8.8 ± 0.7
	<2.0	8.06 ± 0.05		0.93 ± 1.86		28 ± 1	9.3 ± 0.9

In thread maturation experiments, freshly made plaques were cured in fluctuating pH and dissolved oxygen treatments, with extreme pH or oxygen excursions either occurring early (day 1) or later (day 8) in the maturation process. Seawater parameters were held constant for 7 days during thread production experiments. Seawater pH, dissolved oxygen (O_2), temperature (T), and salinity (Sal) were measured in each treatment at 10-min intervals.

supports dense epifaunal communities, often comprising hundreds of other species (Tenore & Gonzalez 1976). Regardless of the cause, the co-occurrence of pH and oxygen excursions necessitates that the interaction between multiple factors be considered in future studies.

Although the seawater between culture lines represented a significant departure from conditions found in the open ocean, even the most extreme values of pH and oxygen observed within these spaces were overshadowed by the mean conditions observed within mussel aggregations. At depth during the summer, seawater pH routinely fell 2–4 units below values recorded by raft sensors placed less than one meter away while dissolved oxygen decreased by 2–6 mg L^{-1}. These kinds of excursions typically lasted only a few hours, with the longest persisting just over 5 days, exposing threads to both pH and oxygen conditions that have been deemed to be harmful to the curing process (George & Carrington 2018, George et al. 2018, this study). Although not directly measured in this study, one possible explanation that could account for such a dramatic decrease in pH and oxygen is the formation of thick diffusive boundary layers (DBL) around aggregations. Diffusive boundary layers are created on surfaces as fluids flow over them, forming a zone where molecular diffusion is the dominant transport mechanism for dissolved material (Gundersen & Jorgensen 1990). Given enough flow reduction through culture lines, increased DBL thickness could limit the diffusion of CO_2 away from the organisms, effectively trapping conditions along the rope line. As a point of reference, increases in DBL thickness have been shown to decrease oxygen

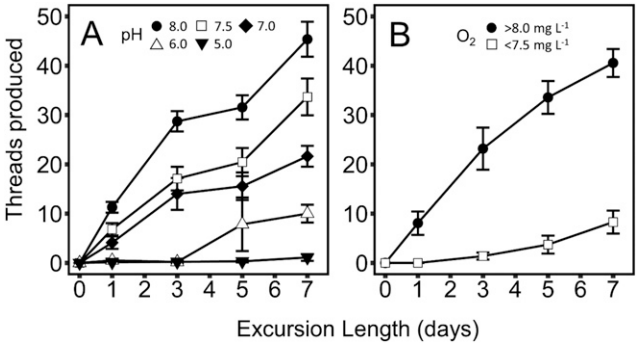

Figure 6. Byssal thread production during excursions of different levels of pH (A; pH targets = 8.0, 7.5, 7.0, 6.0, and 5.0) and dissolved oxygen concentration (B; O_2 targets = >8.0, <2.0 mg L^{-1}). Mussels were sampled after 1, 3, 5, or 7 days, counting the number of threads produced by each individual and averaging (± SE) by treatment. At pH 5, 6, and a dissolved oxygen concentration of less than 2 mg L^{-1}, most mussels remained closed for the first 3 days of exposure.

TABLE 6.

Results of linear mixed-effects models investigating the effect of pH or oxygen treatment on the number of threads produced over the course of 7-day excursions.

Source of error	DF_{num}	DF_{den}	*F*	*P* value
pH experiment				
Intercept	1	361	764.0	<0.001*
pH	4	361	103.3	<0.001*
Time	3	361	46.0	<0.001*
pH × time	12	361	5.1	<0.001*
Oxygen experiment				
Intercept	1	125	272.4	<0.001*
O_2	1	125	167.9	<0.001*
Time	3	125	23.0	<0.001*
O_2 × time	3	125	7.5	<0.001*

* Significant factors (alpha = 0.050).

availability surrounding corals and barnacles (Shashar et al. 1993, Nishizaki & Carrington 2014), decreasing the respiration rate and primary production of reefs (Patterson et al. 1991), whereas the efflux of O_2 away from seagrass has been found to be reliant on the hydrodynamic thinning of the DBL (Larkum et al. 1989, Koch 1994), ultimately leading to an increase in the photosynthetic rate (Mass et al. 2010).

Whereas a promising explanation for hourly pH and oxygen excursions, DBL thickness is unlikely to result in the persistent hypoxia and acidification observed in this study. Within mussel aggregations, the most extreme excursion in pH (<7.0) and oxygen (<5.0) was maintained for more than 5 days in the summer at depth, requiring a flow reduction for much longer than has been reported underneath aquaculture rafts (Blanco et al. 1996, Grant & Bacher 2001, Aguiar et al. 2017) or in tidally driven nearshore systems (Cahalan et al. 1989, Leonard et al. 1998). Alternatively, the fact that pH and oxygen excursions of this magnitude almost exclusively occurred at 7 m during the late summer could be explained by an accelerated accumulation of particulate organic matter, driven by an increase in primary productivity (Wieters et al. 2003). When feeding on phytoplankton blooms near the surface (Winter et al. 1975), mussel feces and pseudofeces can also accumulate on the seafloor, forming a layer of ''mussel mud'' that can be several meters thick at the end of a growing season (Davies et al. 1980, Chamberlain et al. 2001). Without adequate flow to remove sediments, high summer seawater temperatures at depth can accelerate the decomposition of mussel mud, promoting the growth of bacterial communities (Dahlbäck & Gunnarsson 1981) and eventually leading to hypoxic and acidic conditions (Pearson & Rosenberg 1978). Further investigations in the bacterial community structure and flow environment present within mussel aggregations are needed to identify whether the environmental variation observed in this study is the result of geophysical or biological forces.

