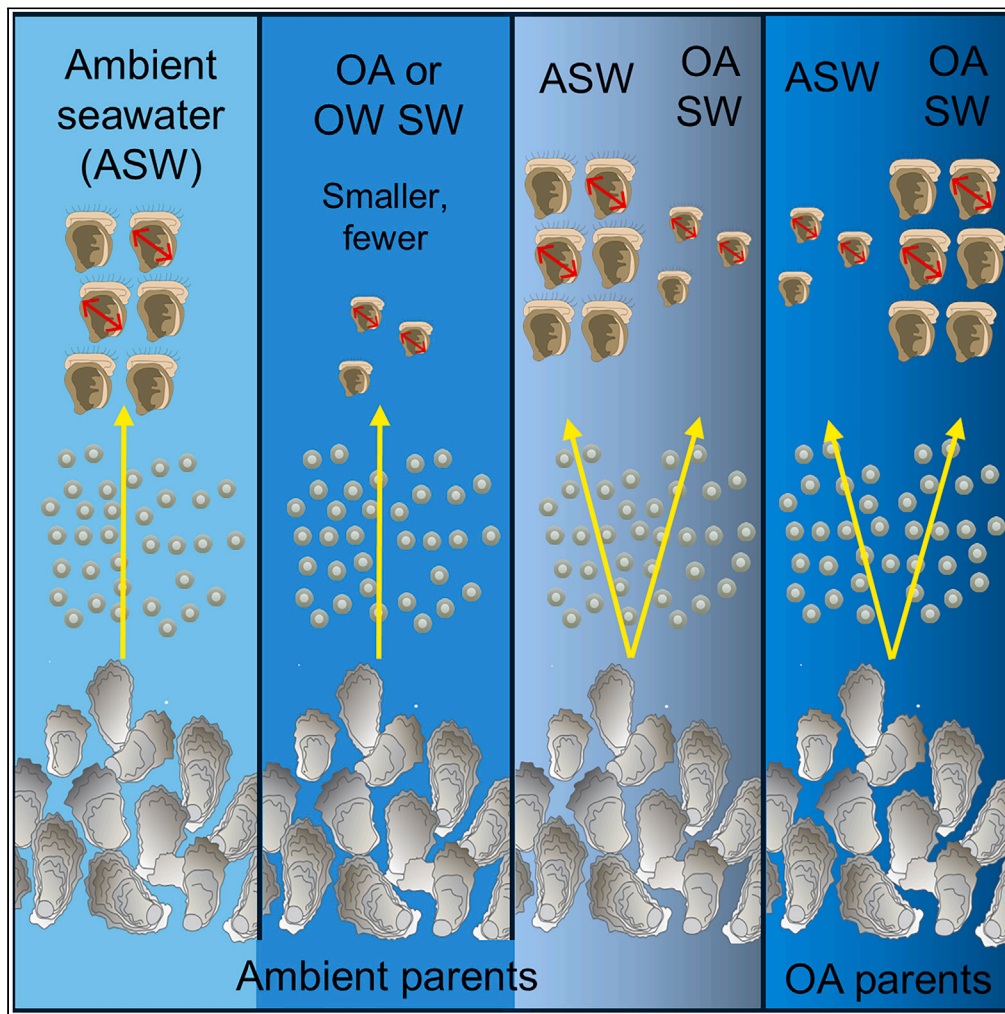


Article

# Meta-analyses reveal climate change impacts on an ecologically and economically significant oyster in Australia



Pauline M. Ross,  
Christopher Pine,  
Elliot Scanes,  
Maria Byrne,  
Wayne A.  
O'Connor,  
Mitchell Gibbs,  
Laura M. Parker

pauline.ross@sydney.edu.au

**Highlights**

Meta-analyses indicate significant ocean acidification (OA) and warming (OW) impacts

OA and OW negatively impact the offspring size and mortality of *Saccostrea glomerata*

Transgenerational plasticity (TGP) to OA positively benefits offspring size

Research gaps include presence and persistence of TGP response to OA and OW

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## Article

## Meta-analyses reveal climate change impacts on an ecologically and economically significant oyster in Australia

Pauline M. Ross,<sup>1,6,\*</sup> Christopher Pine,<sup>1</sup> Elliot Scanes,<sup>1,2</sup> Maria Byrne,<sup>1</sup> Wayne A. O'Connor,<sup>3</sup> Mitchell Gibbs,<sup>4</sup> and Laura M. Parker<sup>3,5</sup>

