



Using meta-analysis to explore the roles of global upwelling exposure and experimental design in bivalve responses to low pH

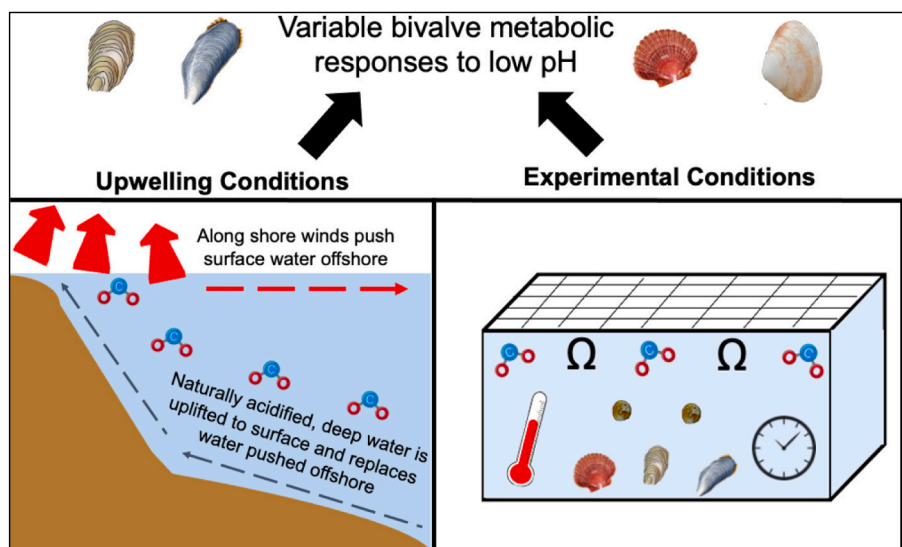
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HIGHLIGHTS

- Meta-analysis was used to assess bivalve responses to low pH.
- Strong upwelling regions may yield bivalves that are less sensitive to low pH.
- Upwelling explains up to 49 % variability of bivalve metabolic responses to low pH.
- Larger carbonate chemistry deltas in experiments yield stronger responses.

GRAPHICAL ABSTRACT



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ABSTRACT

Low pH conditions, associated with ocean acidification, represent threats to many commercially and ecologically important organisms, including bivalves. However, there are knowledge gaps regarding factors explaining observed differences in biological responses to low pH in laboratory experiments. Specific sources of local adaptation such as upwelling exposure and the role of experimental design, such as carbonate chemistry parameter changes, should be considered. Linking upwelling exposure, as an individual oceanographic phenomenon, to responses measured in laboratory experiments may further our understanding of local adaptation to global change. Here, meta-analysis is used to test the hypotheses that upwelling exposure and experimental design affect outcomes of individual, laboratory-based studies that assess bivalve metabolic (clearance and respiration rate) responses to low pH. Results show that while bivalves generally decrease metabolic activity in

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response to low pH, upwelling exposure and experimental design can significantly impact outcomes. Bivalves from downwelling or weak upwelling areas decrease metabolic activity in response to low pH, but bivalves from strong upwelling areas increase or do not change metabolic activity in response to low pH. Furthermore, experimental temperature, exposure time and magnitude of the change in carbonate chemistry parameters all significantly affect outcomes. These results suggest that bivalves from strong upwelling areas may be less sensitive to low pH. This furthers our understanding of local adaptation to global change by demonstrating that upwelling alone can explain up to 49 % of the variability associated with bivalve metabolic responses to low pH. Furthermore, when interpreting outcomes of individual, laboratory experiments, scientists should be aware that higher temperatures, shorter exposure times and larger changes in carbonate chemistry parameters may increase the chance of suppressed metabolic activity.

1. Introduction

As anthropogenic disturbances increase in frequency and intensity, the biosphere will continue to undergo physical, chemical and biological alterations due to climate change. Manipulative laboratory experiments seek to understand how organisms may respond to climate change phenomena. Often, studies that assess impacts of the same climate change phenomena on similar taxa but with different populations or species arrive at different outcomes. For example, for larvae of the Caribbean stony coral, *Porites astreoides*, Serrano et al. (2018) found that ocean warming decreased survivorship but Olsen et al. (2015) found that ocean warming did not affect survivorship. Recent work shows that local adaptation may explain such differences and affect how organisms respond to simulated climate change scenarios, highlighting the need to explore how environmental preconditioning may impact experimental studies (Vargas et al., 2017; Hollarsmith et al., 2020; Thomas et al., 2022; Vargas et al., 2022). Furthermore, for manipulative laboratory experiments, experimental design (e.g., how long organisms are exposed to simulated climate change scenarios) may impact outcomes of individual experimental studies (Kuppler and Kotowska, 2021; Hoppit and Schmidt, 2022).

One oceanographic phenomenon that has gained interest in the last two decades and is often associated with climate change is ocean acidification. The increased oceanic uptake of carbon dioxide leads to ocean acidification, which results in a shift in carbonate chemistry equilibria, including (but not limited to) decreases in aragonite and calcite saturation state (Ω , omega), decreases in pH, and increases in $p\text{CO}_2$ (μatm). Ocean acidification is often associated with climate change, as it is a consequence of increased atmospheric CO_2 accumulation. Therefore, ocean acidification will hereafter be referred to in the context of global change, rather than climate change. By the year 2100 under the “business-as-usual-path” shared socio-economic pathway (SSP 8.5), atmospheric carbon dioxide concentrations may increase to 1090 μatm and seawater pH may decrease by 0.4 (Pörtner et al., 2022). While many organisms are projected to respond negatively to ocean acidification (e.g., decreased growth, Chan and Connolly, 2013; Clements and Darrow, 2018), some studies have found that marine organisms may exhibit no response (e.g., no change in growth, Kroeker et al., 2010; Schram et al., 2016) or respond positively (e.g., increased growth, Lowder et al., 2017) to ocean acidification. This response variability is further reflected in reviews and meta-analyses that emphasize local adaptation as a driver for such variability (Gazeau et al., 2013; Gaitán-Espitia et al., 2017; Vargas et al., 2017; Vargas et al., 2022). These qualitative and quantitative reviews emphasize that a “true” ocean acidification study accounts for natural carbonate chemistry variability by assessing projected pH declines relative to variable, present day, low pH conditions. This approach opposes studying the impact of pH conditions within the present range of natural variability and of plasticity in present conditions. Therefore, the phrase “ocean acidification” is used in a broader context, and the phrase “low pH” is used when referring to individual studies.

Biological traits (e.g., presence of calcium carbonate shells) may dictate how organisms respond to ocean acidification (Ries et al., 2009; Kroeker et al., 2013; Cattano et al., 2018), but local adaptation due to

preconditioning to naturally acidified waters may alter organism responses to ocean acidification. Vargas et al. (2022) recently found that the range of $p\text{CO}_2$ that marine invertebrates experience in their natural habitat can affect ocean acidification sensitivity and help explain variable responses, suggesting local adaptation. As a specific example, Thomsen et al. (2017) found that mussels from naturally acidified environments produce offspring that may be less sensitive to low pH. Such studies have demonstrated that the range of carbonate chemistry conditions that organisms naturally experience in their environment affects how such organisms respond to low pH in lab experiments. However, multiple oceanographic phenomena contribute to natural carbonate chemistry variability including wind-induced coastal upwelling (hereafter, just referred to as “upwelling”) (Vargas et al., 2016; Sridevi and Sarma, 2021). Upwelling may naturally acidify waters by supplying deep, undersaturated waters rich in dissolved inorganic carbon to the shallow coast (Thomsen et al., 2010). For example, coastal waters in the California Current Upwelling System can currently experience a pH of 7.6, $p\text{CO}_2$ of 1100 μatm and saturation state (aragonite) of 0.8 (Feely et al., 2008). Previous studies have documented that organisms living in high upwelling regions can exhibit characteristics similar to those of organisms exposed to simulated acidified conditions in laboratory experiments. For example, Mekkes et al. (2021) found that pteropods in the California Current Upwelling System exhibit thinner shells in regions of higher upwelling intensity and therefore lower saturation state and pH. Relatedly, Hofmann et al. (2014) suggested that organisms from environments of high variability in carbonate chemistry, as seen in upwelling regions, may be locally adapted to anthropogenic low pH, specifically at larger spatial (i.e., global) and taxonomic (i.e., across many species) scales. Gaitán-Espitia et al. (2017) highlighted that natural populations along upwelling systems should be able to cope with projected acidification due to natural selection, however, the impact of upwelling in shaping organismal responses to low pH conditions remains to be quantified.

