Ocean acidification and bivalve byssus: explaining variable responses using meta-analysis

Jeff C. Clements[1]*, Matthew N. George[2]

[1] Fisheries and Oceans Canada, Gulf Region, Moncton, NB, Canada

[2] School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA

***Correspondence:** Jeff C. Clements, PhD
Fisheries and Oceans Canada
343 Avenue Université, Moncton, NB E1B 9B6, Canada
jeffery.clements@dfo-mpo.gc.ca

Abstract

1. Many bivalve molluscs settle and attach to surfaces using adhesive byssal threads – proteinaceous fibers that together form a network known as the byssus. Since these bivalves rely on byssus for survival, strong byssal attachment promotes a myriad of broad ecological services, including water filtration, nutrient extraction, sediment stabilization, and enhancing biodiversity through habitat creation.

2. Numerous studies have documented weakened byssal attachment strength under ocean acidification (OA); however, a comparable number report no effect, even within the same species. Consequently, whether elevated CO_2 levels expected under near-future OA will affect byssal attachment strength in nature remains hotly contested.

3. We used a systematic literature search and meta-analysis to explore factors that could potentially explain observed effect size variation in byssal attachment strength following OA exposure.

4. A systematic literature search uncovered 20 studies experimentally testing the impact of OA on byssal attachment strength (or some proxy thereof). Meta-analysis revealed that body size (mean shell length) was the strongest predictor of effect size variation, with no significant effect of climate, species, year, study temperature, study location, exposure time, food amount, and pH offset. Functionally, a negative linear relationship was observed between body size and effect size.

5. Our finding that the byssal strength of larger bivalves is more susceptible to negative OA effects runs counter to prevailing wisdom that larger, older animals of a given species are more robust to OA than earlier life history stages.

6. This highlights that body size and age may be important factors that determine OA sensitivity in adult calcifiers. In addition to body size, a critical review of each study revealed commonly neglected factors that could influence byssal thread attachment strength which we highlight to provide suggestions for future research in this area.

Keywords: benthic ecology, environmental stress, global change biology, log response ratio, *Mytilus*

Introduction

Bivalve molluscs are unique in that they settle and adhere to surfaces using adhesive byssal threads – proteinaceous fibers that are secreted by the foot and together form a network known as the byssus. The byssus functions by anchoring bivalves to rocky substrates, with individual threads collectively tethered to each other and the animal's retractor muscle at their proximal end (Waite, 1992). At the distal end, a powerful biological adhesive plaque moors each thread in place (Waite, 2017). While all bivalves appear to produce byssus as larvae, only select species – predominantly mussels and scallops – retain functional byssus as adults. Within these species, byssus plays a vital role in the ecology of adults, preventing dislodgement in high-energy wave environments (Bell & Gosline, 1997).

Robust byssus attachment mitigates a bivalve's dislodgement risk, directly contributing to their survival and fitness (Carrington et al., 2015; Sebens et al., 2018). As ecosystem engineers, byssus-producing bivalves modify their environment through the formation of aggregations or 'beds' (Paine & Levin, 1981). These beds and the bivalves within them provide a multitude of ecosystem services, including nutrient extraction (Nielsen et al., 2016), sediment stabilization (Buschbaum et al., 2009), water filtration (Lindahl et al., 2005), and promoting biodiversity through the creation of habitat for smaller invertebrates and fish (Li et al., 2015; Sui et al., 2015; zu Ermgassen et al., 2020). Given this ecological importance of byssus, a growing number of studies have investigated the impact that marine global change stressors such as ocean acidification (OA) have on byssus attachment. Ocean acidification refers to the decrease in seawater pH (and alteration to other marine carbonate system parameters) due to the oceanic uptake of excess anthropogenic CO_2 from the atmosphere. Numerous studies have reported significant negative effects of experimental OA (i.e., laboratory experiments mimicking near-future OA conditions by increasing seawater CO_2) on bivalve byssus attachment strength. For example, O'Donnell et al. (2013) reported that laboratory exposure to elevated CO_2 reduced the attachment strength of byssal threads in mussels (*Mytilus trossulus*) from Washington State by negatively impacting adhesive plaque strength, a finding that has been further supported in follow up studies (George & Carrington, 2018). Similarly, (Li et al., 2017a) reported negative effects of elevated CO_2 on the byssal attachment strength of oysters (*Pinctada fucata*) from China, and (Zhao et al., 2017) found negative effects for mussels (*Mytilus coruscus*) from China.

While multiple studies suggest OA can weaken the attachment strength of bivalve byssus, a comparable number of studies report conflicting trends, even within the same species. For example, while (Zhao et al., 2017) reported strong negative effects of experimental OA on byssus strength in *M. coruscus*, (Sui et al., 2017) found no effect of experimental OA on the same species. Similarly, (Welladsen et al., 2011) reported little effect of elevated CO_2 on byssus characteristics of *P. fucata* from Australia despite significant negative effects being reported for this species by (Li et al., 2017a). Recent studies on blue mussels (*Mytilus edulis*) in the northwest Atlantic also reported no effect of experimental OA on byssal attachment strength (Clements et al., 2018; Dickey et al., 2018), and (Babarro et al., 2018) reported weak effects in native (*Mytilus galloprovincialis*) and introduced (*Xenostrobus securis*) mussels in northwestern Spain. Consequently, whether elevated CO_2 levels expected under near-future OA will affect byssal attachment strength in nature remains hotly contested.

OA exposure has the potential to weaken attachment strength both directly and indirectly, by interrupting the function of byssal proteins (George & Carrington, 2018) and/or decreasing energy allocation to byssogenesis (Clements et al., 2018). Alternatively, many bivalves have been shown to be resilient to OA in the absence of food limitation (Brown et al., 2018; Büscher et al., 2017; Pansch et al., 2014; Ramajo et al., 2016; Thomsen et al., 2013) and are capable of strengthening attachment in response to stress (e.g., predation: Côté, 1995; Cheung et al., 2006; Brown et al., 2011); flow: Carrington *et al.* 2008). Given the variable and conflicting results of studies testing the effects of experimental OA on byssal attachment strength in marine bivalves, it is important to explore potential sources of such variation if we are to understand the true nature of how elevated CO_2 might affect byssal attachment strength. As such, we conducted a meta-analysis to explore some of the potential factors that explain between-study effect size variation to reconcile the puzzling contrasts between studies testing for experimental OA effects on byssus strength.

Materials and Methods

Systematic literature search

A systematic literature search was used to obtain relevant studies testing for the effects of experimental OA on the byssal attachment strength of marine bivalves according to PRISMA guidelines (Page et al., 2021). Search results for each stage of the PRISMA workflow are provided in **Figure 1**; further study-by-study details provided in Table S1.

An initial database search was conducted in both Scopus and Web of Science using 10 different keyword strings (**Figure 1**). Search results for each keyword string were exported as CSV files, formatted for consistency (i.e., with respect to columns and relevant information), and merged into a single Microsoft Excel file after which duplicates were removed.

Each article was then screened for relevance by reading the titles and abstracts and selecting only those studies that experimentally tested the effect of seawater CO_2 and/or pH on characteristics of marine bivalve byssus (i.e., had a low CO_2 control and at least one high CO_2 group). Byssus-related endpoints were then assessed for eligibility, including only those studies (or experiments within studies) that directly measured whole animal byssus attachment strength or a proxy thereof (see Table S2 for a full list of included endpoints). We further excluded studies, or experiments within studies, if the experimental pH condition was not relevant to near-future coastal OA conditions, which we defined as an experimental treatment being >1.0 pH units lower than the control treatment. This definition was based on an upper projected pH decrease of 0.4 units under the IPCC RCP8.5 projection (Hoegh-Guldberg et al., 2014) plus a further 0.6 pH unit drop to encapsulate extreme natural variability. While we recognize that an experimental decrease of 1.0 pH units is extreme in the context of OA, such a decrease is not outside of the scope of natural coastal pH variability (George et al., 2019a; Lowe et al., 2019). Furthermore, the magnitude of pH decrease was not related to effect size within this range (Figure S1; also see results of AIC model selection below). Studies (or experiments within studies) were also selected based on exposure time, excluding studies or experiments with a pH exposure time of <3 days. While exposure times varied across studies, there was no relationship between effect size and exposure time across the range of exposure times included in our data set (Figure S1; also see results of AIC model selection below). This selection process resulted in 16 studies eligible for the meta-analysis.

A cited reference search of the 16 studies selected above was subsequently performed, whereby the reference lists and online lists of citing articles for each study were checked for additional studies that may have been missed in the database search. Herein, potentially relevant articles were initially identified based on their titles and subsequently screened according to the criteria described above for the database search. This resulted in an additional three studies being added to the database. Finally, a paper published on 29 September, 2021 with a relevant title (Jahnsen-Guzmán et al., 2021) appeared in A1’s Google Scholar alerts and was added to the database after evaluation for the above criteria, resulting in a total of 20 studies included in the meta-analysis; studies are summarized in **Table 1**.