## SUMMARY

**Global oceans are warming and acidifying because of increasing greenhouse gas emissions that are anticipated to have cascading impacts on marine ecosystems and organisms, especially those essential for biodiversity and food security. Despite this concern, there remains some skepticism about the reproducibility and reliability of research done to predict future climate change impacts on marine organisms. Here, we present meta-analyses of over two decades of research on the climate change impacts on an ecologically and economically valuable Sydney rock oyster, *Saccostrea glomerata*. We confirm with high confidence that ocean acidification (OA) has a significant impact on the size and mortality of offspring of *S. glomerata*, ocean warming (OW) impacts size, and transgenerational exposure of adults to OA has positive benefits for offspring. These meta-analyses reveal gaps in understanding of OW and transgenerational plasticity on an ecologically and economically significant oyster species to ensure sustainability of this iconic oyster in Australia.**

## INTRODUCTION

Climate-driven greenhouse gas emissions are warming and acidifying global oceans.<sup>1,2</sup> Climate models predict that ocean warming (OW) and ocean acidification (OA) are *virtually certain* and that sea-surface temperatures (SSTs) will rise by 1.51°C (Shared Socioeconomic Pathway SSP1-2.6) up to 2.89°C (SSP5-8.5 range 2.01°C–4.07°C), and oceans will acidify by –0.1 pH in the best case scenario (SSP1-2.6) to –0.45 pH units in the worst-case scenario (SSP5-8.5).<sup>3</sup>

There has been substantial concern for at least two decades that OW and OA will have cascading impacts on the health of biodiversity and ecosystems worldwide and on marine ecosystems and organisms across all life history stages.<sup>4–13</sup> Reviews and meta-analyses done across a wide range of taxa have found that marine molluscs are particularly vulnerable to OA especially during the early life history larval stages.<sup>7,11,14–17</sup> Mollusc larvae experience developmental delays, increased abnormalities, and decreased growth and survival,<sup>18–20</sup> the extent of which can vary depending on the genotype.<sup>21,22</sup> OA will make it energetically more costly for many, but not all marine calcifying molluscs, to maintain and regulate acid-base balance and build calcium carbonate shells. Potential consequences include reduced shell growth and increased shell malformation and dissolution.<sup>23–27</sup> It is also predicted that for many marine molluscs there will be energetic trade-offs with less energy available for reproduction, which may affect gonad development, reduce fertilization, and alter sex determination with implications for reduced offspring production.<sup>28,29</sup> It is clear, however, that some molluscs, calcifying or not, have the potential for adaptive capacity and resilience against the impacts of OA.<sup>25,30,31</sup> For example, Thomsen et al.<sup>24</sup> found that *Mytilus edulis* from Kiel Fjord could grow and recruit when the CO<sub>2</sub> level of seawater was 2,300 μatm (pH < 7.5 pH). Further, Wernberg et al.<sup>32</sup> stated that bivalve reefs composed of oysters persist in highly acidified estuaries. However, although they may persist in low pH conditions, this can compromise biomineral production in wild oysters.<sup>33</sup>

OW in combination with OA is forecast to increase the vulnerability of some marine calcifiers or alternatively ameliorate impacts, depending on stressor levels.<sup>17,34,35</sup> Ocean warming, and in particular extreme events such as marine heatwaves (MHWs), are emerging as the climate change impact of greatest concern for marine ecosystems<sup>36,37</sup> and marine bivalves.<sup>38</sup> Many marine organisms live close to their thermal limits.<sup>39,40</sup> Temperatures beyond thermal limits, as can occur during MHWs, can be lethal to larvae and adults.<sup>41–43</sup> Habitat warming has multiple impacts including shifts in reproductive timing, increased incidence of disease, and stunted growth, likely associated with increases in

<sup>1</sup>School of Life and Environmental Sciences, The University of Sydney, Camperdown, Sydney, NSW 2006, Australia

<sup>2</sup>Climate Change Cluster, University of Technology Sydney, Ultimo, Sydney, NSW 2007, Australia

<sup>3</sup>NSW Department of Primary Industries, Port Stephens Fisheries Institute, Taylors Beach, NSW 2316, Australia

<sup>4</sup>School of Geosciences, The University of Sydney, Camperdown, Sydney, NSW 2006, Australia

<sup>5</sup>School of Biological, Earth and Environmental Sciences, The University of New South Wales, Kensington, Sydney, NSW 2052, Australia

<sup>6</sup>Lead contact

\*Correspondence: pauline.ross@sydney.edu.au

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physiological rates and changes in energetics.<sup>44–47</sup> Extreme events such as MHWs will also have physiological impacts, which will lead to developmental delays, growth reductions,<sup>38</sup> and potentially alter the immune system and nutritional quality.<sup>48</sup>

