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

4 Jeff C. Clements^{1*}, Matthew N. George²

5 ¹ Fisheries and Oceans Canada, Gulf Region, Moncton, NB, Canada

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

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

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18 conceptualization, content, and writing.

19 **Data accessibility**

20 Annotated R-script raw data files, and supplementary data (figures and tables) are available as
21 supplementary material. **NOTE:** For reviewers, all supplementary material can be accessed and
22 commented on at:

23 <https://drive.google.com/drive/folders/1RBhDo9oVe6z6Pep7i6mmH6yQcFj4pk3i?usp=sharing>

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28 **Abstract**

29 Numerous studies have documented weakened byssal attachment strength under ocean
30 acidification (OA); however, a comparable number report no effect, even within the same species.
31 We used meta-analysis to explore factors that could potentially explain observed effect size
32 variation in byssal attachment strength following OA exposure. A systematic literature search
33 uncovered 19 studies experimentally testing the impact of OA on byssal attachment strength (or
34 some proxy thereof). Meta-analysis revealed body size (mean shell length) to be the strongest
35 predictor of effect size variation, with a negative linear relationship observed between body size
36 and effect size. Despite this relationship, no single study or experiment included body size as a
37 moderating factor. Our finding that the byssal strength of larger bivalves is more susceptible to
38 negative OA effects runs counter to prevailing wisdom that larger, older animals of a given species
39 are more robust to OA than earlier life history stages. This highlights that body size and age may
40 be important factors that determine OA sensitivity in adult calcifiers. In addition to body size, a
41 critical review of each study revealed commonly neglected factors that could influence byssal
42 thread attachment strength which we highlight to provide suggestions for future research in this
43 area.

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

46 **Introduction**

47 Bivalve molluscs are unique in that they settle and adhere to surfaces using adhesive byssal
48 threads – proteinaceous fibers that are secreted by the foot and together form a network known as
49 the byssus. The byssus functions by anchoring bivalves to rocky substrates, with individual threads
50 collectively tethered to each other and the animal's retractor muscle at their proximal end (Waite,
51 1992). At the distal end, a powerful biological adhesive plaque moors each thread in place (Waite,
52 2017). While all bivalves appear to produce byssus as larvae, only select species – predominantly
53 mussels and scallops – retain functional byssus as adults. Within these species, byssus plays a
54 vital role in the ecology of adults, preventing dislodgement in high-energy wave environments (Bell
55 and Gosline, 1997).

56 Robust byssus attachment mitigates a bivalve's dislodgement risk, directly contributing to their
57 survival and fitness (Carrington et al., 2015; Sebens et al., 2018). As ecosystem engineers, byssus-
58 producing bivalves modify their environment through the formation of aggregations or 'beds' (Paine
59 and Levin, 1981). These beds and the bivalves within them provide a multitude of ecosystem
60 services, including nutrient extraction (Nielsen et al., 2016), sediment stabilization (Buschbaum et
61 al., 2009), water filtration (Lindahl et al., 2005), and promoting biodiversity through the creation of
62 habitat for smaller invertebrates and fish (Li et al., 2015; Sui et al., 2015; zu Ermgassen et al.,
63 2020). Given this ecological importance of byssus, a growing number of studies have investigated
64 the impact that marine global change stressors such as ocean acidification (OA) have on byssus
65 attachment. Ocean acidification refers to the decrease in seawater pH (and alteration to other
66 marine carbonate system parameters) due to the oceanic uptake of excess anthropogenic CO₂
67 from the atmosphere. Numerous studies have reported significant negative effects of experimental
68 OA (i.e., laboratory experiments mimicking near-future OA conditions by increasing seawater CO₂)
69 on bivalve byssus attachment strength. For example, O'Donnell et al. (2013) reported that
70 laboratory exposure to elevated CO₂ reduced the attachment strength of byssal threads in mussels
71 (*Mytilus trossulus*) from Washington State by negatively impacting adhesive plaque strength, a
72 finding that has been further supported in follow up studies (George and Carrington, 2018).
73 Similarly, (Shiguo Li et al., 2017) reported negative effects of elevated CO₂ on the byssal

74 attachment strength of oysters (*Pinctada fucata*) from China, and (Zhao et al., 2017) found negative
75 effects for mussels (*Mytilus coruscus*) from China.

76 While multiple studies suggest OA can weaken the attachment strength of bivalve byssus, a
77 comparable number of studies report conflicting trends, even within the same species. For
78 example, while (Zhao et al., 2017) reported strong negative effects of experimental OA on byssus
79 strength in *M. coruscus*, (Sui et al., 2017) found no effect of experimental OA on the same species.
80 Similarly, (Welladsen et al., 2011) reported little effect of elevated CO₂ on byssus characteristics of
81 *P. fucata* from Australia despite significant negative effects being reported for this species by
82 (Shiguo Li et al., 2017). Recent studies on blue mussels (*Mytilus edulis*) in the northwest Atlantic
83 also reported no effect of experimental OA on byssal attachment strength (Clements et al., 2018;
84 Dickey et al., 2018), and (Babarro et al., 2018) reported weak effects in native (*Mytilus*
85 *galloprovincialis*) and introduced (*Xenostrobus securis*) mussels in northwestern Spain.
86 Consequently, whether elevated CO₂ levels expected under near-future OA will affect byssal
87 attachment strength in nature remains hotly contested.