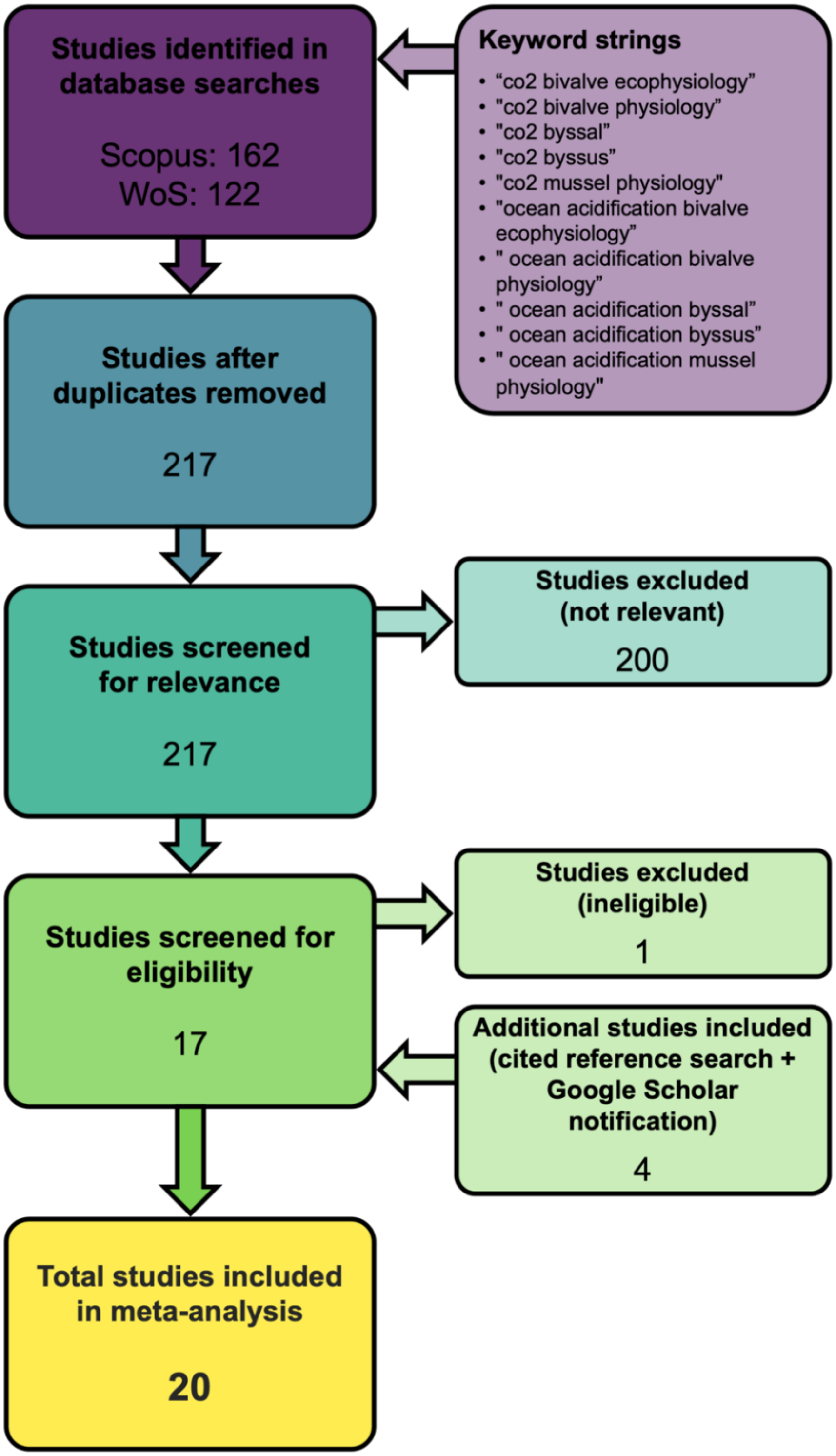

Figure 1. PRISMA flow diagram. Flow diagram depicting the results of each step in the systematic literature search. Detailed information for each unique paper found in the literature search, including reasons for exclusion, are contained in Table S1.

Table 1. Summary of studies. Mean shell length (SL) is depicted in mm and exposure time in days. pH offset is the magnitude difference between the control and the experimental pH treatment in pH units. Studies are ordered chronologically.

Study	Country	Species	Mean SL	Exposure time	pH offset
Welladson et al. (2011)	Australia	*Pinctada fucata*	62.30	5	0.35 - 0.55
O'Donnell et al. (2013)	USA	*Mytilus trossulus*	45.00	20	0.35 - 0.64
Li et al. (2015)	China	*Mytlius coruscus*	27.00	3	0.42 - 0.82
Sui et al. (2015)	China	*Mytlius coruscus*	50.00	3	0.39 - 0.84
Li et al. (2017)	China	*Pinctada fucata*	65.00	30	0.30 - 0.60
Sui et al. (2017)	China	*Mytlius coruscus*	50.00	3	0.40, 0.84
Zhao et al. (2017)	China	*Mytlius coruscus*	97.75	7	0.30 - 0.73
Babarro et al. (2018)	Spain	*Mytilus galloprovincialis,* *Xenostrobus securis*	33.13 (*M. gallo.*) 33.50 (*X. sec.*)	22	0.33
Clements et al. (2018)	Canada	*Mytilus edulis*	37.50	30, 60	0.27 - 0.60
Dickey et al. (2018)	USA	*Mytilus edulis*	65.00	38	0.51
George & Carrington (2018)	USA	*Mytilus trossulus*	47.00	12	1.01
Kong et al. (2018)	China	*Mytilus coruscus,* *Mytilus edulis*	50.00	7	0.40
George et al. (2019)	USA	*Mytilus trossulus*	50.70	3, 5, 7	0.67
Lassoued et al. (2019)	Spain	*Mytilus galloprovincialis*	30.69	15	0.16 - 0.35
Newcomb et al. (2019)	USA	*Mytilus trossulus*	47.40	3	0.28 - 0.84
Shang et al. (2019)	China	*Mytilus coruscus*	52.00	3	0.40
Zhao et al. (2020)	China	*Musculista senhousia*	12.60	196	0.40
Jahnsen-Guzmán et al. (2021)	Chile	*Perumytilus purpuratus*	8.00	60	0.27
Lassoued et al. (2021)	Spain	*Mytilus galloprovincialis*	30.69	15	0.23 - 0.36
Manríquez et al. (2021)	Chile	*Perumytilus purpuratus*	13.50	84	0.34

Data collection

For each of the 20 studies, we collected various qualitative and quantitative attributes (see Table S2). Specifically, we recorded general bibliographic data (authors, year published, title, journal), the species tested, whether the animals were fed during the experiment, the country and climate region where the experimental animals originated, the amount of food fed to the animals during the experiment (% body weight daily), the mean shell length of the animals tested, exposure time, the experimental temperature, and the pH difference between the experimental and control treatments. For each eligible experiment, we also obtained the mean, sample size (*n*), and variance reported for the control and experimental treatment. These values were obtained directly from the text or tables within the article, from graphs within the article (using ImageJ; Schneider et al., 2012), or from raw data shared alongside the article. Measures of variance differed between studies and were thus standardized to reflect standard deviation (SD) prior to analyses. Where multiple stressors or factors were studied (e.g., temperature, oxygen, predation, etc.), we only collected data for OA effects under control treatment of the other variable to isolate OA-only effects for between-study comparability. Likewise, where ecological complexity was included in experiments, data were collected only for treatments that were comparable to other studies (e.g., for mixed versus single-species aggregations as in (Babarro et al., 2018) only single-species aggregations were included). Relevant information regarding these details is contained in the “Notes” column in Table S2.

Meta-analysis

Effect size calculations

We computed effect sizes (natural log response ratio; *lnRR*) and effect size variance for each experiment from the mean, *n*, and SD. The *lnRR* was calculated as,

$$lnRR = \left(\frac{\bar{x}_E}{\bar{x}_C}\right)$$

where $\bar{x}_E$ and $\bar{x}_C$ are the mean byssal attachment strength of the experimental and control OA treatments, respectively. A *lnRR* = 0 signifies no effect, while negative and positive values indicate negative and positive functional effects, respectively.
Effect size variance (*v*) was calculated as,

$$v = \frac{(S_E)}{n_E \bar{x}_E^2} + \frac{(S_C)}{n_C \bar{x}_C^2}$$

where *S* and *n* are the standard deviation and sample size, respectively, for a given experimental treatment (denoted by the subscripts C [control] and E [experimental, i.e., OA]); $\bar{x}_E$ and $\bar{x}_C$ are defined as above. Effect sizes and their variance for each experiment were calculated using the escalc() function in the *metafor* package (Viechtbauer, 2010) in R version 4.1.1 (R Core Team, 2021).