Recent meta-analyses and reviews suggest that ocean warming will also have impacts on species abundance, behavior, distribution, and ultimately ecosystem processes across trophic levels and that there will be “winners” and “losers.” Losers will include tropical fish, reef building corals, mangroves, and seagrass, all declining in abundance with an increase of warming in their habitats.<sup>49,50</sup> Winners will be those marine organisms that benefit from the warmer ocean including species of phytoplankton, sponges, seagrass, algae, fish, and some marine reptiles and mammals<sup>49,50</sup> and those with a migratory phase that can follow favorable isotherms as seen in the poleward migration of many species.<sup>51,52</sup>

While there remains some controversy about the absolute impacts of OW and OA on marine biodiversity and ecosystems, especially where those conclusions are based solely on laboratory experiments, what remains is a solid consensus that when OW and OA combine with other stressors the impacts of climate change are more evident and impacts, in general, are more negative.<sup>31,53–55</sup> Many studies emphasize that when OW and OA combine with other stressors such as deoxygenation (OD), pollution, altered salinity, and low food availability, there will be additive, synergistic, and/or antagonistic effects on marine organisms and ecosystems,<sup>56–58</sup> OW, OA, and OD being the deadly trio.<sup>59,60</sup> These impacts may even lead to extinctions of major groups.<sup>61</sup> Even those molluscs with resilience to OA may not persist when periods of acidified seawater combine with low food availability.<sup>54</sup> However, when periods of acidified seawater combine with abundant food availability, they can tolerate low pH and even outperform other species.<sup>62</sup>

Such broad concerns have increased efforts to determine the magnitude and certainty of the impacts of climate change on marine organisms. Early meta-analyses synthesizing 139 and 228 studies found decreased survival, calcification, growth, and development across a wide range of marine organisms, with enhanced vulnerability of early life history stages.<sup>7,8</sup> In these analyses, molluscs were particularly vulnerable with uneven impacts across other taxonomic groups and life history stages. Other meta-analyses synthesizing 167 studies across a range of taxa identified coral, echinoderms, and molluscs to be more sensitive than crustaceans, with larval fishes perhaps being more sensitive than invertebrates.<sup>13</sup> Further, studies to determine the impact of OA in the southern ocean synthesizing 20 studies and 25 response ratios found negative responses to OA in marine calcifiers, and high vulnerability of echinoderms, brachiopods, pteropods, bivalves, and stony corals, and mid-vulnerability of coccolithophores although there was greater variability than by chance alone between studies.<sup>17</sup> More recent meta-analyses synthesizing results from 985 studies found that many calcifiers (e.g., echinoderms, crustaceans, corals, and cephalopods) were more tolerant to near-future OA (i.e., pH 7.8 by 2,100) than reported previously, but bivalves and gastropods along with pteropods were identified to be particularly vulnerable.<sup>11</sup> In perhaps the most complete examination of bivalve responses to date by Kruff Welton et al.,<sup>63</sup> analysis of 203 unique experimental setups found negative impacts of climate change (OW, OA, OD, and salinity) on growth and size of bivalves, especially *Ostreidae*.

It has been suggested that the reporting in the literature of the effects of ocean change stressors, especially acidification, has been inflated due to a confirmatory view.<sup>11</sup> Factors including publication bias toward negative effects, early experiments that exposed marine organisms to extremely low pH levels outside the range predicted by near-future climate change,<sup>64,65</sup> the use of mineral acid in early experiments to manipulate pH rather than CO<sub>2</sub>,<sup>66</sup> and the acute exposure of species across a few days or weeks have been done without consideration of the capacity for species to acclimate or adapt over longer periods.

Leung et al.<sup>11</sup> has drawn attention to the increasing uncertainty around the severity of OA and its effects on marine organisms. Others have also communicated increasing skepticism.<sup>67,68</sup> Clements et al.<sup>68</sup> suggested that this is in part due to groundbreaking scientific discoveries often having inflated large effect sizes with evidence that subsequent follow-up replicated studies report smaller effect sizes and declines. This may not necessarily need to be interpreted as a crisis but rather as part of the scientific process by which “limitations are explored.”<sup>69</sup> Nonetheless, it appears there is a problem reproducing many of the previously reported results.