88 OA exposure has the potential to weaken attachment strength both directly and indirectly, by
89 interrupting the function of byssal proteins (George and Carrington, 2018) and/or decreasing
90 energy allocation to byssogenesis (Clements et al., 2018). Alternatively, many bivalves have been
91 shown to be resilient to OA in the absence of food limitation (Brown et al., 2018; Büscher et al.,
92 2017; Pansch et al., 2014; Ramajo et al., 2016; Thomsen et al., 2013) and are capable of
93 strengthening attachment in response to stress (e.g., predation: Côté, 1995; Cheung et al., 2006;
94 Brown et al., 2011); flow: Carrington *et al.* 2008). Given the variable and conflicting results of studies
95 testing the effects of experimental OA on byssal attachment strength in marine bivalves, it is
96 important to explore potential sources of such variation if we are to understand the true nature of
97 how elevated CO₂ might affect byssal attachment strength. As such, we conducted a meta-analysis
98 to explore some of the potential factors that explain between-study effect size variation to reconcile
99 the puzzling contrasts between studies testing for experimental OA effects on byssus strength.

100 **Materials and Methods**

101 *Systematic literature search*

102 A systematic literature search was used to obtain relevant studies testing for the effects of
 103 experimental OA on the byssal attachment strength of marine bivalves according to PRISMA
 104 guidelines (Page et al., 2021). Search results for each stage of the PRISMA workflow are provided
 105 in **Figure 1**; study-specific details provided in **Table 1** and all raw data for each experiment in Table
 106 S1.

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

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	<i>Pinctada fucata</i>	62.30	5	0.35 - 0.55
O'Donnell et al. (2013)	USA	<i>Mytilus trossulus</i>	45.00	20	0.35 - 0.64
Li et al. (2015)	China	<i>Mytilus coruscus</i>	27.00	3	0.42 - 0.82
Sui et al. (2015)	China	<i>Mytilus coruscus</i>	50.00	3	0.39 - 0.84
Li et al. (2017)	China	<i>Pinctada fucata</i>	65.00	30	0.30 - 0.60
Sui et al. (2017)	China	<i>Mytilus coruscus</i>	50.00	3	0.40, 0.84
Zhao et al. (2017)	China	<i>Mytilus coruscus</i>	97.75	7	0.30 - 0.73
Babarro et al. (2018)	Spain	<i>Mytilus galloprovincialis</i>	33.13	22	0.33
		<i>Xenostrobus securus</i>	33.50	22	0.33
Clements et al. (2018)	Canada	<i>Mytilus edulis</i>	37.50	30, 60	0.27 - 0.60
Dickey et al. (2018)	USA	<i>Mytilus edulis</i>	65.00	38	0.51
George & Carrington (2018)	USA	<i>Mytilus trossulus</i>	47.00	12	1.01
Kong et al. (2018)	China	<i>Mytilus coruscus</i>	50.00	7	0.40
		<i>Mytilus edulis</i>	50.00	7	0.40
George et al. (2019)	USA	<i>Mytilus trossulus</i>	50.70	3, 5, 7	0.67
Lassoued et al. (2019)	Spain	<i>Mytilus galloprovincialis</i>	30.69	15	0.16 - 0.35
Newcomb et al. (2019)	USA	<i>Mytilus trossulus</i>	47.40	3	0.28 - 0.84
Shang et al. (2019)	China	<i>Mytilus coruscus</i>	52.00	3	0.40
Zhao et al. (2020)	China	<i>Musculista senhousia</i>	12.60	196	0.40
Jahnsen-Guzmán et al. (2021)	Chile	<i>Perumytilus purpuratus</i>	8.00	60	0.27
Lassoued et al. (2021)	Spain	<i>Mytilus galloprovincialis</i>	30.69	15	0.24 - 0.36
	Africa	<i>Mytilus galloprovincialis</i>	30.69	15	0.23 - 0.36
Manríquez et al. (2021)	Chile	<i>Perumytilus purpuratus</i>	13.50	84	0.34