The organisms that are especially at risk to ocean acidification include bivalve mollusks, likely due to their reduced capacity for acid-base regulation (a byproduct of being an osmo-conformer) (Gazeau et al., 2013), the energetic demands associated with producing and maintaining a calcium carbonate shell (Kroeker et al., 2013), and the increased shell dissolution rates resulting from reduced pH (Fabry et al., 2008). Not only do bivalves provide a variety of ecosystem functions such as habitat formation (Gutiérrez et al., 2003; Sorte et al., 2017), benthic-pelagic coupling (Coen et al., 2007; Gallardi, 2014), and nutrient recycling (Menge, 1992; Newell, 2004), but they also support worldwide fisheries (FAO, 2016). Given their widespread distribution and importance, understanding and projecting bivalve responses to ocean acidification is critical for future ecosystem functioning and maintenance of fisheries and aquaculture.

Bivalves have been the subject of previous ocean acidification meta-analyses and reviews (Gazeau et al., 2013; Clements and Comeau, 2019; Tan et al., 2020; Tan and Zheng, 2020; Clements and George, 2022), but there are still remaining knowledge gaps, including the roles of upwelling exposure and experimental design. Furthermore, previous meta-analyses have established that bivalves generally decrease growth in response to ocean acidification (Hendriks et al., 2010); however,

underlying processes and specific metabolic responses such as clearance rate (CR) and respiration rate (RR) have received less attention. These responses may provide mechanistic insight regarding growth rate changes, as clearance rate represents energy intake (i.e., potential food from the surrounding water) and respiration rate largely determines energy devoted to maintenance. Additionally, bivalves represent an ideal taxon to examine the role of upwelling exposure at a global scale, as bivalves inhabit most, if not all, major coastlines, with many bivalves (and bivalve studies) occurring in high upwelling areas (e.g., the Peru-Chile Upwelling system).

Therefore, the present study used meta-analysis techniques and bivalves as model organisms to address three objectives. The first objective was to compute summary effects (for both CR and RR) to assess consensus metabolic responses. It was hypothesized that bivalves would generally decrease metabolic activity in response to low pH. The second objective was to determine whether upwelling exposure alone may significantly impact study outcomes. It was hypothesized that bivalves from high upwelling areas would be less sensitive to low pH, and therefore would not change or would increase metabolic activity in response to low pH. The third objective was to determine whether aspects of experimental design including exposure time, temperature and the change in carbonate chemistry parameters (change in pH, $p\text{CO}_2$ or saturation state) may significantly affect study outcomes. It was hypothesized that higher temperatures, shorter exposure times and larger changes in carbonate chemistry parameters would lead to a larger decrease in metabolic activity. As previously demonstrated in several meta-analyses (Kroeker et al., 2013; Kelley and Lunden, 2017; Clements and Darrow, 2018; Hancock et al., 2020) life stage affects how mollusks respond to low pH and was consequently assessed as an additional factor. In this framework, while a meta-analysis may group bivalve genera with different physiological characteristics relevant to the questions of interest (e.g., feeding mode and shell mineralogy), proper meta-regression techniques (e.g., mixed effects models that account for genus specific artifacts) and context can allow for broad conclusions that will provide insight regarding organismal responses to global change.

2. Materials and methods

2.1. Data collection

The objectives were explicitly stated to include studies that do not only assess ocean acidification responses, but also assess plastic responses to present day low pH variability. Therefore, while the literature search was geared towards “ocean acidification” studies, this meta-analysis and its methods are presented in the framework of responses to low pH. A multiple phase data collection process was conducted to collect values needed for effect size analyses. A fine literature search was conducted (up until April 2022) via Web of Sciences (advanced search feature) using the key words ocean acid* AND feed* AND clearance rate* AND bivalve* AND respiration*. A coarser literature search was also conducted in Google Scholar using the key words: “ocean acidification” “bivalve” “feeding” “clearance rate” “respiration rate” and in the Ocean Acidification International Coordination Center bibliographic database using the same keywords via the “advanced search” feature including “all fields”. Overlap between databases was checked and article titles and abstracts were read to assess relevance. For example, title and abstract screening allowed for removal of articles that examined species other than filter feeding bivalves and/or examined physiological metrics other than clearance or respiration rates. Articles were then assessed for usable data and potential exclusion criteria, such as if effect size data (i.e., mean, variance estimates and sample sizes) were not available, if confounding experimental designs were employed (e.g., non-replicated designs, non-factorial designs or potential pseudoreplication) and/or if only one carbonate chemistry parameter (e.g., pH) was reported. These exclusion criteria were used in accordance with ocean acidification best practices (Riebesell et al., 2011). Additionally,

without true replicates, effect size estimates may be unreliable and without more than one carbonate chemistry parameter, moderator variable analyses may be unreliable (see upcoming section). All studies were checked to verify that CO_2 bubbling, as opposed to acid addition, was used to manipulate carbonate chemistry. When necessary, effect size data were extracted from graphs using ImageJ. When effect size data were missing, attempts were made to contact authors for the desired data. Unlike previous ocean acidification meta-analyses which may exclude studies with low treatment exposure times (e.g., < 3 days) or treatments with pH changes >1.0 (Clements and George, 2022), the decision was made to not exclude these studies, as exposure time and magnitude of pH change were variables of interest. Furthermore, the greatest pH change used in this study was 0.96, which is not outside the scope of natural pH variability in coastal systems (George et al., 2019; Lowe et al., 2019). For studies that used multi-stressor approaches (e.g., ocean acidification and hypoxia), data were only used at ambient levels of the additional stressor (e.g., dissolved oxygen concentration of 6.0 mg/L and not 2.0 mg/L). All data were collected by one author (RC). These processes and criteria were conducted according to PRISMA guidelines (Appendix 1: PRISMA checklist).

2.2. Effect size analysis

Hedge's d effect sizes (Eq. (1), Hedges and Olkin, 2014), also referred to as Hedge's g in sociological and medical literatures, were calculated for each observation per Gurevitch et al. (2000). Hedge's d was chosen because it not only accounts for error estimates (unlike other effect size calculations such as the natural log ratio), but the calculation for pooled standard deviation also includes a correction factor for small sample sizes (Rosenberg et al., 2013), a common feature of laboratory, microcosm-based global change experiments. For each effect size calculation, Group One was treated as the group with higher pH (i.e., the control) and Group Two was treated as the group with lower pH, such that a negative effect size means an increase in CR or RR in response to low pH, and a positive effect size means a decrease in CR or RR in response to low pH. For effect size calculations, SE (Standard Error) and interquartile ranges (IQR) were converted to SD (Standard Deviation) (Eqs. (2)–(3)). When IQR and median were reported, a normal distribution was assumed and the median was treated as the mean. Effect sizes were calculated in R version 4.0.2 (2020-06-22) using the ‘metafor’ package and the ‘escalc’ function. All proceeding analyses were also conducted in R.

$$\text{Hedge's } d \text{ Effect Size} = \frac{M_1 - M_2}{SD_{\text{Pooled}}} \quad (1)$$

Equation 1: Calculation for Hedge's d where M_1 is the mean of the control group (i.e., Group 1), M_2 is the mean of the lower pH group (i.e., Group 2), and SD_{Pooled} is the pooled standard deviation of both groups, corrected for small sample size.

$$SD = SE \cdot \sqrt{N} \quad (2)$$

Equation 2: Calculation to convert SE (Standard Error) to SD (Standard Deviation) for effect size calculations, where N is the sample size.

$$SD = \text{IQR} / 1.35 \quad (3)$$

Equation 3: Calculation to convert IQR (Interquartile Range) to SD (Standard Deviation) for effect size calculations.