It is not solely the lack of reproducibility, however, that has increased skepticism about whether there should be concern about the impacts of climate change on marine organisms. It has been suggested that the bulk of prior research has neglected the fact that marine organisms have adaptive capacity. Therefore, studies that report negative impacts can be somewhat discounted because if transgenerational plasticity is considered, then the impacts of OA on marine organisms may not be as severe.<sup>11</sup>

Transgenerational plasticity (TGP) is a non-genetic inheritance process, where parents’ experience of stress can alter the behavior, morphology, or physiology phenotypes of their offspring without modifying the genotype.<sup>17,70–72</sup> Such phenotypic plasticity is not always positive, and not always without trade-offs, but it is a rapid response that may buy the time needed for genetic adaptation to occur.<sup>31,71</sup> Genetic adaptation is a much slower process and involves the selection of genotypes toward peak fitness across many generations. Leung et al.<sup>11</sup> state that calcifiers such as oysters and mussels may be able to modify their phenotypes across generations and rapidly adapt to OA because of parental provisioning (p. 16). Parental exposure and TGP have long been discussed in OA research in creating offspring with more resilience, which can persist across generations<sup>73,74</sup> but not in the presence of multiple stressors<sup>54</sup> although there are differences between species responses.<sup>75</sup>

On the other hand, studies have suggested that cross- and transgenerational plasticity will not be the panacea to rescue progeny from negative responses to OA.<sup>71</sup> It appears that TGP may be an insufficient buffer, if it does not persist over time and thus does not protect each life history and “buy time” to allow marine organisms time to adapt.<sup>71</sup> Moreover, for some species, TGP effects have been found to be neutral or even negative.<sup>70,71,76,77</sup> Thus, TGP cannot be broadly relied on as a solution to the potential crisis that marine ecosystems and organisms may face.

It is unsurprising given this background that predictions about whether OA is really a threat to marine calcifiers have been recently called into question.<sup>11,68</sup> As stated previously, there have been concerns expressed about publication bias and the overreporting of negative impacts in the literature and the potential of moderating factors such as TGP rescue.<sup>11</sup> Moreover, there have been concerns about the robustness of scientific evidence and reproducibility of experiments especially for fish<sup>68,69,78</sup> and citation bias (the practice of selective/high citations of early studies) and inflation of effect sizes.<sup>68</sup> More broadly, there is acknowledgment in science of a “decline” effect and a gravitation toward consensus.<sup>69</sup> Nonetheless, accurately understanding the impacts of climate change remains a priority, particularly for key habitat-forming species and/or species that form the basis of aquaculture industries and food source. Such understanding is essential to determine whether strategies are needed to prevent significant losses in these species and sustain them, along with the ecosystems, industries, and livelihoods that they support, into the future.

One way to guard or mitigate against the exaggeration of effect sizes is through systematic review and meta-analysis to empirically determine the robustness of the evidence. On one hand, it is known that bias in original studies and meta-analysis of literature can lead to misleading outcomes, especially if there is selective inclusion of studies. On the other hand, meta-analyses can detect bias. Here, we present meta-analyses on the two decades of studies on the Sydney rock oyster, *Saccostrea glomerata*, in a synthesis to provide robust evidence on the impacts of climate change and the future for sustainability of this ecologically and economically important species in Australia.<sup>19,27</sup> Sydney rock oysters are a valuable aquacultural and iconic native species with Indigenous cultural significance.<sup>79</sup> Oyster aquaculture products in NSW are valued at over \$50 million annually, with a total value of ~\$300 million when considering the social and economic benefits.<sup>80,81</sup> Over the last decade there has been significant effort and investment to restore Sydney rock oyster reefs, in light of loss of approximately 95% of these reefs and to restore important ecosystem services and cultural significance.<sup>79,82</sup>

Unlike most meta-analyses that span multiple taxa and laboratories, importantly the aim of these meta-analyses are to analyze two decades of multiple experimental research focus on one species. The goal was to seek greater certainty and predictability about the future capacity of Sydney rock oysters to cope with OA and OW, test for any moderating factors such as parental exposure and phenotypic plasticity to ameliorate these impacts, and rescue the species. We identify limitations of experiments done to date and suggest an experimental pathway forward for this iconic species and other marine organisms faced with the threat of climate change.