Explaining effect size variation

We explored a subset of the qualitative and quantitative factors collected from each experiment as potential explainers of effect size variation. Categorical factors included study, food amount (% body weight per day), country, climate region, and species (see **Table S2** for factor categories). Continuous factors included year of publication, mean shell length, experimental temperature, exposure time, and pH offset (i.e., difference between control and experimental pH).

To determine which factors contributed most to effect size variation, we employed Akaike's Information Criterion (AIC0 model selection and multimodal inference using the *metafor* package in combination with the *glmulti* package (Calcagno, 2020) according to (Viechtbauer, 2021). AIC model selection was performed by fitting all possible additive models with the categorical and continuous factors listed above using the glmulti() function. Because this exercise resulted in multiple "top" models, we visually assessed the relative importance of each individual factor that showed up in any of the top models and subsequently used multimodal inference to determine which of those factors were significantly important; multimodal inference was again conducted using *metafor* in combination with *glmulti* according to (Viechtbauer, 2021). We then constructed three-level meta-regression models for each of the factors found to be significantly important in the multimodal inference exercise using the rma.mv() function in *metafor* to verify significance' meta-regression models included experiment nested within study as a random factor. For each meta-regression model, funnel plots were constructed to visually check for publication bias.

Finally, we used sensitivity analysis to determine if particular studies contributed to the overall results from the full dataset. Herein, we re-ran the analysis on a second database excluding studies on scallops (*Pinctada fucata*, two studies; (Li et al., 2017b; Welladsen et al., 2011) to verify that taxonomy (i.e., mussels *vs.* scallops) had no influence on the dataset. Likewise, we re-ran the analysis on a third dataset excluding the study showing the most extreme effect sizes (Zhao et al., 2017) to ensure that this study alone did not drive the overall results.

Results

A systematic literature search identified a total of 20 studies empirically testing for effects of OA on the byssal attachment strength (or a relevant proxy thereof) from which data could be collected (**Figure 1, Table 1)**. Weighted mean effect sizes and their variance for each experiment are depicted in **Figure 2a**.

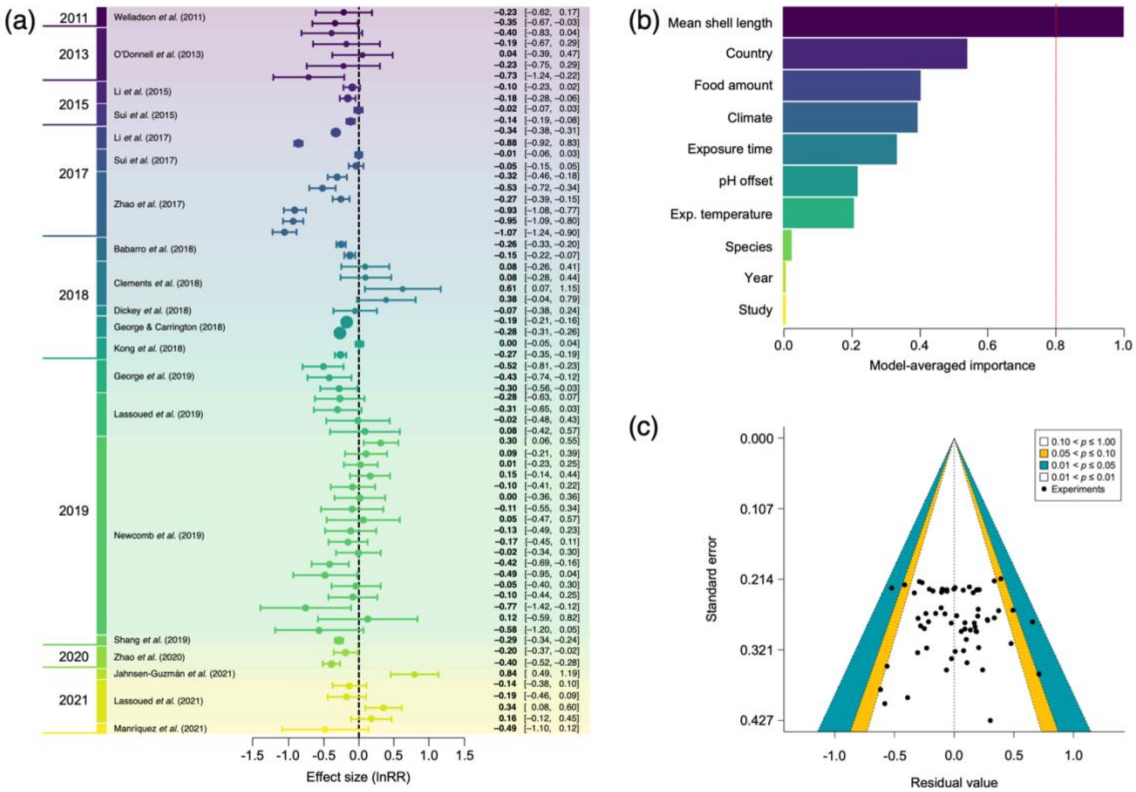

Figure 2. Meta-analysis results. (a) Forest plot of effect sizes and their estimated variance for each experiment (within each study) included in the analysis. Values on the right side of the plot denote the mean effect size estimate (bold) with upper and lower confidence bounds in squared parentheses. **(b)** Relative importance of moderators included in the AIC model selection exercise. Dashed red line depicts an arbitrary importance of 0.80, which is a common cutoff for this approach (Viechtbauer, 2021). **(c)** Funnel plot depicting the effect size of each experiment (x-axis) in relation to the precision of that experiment (SE; y-axis). Points are scattered symmetrically within the funnel, suggesting a lack of publication bias. Note that the color scheme in panels a and b are not functionally linked.

AIC model selection revealed five candidate “top” models which included five explanatory factors: mean shell length, exposure time, food amount, country, and climate (Table S3). Among these factors, mean shell length was captured in all five candidate models, while country, climate, and food amount were each captured in two models, and exposure time in one (Table S3). Multimodal inference indicated that mean shell length was the only significantly important factor (Importance measure = 0.998, $p < 0.001$), while all other factors were of non-significant importance (Importance measures ≤ 0.536, $p \geq 0.000$) **(Figure 2b**; Table S4). Publication bias was not apparent according to visual inspection of the funnel plot (i.e., data points were symmetrically scattered within the funnel; **Figure 2c**).

A three-level meta-regression model including mean shell length as a continuous moderator indicated significant residual heterogeneity (df = 63, $Q_E = 1039.001$, $p < 0.001$) and a significant overall model fit (df = 1, $Q_M = 20.459$, $p < 0.001$). Herein, mean shell length displayed a negative overall effect size (estimate [CI_{lower}, CI_{upper}] = –0.009 [–0.013, –0.005], $p < 0.001$) (see Table S5 for full model results), and a negative relationship between mean shell length and effect size was apparent (**Figure 3**).

Re-running the analysis on the dataset without scallop studies yielded slightly different top models in the AIC selection process (Table S6) but did not change results of the multimodal inference for moderator importance (Table S7) nor the overall meta-regression model results (Table S8) (although note that the importance of “Country” increased beyond 0.8). This was the same for the sensitivity analysis when the study with the most extreme effect sizes was removed from the analysis (Tables S9-S11).