2.3. Moderator variables and upwelling index calculations

Multiple moderator variables were considered, including upwelling

index values, exposure time, temperature, changes in three carbonate chemistry parameters ($p\text{CO}_2$, pH and saturation state) and life stage. All moderator variables, other than upwelling indices, were directly obtained from each study. Upwelling indices were calculated via the approach of Sellers et al. (2020). For each study, Bakun Upwelling Indices (BUIs) were calculated as the water flux (cubic meters per second per 100 m of coastline) away from the coast such that a highly positive BUI equals strong upwelling conditions, a highly negative BUI equals strong downwelling conditions and a BUI of zero equals no net, wind-induced, across-shore Ekman transport of surface waters (Bakun, 1973; Menge and Menge, 2013). This index is commonly used for studies linking ecological processes to upwelling conditions (Freidenburg et al., 2007; Menge and Menge, 2013). BUI data were obtained online (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/erdlasFnWPr.html>).

These data are available at a temporal resolution of 6 h and a spatial resolution of 0.5° . For each study, the mean BUI was calculated for 100 km of coastline surrounding each bivalve collection site for the maximum temporal span available (i.e., 1967–2020). When bivalves were provided via hatchery facilities, the mean BUI was calculated surrounding the hatchery facility. Studies that used bivalves from small, remote island, latitudes $<25^\circ$, complex coastlines or small embayments that are distant from shelf waters were not included in this analysis, as these location characteristics provide unreliable estimates of BUI-based upwelling index strength (Bakun and Agostini, 2001). Such studies include Meseck et al. (2020) (small embayment/complex coastline), Brahmi et al. (2021) (small, remote island) and Awad et al. (2019) (small embayment/complex coastline). The BUI values used in this study align well with global patterns of upwelling (e.g., high BUI values near the Peruvian-Chilean upwelling system) (Fig. 1), supporting the use of BUI values to assess Objective Two. Exposure time was defined as the amount of time between the start of the experiment and the time of CR and/or RR measurements. Temperature was defined as the temperature

at which the experiment was conducted. Changes in carbonate chemistry parameters include $p\text{CO}_2$, pH, aragonite saturation state and calcite saturation state. These moderators were defined as the numerical difference in carbonate chemistry of interest between the two treatments used in that effect size calculation. For example, an effect size comparing the CR means between a control treatment of pH of 8.0 and an experimental treatment of 7.6 would yield a change in pH of 0.4. Total alkalinity and dissolved inorganic carbon were considered but not included as less than half of the studies in this meta-analysis did not report these parameters.

2.4. Modeling approaches

Weighted mixed effects models (using restricted maximum likelihood estimation) were used to assess how moderator variables (see next subsection for specific moderator variables) were related to CR and RR effect sizes using the 'rma.mva' function in the 'metafor' package. Instead of re-analyzing the data with omitted genera (i.e., genus-specific sensitivity analyses), genus was included as a random factor to account for phylogenetic nonindependence (e.g., potential differences and similarities in feeding modes of different genera). Study ID (i.e., each individual manuscript) was also used as a random factor to account for nonindependence for effect sizes from the same study (i.e., many studies yielded multiple effect sizes due to measuring CR and RR at multiple time points, using multiple species and using multiple pH levels), thereby incorporating within-study and between-study variances (Mengersen et al., 2013). All moderator variables were included as fixed factors.

Models were weighted by sample size, as weighting by the inverse variance may yield strong biases (Buck et al., 2022), especially when sample sizes within experiments are low, as is the case in this meta-analysis (Hamman et al., 2018). For Objective One, model summary

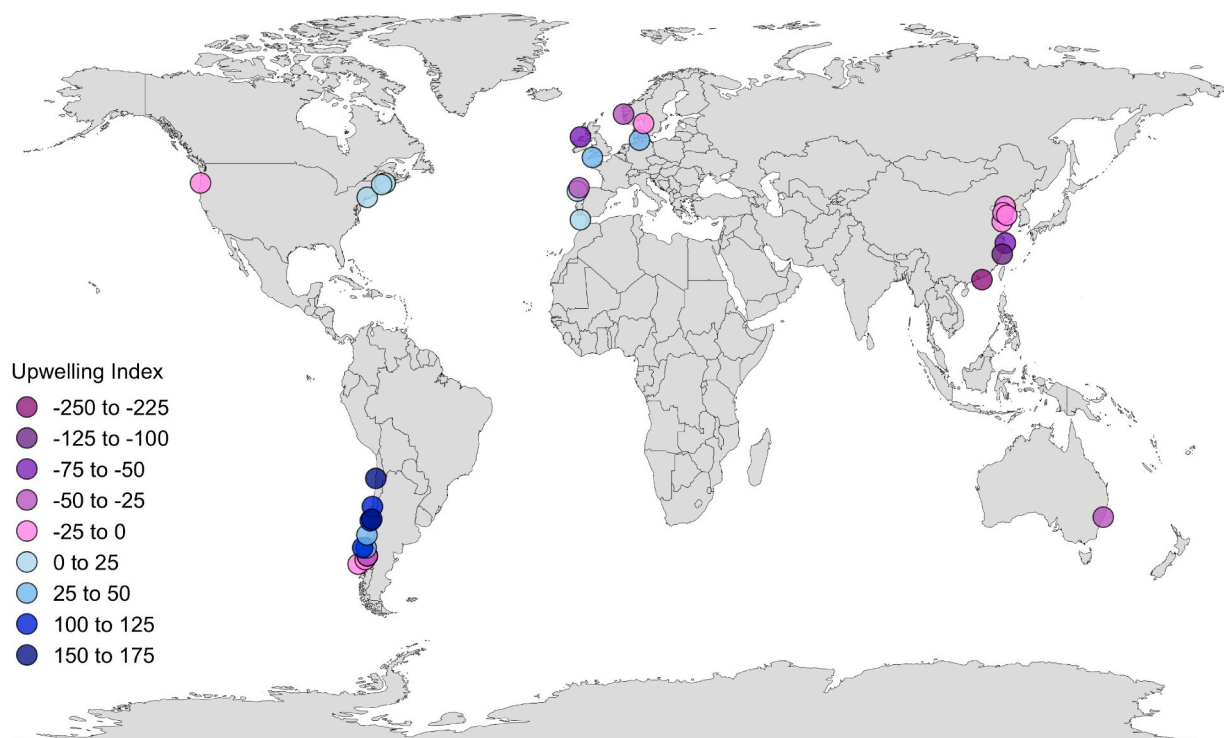


Fig. 1. Global distribution of BUI (Upwelling Index) values from locations of individual studies. The color of each point reflects the relative strength of upwelling or downwelling, such that dark blue colors represent strong upwelling (highly positive BUI values), dark purple colors represent strong downwelling (highly negative BUI values) and lighter colors represent neither strong upwelling or downwelling.

effects were considered non-neutral if confidence intervals did not overlap with zero (Koricheva et al., 2013). Orchard plots were used to visualize summary effects, the distribution of individual effect sizes, confidence intervals, and prediction intervals using the package ‘orchardR’ and the function ‘orchard_plot’. For Objectives Two and Three, because specific a priori hypotheses were postulated, a hypothesis testing approach was used to assess potential significant relationships between individual moderator variables and effect sizes. More specifically, the Q statistic (Q_M) for each moderator variable was assessed to see if that moderator variable significantly (alpha of 0.05) explained variation in effect sizes. However, because different carbonate chemistry parameters are highly correlated, an information criterion (Akaike Information Criterion, AIC) approach was used to assess which combination of carbonate chemistry parameters yielded the best fit for CR and RR effect sizes. While it may be unconventional to use both hypothesis testing and information criterion approaches in the same study, it is the belief of these authors that such an approach may be used when addressing separate questions. For all variables, marginal R^2 values are reported to assess the ability of moderator variables to explain response variability. To check for publication bias, funnel plots were visually assessed and Rosenthal’s Fail-Safe Number was used via the ‘fsn’ function in the ‘metafor’ package, such that publication bias is unlikely if the fail-safe number was $>5k + 10$, where k is equal to the total number of data points used in the analyses (Fragkos et al., 2014; Clements and George, 2022). Effect sizes that were designated as extreme outliers (>2.5 SDs from the mean) were not used in analyses (Kristensen et al., 2020). Sensitivity analyses were performed such that all analyses were repeated excluding two studies (Zhao et al., 2017; Jahnsen-Guzmán et al., 2022), as these studies had the highest sample sizes and therefore the highest weights in each model. Sensitivity analyses, combined with a sample size dependent weighting scheme, help to address potential bias of included studies.