## RESULTS

### Effect of OA on size

In total the systematic search strategy on three databases; Web of Science Core Collection, Scopus and ProQuest Central identified 35 eligible studies, with 284 effect sizes<sup>83–89</sup> (Table 1). There were 11 studies with 94 effect sizes to estimate the influence of OA on Sydney rock oyster shell size and eight studies with 47 effect sizes to estimate the influence of OA on mortality (Tables 1 and 2; Figures S1 and S2). To estimate the influence of transgenerational exposure to OA, there were six studies with 76 effect sizes and five studies with 44 effect sizes to estimate mortality (Tables 1 and 2; Figures S3 and S4). There were fewer studies done on the influence of OW on size and mortality i.e., there were four studies with 21 effect sizes to estimate the influence of OW on Sydney rock oyster size and only one study with two effect sizes to estimate the influence of OW on mortality (Tables 1 and 2; Figures S5 and S6). This low number of studies reflect a clear gap in knowledge in this area and in interpretation.<sup>90–92</sup> There were no studies conduct a meta-analysis on the influence of transgenerational exposure to OW on size or mortality (Table 1; Figures S7 and S8). There were a range of moderators used in the metanalyses including parental and larval treatment, feeding and fertilization regime, family lines of oysters, salinity, life history stage and age of larvae (Tables 3, 4, and 5). There were no studies to estimate the influence of TGP to OW on shell size or mortality (Table 1).

The results of the meta-analysis showed that there was a significant large negative association between increasing OA and shell size ( $R = -0.95$ ;  $p = 0.0001$ ; Figure 1; Table 6). The total variance unaccounted for by sampling error within the adopted model was approximately 59.22%. Within-study variance ( $I^2_{\text{level } 2}$ ) explained 6.64% of the residual variance not attributable to sampling error. The between-study variance was greater than the within-study variance ( $I^2_{\text{level } 3} = 34.14\%$ ), indicating that there may be some differences within studies contributing to the overall variance that is not being accounted for in this model (Figure S9).

### Results of moderator analysis

Results of an omnibus test on the multi-moderator model indicated that at least one of the moderators significantly influenced the effect of OA on size ( $F = 8.3219$ ,  $p < 0.0001$ ,  $df_1 = 6$ ,  $df_2 = 85$ ). However, further analysis of the coefficients after the RVE procedure showed that there were no significant moderators of the effect of OA on size ( $p > 0.05$ ). Despite this, the inclusion of moderators did reduce the overall model heterogeneity relative to the base model ( $I^2 = 11.32$  compared to  $I^2 = 59.22$ ), alongside a reduction in both the within-study variance ( $I^2_{\text{level } 2} = 11.16$ ) and between-study variance ( $I^2_{\text{level } 3} = 0.15$ ; Figure S10).

### Effect of OA on mortality

There was a significant overall negative impact of OA on oyster mortality ( $R = 0.688$ ,  $p = 0.0449$ ; Figure 2; Table 6). Around 43.97% of the total variance within the model was attributed to sampling error within the studies (i.e., individual studies within each paper). There was a similar amount of heterogeneity within clusters ( $I^2_{\text{level } 2} = 40.04\%$ , i.e., within each paper), whereas the amount of heterogeneity between studies ( $I^2_{\text{level } 3}$ ; i.e., individual studies within each paper) was 15.99% (Figure S11), indicating that there are potentially other factors between individual studies and clusters that are significantly influencing the model that are potentially unaccounted for.