Regardless of their cause, when exposed to excursions in seawater pH and dissolved oxygen directly, mussels refrained from making threads as conditions got progressively worse. Thread production stopped for the first 3 days of exposure at a pH of 6.0, with almost no mussels producing threads at pH 5.0 over the entire 7-day exposure. A similar result was seen under hypoxia, with mussels refraining from making threads for the first day and producing minimal threads thereafter. Likely a behavioral response to stressful physiological conditions (Gudimov 2006), intertidal mussels routinely remain closed for extended periods of time during low tide to lower predation risk and water loss (Lent 1968). In subtidal habitats, mussels close the valves of their shell when the chemical conditions of the ocean are physiologically harmful (Gleason et al. 2017), perhaps as an attempt to wait out stressful conditions before reopening. It is important to note that this strategy may not be effective long term, as the functionality of the abductor muscle diminishes after high temperature stress (Dowd & Somero 2013), decreasing the ability of mussels to resist damage from further excursions. Either way, mussels cannot make byssal threads without opening the valves of their shells to extend their foot and cannot protect the external, acellular fibers from unfavorable conditions after they are made. As a result, even when byssal threads are made under favorable pH and oxygen conditions, attachment strength can remain low if excursions occur during the adhesive cure window (1–8 days after deposition).

Although seawater pH and dissolved oxygen had a significant effect on plaque adhesion strength and thread production in this study, additional work is needed before the environmental conditions that mussels experience can be directly linked to the fall-off events that are commonly seen in mussel mariculture. For example, higher frequency measurements of pH and oxygen conditions in mussel aggregations are needed to ascertain whether unfavorable conditions for plaque adhesion persist for days at a time without occasional increases back to baseline conditions. Given the ability of the plaque to recover and continue strengthening after curing is arrested, even intermittent changes in flow through rafts could serve to rescue mussel attachment by increasing the flux of oxygen to the byssus. This process may occur naturally, as mussels in high densities often undergo self-thinning where competition-driven mortality causes weaker mussels to fall off when conditions are too stagnant, increasing the space between the individuals that remain (Fréchette & Lefaivre 1995, Lachance-Bernard et al. 2010).

Byssal thread strength has been shown to vary seasonally, with whole mussel tenacity peaking in the spring before crashing to a yearly low in the fall (Carrington et al. 2015). One explanation is a seasonal energetic trade-off between byssus production and reproduction (Sebens et al. 2018), with mussels building large stores of gametes before releasing them in one massive energy expenditure in late spring (Carrington 2002). Although the reproductive state undoubtedly plays a role (Babarro & Reiriz 2010), there is mounting evidence that environmental changes can affect mussel attachment when adult mussels experience stressful environmental conditions. Byssal thread production and strength both decrease with increased seawater temperature (Stern & Achituv 1978, Paul 1980, Newcomb 2015, Clements et al. 2018), although mixed results have been observed in the case of ocean acidification (O'Donnell et al. 2013, Newcomb 2015, Zhao et al. 2017, Clements et al. 2018). Strength differences observed in this study represent a tangential line of inquiry that investigates the direct interaction between the adhesive plaque and the environment, circumventing any effect of acidification or hypoxia on mussel physiology. It should be noted that although this approach was useful to isolate the effects outlined here, physiological changes in response to environmental stress could act to modulate the plaque-curing process, particularly when it remains unclear how much compositional flexibility mussels have when making threads (Waite & Broomell 2012, Waite 2017).

As recorded in mussel aggregations in this study, the greatest excursions in dissolved oxygen and seawater pH were observed during the warmest time of the year, seven meters below the surface, and dwarfed the magnitude of treatments used in most ocean acidification or warming studies. Although it may be tempting to ascribe mussel fall-off events to these conditions, the extent to which temperature-driven increases in mussel metabolism (Newell 1969), in combination with increases in food availability (Thomsen et al. 2013), could negate any impact of the environment on byssal thread strength is unknown. As such, a comprehensive investigation of all these factors, in combination with mussel survivorship and farming yields, is needed to answer whether the timing and magnitude of the excursions observed here are responsible for mussel falloff, particularly as the frequency and magnitude of excursions in

seawater temperature (Roemmich et al. 2015, Wijffels et al. 2016), pH (Woosley et al. 2016), and dissolved oxygen (Stramma et al. 2010, Gobler & Baumann 2016) continue to increase.

AUTHOR CONTRIBUTIONS

MNG designed the study, calibrated water quality sensors, conducted laboratory experiments, performed mechanical testing, and wrote the manuscript. J. A. and J. H. preformed tensometer testing and aided in experimental design of fluctuating pH and oxygen experiments. E. C. aided in data analysis and edited the manuscript. All authors gave final approval for publication.

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DISCLAIMER

Any opinion, findings, conclusions and/or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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