3. Results

3.1. Overview of included studies (objective one)

After screening, a total of 38 studies were assessed for eligibility,

with only four studies not meeting eligibility criteria (Supp. Table 1). Out of the 34 studies that met eligibility criteria, 31 yielded CR effect sizes and 27 yielded RR effect sizes (Appendix 2: Summary Table). Summary effects showed a statistically significant outcome for CR and RR, as confidence intervals did not overlap with zero (Fig. 2). Summary effects were significantly positive, indicating that bivalves generally decrease CR ($p = 0.003$) and RR ($p = 0.003$) in response to low pH. Summary effect outcomes did not change when sensitivity analyses were performed (Supp. Table 2). Rosenthal’s Fail Safe number revealed no significant publication bias (CR: $8276 > 2015$, RR: $4380 > 675$). Funnel plots confirmed a lack of strong publication bias (Supp. Fig. 1).

3.2. Moderator analyses (objectives two and three)

Out of 34 studies, 31 were included for upwelling moderator analysis, as three studies were conducted in regions not appropriate for upwelling index calculations (Awad et al., 2019; Meseck et al., 2020; Brahmi et al., 2021). Upwelling Index values yielded a significant, negative relationship with effect sizes for both CR ($R^2_{\text{marginal}} = 0.49$, Fig. 3A, Table 1) and RR ($R^2_{\text{marginal}} = 0.33$, Fig. 3B, Table 1). One study was excluded from temperature moderator analysis, as it did not specify the temperature of the experiment (Awad et al., 2019). Temperature yielded a significant, positive relationship with effect sizes for both CR ($R^2_{\text{marginal}} = 0.68$, Fig. 4A, Table 1) and RR ($R^2_{\text{marginal}} = 0.23$, Fig. 4B, Table 1). Exposure time did not yield a significant relationship for CR ($R^2_{\text{marginal}} = 0.05$, Fig. 5A, Table 1) but did yield a significant, negative relationship for RR ($R^2_{\text{marginal}} = 0.21$, Fig. 5B, Table 1). Life Stage yielded a significant effect for CR ($R^2_{\text{marginal}} = 0.34$), but not RR ($R^2_{\text{marginal}} = 0.10$, Table 1), with adults having significantly reduced CR, in response to low pH, compared to juveniles (Supp. Fig. 2). Sensitivity analyses revealed that omitting two studies no longer yielded a significant relationship between temperature and RR effect sizes, but all other relationships and outcomes did not change under sensitivity analyses (not including carbonate chemistry parameters, see next paragraph) (Supp. Table 2).

Changes in all four carbonate chemistry parameters (Ω_{Ar} , Ω_{Ca} , pH and pCO_2) yielded significant relationships for both CR and RR (Table 2), with larger carbonate chemistry parameter changes yielding

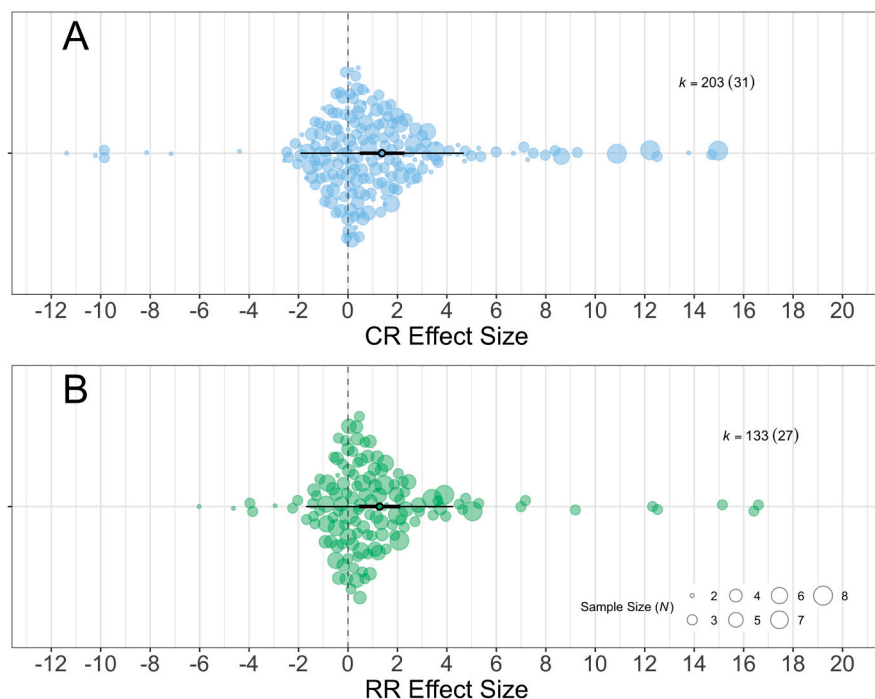


Fig. 2. Orchard plot displaying mixed effects models and all effect sizes for CR (A) and RR (B). Positive effect sizes indicate a decrease in CR or RR in response to acidified conditions, and negative effect sizes indicate an increase in CR or RR in response to acidified conditions. k indicates the number of data points in the model and the corresponding number in parentheses equals the number studies in the model. The size of the bubble indicates the sample size of that effect size. The black outlined circles represent summary effects, 1.38 for CR and 1.28 for RR. The thick black lines extending from summary effects represent 95 % confidence intervals displaying upper and lower bounds for CR as 2.28 and 0.47, respectively, and for RR as 2.12 and 0.44. The thin black lines extending from summary effects represent model prediction intervals.