**Table 1. Search strings used in the meta-analysis topic of interest and number of eligible studies and effect sizes**

Meta analysis Topic of Interest	Search Strings	Number of Eligible Studies	Number of Effect Sizes
OA + Shell Size	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (acid* OR pCO <sub>2</sub> OR CO <sub>2</sub> OR pH?) AND (size? OR length? OR development OR growth OR tolerance? OR effect? OR response? OR influence?)	11	94
OA + Mortality	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (acid* OR pCO <sub>2</sub> OR CO <sub>2</sub> OR pH?) AND (development OR tolerance? OR effect? OR response? OR influence? OR mortality OR survival OR death OR die OR dead OR loss)	8	47
OW + Shell Size	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (development OR tolerance? OR effect? OR response? OR influence? OR mortality OR survival OR death OR die OR dead OR loss) AND (temperature? OR acid* OR warm* OR heat* OR "thermal stress" OR "thermal tolerance" OR "thermal shock" OR warm* OR heat* OR "high temperature" OR "low temperature" OR "extreme temperature" OR thermal)	4	21
OW + Mortality	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (temperature? OR acid* OR warm* OR heat* OR "thermal stress" OR "thermal tolerance" OR "thermal shock" OR warm* OR heat* OR "high temperature" OR "low temperature" OR "extreme temperature" OR thermal) AND (size? OR length? OR width OR development OR growth OR tolerance? OR effect? OR response? OR influence?)	1	2
Transgenerational Exposure to OA + Shell Size	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (acid* OR pCO <sub>2</sub> OR CO <sub>2</sub> OR pH?) AND (Transgen* OR "adult exposure" OR carryover OR persistence OR "parental exposure") AND (size? OR length? OR development OR growth OR tolerance? OR effect? OR response? OR influence?)	6	76
Transgenerational Exposure to OA + Mortality	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (acid* OR pCO <sub>2</sub> OR CO <sub>2</sub> OR pH OR pHs OR ph OR phs) AND (transgen* OR "adult exposure" OR "exposed adults" OR "adults exposed" OR carryover OR persistence OR "parental exposure" OR "exposed parents" OR "parents exposed") AND (develop* OR tolerance? OR effect? OR response? OR influence? OR mortality OR surviv* OR death OR die OR dead OR loss)	5	44
Transgenerational Exposure to OW + Shell Size	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (transgen* OR "adult exposure" OR "exposed adults" OR "adults exposed" OR carryover OR persistence OR "parental exposure" OR "exposed parents" OR "parents exposed") AND (temperature? OR acid* OR warm* OR heat* OR "thermal stress" OR "thermal tolerance" OR "thermal shock" OR warm* OR "heat shock" OR "high temperature" OR "low temperature" OR "extreme temperature" OR thermal) AND (size? OR length? OR development OR growth OR tolerance? OR effect? OR response? OR influence?)	0	0
Transgenerational Exposure to OW + Mortality	("Saccostrea glomerata" OR "Sydney rock oyster" OR "S. glomerata") AND (transgen* OR "adult exposure" OR "exposed adults" OR "adults exposed" OR carryover OR persistence OR "parental exposure" OR "exposed parents" OR "parents exposed") AND (temperature? OR warm* OR heat* OR "thermal stress" OR "thermal tolerance" OR "thermal shock" OR warm* OR "heat shock" OR "high temperature" OR "low temperature" OR "extreme temperature" OR thermal) AND (develop* OR tolerance? OR effect? OR response? OR influence? OR mortality OR surviv* OR death OR die OR dead OR loss)	0	0

**Table 2. Publications which were included in the five meta-analyses on the influence of OA and OW on size and mortality and TGP to OA on Sydney rock oysters**

Authors	Title	Number of Effect Sizes
	<b>Influence of OA on Sydney Rock Oyster Shell Size</b>	
Watson et al. <sup>93</sup>	Early larval development of the Sydney rock oyster <i>Saccostrea glomerata</i> under near-future predictions of CO <sub>2</sub> -driven ocean acidification.	2
Parker et al. <sup>19</sup>	The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster <i>Saccostrea glomerata</i> (Gould 1850).	20
Parker, Ross & O'Connor, <sup>18</sup>	Comparing the effect of elevated pCO <sub>2</sub> and temperature on the fertilization and early development of two species of oysters.	10
Parker et al. <sup>54</sup>	Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster <i>Saccostrea glomerata</i> in the presence of multiple stressors.	2
Parker et al. <sup>74</sup>	Adult exposure influences offspring response to ocean acidification in oysters.	4
Parker et al. <sup>73</sup>	Persistence of Positive Carryover Effects in the Oyster, <i>Saccostrea glomerata</i> , following Transgenerational Exposure to Ocean Acidification.	2
Parker et al. <sup>29</sup>	Ocean acidification but not warming alters sex determination in the Sydney rock oyster, <i>Saccostrea glomerata</i> .	2
Gibbs et al. <sup>94</sup>	Adult exposure to ocean acidification and warming leads to limited beneficial responses for oyster larvae.	20
Gibbs et al. <sup>95</sup>	Energetic lipid responses of larval oysters to ocean acidification.	2
Gibbs et al. <sup>94</sup>	Adult exposure to ocean acidification and warming remains beneficial for oyster larvae following starvation.	22
Parker et al. <sup>72</sup>	Transgenerational plasticity responses of oysters to ocean acidification differ with habitat.	8
<b>Total</b>		<b>94</b>
	<b>Influence of OA on Sydney Rock Oyster Mortality</b>	
Watson et al. <sup>93</sup>	Early larval development of the Sydney rock oyster <i>Saccostrea glomerata</i> under near-future predictions of CO <sub>2</sub> -driven ocean acidification.	2
Parker et al. <sup>74</sup>	Adult exposure influences offspring response to ocean acidification in oysters.	2
Parker et al. <sup>73</sup>	Persistence of Positive Carryover Effects in the Oyster, <i>Saccostrea glomerata</i> , following Transgenerational Exposure to Ocean Acidification.	1
Parker et al. <sup>54</sup>	Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster <i>Saccostrea glomerata</i> in the presence of multiple stressors.	2
Parker et al. <sup>29</sup>	Ocean acidification but not warming alters sex determination in the Sydney rock oyster, <i>Saccostrea glomerata</i> .	2
Gibbs et al. <sup>96</sup>	Adult exposure to ocean acidification and warming leads to limited beneficial responses for oyster larvae	20
Gibbs et al. <sup>95</sup>	Energetic lipid responses of larval oysters to ocean acidification.	2
Gibbs et al. <sup>94</sup>	Adult exposure to ocean acidification and warming remains beneficial for oyster larvae following starvation.	16
<b>Total</b>		<b>47</b>
	<b>Influence of OW on Sydney Rock Oyster Shell Size</b>	
Parker et al. <sup>19</sup>	The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster <i>Saccostrea glomerata</i> (Gould 1850).	12
Parker, Ross & O'Connor, <sup>18</sup>	Comparing the effect of elevated pCO <sub>2</sub> and temperature on the fertilization and early development of two species of oysters.	6
Parker et al. <sup>54</sup>	Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster <i>Saccostrea glomerata</i> in the presence of multiple stressors.	1
Parker et al. <sup>29</sup>	Ocean acidification but not warming alters sex determination in the Sydney rock oyster, <i>Saccostrea glomerata</i> .	2