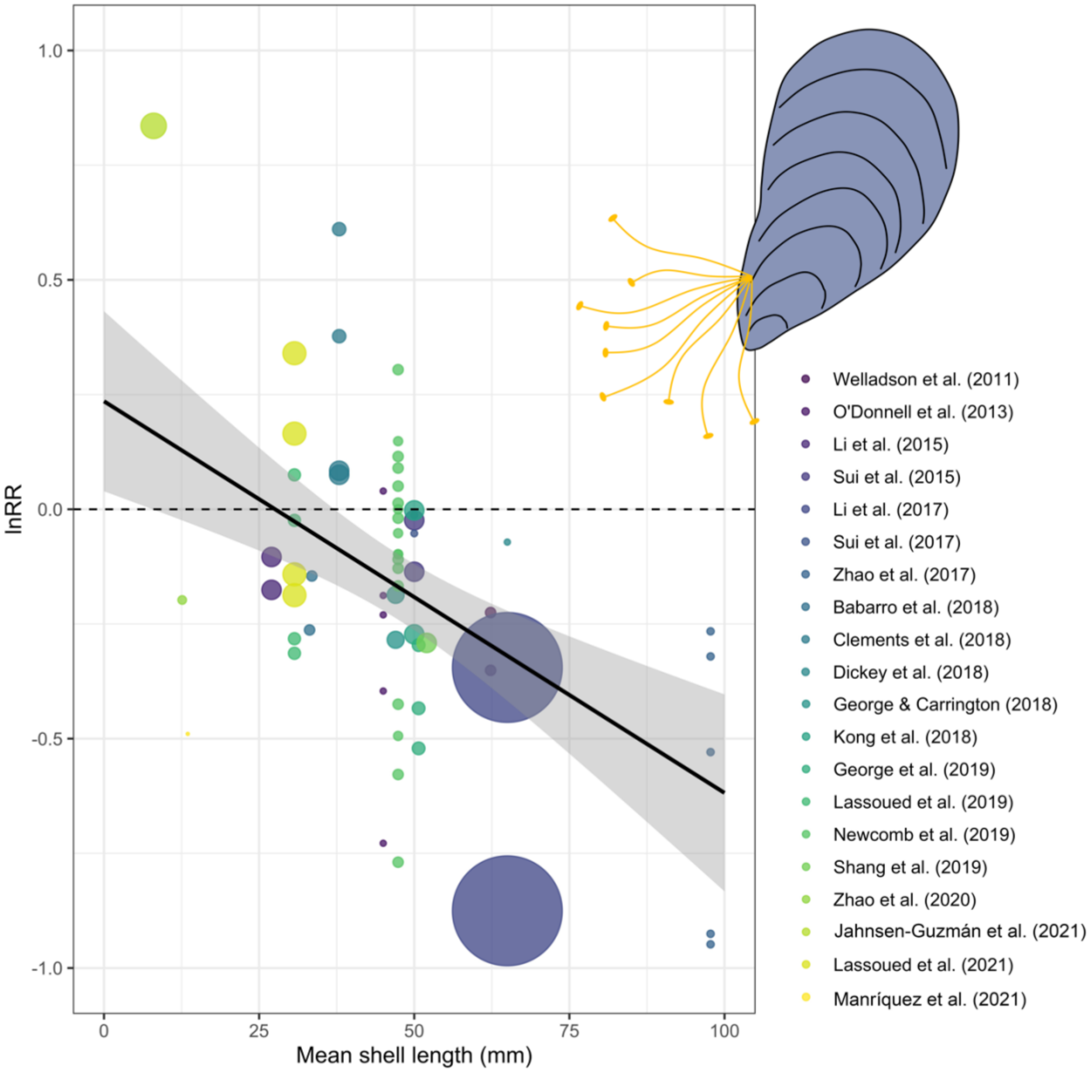

Figure 3. Larger bivalves are more susceptible to OA effects on byssal attachment strength. Effect size (lnRR) as a function of mean shell length. Each data point represents a single experiment, with colors denoting different studies. The size of each point represents the relative sample size of the experiment. See Figure 2a and Table S2 for experiment-specific data.

Discussion

Body size as a moderator of OA effects on byssal attachment strength

Our results suggest that the byssal attachment strength of larger bivalves is more vulnerable to experimental OA than that of smaller bivalves. This result runs counter to the prevailing wisdom that larger, older animals of a given species are more robust to the effects of OA than earlier life history stages (i.e., larvae and juveniles; Kroeker et al., 2013; Harvey & Moore 2017; Clements & Darrow, 2018). While no studies included in our database assessed the impact of OA on larvae, our results do suggest that larger adults display weakened attachment under OA when compared to smaller individuals. This finding underscores that size and/or age may act as determinants of OA sensitivity in adult-stage organisms – a moderator that has received little attention in the context of OA impacts. Furthermore, size and/or age may moderate byssus responses to the myriad other environmental stressors, both natural and anthropogenic. We suggest that studies assessing the impact of stressors, broadly, should consider size and age (both independently and combined, i.e., size-at-age) as moderating factors in explaining observed responses.

Out of the variables tested in this study, body size was the only variable of interest that could adequately explain effect size variability, with no significant influence of climate, species, year, study temperature, study location (country), exposure time, food amount, or pH offset. Larger mussels typically secrete fewer (Babarro et al., 2008; Van Winkle, 1970), but larger, mechanically superior threads than smaller individuals (Babarro & Carrington, 2013), and generally display weaker size-corrected whole animal tenacity (Babarro et al., 2020). However, relationships between animal size and byssus are often site-specific (Babarro & Carrington, 2011), and environmental conditions are known to affect thread attachment strength and byssogensis, including water flow (Babarro & Carrington, 2013; Lee et al., 1990), predation (K. Brown et al., 2011; Cheung et al., 2006; Côté, 1995), hypoxia (M. George et al., 2018), and temperature (Clements et al., 2018; Newcomb et al., 2019). None of the studies obtained in our literature search included size as a variable of interest in moderating OA effects on byssus. Indeed, size has been suggested as a potentially important factor influencing byssus function (Babarro & Carrington, 2013). We suggest that future studies place an emphasis on understanding how size modulates OA effects on byssus, in combination with other environmental conditions.

Animals surviving past climate-related extinction events have been found to be smaller than animals succumbing to such events (Morten & Twitchett, 2009; Twitchett, 2007) — a phenomenon known as the Lilliput Effect (Urbanek, 1993). With respect to OA, gastropods adapted to high CO_2 conditions at CO_2 seeps were found to be smaller than those at reference sites, suggesting that smaller animals are better adapted to survive prolonged exposures to high CO_2 (Garilli et al., 2015). Therein, smaller organisms exhibited lower whole-animal metabolic demand, allowing them to maintain key biological functions to survive under high CO_2 conditions. It is thus possible that smaller bivalves with a lower whole-animal metabolic demand are better able to maintain byssus under high CO_2 conditions as compared to larger bivalves. However, this effect has yet to be empirically demonstrated for byssus attachment strength under OA and warrants future study.

Hidden cofactors: energy and seasonality

As a non-living external biomaterial, byssal threads form a network to ensure robust attachment through the absorption and distribution of wave energy. Bivalves have the ability to dynamically modify byssus tenacity, whether by increasing the size of the byssal network (thread number), increasing thread size (i.e., diameter, length), or modifying thread mechanical properties (e.g., stiffness, extensibility). Out of the 13 studies that measured thread number in our analysis, 10 reported decreased thread production under OA, with 5 noting significant interactions between OA and temperature or predator cues (although such additional variables were ignored in our analysis).

While thread number can have an impact on attachment strength, thread quality is the primary driver of whole animal tenacity (Carrington et al., 2015; Moeser et al., 2006; Moeser & Carrington, 2006). Perhaps nowhere is this more evident than in the regular field monitoring of several Mytilid species, where 2-3fold fluctuations in tenacity are regularly observed within a given year, irrespective of thread number (Carrington, 2002; Hunt & Scheibling, 2001; Price, 1980; Zardi et al., 2007). Seasonal variability of this magnitude is likely related, at least in part, to energetic limitations imposed by reproduction, a significant energy expenditure that corresponds with a measurable decrease in attachment strength (Babarro et al., 2008; Babarro & Fernández Reiriz, 2010). Energy allocation to byssogenesis is constitutive (Hawkins & Bayne, 1985) and prioritized over growth (Clarke, 1999; Roberts et al., 2021), indicating that adult mussels employ a physiological strategy that minimizes dislodgement risk and maximizes overall fitness (Sebens et al., 2018). However, it remains unclear to what extent adult size, seasonality, and energy allocation interact to influence tenacity (Roberts et al., 2021), particularly within the context of OA. Unfortunately, only nine studies included in our database reported when animals were collected, representing an even split across seasons (except for winter, which had three). Such a small sample precludes any confident analyses. To resolve this discrepancy, future studies would benefit from reporting when animals are collected, as well as performing temporal replicates with multiple cohorts from the same location across seasons.

An integrated approach: the role of byssal thread mechanics

A reduction in oceanic pH can potentially impact byssal thread quality through one of two mechanisms: first, by directly harming the material itself, and/or second, by indirectly compromising thread quality by imposing a physiological stress on the animal (Waite & Broomell, 2012). Included in our database are studies that investigate both avenues. Addressing the first, two studies observed a direct negative effect of OA on the attachment strength of byssal threads produced by unstressed mussels (*Mytilus trossulus*: O’Donnell et al., 2013; George et al., 2019) an effect that has since been confirmed within another species (*Mytilus californianus*, Bernstein et al., 2020). These studies suggest that the direct impact of OA on byssus is localized to the adhesive plaque, and is a consequence of the material utilizing oceanic pH (~8.1) as a molecular trigger to facilitate the crosslinking of DOPA (3,4-dihydroxyphenyl-l-alanine) rich proteins during plaque curing (Martinez Rodriguez et al., 2015; Waite, 2017). However, it should be noted that the arrested development that the plaque experiences under OA requires pH be maintained at a level much lower than oceanic conditions (pH ~7.0 or lower), and doesn’t appear to be permanent if conditions improve (George et al., 2019). As such, under near future OA conditions animal physiology may play a more important role than direct effects on byssal mechanics. Given that the physiological effects of OA can be size-dependent (Garilli et al., 2015), studies linking physiology and byssus strength under OA across a range of body sizes are needed.