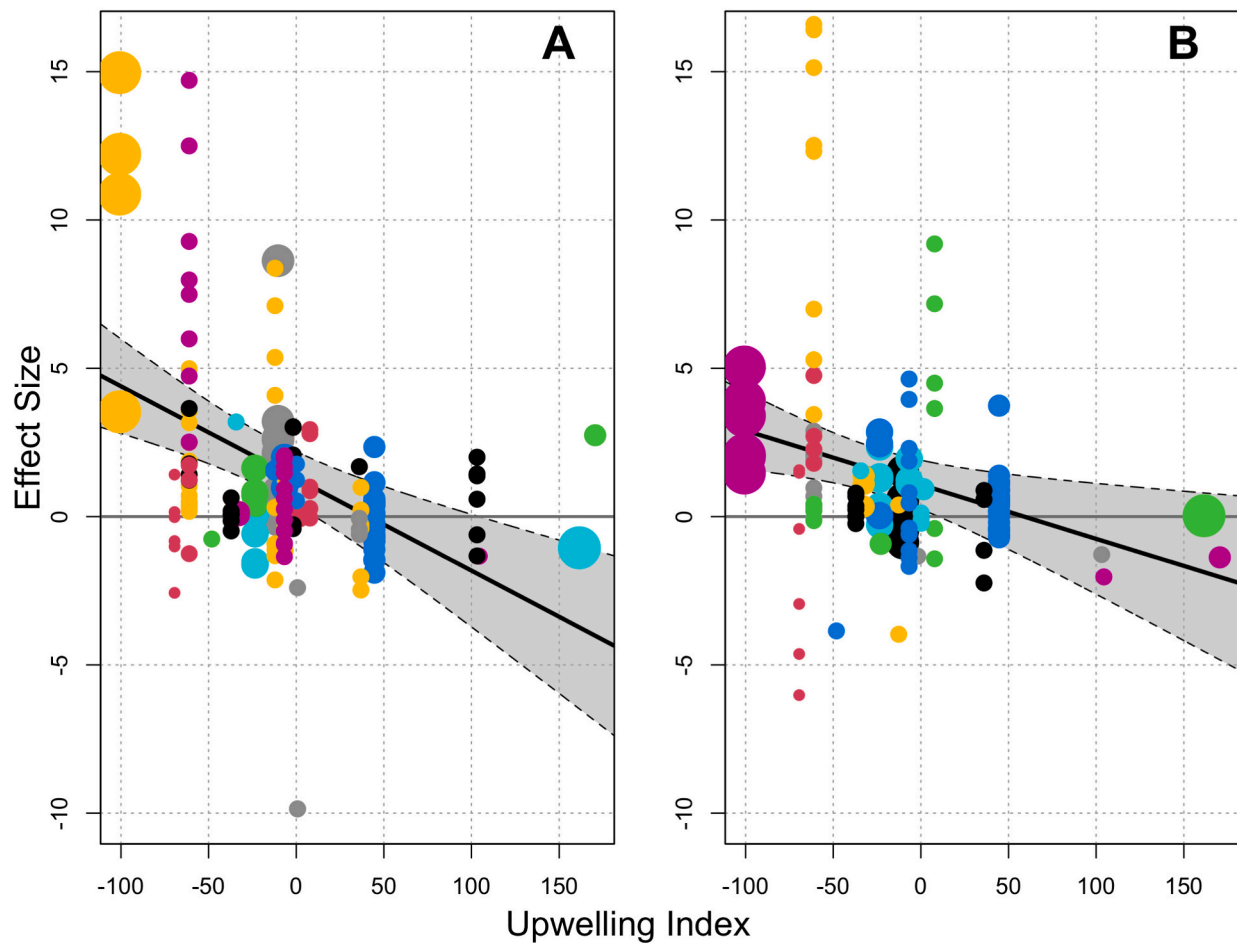


Fig. 3. Modeled linear relationship between effect sizes and Upwelling Index values for CR (A, slope = -0.031 , $p < 0.0001$) and RR (B, slope = -0.018 , $p = 0.010$). Shaded regions represent 95 % CIs. Positive effects sizes indicate a decrease in CR or RR in response to acidified conditions, and negative effect sizes indicate an increase in CR or RR in response to acidified conditions. The color and size of the bubbles (effect sizes) represent study IDs and study sample sizes, respectively.

Table 1

Model output and relationships between effect sizes for clearance (CR) and respiration (RR) rates and continuous moderator variables (not including carbonate chemistry parameters). * denotes statistically significant relationships.

Response	Parameter(s)	Q_M	p -value	Slope	R^2_{marginal}
CR	Upwelling Index	16.390	* < 0.0001	-0.031	0.489
	Temperature	39.656	* < 0.001	0.459	0.677
	Exposure Time	1.364	0.243	-0.007	0.0523
	Life Stage	7.683	*0.022	n/a	0.338
RR	Upwelling Index	6.659	*0.010	-0.018	0.330
	Temperature	5.283	*0.022	0.176	0.227
	Exposure Time	5.453	*0.020	-0.012	0.209
	Life Stage	1.612	0.447	n/a	0.100

larger effect sizes (Supp. Figs. 3–6). For CR, the combination of ΩAr , ΩCa and pCO_2 yielded the highest R^2_{marginal} of 0.45 (Table 2). For RR, the combination of ΩAr and pH yielded the highest R^2_{marginal} of 0.39 (Table 2). For CR, ΩAr alone yielded the lowest AIC, with a R^2_{marginal} of 0.22 (Table 2). For RR, four models with the lowest AIC values yielded AIC values that did not differ by >2.0 , suggesting that these models were not substantially different from one another (Table 2). These four models included ΩAr , which alone yielded a R^2_{marginal} of 0.30 (Table 2). Sensitivity analyses did not change any carbonate chemistry outcomes for CR (Supp. Tables 2 and 3). However, sensitivity analyses for RR revealed that omitting two studies no longer yielded a significant effect of changes in pH and pCO_2 (Supp. Table 2). AIC outcomes for RR

sensitivity analyses did not change as the two best models determined from sensitivity analyses were two of the four best models from the original analyses (Supp. Table 3).

4. Discussion

4.1. General outcomes and biases

The results of this meta-analysis suggest that broadly, bivalves decrease metabolic activity (CR and RR) in response to low pH (Objective One). While summary effects were significantly positive (indicating decreases in CR and RR in response to low pH), observed variability (up to 49 %) can be related to upwelling exposure (Objective Two), and experimental design (Objective Three) including exposure time, experimental temperature, and magnitude of changes in carbonate chemistry parameters. More specifically, bivalves from high upwelling areas appear to be less sensitive to low pH and broadly, shorter exposure times, higher temperature and larger changes in carbonate chemistry parameters may lead to stronger negative responses. Minor differences from sensitivity analyses suggest limited biases in this meta-analysis. While observed biases may be negligible, other limitations should be considered. For example, analyses regarding the role of upwelling exposure did not include data from the California Current System and the Benguela Current System (due to no eligible studies being conducted in these areas). Evidence of low bivalve sensitivity to low pH from these areas would further support the conclusion of global-scale upwelling-