(Continued on next page)



Table 2. Continued

Authors	Title	Number of Effect Sizes
Total		21
	<b>Influence of OW on Sydney Rock Oyster Mortality</b>	
Parker et al. <sup>29</sup>	Ocean acidification but not warming alters sex determination in the Sydney rock oyster, <i>Saccostrea glomerata</i> .	2
Total		2
	<b>Transgenerational Exposure to OA on Sydney Rock Oyster Shell Size</b>	
Parker et al. <sup>74</sup>	Adult exposure influences offspring response to ocean acidification in oysters	22
Parker et al. <sup>73</sup>	Persistence of Positive Carryover Effects in the Oyster, <i>Saccostrea glomerata</i> , following Transgenerational Exposure to Ocean Acidification	2
Parker et al. <sup>54</sup>	Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster <i>Saccostrea glomerata</i> in the presence of multiple stressors	2
Gibbs et al. <sup>96</sup>	Adult exposure to ocean acidification and warming leads to limited beneficial responses for oyster larvae	22
Gibbs et al. <sup>94</sup>	Adult exposure to ocean acidification and warming remains beneficial for oyster larvae following starvation	20
Parker et al. <sup>72</sup>	Transgenerational plasticity responses of oysters to ocean acidification differ with habitat	8
Total		76
	<b>Transgenerational Exposure to OA influence on Sydney Rock Oyster Mortality</b>	
Parker et al. <sup>74</sup>	Adult exposure influences offspring response to ocean acidification in oysters	4
Parker et al. <sup>73</sup>	Persistence of Positive Carryover Effects in the Oyster, <i>Saccostrea glomerata</i> , following Transgenerational Exposure to Ocean Acidification	2
Parker et al. <sup>54</sup>	Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster <i>Saccostrea glomerata</i> in the presence of multiple stressors	2
Gibbs et al. <sup>96</sup>	Adult exposure to ocean acidification and warming leads to limited beneficial responses for oyster larvae	16
Gibbs et al. <sup>94</sup>	Adult exposure to ocean acidification and warming remains beneficial for oyster larvae following starvation	20
Total		44

### Results of moderator analysis

Varying levels of fertilization regimes, pH, feeding regimes, oyster lines, and the age of individuals measured did not reduce the variance within the model and therefore were not included in the moderator analysis.

When temperature and parental treatments were added to the model, overall heterogeneity within the model ( $I^2 = 1.02\%$ ) decreased compared to the overall model ( $I^2 = 56.03\%$ ) (Figures S11 and S12). Within-study heterogeneity decreased to 1.02%, whereas the between-study heterogeneity decreased to 0%.

Results of an omnibus test on the multi-moderator model indicated that at least one of the moderators significantly influenced the effect of OA on mortality. Further analysis of the coefficients showed that there was a moderating effect of parental exposure to OA and parental exposure to OW (but not OW + OA) on the magnitude of the effect of increased OA and mortality when oysters with the same parentage were compared. TGP to OA decreased the pooled effect of OA on mortality by  $-0.405$  compared to the overall pooled effect, and TGP to OW decreased the pooled effect of OA on mortality by  $-0.902$  (Table 4). Additionally, treatment temperature was shown to have a significant impact on the effect of OA on mortality. The results indicate that for every 1-degree increase in temperature, there seems to be a  $-0.169$  decrease in the magnitude of the effect of OA on mortality (Table 4). This was consistent for every level of pH and for all parental treatments.