Most studies (17 out of 19) included in our analysis investigated whether OA impacted attachment by subjecting whole animals to elevated CO_2, thirteen of which measured attachment strength directly. Out of that subset, six reported negative effects of OA, two reported mixed or context dependent effects, one reported a positive effect, and four reported no effects. As a complex biomaterial, byssal threads consist of three distinct thread regions (proximal region, distal region, and adhesive plaque) that have different material properties (Bell & Gosline, 1996). For this reason, a weakness in one thread region can act like a weak link in a chain (Newcomb et al., 2019), resulting in structural failure before threads can adequately ‘yield’ and distribute force across the thread network (Carrington & Gosline, 2004). For this reason, it is important to isolate the impact that environmental factors can have on each region to determine the magnitude of their effect. While the byssus metrics included in our database are all proxies of attachment strength, the specific measures vary between studies. Four studies included in our analysis investigated region-specific impacts of OA (George & Carrington, 2018; George et al., 2019a; Newcomb et al., 2019; O’Donnell et al., 2013), while two attempted to link gene expression levels of byssal proteins to mechanical performance (Li et al., 2017b; Zhao et al., 2017). While the integration of genomics, proteomics, and thread mechanics represents a powerful approach for teasing apart the impact of OA on byssus, we acknowledge this route can be cost-prohibitive. As such, we suggest that future studies include the following five metrics to increase cross-study comparability: byssal thread number, thread diameter within the distal region, plaque cross-sectional area, single-thread attachment force, and thread failure location.

Nutrition, transgenerational exposure, and local adaptation

Body size notwithstanding, it is puzzling that other factors known to influence biological responses under OA were not important explainers of byssus weakening. For example, food supply is often considered one of the most important factors moderating experimental OA effects on calcifying organisms (Brown et al., 2018; Büscher et al., 2017; Pansch et al., 2014; Ramajo et al., 2016; Thomsen et al., 2013). While food supply appeared in three of the six “top” models in our AIC model selection exercise, its explanatory power was low. One possible explanation for this mismatch may be that although animals were fed while under laboratory conditions, energetic limitations were imposed on some treatment groups due to inadequate nutritional support. Matching the complex algal diet of adult bivalves can be challenging, even in a hatchery setting, and most studies feed animals commercially available algal pastes that may not represent the algal or bacterial diversity seen in a natural setting (Coffin et al., 2021; Timmins-Schiffman et al., 2021). Additionally, condition indices can vary between species and populations, and when reported in isolation are unlikely to provide a holistic measure of a bivalve's nutritional state even when differences in tenacity are observed (Babarro et al., 2020). To resolve this issue, we suggest that future studies employ more rigorous histological and/or biochemical characterizations of nutritional status (Grkovic et al., 2019), integrating information from multiple tissues and/or macronutrients when possible (see Clements et al., 2018).

Exposure duration and the magnitude of pH change are also thought to modulate responses to OA, as chronic and acute exposures can yield contrasting responses and effects can be more severe when pH decreases are larger (Dupont et al., 2010; Shaw et al., 2013). However, neither exposure time nor pH offset were significantly important moderators in our analysis. This is not particularly surprising given that we restricted exposure times to ≥3 days and pH offsets ≤1.0 pH units. While more acute exposures and larger pH offsets may have yielded different results for our meta-analysis, these are not applicable to near-future OA scenarios. As such, in the context of near-future OA, this magnitude of change is unlikely to influence byssal attachment strength responses. Of note, however, is that only a single transgenerational study was identified in our review, reporting positive effects of parental exposure to OA on offspring byssus attachment (Zhao et al., 2020). However, parental exposure to OA may be species specific, with reports of negative (Griffith & Gobler, 2017), positive (Kong et al., 2018), and null (Clements et al., 2021) effects on offspring responses. While we recognize that transgenerational studies across the full ontogeny of byssus producing bivalves requires considerable time and effort, such studies would be beneficial to better understanding how byssus may be impacted by OA in the future.

Local adaptation and geography have also been suggested as potentially important moderators of OA effects (Vargas et al., 2017). In our analysis, geographic moderators (country and climate) did not significantly contribute to any of the top AIC models identified in our meta-analysis. Interestingly, however, "country" was consistently the second most important moderator identified in the multimodal inference exercise (Tables S4, S7, S10). Herein, bivalves from China and the USA displayed a significant negative effect size (Figure S2). This suggests that there is perhaps a geographical element to OA effects on byssal attachment strength, although more detailed conclusions are not currently possible. Furthermore, while factors other than shell length and country were not identified as important in our global meta-analysis, they very well may be important in more specific contexts. As such, while body size appears most important in explaining OA effects on byssal strength across a broad range of bivalve taxa, other factors may serve as important moderators in a context-specific manner. Studies assessing the effects of geography, particularly in the context of local adaptation, would be beneficial.

Conclusions

Out of the factors included in our analysis, shell length was the only significant moderator of OA effects on byssus attachment strength. Unfortunately, no studies to date have included animal size as a treatment variable. We argue that future studies examining OA effects on byssal attachment strength should incorporate tests of animal size to uncover potential mechanisms for any observed patterns. Furthermore, studies have largely neglected other important co-variables that can potentially impact byssal thread strength, including aspects of thread morphology, animal nutritional status, and the impact of seasonality. With respect to thread morphology, we suggest the measurement of a more comprehensive suite of thread properties to facilitate cross-study comparability, which at least include byssal thread number, distal region diameter, plaque cross-sectional area, single-thread attachment force, and thread failure location. Future studies would also benefit from a more robust characterization of animal nutritional status, which includes a biochemical characterization of multiple tissues when possible. Finally, performing temporal replicates with multiple cohorts from the same location across seasons would go a long way to resolving the influence of seasonality on interstudy variability. Studies incorporating the above suggestions will help to resolve the current lack of agreement regarding OA impacts on byssal attachment strength. Given the substantial ecological significance of byssus-producing bivalves and their roles as "ecosystem engineers", these studies are necessary for predicting the ecological consequences of a more acidic ocean.

Acknowledgments

We thank Dr. José M.F. Babarro of the Spanish National Research Council in Vigo, Spain for discussions regarding the effects of OA on bivalve byssus. We also thank Dr. Emily Carrington for providing size data for our dataset. Thanks to Dr. Robert Ellis of Plymouth University for providing constructive feedback on an earlier draft of this manuscript

.

Author Contributions: JCC conceptualized the study, conducted the literature search, collected and analyzed the data, and wrote and revised the manuscript. MNG contributed to manuscript conceptualization, content, and writing.

Data accessibility

Annotated R-script raw data files, and supplementary data (figures and tables) are available as supplementary material and can be accessed at: https://osf.io/kusyc/.

Competing Interest Statement: We declare no competing interests.

Funding

This study was partially funded by a Marie Skłodowska-Curie Individual Fellowship funded through the European Union Horizon 2020 program (project number 752813 to JCC).

References

Babarro, J., & Carrington, E. (2011). Byssus secretion of *Mytilus galloprovincialis*: Effect of site at macro- and micro-geographical scales within Ría de Vigo (NW Spain). *Marine Ecology Progress Series*, *435*, 125–140. https://doi.org/10.3354/meps09200

Babarro, J., & Carrington, E. (2013). Attachment strength of the mussel *Mytilus galloprovincialis*: Effect of habitat and body size. *Journal of Experimental Marine Biology and Ecology*, *443*, 188–196. https://doi.org/10.1016/j.jembe.2013.02.035

Babarro, J., & Fernández Reiriz, M. (2010). Secretion of byssal threads in *Mytilus galloprovincialis*: Quantitative and qualitative values after spawning stress. *Journal of Comparative Physiology B*, *180*, 95–104. https://doi.org/10.1007/s00360-009-0392-y

Babarro, J., Fernández Reiriz, M., & Labarta, U. (2008). Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: The influence of size and food availability. *Journal of the Marine Biological Association of the United Kingdom*, *88*, 783–791. https://doi.org/10.1017/S0025315408001367

Babarro, J., Filgueira, R., Padín, X., & Longa Portabales, M. (2020). A novel index of the performance of *Mytilus galloprovincialis* to improve commercial exploitation in aquaculture. *Frontiers in Marine Science*, *7*, 719. https://doi.org/10.3389/fmars.2020.00719