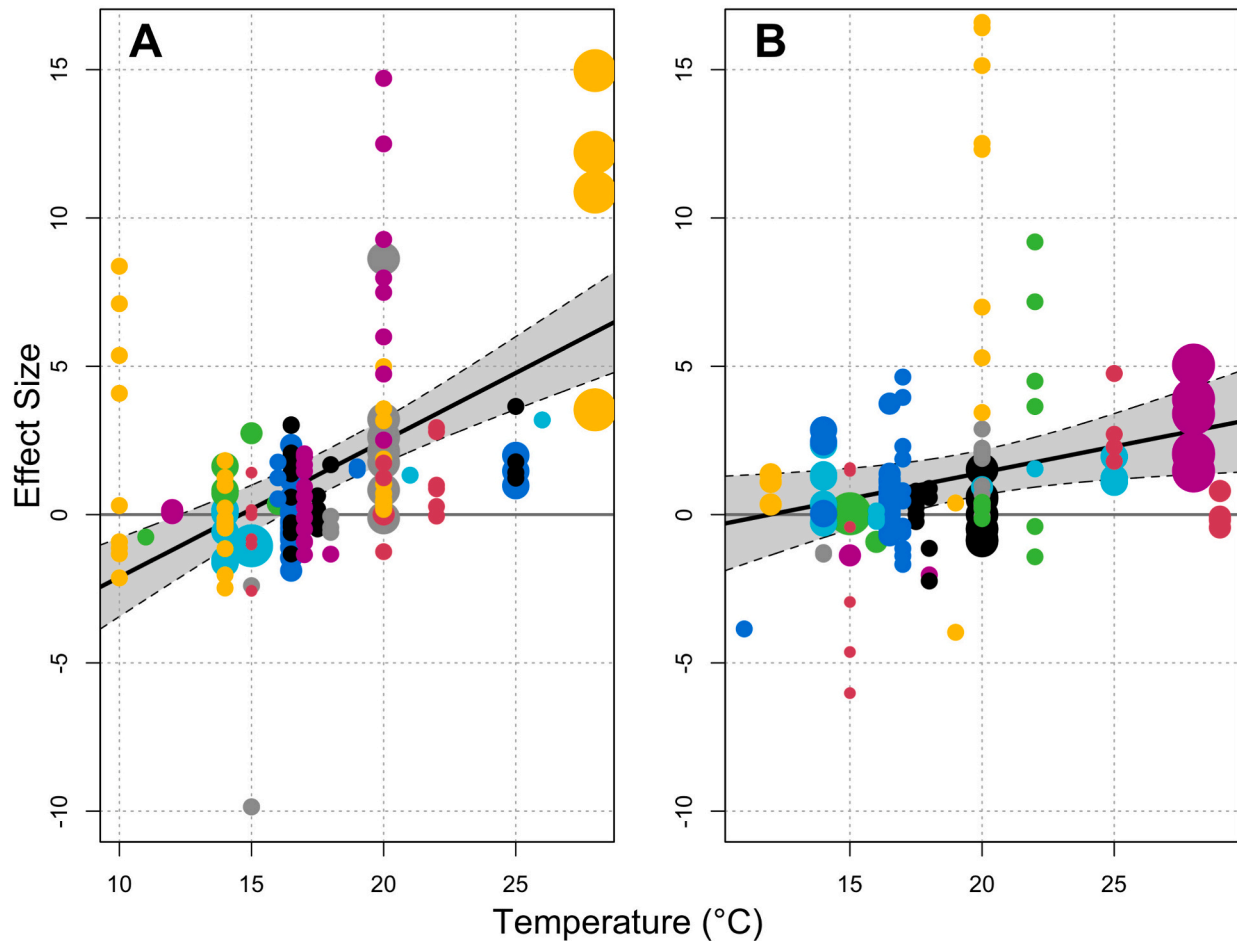


Fig. 4. Modeled, linear relationship between effect sizes and temperature for CR (A, slope = 0.459, $p < 0.001$) and RR (B, slope = 0.176, $p = 0.022$). Shaded regions represent 95 % CIs. Positive effects sizes indicate a decrease in CR or RR in response to acidified conditions, and negative effect sizes indicate an increase in CR or RR in response to acidified conditions. The color and size of the bubbles (effect sizes) represent study IDs and study sample sizes, respectively.

based local adaptation, as they present two major upwelling areas. Nevertheless, the BUI values representing locations in these upwelling areas likely fall in the range of BUI values represented in the present study. Therefore, while these areas may not be represented from a biological perspective, they are likely represented from a quantitative perspective. As another potential bias, the lowest temperature observed in this meta-analysis was 10 °C. To more accurately assess the role of temperature, lower temperatures should be included (further discussed below). Although not a bias per se, the present study assessed metabolic responses (clearance and respiration rate). While metabolism can be directed related to survivorship and fecundity, and therefore fitness, altered metabolic activity may also be a short term, environmentally-sensitive expression of adaptive plasticity that does not always correlate with fitness (Angilletta Jr, 2009; Applebaum et al., 2014; Sebens et al., 2018). Therefore, while the scope of the present study provides new insight on bivalve metabolic responses, caution should be used when extrapolating these outcomes to fitness.

4.2. Bivalve metabolism

The amount of energy allocated for growth is largely determined by acquired energy (i.e., feeding) and energy used for maintenance (e.g., respiration) after reproduction. Therefore, bivalve CR and RR may indicate how much energy is available for growth (Bayne and Newell, 1983; Griffiths and Griffiths, 1987). The present study found that bivalves generally decrease CR in response to low pH, potentially

explaining why previous meta-analyses have found that bivalves generally decrease growth in response to low pH (Hendriks et al., 2010). These results are not surprising as suspension feeding (the most common mode by which bivalves take up particles) is controlled by ciliary activity (Jørgensen, 1990), an energy demanding process that is sensitive to environmental conditions and may also decrease in response to low pH (Meseck et al., 2020). In accordance with decreased CR, the present study found that bivalves decrease RR in response low pH. From a strictly energy budget perspective, suppressed (but maintained) oxygen consumption in the long term as an adaptive response could serve to protect against high metabolic rates, suggesting potential energy conservation and benefits (Bayne and Newell, 1983; Pörtner, 2002). However, decreased RR in response to low pH may be interpreted as a necessary acute tradeoff to conserve energy in response to altered environmental conditions, providing a short-term solution to altered metabolic demands (Rosa et al., 2014), as long-term suppressed metabolic activity can also lead to sustained anaerobic mitochondrial metabolism (Pörtner, 2002). Such metabolic reductions in response to low pH have been attributed to uncompensated increases in extracellular pH (Stumpp et al., 2011). Furthermore, downregulation of genes involved in metabolic activity (e.g., genes associated with the electron transport chain and the tricarboxylic acid cycle, Kaniewska et al., 2012) typically leads to suppressed feeding and respiration rates. Such links between cell-level and organism-level processes may not only provide mechanistic insights, but may also aid in identifying mechanisms of ocean acidification sensitivity. Model results also showed no significant life

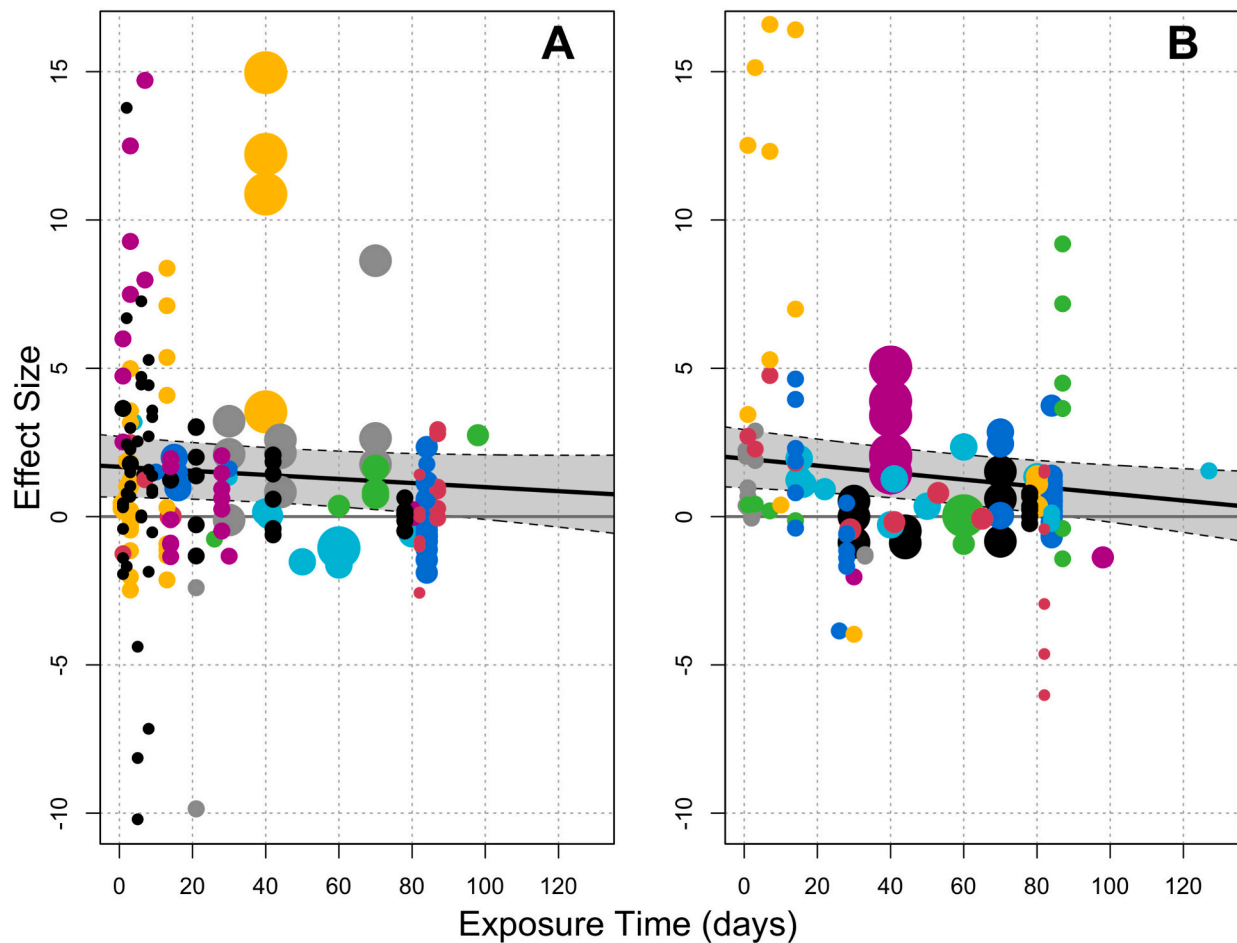


Fig. 5. Modeled, linear relationship between effect sizes and exposure time for CR (A, slope = -0.007 , $p = 0.243$) and RR (B, slope = -0.012 , $p = 0.020$). Shaded regions represent 95 % CIs. Positive effects sizes indicate a decrease in CR or RR in response to acidified conditions, and negative effect sizes indicate an increase in CR or RR in response to acidified conditions. The color and size of the bubbles (effect sizes) represent study IDs and study sample sizes, respectively.