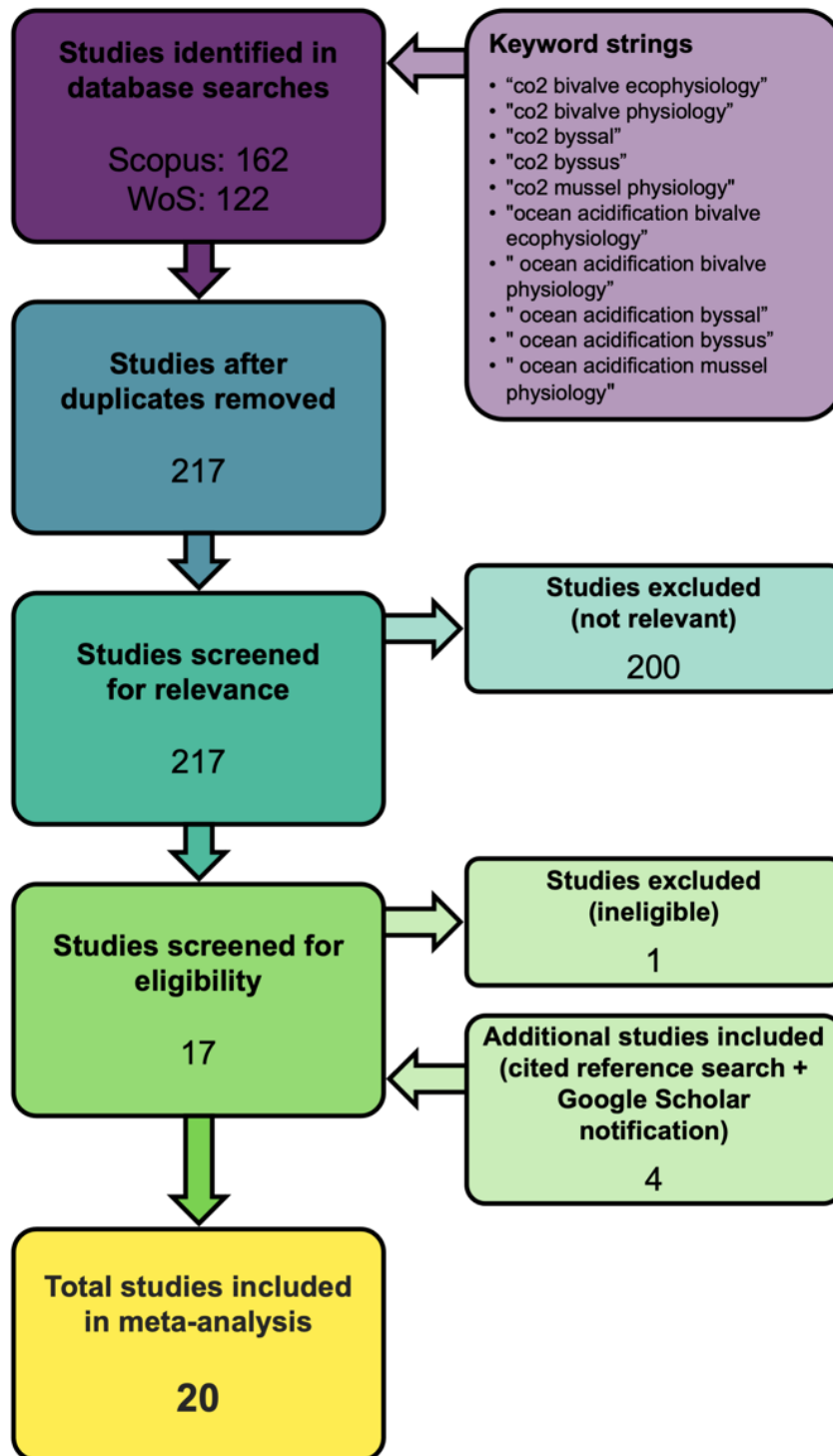


Figure 1. PRISMA flow diagram. Flow diagram depicting the results of each step in the systematic literature search. Detailed information for each unique paper found in the literature search, including reasons for exclusion, are contained in Table S1.

111 Each article was then screened for relevance by reading the titles and abstracts and selecting only
112 those studies that experimentally tested the effect of seawater CO₂ and/or pH on characteristics of
113 marine bivalve byssus (i.e., had a low CO₂ control and at least one high CO₂ group). Byssus-related
114 endpoints were then assessed for eligibility, including only those studies (or experiments within
115 studies) that directly measured whole animal byssus attachment strength or a proxy thereof (see
116 Table S2 for a full list of included endpoints). We further excluded studies, or experiments within
117 studies, if the experimental pH condition was not relevant to near-future coastal OA conditions,
118 which we defined as an experimental treatment being >1.0 pH units lower than the control
119 treatment. This definition was based on an upper projected pH decrease of 0.4 units under the
120 IPCC RCP8.5 projection (Hoegh-Guldberg et al., 2014) plus a further 0.6 pH unit drop to
121 encapsulate extreme natural variability. While we recognize that an experimental decrease of 1.0
122 pH units is extreme in the context of OA, such a decrease is not outside of the scope of natural
123 coastal pH variability (M. N. George et al., 2019; Lowe et al., 2019). Furthermore, the magnitude of
124 pH decrease was not related to effect size within this range (Figure S1; also see results of AIC
125 model selection below). Studies (or experiments within studies) were also selected based on
126 exposure time, excluding studies or experiments with a pH exposure time of <3 days. While
127 exposure times varied across studies, there was no relationship between effect size and exposure
128 time across the range of exposure times included in our data set (Figure S1; also see results of
129 AIC model selection below). This selection process resulted in 16 studies eligible for the meta-
130 analysis.