Babarro, J. M. F., Abad, M. J., Gestoso, I., Silva, E., & Olabarria, C. (2018). Susceptibility of two co-existing mytilid species to simulated predation under projected climate change conditions. *Hydrobiologia*, *807*, 247–261. https://doi.org/10.1007/s10750-017-3397-7

Bell, E. C., & Gosline, J. M. (1996). Mechanical design of mussel byssus: Material yield enhances attachment strength. *Journal of Experimental Biology*, *199*, 1005–1017. https://doi.org/10.1242/jeb.199.4.1005

Bell, E., & Gosline, J. (1997). Strategies for life in flow: Tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. *Marine Ecology Progress Series*, *159*, 197–208. https://doi.org/10.3354/meps159197

Bernstein, J., Filippidi, E., Waite, J., & Valentine, M. (2020). Effects of sea water pH on marine mussel plaque maturation. *Soft Matter*, *16*, 9339–9346. https://doi.org/10.1039/D0SM01237H

Brown, K., Aronhime, B., & Wang, X. (2011). Predatory blue crabs induce byssal thread production in hooked mussels: Predator-induced responses of hooked mussels. *Invertebrate Biology*, *130*, 43–48. https://doi.org/10.1111/j.1744-7410.2011.00223.x

Brown, N. E. M., Bernhardt, J. R., Anderson, K. M., & Harley, C. D. G. (2018). Increased food supply mitigates ocean acidification effects on calcification but exacerbates effects on growth. *Scientific Reports*, *8*, 9800. https://doi.org/10.1038/s41598-018-28012-w

Buschbaum, C., Dittmann, S., Hong, J.-S., Hwang, I.-S., Strasser, M., Thiel, M., Valdivia, N., Yoon, S.-P., & Reise, K. (2009). Mytilid mussels: Global habitat engineers in coastal sediments. *Helgoland Marine Research*, *63*, 47–58. https://doi.org/10.1007/s10152-008-0139-2

Büscher, J., Form, A., & Riebesell, U. (2017). Interactive effects of ocean acidification and warming on growth, fitness and survival of the cold-water coral *Lophelia pertusa* under different food availabilities. *Frontiers in Marine Science*, *4*, 101. https://doi.org/10.3389/fmars.2017.00101

Calcagno, V. (2020). *glmulti: Model Selection and Multimodel Inference Made Easy*. R package version 1.0.8. https://CRAN.R-project.org/package=glmulti

Carrington, E. (2002). Seasonal variation in the attachment strength of blue mussels: Causes and consequences. *Limnology and Oceanography*, *47*, 1723–1733. https://doi.org/10.4319/lo.2002.47.6.1723

Carrington, E., & Gosline, J. (2004). Mechanical design of mussel byssus: Load cycle and strain rate dependence. *American Malacological Bulletin*, *18*, 135–142.

Carrington, E., Moeser, G., Thompson, S., Coutts, L., & Craig, C. (2008). Mussel attachment on rocky shores: The effect of flow on byssus production. *Integrative and Comparative Biology*, *48*, 801–807. https://doi.org/10.1093/icb/icn078

Carrington, E., Waite, J., Sarà, G., & Sebens, K. (2015). Mussels as a model system for integrative ecomechanics. *Annual Review of Marine Science*, *7*, 443–469. https://doi.org/10.1146/annurev-marine-010213-135049

Cheung, S., Luk, K., & Shin, P. (2006). Predator-labeling effect on byssus production in marine mussels *Perna viridis* (L.) and *Brachidontes variabilis* (Krauss). *Journal of Chemical Ecology*, *32*, 1501–1512. https://doi.org/10.1007/s10886-006-9065-4

Clarke, M. (1999). The effect of food availability on byssogenesis by the zebra mussel (*Dreissena polymorpha* Pallas). *Journal of Molluscan Studies*, *65*, 327–333. https://doi.org/10.1093/mollus/65.3.327

Clements, J. C., & Darrow, E. S. (2018). Eating in an acidifying ocean: A quantitative review of elevated CO_2 effects on the feeding rates of calcifying marine invertebrates. *Hydrobiologia*, *820*, 1–21. https://doi.org/10.1007/s10750-018-3665-1

Clements, J. C., Hicks, C., Tremblay, R., & Comeau, L. A. (2018). Elevated seawater temperature, not $p$$CO_2$, negatively affects post-spawning adult mussels (*Mytilus edulis*) under food limitation. *Conservation Physiology*, *6*, cox078. https://doi.org/10.1093/conphys/cox078

Clements, J., Carver, C., Mallet, M., Comeau, L., & Mallet, A. (2021). CO_2-induced low pH in an eastern oyster (*Crassostrea virginica*) hatchery positively affects reproductive development and larval survival but negatively affects larval shape and size, with no intergenerational linkages. *ICES Journal of Marine Science*, *78*, 349–359. https://doi.org/10.1093/icesjms/fsaa089

Coffin, M., Clements, J., Comeau, L., Guyondet, T., Maillet, M., Steeves, L., Winterburn, k, Babarro, J., Mallet, L., Haché, R., Poirier, L., Deb, S., & Filgueira, R. (2021). The killer within: Endogenous bacteria accelerate oyster mortality during sustained anoxia. *Limnology and Oceanography*, *66*, 2885–2900. https://doi.org/10.1002/lno.11798

Côté, I. (1995). Effects of predatory crab effluent on byssus production in mussels. *Journal of Experimental Marine Biology and Ecology*, *188*, 233–241. https://doi.org/10.1016/0022-0981(94)00197-L

Dickey, G., Preziosi, B. M., Clark, C. T., & Bowden, T. J. (2018). The impact of ocean acidification on the byssal threads of the blue mussel (*Mytilus edulis*). *PLOS One*, *13*(10), e0205908. https://doi.org/10.1371/journal.pone.0205908

Dupont, S., Ortega-Martínez, O., & Thorndyke, M. (2010). Impact of near-future ocean acidification on echinoderms. *Ecotoxicology*, *19*, 449–462. https://doi.org/10.1007/s10646-010-0463-6

Garilli, V., Rodolfo-Metalpa, R., Scuderi, D., Brusca, L., Parrinello, D., Rastrick, S., Foggo, A., Twitchett, R., Hall-Spencer, J., & Milazzo, M. (2015). Physiological advantages of dwarfing in surviving extinctions in high-CO_2 oceans. *Nature Climate Change*, *5*, 678–682. https://doi.org/10.1038/nclimate2616

George, M., Andino, J., Huie, J., & Carrington, E. (2019). Microscale pH and dissolved oxygen fluctuations within mussel aggregations and their implications for mussel attachment and raft aquaculture. *Journal of Shellfish Research*, *38*, 795. https://doi.org/10.2983/035.038.0329

George, M., & Carrington, E. (2018). Environmental post-processing increases the adhesion strength of mussel byssus adhesive. *Biofouling*, *34*, 388–397. https://doi.org/10.1080/08927014.2018.1453927

George, M., Pedigo, B., & Carrington, E. (2018). Hypoxia weakens mussel attachment by interrupting DOPA cross-linking during adhesive plaque curing. *Journal of The Royal Society Interface*, *15*, 20180489. https://doi.org/10.1098/rsif.2018.0489

Griffith, A., & Gobler, C. (2017). Transgenerational exposure of North Atlantic bivalves to ocean acidification renders offspring more vulnerable to low pH and additional stressors. *Scientific Reports*, *7*, 11394. https://doi.org/10.1038/s41598-017-11442-3

Grkovic, N., Dimitrijevic, M., Teodorovic, V., Karabasil, N., Vasilev, D., Stajkovic, S., & Velebit, B. (2019). Factors influencing mussel (*Mytilus galloprovincialis*) nutritional quality. *IOP Conference Series: Earth and Environmental Science*, *333*, 012062. https://doi.org/10.1088/1755-1315/333/1/012062

Harvey, B. P., & Moore, P. J. (2017). Ocean warming and acidification prevent compensatory response in a predator to reduced prey quality. *Marine Ecology Progress Series*, *563*, 111–122. https://doi.org/10.3354/meps11956

Hawkins, A., & Bayne, B. (1985). Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: Budgets, conversion efficiencies and maintenance requirements. *Marine Ecology Progress Series*, *25*, 181–188. https://doi.org/10.3354/meps025181

Hoegh-Guldberg, O., Cai, R., Poloczanska, E., Brewer, P., Sundby, S., Hilmi, K., Fabry, V., & Jung, S. (2014). The ocean. In *Climate change 2014: Impacts, adaptation, and vulnerability. Part B: regional aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1655–1731). Cambridge University Press.