stage effects for RR, but showed that adult CR effect sizes were significantly greater than that of juveniles, suggesting adults may be more sensitive to low pH than juveniles. This outcome is surprising, as it contradicts previous meta-analyses including Clements and Darrow (2018), who found that for calcifying marine invertebrates, larvae and juvenile feeding rates may be more sensitive to low pH than adults. The differences in outcomes between studies may be attributed to separating bivalves from other calcifying invertebrates, higher sample sizes in the current study, and distinguishing juveniles from adults. Additionally, higher net energetic demands for larger organisms (i.e., adults) may explain why adult CR was more sensitive to low pH than juvenile CR. Interestingly, Clements and George (2022) found that when assessing byssus production, larger bivalves may be more sensitive to low pH, aligning with the outcomes of the present study.

4.3. Upwelling exposure

Previous studies suggest that organisms in areas that experience regular, strong upwelling may be less sensitive to low pH (Gaitán-Espitia et al., 2017). For example, Evans et al. (2013) found that purple urchin, *Strongylocentrotus purpuratus*, larvae from a high upwelling region in the north Pacific demonstrated an enhanced ability to cope with more intense pH fluctuations. Indeed other experimental studies have demonstrated potential low pH local adaptation (often in an upwelling context) for sea urchins (Kelly et al., 2013; Evans et al., 2017; Kapsenberg et al., 2017; Wong et al., 2018; Wong et al., 2019) and other taxa (e.

g., corals, Griffiths et al., 2019). As a more taxonomically relevant example, Vargas et al. (2015) exposed two populations of juvenile Chilean mussels, *Mytilus chilensis*, one from a high upwelling area and one from a low upwelling area, to different $p\text{CO}_2$ levels and then measured CR. After six weeks of exposure, mussels from the high upwelling site did not have significant differences in CR, but mussels from the low upwelling site exhibited significantly lower CR at the highest $p\text{CO}_2$ level. More recently, Rose et al. (2020) found that along the west coast of the United States, California mussels, *Mytilus californianus*, from sites with upwelling-induced high $p\text{CO}_2$ variability may respond differently to low pH than those from sites with less $p\text{CO}_2$ variability. These outcomes, as well as other meta-analyses and reviews that have linked ocean acidification sensitivity to natural $p\text{CO}_2$ variability (Vargas et al., 2017; Vargas et al., 2022), align with the results of the present study. However, the results of the present study are the first to directly link low pH sensitivity to upwelling exposure, as an individual process, at a global scale. This relationship not only provides valuable information regarding the global change basic science, but may lead to potential applied science value. For example, aquaculture may benefit from related selective breeding efforts (i.e., from using genetic lines derived from upwelling systems), as to limit negative impacts of ocean acidification on aquaculture production. In fact, recent reviews have identified selective breeding as an area of need for bivalve aquaculture in response to ocean acidification (Tan et al., 2020; Tan and Zheng, 2020). Indeed selective breeding to combat ocean acidification has been implemented in the Pacific Northwest of North America, an area that experiences

Table 2

Model output and relationships between effect sizes for clearance (CR) and respiration (RR) rates and Δ carbonate chemistry parameters. Each Δ parameter refers to the change in parameter (e.g., pH = the change in pH). Ω_{Ar} and Ω_{Ca} denote aragonite and calcite saturation states, respectively. All p -values are statistically significant. * denotes the models with the highest R^2_{marginal} and the lowest AIC for each response. AIC differences <2.0 are not considered significantly different, therefore, multiple RR models contain AIC asterisks.

Response	Δ Parameter(s)	Q_M	p -value	Slope	R^2_{marginal}	AIC	
CR	Ω_{Ar}	6.390	0.012	0.925	0.217	*576.87	
	Ω_{Ca}	7.356	0.007	0.655	0.250	578.94	
	pH	9.971	0.002	5.298	0.384	595.19	
	pCO_2	11.628	0.001	-0.002	0.405	587.35	
	$\Omega_{Ar} + pH$	9.962			0.387	595.38	
	$\Omega_{Ar} + pCO_2$	12.299			0.409	587.42	
	$\Omega_{Ar} + \Omega_{Ca}$	9.935			0.321	583.05	
	$pH + \Omega_{Ca}$	9.895			0.383	594.94	
	$pCO_2 + \Omega_{Ca}$	12.075			0.410	584.89	
	$pH + pCO_2$	11.850			0.418	588.32	
	$\Omega_{Ca} + pH + pCO_2$	11.724			0.411	590.12	
	$\Omega_{Ar} + \Omega_{Ca} + pCO_2$	13.019			*0.445	584.17	
	$\Omega_{Ar} + pH + \Omega_{Ca}$	11.055			0.418	592.45	
	$\Omega_{Ar} + pH + pCO_2$	12.028			0.416	592.30	
	$\Omega_{Ar} + \Omega_{Ca} + pH + pCO_2$	12.748			0.441	587.37	
	RR	Ω_{Ar}	8.186	0.004	1.324	0.295	*513.99
		Ω_{Ca}	9.130	0.003	0.878	0.325	520.04
pH		5.228	0.022	4.708	0.260	533.31	
pCO_2		5.821	0.016	-0.001	0.274	535.21	
$\Omega_{Ar} + pH$		7.896			*0.387	520.29	
$\Omega_{Ar} + pCO_2$		8.426			0.316	*515.49	
$\Omega_{Ar} + \Omega_{Ca}$		6.207			0.237	526.80	
$pH + \Omega_{Ca}$		9.468			0.319	522.84	
$pCO_2 + \Omega_{Ca}$		9.646			0.342	518.35	
$pH + pCO_2$		5.611			0.280	532.96	
$\Omega_{Ca} + pH + pCO_2$		9.215			0.324	*514.68	
$\Omega_{Ar} + \Omega_{Ca} + pCO_2$		6.946			0.248	522.24	
$\Omega_{Ar} + pH + \Omega_{Ca}$		6.777			0.230	527.40	
$\Omega_{Ar} + pH + pCO_2$		7.406			0.280	*513.51	
$\Omega_{Ar} + \Omega_{Ca} + pH + pCO_2$		6.981			0.245	518.13	

strong seasonal upwelling, although it remains to be seen if upwelling can be directly linked to the developed ocean acidification tolerant stocks (Barton et al., 2015). While upwelling exposure explained 49 % of the variability in CR responses, CR may not necessarily or solely provide a concrete indicator of fitness. Therefore, results from the present study may not necessarily translate to increased bivalve production. Additionally, other studies have found that extreme upwelling events can lead to severe losses in shellfish hatchery production (Barton et al., 2012; Barton et al., 2015), suggesting that upwelling based local adaptation may be constrained (see references within Clements and Chopin, 2017; Tan and Zheng, 2020). Indeed, additional work is needed to assess application value of selective breeding efforts in an upwelling-based local adaptation context.