131 A cited reference search of the 16 studies selected above was subsequently performed, whereby
132 the reference lists and online lists of citing articles for each study were checked for additional
133 studies that may have been missed in the database search. Herein, potentially relevant articles
134 were initially identified based on their titles and subsequently screened according to the criteria
135 described above for the database search. This resulted in an additional three studies being added
136 to the database. Finally, a paper published on 29 September, 2021 with a relevant title (Jahnsen-
137 Guzmán et al., 2021) appeared in A1's Google Scholar alerts and was added to the database after

138 evaluation for the above criteria, resulting in a total of 20 studies included in the meta-analysis;
139 general attributes of studies are summarized in **Table 1**.

140 *Data collection*

141 For each of the 20 studies, we collected various qualitative and quantitative attributes (see Table
142 S3). Specifically, we recorded general bibliographic data (authors, year published, title, journal),
143 the species tested, whether the animals were fed during the experiment, the country and climate
144 region where the experimental animals originated, the amount of food fed to the animals during the
145 experiment (% body weight daily), the reported mean shell length of the animals tested, exposure
146 time, the experimental temperature, and the pH difference between the experimental and control
147 treatments. For each eligible experiment, we also obtained the mean, sample size (n), and variance
148 reported for the control and experimental treatment. These values were obtained directly from the
149 text or tables within the article, from graphs within the article (using ImageJ; [Schneider et al., 2012](#)),
150 or from raw data shared alongside the article. Measures of variance differed between studies and
151 were thus standardized to reflect standard deviation (SD) prior to analyses. Where multiple
152 stressors or factors were studied (e.g., temperature, oxygen, predation, etc.), we only collected
153 data for OA effects under control treatment of the other variable to isolate OA-only effects for
154 between-study comparability. Likewise, where ecological complexity was included in experiments,
155 data were collected only for treatments that were comparable to other studies (e.g., for mixed
156 versus single-species aggregations as in (Babarro et al., 2018) only single-species aggregations
157 were included). Relevant information regarding these details is contained in the “Notes” column in
158 Table S2.

159 *Meta-analysis*

160 Effect size calculations

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

163

$$\ln RR = \left(\frac{\bar{X}_E}{\bar{X}_C} \right)$$

164 where \bar{X}_E and \bar{X}_C are the mean byssal attachment strength of the experimental and control OA
165 treatments, respectively. A $\ln RR = 0$ signifies no effect, while negative and positive values indicate
166 negative and positive functional effects, respectively.

167 Effect size variance (v) was calculated as,

168

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

169 where S and n are the standard deviation and sample size, respectively, for a given experimental
170 treatment (denoted by the subscripts C [control] and E [experimental, i.e., OA]); \bar{X}_E and \bar{X}_C are
171 defined as above. Effect sizes and their variance for each experiment were calculated using the
172 `escalc()` function in the *metafor* package (Viechtbauer, 2010) in R version 4.1.1 (R Core Team,
173 2021).

174 Explaining effect size variation

175 We explored a subset of the qualitative and quantitative factors collected from each experiment as
176 potential explainers of effect size variation. Categorical factors included study, food amount (%
177 body weight per day), country, climate region, and species (see Table S3 for factor categories).
178 Continuous factors included year of publication, mean shell length, experimental temperature,
179 exposure time, and pH offset (i.e., difference between control and experimental pH).

180 To determine which factors contributed most to effect size variation, we employed Akaike's
181 Information Criterion (AIC) model selection and multimodal inference using the *metafor* package in
182 combination with the *glmulti* package (Calcagno, 2020) according to (Viechtbauer, 2021). AIC
183 model selection was performed by fitting all possible additive models with the categorical and
184 continuous factors listed above using the `glmulti()` function; the code automatically drops redundant
185 (correlated) factors from available models. Because this exercise resulted in multiple "top" models,
186 we visually assessed the relative importance of each individual factor that showed up in any of the
187 top models and subsequently used multimodal inference to determine which of those factors were

188 significantly important; multimodal inference was again conducted using *metafor* in combination
 189 with *glmulti* according to (Viechtbauer, 2021). We then constructed three-level meta-regression
 190 models for each of the factors found to be significantly important in the multimodal inference
 191 exercise using the `rma.mv()` function in *metafor* to verify significance' meta-regression models
 192 included experiment nested within study as a random factor. For each meta-regression model,
 193 funnel plots were constructed to visually check for publication bias, and analysis of Rosenthal's
 194 Fail-Safe Number was used to confirm whether publication bias was probable using the `fsn()`
 195 function in *metafor*; publication bias was deemed improbable if the computed fail-safe number
 196 exceeded $5k + 10$, where k is the total number of experiments in the dataset (Fragkos et al., 2014;
 197 $k = 71$ for our dataset).