Hunt, H., & Scheibling, R. (2001). Predicting wave dislodgment of mussels: Variation in attachment strength with body size, habitat, and season. *Marine Ecology Progress Series*, *213*, 157–164. https://doi.org/10.3354/meps213157

Jahnsen-Guzmán, N., Lagos, N. A., Quijón, P. A., Manríquez, P. H., Lardies, M. A., Fernández, C., Reyes, M., Zapata, J., García-Huidobro, M. R., Labra, F. A., & Duarte, C. (2021). Ocean acidification alters anti-predator responses in a competitive dominant intertidal mussel. *Chemosphere*, *288*, 132410. https://doi.org/10.1016/j.chemosphere.2021.132410

Kong, H., Jiang, X., Clements, J. C., Wang, T., Huang, X., Shang, Y., Chen, J., Hu, M., & Wang, Y. (2018). Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. *Marine Environmental Research*, *145*, 73–80. https://doi.org/10.1016/j.marenvres.2019.02.011

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., & Gattuso, J. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*, 1884–1896. https://doi.org/10.1111/gcb.12179

Lee, C., Lim, S., & Owen, M. (1990). The rate and strength of byssal reattachment by blue mussels (*Mytilus edulis* L.). *Canadian Journal of Zoology*, *68*, 2005–2009. https://doi.org/10.1139/z90-282

Li, L., Lu, W., Sui, Y., Wang, Y., Gul, Y., & Dupont, S. (2015). Conflicting effects of predator cue and ocean acidification on the mussel *Mytilus coruscus* byssus production. *Journal of Shellfish Research*, *34*, 393–400. https://doi.org/10.2983/035.034.0222

Li, S., Liu, C., Zhan, A., Xie, L., & Zhang, R. (2017a). Influencing mechanism of ocean acidification on byssus performance in the pearl oyster *Pinctada fucata*. *Environmental Science & Technology*, *51*, 7696–7706.

Li, S., Liu, C., Zhan, A., Xie, L., & Zhang, R. (2017b). Influencing mechanism of ocean acidification on byssus performance in the pearl oyster *Pinctada fucata*. *Environmental Science & Technology*, *51*, 7696–7706. https://doi.org/10.1021/acs.est.7b02132

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.-O., Olrog, L., Rehnstam-Holm, A.-S., Svensson, J., Svensson, S., & Syversen, U. (2005). Improving marine water quality by mussel farming: A profitable solution for Swedish society. *AMBIO: A Journal of the Human Environment*, *34*, 131–138. https://doi.org/10.1579/0044-7447-34.2.131

Lowe, A., Bos, J., & Ruesink, J. (2019). Ecosystem metabolism drives pH variability and modulates long-term ocean acidification in the Northeast Pacific coastal ocean. *Scientific Reports*, *9*, 963. https://doi.org/10.1038/s41598-018-37764-4

Martinez Rodriguez, N., Das, S., Kaufman, Y., Israelachvili, J., & Waite, J. (2015). Interfacial pH during mussel adhesive plaque formation. *Biofouling*, *31*, 221–227. https://doi.org/10.1080/08927014.2015.1026337

Moeser, G., & Carrington, E. (2006). Seasonal variation in mussel byssal thread mechanics. *Journal of Experimental Biology*, *209*, 1996–2003. https://doi.org/10.1242/jeb.02234

Moeser, G., Leba, H., & Carrington, E. (2006). Seasonal influence of wave action on thread production in *Mytilus edulis*. *Journal of Experimental Biology*, *209*, 881–890. https://doi.org/10.1242/jeb.02050

Morten, S., & Twitchett, R. (2009). Fluctuations in the body size of marine invertebrates through the Pliensbachian–Toarcian extinction event. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *284*, 29–38. https://doi.org/10.1016/j.palaeo.2009.08.023

Newcomb, L. A., George, M. N., O'Donnell, M. J., & Carrington, E. (2019). Only as strong as the weakest link: Structural analysis of the combined effects of elevated temperature and $p\mathrm{CO}_2$ on mussel attachment. *Conservation Physiology*, *7*, coz068. https://doi.org/10.1093/conphys/coz068

Nielsen, P., Cranford, P., Maar, M., & Petersen, J. (2016). Magnitude, spatial scale and optimization of ecosystem services from a nutrient extraction mussel farm in the eutrophic Skive Fjord, Denmark. *Aquaculture Environment Interactions*, *8*, 311–329. https://doi.org/10.3354/aei00175

O'Donnell, M., George, M., & Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. *Nature Climate Change*, *3*, 587–590. https://doi.org/10.1038/nclimate1846

Page, M., McKenzie, J., Bossuyt, P., Boutron, I., Hoffmann, T., Mulrow, C., Shamseer, L., Tetzlaff, J., Akl, E., Brennan, S., Chou, R., Glanville, J., Grimshaw, J., Hróbjartsson, A., Lalu, M., Li, T., Loder, E., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*, *372*, n71. https://doi.org/10.1136/bmj.n71

Paine, R., & Levin, S. (1981). Intertidal landscapes: Disturbance and the dynamics of pattern. *Ecological Monographs*, *51*, 145–178. https://doi.org/10.2307/2937261

Pansch, C., Schaub, I., Havenhand, J., & Wahl, M. (2014). Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. *Global Change Biology*, *20*, 765–777. https://doi.org/10.1111/gcb.12478

Price, H. (1980). Seasonal variation in the strength of byssal attachment of the common mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom*, *60*, 1035–1037. https://doi.org/10.1017/S0025315400042065

R Core Team. (2021). *R: A language and environment for statistical computing.* (4.1.1) [Computer software]. R Foundation for Statistical Computing.

Ramajo, L., Marbà, N., Prado, L., Peron, S., Lardies, M., Rodriguez-Navarro, A., Vargas, C., Lagos, N., & Duarte, C. (2016). Biomineralization changes with food supply confer juvenile scallops (*Argopecten purpuratus*) resistance to ocean acidification. *Global Change Biology*, *22*, 2025–2037. https://doi.org/10.1111/gcb.13179

Roberts, E. A., Newcomb, L. A., McCartha, M. M., Harrington, K. J., LaFramboise, S. A., Carrington, E., & Sebens, K. P. (2021). Resource allocation to a structural biomaterial: Induced production of byssal threads decreases growth of a marine mussel. *Functional Ecology, 35,* 1222–1239. https://doi.org/10.1111/1365-2435.13788

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Sebens, K., Sarà, G., & Carrington, E. (2018). Estimation of fitness from energetics and life-history data: An example using mussels. *Ecology and Evolution*, *8*, 5279–5290. https://doi.org/10.1002/ece3.4004

Shaw, E., Munday, P., & McNeil, B. (2013). The role of CO_2 variability and exposure time for biological impacts of ocean acidification. *Geophysical Research Letters*, *40*, 4685–4688. https://doi.org/10.1002/grl.50883

Sui, Y., Hu, M., Huang, X., Wang, Y., & Lu, W. (2015). Anti-predatory responses of the thick shell mussel *Mytilus coruscus* exposed to seawater acidification and hypoxia. *Marine Environmental Research*, *109*, 159–167. https://doi.org/10.1016/j.marenvres.2015.07.008

Sui, Y., Liu, Y., Zhao, X., Dupont, S., Hu, M., Wu, F., Huang, X., Li, J., Lu, W., & Wang, Y. (2017). Defense responses to short-term hypoxia and seawater acidification in the thick shell mussel *Mytilus coruscus*. *Frontiers in Physiology*, *8*, 145. https://doi.org/10.3389/fphys.2017.00145

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A., & Melzner, F. (2013). Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: Laboratory and field experiments. *Global Change Biology*, *19*, 1017–1027. https://doi.org/10.1111/gcb.12109

Timmins-Schiffman, E., White, S., Thompson, R., Vadopalas, B., Eudeline, B., Nunn, B., & Roberts, S. (2021). Coupled microbiome analyses highlights relative functional roles of bacteria in a bivalve hatchery. *Environmental Microbiome*, *16*, 7. https://doi.org/10.1186/s40793-021-00376-z

Twitchett, R. (2007). The Lilliput effect in the aftermath of the end-Permian extinction event. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *252*, 132–144. https://doi.org/10.1016/j.palaeo.2006.11.038

Urbanek, A. (1993). Biotic crises in the history of Upper Silurian graptoloids: A Palaeobiological model. *Historical Biology*, *7*, 29–50. https://doi.org/10.1080/10292389309380442

Van Winkle, W. (1970). Effect of environmental factors on byssal thread formation. *Marine Biology*, *7*, 143–148. https://doi.org/10.1007/BF00354918

Vargas, C. A., Lagos, N. A., Lardies, M. A., Duarte, C., Manríquez, P. H., Aguilera, V. M., Broitman, B., Widdicombe, S., & Dupont, S. (2017). Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. *Nature Ecology & Evolution*, *1*, 0084. https://doi.org/10.1038/s41559-017-0084

Viechtbauer, W. (2010). Conducting Meta-Analyses in *R* with the **metafor** Package. *Journal of Statistical Software*, *36*, 1–48. https://doi.org/10.18637/jss.v036.i03

Viechtbauer, W. (2021). *Model selection using the glmulti and MuMIn packages*. https://www.metafor-project.org/doku.php/tips:model_selection_with_glmulti_and_mumin

Waite, J. (1992). The formation of mussel byssus: Anatomy of a natural manufacturing process. In S. Case (Ed.), *Structure, Cellular Synthesis and Assembly of Biopolymers* (Vol. 19, pp. 27–54). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-47207-0_2

Waite, J. (2017). Mussel adhesion – essential footwork. *Journal of Experimental Biology*, *220*, 517–530. https://doi.org/10.1242/jeb.134056

Waite, J. H., & Broomell, C. C. (2012). Changing environments and structure–property relationships in marine biomaterials. *Journal of Experimental Biology*, *215*, 873–883.