While this study highlights upwelling-based local adaptation in bivalve responses to low pH, the relative strength and roles of acclimation and/or adaptation mechanisms remain unknown (Sunday et al., 2014). Gene expression studies may highlight genes or pathways that are upregulated for different populations in response to low pH, as previous studies have found that bivalve genes related to metabolism may be differentially expressed in response to low pH (Hüning et al., 2013; Schwaner et al., 2022). While the present study cannot make any

conclusions regarding potential evolutionary adaptation, future work can consider using molecular approaches at the genome scale (e.g., next generation DNA and RNA sequencing) to unravel the capacity for evolutionary adaptation (Sunday et al., 2014). For example, DNA sequencing may reveal different genetic structures (e.g., allele frequencies) between populations that exhibit different responses to ocean acidification, which can suggest that different lineages have evolved in response to different environmental conditions (Vendrami et al., 2019; Schwaner et al., 2022), or, in the appropriate context, can suggest allele-specific survival (Pespeni et al., 2013).

It is also important to consider the potential shortcomings of experimental studies in high upwelling areas. More specifically, Vargas et al. (2017) suggested that experimental studies that use organisms that occupy areas with high pCO_2 variability (e.g., strong upwelling zones) may be underestimating ocean acidification impacts. The pCO_2 treatments used in such experiments may represent observed natural variability, as opposed to forecasted increases in pCO_2 levels. Therefore, while the results of the present meta-analysis suggest that bivalves in strong upwelling areas may be less sensitive to low pH, it is possible that when faced with larger increases in pCO_2 , (i.e., more realistic ocean acidification scenarios for upwelling areas), bivalve responses may be more sensitive to ocean acidification.

4.4. Experimental design implications and further future directions

When designing ocean acidification experiments, it is important to consider characteristics of the system and species of interest. For example, local projections of carbonate chemistry parameters should be used to set carbonate chemistry treatments. Additionally, temperatures experienced by the organism in its natural environment should be used. Therefore, the results of this meta-analysis do not aim to guide decision making for ocean acidification experiments, but instead aim to provide context for such experiments. For example, if an experiment finds a strong response when exposing organisms to a pH of 7.6 relative to a control of 7.8, the result of the present meta-analysis would highlight the unexpected nature of such response, as a change in pH of 0.2 should yield a weaker response (according to the models of the present meta-analysis). Therefore, such a hypothetical study might conclude that the organisms in question may be particularly sensitive to low pH. Model results also suggest that at short and long exposure times, CR is likely to decrease in response to low pH, but at long exposure times, RR is not likely to change in response to low pH. The latter of which aligns with previous meta-analyses including Cattano et al. (2018) who found that at short exposure times, fish growth was likely to decrease in response to low pH but at long exposure times, fish growth was likely to not change in response to low pH.

Not surprisingly, larger changes in carbonate chemistry parameters led to larger effect sizes. These results suggest that regions experiencing more rapid rates of ocean acidification may encounter more intense, negative responses for bivalves. For example, in the North Atlantic, the Gulf of Maine (GoM) has been identified as being particularly vulnerable to anthropogenic CO_2 forcing and large aragonite saturation state declines (Cai et al., 2020; Balch et al., 2022). The GoM hosts many bivalve species, including ecologically important foundation species such as the blue mussel, *Mytilus edulis*, (Menge, 1976; Sorte et al., 2017) and commercially important fishery species, such as the sea scallop, *Placopecten magellanicus*, and the softshell clam, *Mya arenaria* (Wallace, 1997). Therefore, these GoM bivalves may experience larger declines in metabolic activity than bivalves in other regions. Furthermore, this idea complements recent conclusions stating that ecological implications of global change will depend on the pace of global change and species adaptability (Kleisner et al., 2017). Carbonate chemistry models also showed that aragonite saturation state, as an individual predictor variable, yielded the best fit models for both CR and RR. This suggests that aragonite saturation state, rather than calcite saturation state, pH or pCO_2 , can best predict effect sizes. Saturation state is believed to drive

the energetic cost of the kinetics of shell formation (Waldbusser et al., 2013), particularly in mollusk larvae, potentially explaining the observed outcome. Additionally, most mollusk larvae contain aragonite in similar crystalline structures (Weiss et al., 2002), potentially explaining the ubiquitous effects of aragonite saturation state. From a non-larval-only perspective, the relatively unstable structure of aragonite and predominance of aragonite over calcite in adult shells (in both the outer prismatic layer and inner nacreous layer, Addadi et al., 2006) may drive the importance of aragonite saturation state in predicting responses of all bivalve life stages. These results contribute to the ongoing body of support that for calcifying organisms such as mollusks, physiological responses to global change-induced alterations in carbonate chemistry are most directly linked to saturation state (Waldbusser et al., 2015; Gray et al., 2017).

Regarding the observed positive relationships between temperature and effects sizes, it would be tempting to conclude that bivalves in warmer areas may be more sensitive to low pH. This conclusion agrees with previous studies such as Watson et al. (2012) who found that low latitude mollusks, brachiopods, and echinoids produce thinner shells than those of high latitudes and with other meta-analyses results that identified that higher temperatures may exacerbate negative responses to low pH (Harvey et al., 2013; Kroeker et al., 2013; Lefevre, 2016). However, such conclusions may be premature, as this meta-analysis used absolute temperature as a predictor variable. To truly assess if bivalves from warm areas are more sensitive to ocean acidification, temperature needs to be standardized in the context of present variability (Vargas et al., 2022). To aid such efforts, future work may consider increased temporal and spatial resolution (multiple dimensions) of temperature monitoring, coupled with increased reporting of environmental conditions by studies that use organisms captured from the field. Nevertheless, the present results suggest that when experiments use higher temperatures, bivalves are likely to show stronger declines in metabolic activity in response to low pH.

While upwelling alone explained nearly 50 % of the variability in CR results, significant unexplained variability remains. As previously described, temperature may play a role in remaining variability. However, other oceanographic phenomena and local environmental processes, including ecosystem respiration and river outflow, which also contribute to pCO₂ variability, may also explain remaining response variability (Vargas et al., 2016; Sridevi and Sarma, 2021). As a relevant example of the potential influence of other local environmental processes on low pH tolerances, Clements et al. (2021) found that larval oysters, *Crassostrea virginica*, in New Brunswick were tolerant of low pH (7.5) conditions. They postulated that this tolerance may be due to local adaptation shaped by acidic runoff from local peat bogs. Therefore, future studies may quantify the relative contribution of additional individual environmental processes to variable organism responses to ocean acidification. Additionally, if future ocean acidification experiments adjust experimental designs to allow for calculations of carbonate chemistry performance curves (e.g., a continuous gradient of conditions instead of categorical scenarios, see Lawlor and Arellano, 2020), meta-analyses may not only resolve complexities of variable responses, but may compare tolerance curves among different studies and taxa.

4.5. Conclusions

Negative impacts of climate change on organisms are likely inevitable. Nevertheless, it is important to understand what factors drive responses, response variability and may alleviate negative responses from both an experimental and global, ecological perspective. As a quantitative tool, meta-analyses allow for addressing such questions. This meta-analysis showed that while most bivalves decrease metabolic activity in response to low pH, neutral or positive responses are also observed. Bivalves from strong upwelling areas, globally, tended to not change or increase metabolic activity in response to low pH. This suggests potential local adaptation for organisms that already experience

naturally acidified conditions. Furthermore, results also showed that lower temperatures, longer exposure times and smaller changes in carbonate chemistry parameters may also allow bivalves to not change or increase metabolic activity in response to low pH. These results not only provide context for future experimental designs, but have implications for the natural environment. For example, organisms in regions that experience higher temperature and more rapid rates of ocean acidification may experience greater declines in metabolic activity. This research contributes to the ongoing investigations of local adaptation to ocean acidification. While results cannot immediately be applied for bivalve restoration, fisheries or aquaculture efforts, results do suggest that populations from strong upwelling areas are less sensitive to low pH, providing a foundation for future efforts that wish to more thoroughly test the ability of strong upwelling areas to supply ocean acidification tolerant organisms.

CRedit authorship contribution statement

Raymond Czaja: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **Emmanuelle Pales-Espinosa:** Funding acquisition, Project administration, Resources, Writing – review & editing. **Robert M. Cerrato:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Kamazima Lwiza:** Funding acquisition, Investigation, Resources, Writing – review & editing. **Bassem Allam:** Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

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Data availability

Data will be made available on request.

Appendices. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165900>.

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