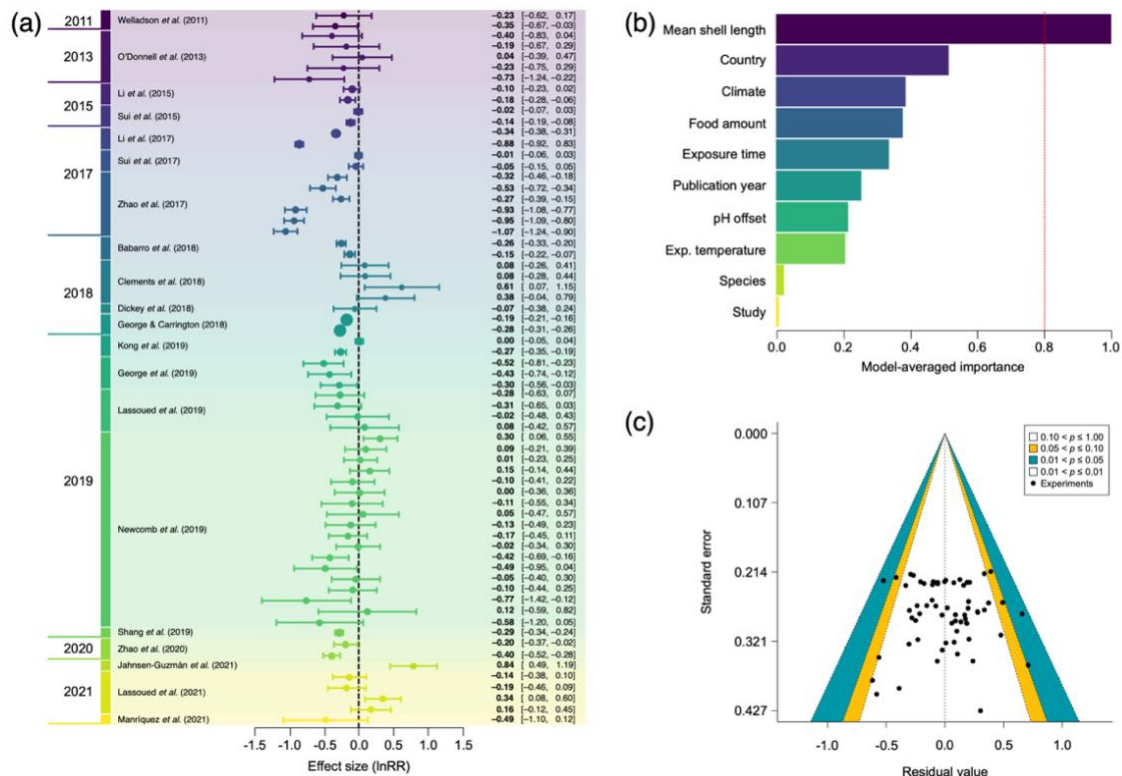


Figure 2. Meta-analysis results. (a) Forest plot of effect sizes and their estimated variance for each experiment (within each study) included in the analysis. Values on the right side of the plot denote the mean effect size estimate (bold) with upper and lower confidence bounds in squared parentheses. (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.

198 Finally, we used sensitivity analysis to determine if particular studies contributed to the overall
199 results from the full dataset. Herein, we re-ran the analysis on a second database excluding studies
200 on scallops (*Pinctada fucata*, two studies; (S Li et al., 2017; Welladsen et al., 2011) to verify that
201 taxonomy (i.e., mussels vs. scallops) had no influence on the dataset. Likewise, we re-ran the
202 analysis on a third dataset excluding the study showing the most extreme effect sizes (Zhao et al.,
203 2017) to ensure that this study alone did not drive the overall results.

204 Results

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

209 AIC model selection revealed seven candidate “top” models which included seven explanatory
210 factors: mean shell length, food amount, country, climate, exposure time, experimental
211 temperature, and year of publication (Table S4). Among these factors, mean shell length was
212 captured in all candidate models, while country, climate, and food amount were each captured in
213 two models, and exposure time, experimental temperature, and year of publication in one model
214 (Table S4). Multimodal inference indicated that mean shell length was the only significantly
215 important factor (Importance measure = 0.998, $p < 0.001$), while all other factors were of non-
216 significant importance (Importance measures ≤ 0.512 , $p \geq 0.385$) (**Figure 2b**; Table S5). While
217 there appeared to be some experiments missing from the left side of the funnel plot (**Figure 2c**),
218 analysis Rosenthal’s Fail-Safe Number indicated that publication bias was highly unlikely (i.e., Fail-
219 Safe Number = 16,357, which far exceeds $5k + 10$ for our dataset).

220 A three-level meta-regression model including mean shell length as a continuous moderator
221 indicated significant residual heterogeneity ($df = 63$, $Q_E = 1039.001$, $p < 0.001$) and a significant
222 overall model fit ($df = 1$, $Q_M = 20.459$, $p < 0.001$). Herein, mean shell length displayed a negative
223 overall effect size (estimate [CI_{lower} , CI_{upper}] = -0.009 [-0.013 , -0.005], $p < 0.001$) (see Table S6 for

224 full model results), and a negative relationship between mean shell length and effect size was
225 apparent (**Figure 3**).