Welladsen, H. M., Heimann, K., & Southgate, P. C. (2011). The effects of exposure to near-future levels of ocean acidification on activity and byssus production of the Akoya pearl oyster, *Pinctada fucata*. *Journal of Shellfish Research*, *30*, 85–88.

Zardi, G., McQuaid, C., & Nicastro, K. (2007). Balancing survival and reproduction: Seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Marine Ecology Progress Series*, *334*, 155–163. https://doi.org/10.3354/meps334155

Zhao, L., Liang, J., Liang, J., Liu, B., Deng, Y., Sun, X., Li, H., Lu, Y., & Yang, F. (2020). Experimental study of transgenerational effects, pH and predation risk on byssus production in a swiftly spreading invasive fouling Asian mussel, *Musculista senhousia* (Benson). *Environmental Pollution*, *260*, 114111.

Zhao, X., Guo, C., Han, Y., Che, Z., Wang, Y., Wang, X., Chai, X., Wu, H., & Liu, G. (2017). Ocean acidification decreases mussel byssal attachment strength and induces molecular byssal responses. *Marine Ecology Progress Series*, *565*, 67–77. https://doi.org/10.3354/meps11992

zu Ermgassen, P., Thurstan, R., Corrales, J., Alleway, H., Carranza, A., Dankers, N., DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., & Sanderson, W. (2020). The benefits of bivalve reef restoration: A global synthesis of underrepresented species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*, 2050–2065. https://doi.org/10.1002/aqc.3410

Data sources

Babarro, J. M. F., Abad, M. J., Gestoso, I., Silva, E., & Olabarria, C. (2018). Susceptibility of two co-existing mytilid species to simulated predation under projected climate change conditions. *Hydrobiologia*, *807*, 247–261. https://doi.org/10.1007/s10750-017-3397-7

Clements, J. C., Hicks, C., Tremblay, R., & Comeau, L. A. (2018). Elevated seawater temperature, not pCO_2, negatively affects post-spawning adult mussels (*Mytilus edulis*) under food limitation. *Conservation Physiology*, *6*, cox078. https://doi.org/10.1093/conphys/cox078

Dickey, G., Preziosi, B. M., Clark, C. T., & Bowden, T. J. (2018). The impact of ocean acidification on the byssal threads of the blue mussel (*Mytilus edulis*). *PLOS One*, *13*, e0205908. https://doi.org/10.1371/journal.pone.0205908

George, M., Andino, J., Huie, J., & Carrington, E. (2019). Microscale pH and dissolved oxygen fluctuations within mussel aggregations and their implications for mussel attachment and raft aquaculture. *Journal of Shellfish Research*, *38*, 795. https://doi.org/10.2983/035.038.0329

George, M., & Carrington, E. (2018). Environmental post-processing increases the adhesion strength of mussel byssus adhesive. *Biofouling*, *34*, 388–397. https://doi.org/10.1080/08927014.2018.1453927

Jahnsen-Guzmán, N., Lagos, N. A., Quijón, P. A., Manríquez, P. H., Lardies, M. A., Fernández, C., Reyes, M., Zapata, J., García-Huidobro, M. R., Labra, F. A., & Duarte, C. (2021). Ocean acidification alters anti-predator responses in a competitive dominant intertidal mussel. *Chemosphere*, *288*, 132410. https://doi.org/10.1016/j.chemosphere.2021.132410

Kong, H., Jiang, X., Clements, J. C., Wang, T., Huang, X., Shang, Y., Chen, J., Hu, M., & Wang, Y. (2018). Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. *Marine Environmental Research*, *145*, 73–80. https://doi.org/10.1016/j.marenvres.2019.02.011

Lassoued, J., Babarro, J. M. F., Padín, X. A., Comeau, L. A., Bejaoui, N., Pérez, F. F. (2019). Behavioural and eco-physiological responses of the mussel *Mytilus galloprovincialis* to acidification and distinct feeding regimes. *Marine Ecology Progress Series, 626,* 97–108. https://doi.org/10.3354/meps13075

Lassoued, J., Padín, X. A., Comeau, L. A., Bejaoui, N., Pérez, F. F., Babarro, J. M. F. (2021). The Mediterranean mussel *Mytilus galloprovincialis*: responses to climate change scenarios as a function of the original habitat. *Conservation Physiology, 9,* coaa114. https://doi.org/10.1093/conphys/coaa114

Li, L., Lu, W., Sui, Y., Wang, Y., Gul, Y., & Dupont, S. (2015). Conflicting effects of predator cue and ocean acidification on the mussel *Mytilus coruscus* byssus production. *Journal of Shellfish Research*, *34*, 393–400. https://doi.org/10.2983/035.034.0222

Li, S., Liu, C., Zhan, A., Xie, L., & Zhang, R. (2017b). Influencing mechanism of ocean acidification on byssus performance in the pearl oyster *Pinctada fucata*. *Environmental Science & Technology*, *51*, 7696–7706. https://doi.org/10.1021/acs.est.7b02132

Manríquez, P. H., Jara, M. E., González, C. P., Seguel, M. E., Domenici, P., Watson, S.-A., Anguita, C., Duarte, C., Brokordt, K. (2021). The combined effects of climate change stressors and predatory cues on a mussel species. *Science of the Total Environment, 776,* 145916. https://doi.org/10.1016/j.scitotenv.2021.145916

Newcomb, L. A., George, M. N., O'Donnell, M. J., & Carrington, E. (2019). Only as strong as the weakest link: Structural analysis of the combined effects of elevated temperature and $p\mathrm{CO}_2$ on mussel attachment. *Conservation Physiology*, *7*, coz068. https://doi.org/10.1093/conphys/coz068

O'Donnell, M., George, M., & Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. *Nature Climate Change*, *3*, 587–590. https://doi.org/10.1038/nclimate1846

Shang, Y., Wang, X., Kong, H., Huang, W., Hu, M., Wang, Y. (2019). Nano-ZnO impairs anti-predation capacity of marine mussels under seawater acidification. *Journal of Hazardous Materials, 371,* 521–528. https://doi.org/10.1016/j.jhazmat.2019.02.072

Sui, Y., Hu, M., Huang, X., Wang, Y., & Lu, W. (2015). Anti-predatory responses of the thick shell mussel *Mytilus coruscus* exposed to seawater acidification and hypoxia. *Marine Environmental Research*, *109*, 159–167. https://doi.org/10.1016/j.marenvres.2015.07.008

Sui, Y., Liu, Y., Zhao, X., Dupont, S., Hu, M., Wu, F., Huang, X., Li, J., Lu, W., & Wang, Y. (2017). Defense responses to short-term hypoxia and seawater acidification in the thick shell mussel *Mytilus coruscus*. *Frontiers in Physiology*, *8*, 145. https://doi.org/10.3389/fphys.2017.00145

Zhao, L., Liang, J., Liang, J., Liu, B., Deng, Y., Sun, X., Li, H., Lu, Y., & Yang, F. (2020). Experimental study of transgenerational effects, pH and predation risk on byssus production in a swiftly spreading invasive fouling Asian mussel, *Musculista senhousia* (Benson). *Environmental Pollution*, *260*, 114111.

Zhao, X., Guo, C., Han, Y., Che, Z., Wang, Y., Wang, X., Chai, X., Wu, H., & Liu, G. (2017). Ocean acidification decreases mussel byssal attachment strength and induces molecular byssal responses. *Marine Ecology Progress Series*, *565*, 67–77. https://doi.org/10.3354/meps11992

Welladsen, H. M., Heimann, K., & Southgate, P. C. (2011). The effects of exposure to near-future levels of ocean acidification on activity and byssus production of the Akoya pearl oyster, *Pinctada fucata*. *Journal of Shellfish Research*, *30*, 85–88. https://doi.org/10.2983/035.030.0112