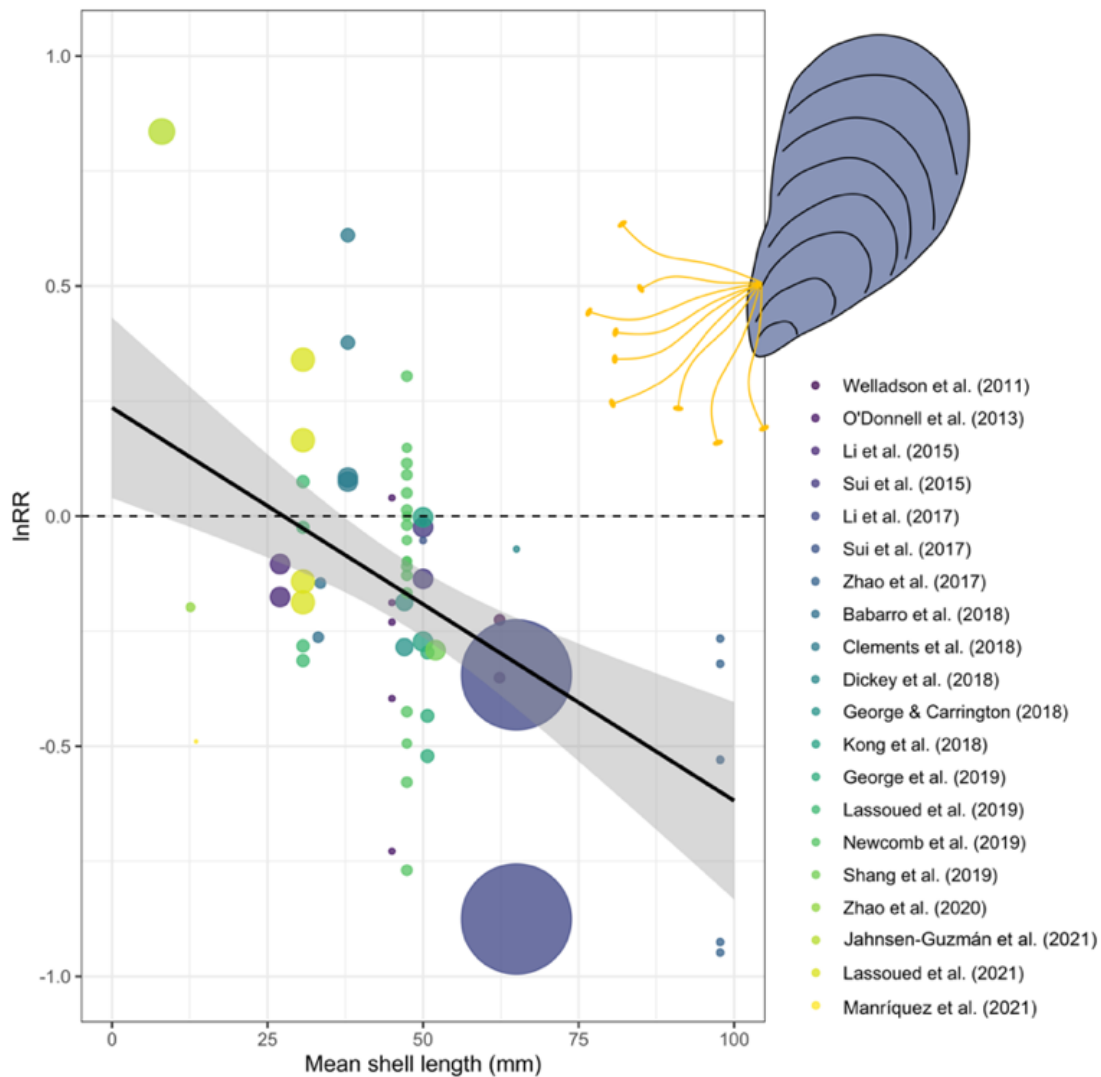


Figure 3. Larger bivalves are more susceptible to OA effects on byssal attachment strength. Effect size (lnRR) as a function of mean shell length. Each data point represents a single experiment, with colors denoting different studies. The size of each point represents the relative sample size of the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

226 Re-running the analysis on the dataset without scallop studies yielded slightly different top models
227 in the AIC selection process (Table S7) but did not change results of the multimodal inference for
228 moderator importance (Table S8) nor the overall meta-regression model results (Table S9). This
229 was generally the same when the analysis was re-run without the study showing the largest effect

230 sizes (Zhao et al., 2017; Table S10), although both Country and Food Amount rose to equal
231 importance with mean shell length (Importance > 0.92; Table S11). The meta-regression model for
232 mean shell length remained significant (Table S12). For Country, China and the USA exhibited
233 significant negative effect sizes based on the meta-regression model, while all other countries
234 (Africa, Australia, Canada, Chile, and Spain) showed non-significant effect sizes (Table S13, Figure
235 S2); however, no individual levels of the Food Amount variable displayed significant effect sizes
236 (Table S14, Figure S3).

237 **Discussion**

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

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

251 Out of the variables tested in this study, body size was the only variable of interest that could
252 adequately explain effect size variability, with no significant influence of climate, species, year,
253 study temperature, study location (country), exposure time, food amount, or pH offset. Larger
254 mussels typically secrete fewer (Babarro et al., 2008; Van Winkle, 1970), but larger, mechanically
255 superior threads than smaller individuals (Babarro and Carrington, 2013), and generally display
256 weaker size-corrected whole animal tenacity (Babarro et al., 2020). However, relationships

257 between animal size and byssus are often site-specific (Babarro and Carrington, 2011), and
258 environmental conditions are known to affect thread attachment strength and byssogenesis,
259 including water flow (Babarro and Carrington, 2013; Lee et al., 1990), predation (Brown et al., 2011;
260 Cheung et al., 2006; Côté, 1995), hypoxia (George et al., 2018), and temperature (Clements et al.,
261 2018; Newcomb et al., 2019). None of the studies obtained in our literature search included size
262 as a variable of interest in moderating OA effects on byssus. Indeed, size has been suggested as
263 a potentially important factor influencing byssus function (Babarro and Carrington, 2013). We
264 suggest that future studies place an emphasis on understanding how size modulates OA effects
265 on byssus, in combination with other environmental conditions.

266 Animals surviving past climate-related extinction events have been found to be smaller than
267 animals succumbing to such events (Morten and Twitchett, 2009; Twitchett, 2007) — a
268 phenomenon known as the Lilliput Effect (Urbanek, 1993). With respect to OA, gastropods adapted
269 to high CO₂ conditions at CO₂ seeps were found to be smaller than those at reference sites,
270 suggesting that smaller animals are better adapted to survive prolonged exposures to high CO₂
271 (Garilli et al., 2015). Therein, smaller organisms exhibited lower whole-animal metabolic demand,
272 allowing them to maintain key biological functions to survive under high CO₂ conditions. It is thus
273 possible that smaller bivalves with a lower whole-animal metabolic demand are better able to
274 maintain byssus under high CO₂ conditions as compared to larger bivalves. However, this effect
275 has yet to be empirically demonstrated for byssus attachment strength under OA and warrants
276 future study.

277 Size- and age-dependent effects of stressors such as OA on adult organisms have the potential for
278 broad-scale ecological ramifications. For example, if older, adult bivalves within a population are
279 more vulnerable to OA effects than younger, smaller bivalves, this could have consequences for
280 the size and age structure of bivalve populations. Such effects could translate to population-level
281 reproductive impacts and influence population replenishment in varied ways depending on the
282 relative reproductive contribution of individuals at different sizes or ages. Filtration rates in mytilids
283 also tend to scale allometrically, with larger mussels having higher filtration rates than smaller
284 mussels (Riisgård et al., 2014). If the size-dependent effects of OA on byssus observed here result

285 in the loss of larger bivalves from a given population, such effects could have ramifications for
286 ecosystem services that bivalves provide, such as enhancing water clarity and quality.
287 Understanding such size- and age-dependent responses of bivalves to various stressors is also
288 important for predicting population-level responses to future global change (Botsford et al., 2011).
289 Given the importance of bivalve populations to coastal marine ecosystems, more research
290 regarding the role of size and age in moderating the effects of OA and other stressors across the
291 ontogeny of juvenile and adult bivalves is most certainly warranted.

292 *Hidden cofactors: energy and seasonality*

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

300 While thread number can have an impact on attachment strength, thread quality is the primary
301 driver of whole animal tenacity (Carrington et al., 2015; Moeser et al., 2006; Moeser and Carrington,
302 2006). Perhaps nowhere is this more evident than in the regular field monitoring of several Mytilid
303 species, where 2-3fold fluctuations in tenacity are regularly observed within a given year,
304 irrespective of thread number (Carrington, 2002; Hunt and Scheibling, 2001; Price, 1980; Zardi et
305 al., 2007). Seasonal variability of this magnitude is likely related, at least in part, to energetic
306 limitations imposed by reproduction, a significant energy expenditure that corresponds with a
307 measurable decrease in attachment strength (Babarro et al., 2008; Babarro and Fernández Reiriz,
308 2010). Energy allocation to byssogenesis is constitutive (Hawkins and Bayne, 1985) and prioritized
309 over growth (Clarke, 1999; Roberts et al., 2021), indicating that adult mussels employ a
310 physiological strategy that minimizes dislodgement risk and maximizes overall fitness (Sebens et
311 al., 2018). However, it remains unclear to what extent adult size, seasonality, and energy allocation
312 interact to influence tenacity (Roberts et al., 2021), particularly within the context of OA.

313 Unfortunately, only nine studies included in our database reported when animals were collected,
314 representing an even split across seasons (except for winter, which had three). Such a small
315 sample precludes any confident analyses. To resolve this discrepancy, future studies would benefit
316 from reporting when animals are collected, as well as performing temporal replicates with multiple
317 cohorts from the same location across seasons.

318 *An integrated approach: the role of byssal thread mechanics*

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

336 Most studies (17 out of 19) included in our analysis investigated whether OA impacted attachment
337 by subjecting whole animals to elevated CO₂, thirteen of which measured attachment strength
338 directly. Out of that subset, six reported negative effects of OA, two reported mixed or context
339 dependent effects, one reported a positive effect, and four reported no effects. As a complex
340 biomaterial, byssal threads consist of three distinct thread regions (proximal region, distal region,

341 and adhesive plaque) that have different material properties (Bell and Gosline, 1996). For this
342 reason, a weakness in one thread region can act like a weak link in a chain (Newcomb et al., 2019),
343 resulting in structural failure before threads can adequately ‘yield’ and distribute force across the
344 thread network (Carrington and Gosline, 2004). For this reason, it is important to isolate the impact
345 that environmental factors can have on each region to determine the magnitude of their effect.
346 While the byssus metrics included in our database are all proxies of attachment strength, the
347 specific measures vary between studies. Four studies included in our analysis investigated region-
348 specific impacts of OA (George and Carrington, 2018; M. N. George et al., 2019; Newcomb et al.,
349 2019; M. O’Donnell et al., 2013), while two attempted to link gene expression levels of byssal
350 proteins to mechanical performance (S Li et al., 2017; Zhao et al., 2017). While the integration of
351 genomics, proteomics, and thread mechanics represents a powerful approach for teasing apart the
352 impact of OA on byssus, we acknowledge this route can be cost-prohibitive. As such, we suggest
353 that future studies include the following five metrics to increase cross-study comparability: byssal
354 thread number, thread diameter within the distal region, plaque cross-sectional area, single-thread
355 attachment force, and thread failure location.

356 *Nutrition, transgenerational exposure, and local adaptation*

357 Body size notwithstanding, it is puzzling that other factors known to influence biological responses
358 under OA were not important explainers of byssus weakening. For example, food supply is often
359 considered one of the most important factors moderating experimental OA effects on calcifying
360 organisms (Brown et al., 2018; Büscher et al., 2017; Pansch et al., 2014; Ramajo et al., 2016;
361 Thomsen et al., 2013). While food supply appeared in three of the six “top” models in our AIC model
362 selection exercise, its explanatory power was low except for when the study with the largest effect
363 sizes was excluded (although the meta-regression model did not detect any significant effect sizes
364 for any of the food amount categories). One possible explanation for this mismatch may be that
365 although animals were fed while under laboratory conditions, energetic limitations were imposed
366 on some treatment groups due to inadequate nutritional support. Matching the complex algal diet
367 of adult bivalves can be challenging, even in a hatchery setting, and most studies feed animals
368 commercially available algal pastes that may not represent the algal or bacterial diversity seen in

369 a natural setting (Coffin et al., 2021; Timmins-Schiffman et al., 2021). Additionally, condition indices
370 can vary between species and populations, and when reported in isolation are unlikely to provide
371 a holistic measure of a bivalve's nutritional state even when differences in tenacity are observed
372 (Babarro et al., 2020). To resolve this issue, we suggest that future studies employ more rigorous
373 histological and/or biochemical characterizations of nutritional status (Grkovic et al., 2019),
374 integrating information from multiple tissues and/or macronutrients when possible (see Clements
375 et al., 2018).

376 Exposure duration and the magnitude of pH change are also thought to modulate responses to OA,
377 as chronic and acute exposures can yield contrasting responses and effects can be more severe
378 when pH decreases are larger (Dupont et al., 2010; Shaw et al., 2013). However, neither exposure
379 time nor pH offset were significantly important moderators in our analysis. This is not particularly
380 surprising given that we restricted exposure times to ≥ 3 days and pH offsets ≤ 1.0 pH units. While
381 more acute exposures and larger pH offsets may have yielded different results for our meta-
382 analysis, these are not applicable to near-future OA scenarios. As such, in the context of near-
383 future OA, this magnitude of change is unlikely to influence byssal attachment strength responses.
384 Of note, however, is that only a single transgenerational study was identified in our review, reporting
385 positive effects of parental exposure to OA on offspring byssus attachment (Zhao et al., 2020).
386 However, parental exposure to OA may be species specific, with reports of negative (Griffith and
387 Gobler, 2017), positive (Kong et al., 2019), and null (Clements et al., 2021) effects on offspring
388 responses. While we recognize that transgenerational studies across the full ontogeny of byssus
389 producing bivalves requires considerable time and effort, such studies would be beneficial to better
390 understanding how byssus may be impacted by OA in the future.

391 Local adaptation and geography have also been suggested as potentially important moderators of
392 OA effects (Vargas et al., 2017). In our analysis, geographic moderators (country and climate) did
393 not significantly contribute to any of the top AIC models identified in our meta-analysis, except for
394 when the study with the largest effect sizes was removed from the dataset (Zhao et al., 2017)d.
395 Furthermore, Country was consistently the second most important moderator identified in the
396 multimodal inference exercise (Tables S4, S7, S10). Herein, bivalves from China and the USA

397 displayed a significant negative effect size (Figure S2, Table S12). This suggests that there is
398 perhaps a geographical element to OA effects on byssal attachment strength, although more
399 detailed conclusions are not currently possible. Furthermore, while factors other than shell length
400 and country were not identified as important in our global meta-analysis, they very well may be
401 important in more specific contexts. As such, while body size appears most important in explaining
402 OA effects on byssal strength across a broad range of bivalve taxa, other factors may serve as
403 important moderators in a context-specific manner. Studies assessing the effects of geography,
404 particularly in the context of local adaptation, would be beneficial.

405 **Conclusions**

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

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