### Effect of OW on size

There was a significant overall negative impact of OW on oyster shell size ( $R = -0.97$ ,  $p = 0.0064$ ; Figure 3; Table 6). Evaluation of the  $I^2$  statistic showed that sampling error explained 37.15% of the variance within the moderator model, and overall heterogeneity was quite high ( $I^2 = 62.85\%$ ; Figure S13). The observed heterogeneity within clusters was extremely low and significantly influenced the results of the model ( $I^2_{\text{level } 2} = 0\%$ ,  $p = 0.0014$ ). Conversely, the heterogeneity between studies explained approximately two-thirds of the observed variance in the model and did not significantly influence the results ( $I^2_{\text{level } 3} = 62.85\%$ ,  $p = 1$ ).

**Table 3. Explanation of study meta data variables used in the meta-analyses**

Study Meta-data Variables	Explanation of Variables
Parental Treatment	Categorical variables representing the groups of parental treatment (e.g., Ambient, Elevated Temperature, Elevated Temperature and pH etc.)
Parental Temperature Exposure	Temperature that the parents were exposed to for testing
Parental pH Exposure	pH that the study groups parents were exposed to for testing
Feeding Regime	How often were the larvae were fed
Fertilisation Regime	If the larvae were fertilised underneath ambient or treatment conditions
Types of Oyster Lines	Specific genetic lines of oysters tested (e.g., Wild, selected, QX resistant etc.)
Larval Treatment Temperature	Temperature that the larvae were exposed to for testing
Larval Treatment pH	pH that the larvae were exposed to for testing
Salinity	Salinity value of the seawater used in the study
Age of Larvae when tested	Age of the larvae tested
Generation	Generation of test larvae (e.g., F <sub>0</sub> , F <sub>1</sub> , F <sub>2</sub> etc.)
Life Stage Tested	Whether larvae or adults were tested

#### Results of the moderator analysis

Results of an omnibus test on the moderator model showed that there were no significant moderating effects of any of the variables on the effect between temperature and size.

#### Effect of transgenerational exposure to OA on shell size

Parental exposure to OA had a moderate significant positive correlation with shell size at the same treatment pH exposure ( $R = 0.79$ ,  $p = 0.0119$ ; Figure 4; Table 6). Evaluation of the  $I^2$  statistic showed that sampling error explained 45.38% of the variance within the model and that overall heterogeneity was quite high ( $I^2 = 54.62\%$ ; Figure S14). The observed heterogeneity within clusters was low ( $I^2_{\text{level } 2} = 16.78\%$ ), whereas the heterogeneity between studies explained roughly one-third of the observed variance within the model ( $I^2_{\text{level } 3} = 37.84\%$ ).

#### Results of the moderator analysis

Results of an omnibus test on the moderator model showed that there were no significant moderating effects of any of the variables on the effect of transgenerational exposure to OA on shell size.

#### Influence of transgenerational exposure to OA on mortality

Parental exposure to OA had a small significant negative correlation with mortality at the same treatment pH exposure ( $R = -0.31$ ,  $p = 0.0121$ ; Figure 5; Table 6). Evaluation of the  $I^2$  statistic showed that sampling error explained 100% of the variance within the model, with no heterogeneity observed between studies and within studies (Figure S15).

#### Results of the moderator analysis

Results of an omnibus test on the moderator model showed that there was a significant moderating effect of larval treatment (e.g., larvae exposed to ambient conditions or elevated conditions). When larval treatments were added to the model, overall heterogeneity within the model remained the same as the full model ( $I^2 = 0\%$ ) (Figure S16). Analysis of the coefficients showed that when larvae of exposed parents were exposed to ambient conditions, there was an increase in mortality with a pooled effect estimate of 0.542 (Table 5). Conversely, when larvae of exposed parents were exposed to elevated conditions, there was a decrease in mortality, with a pooled effect estimate of  $-0.453$ .

**Table 4. Moderator Analysis of temperature and parental treatment on OA influence on *S. glomerata* mortality**

		R value	S.E.	DF (Satt)	Lower 95% CI	Upper 95% CI	t-stat	p-val (Satt)
Temperature		-0.169	0.0389	2.38	1.187	9.5906	-4.34	0.0355
Parental Treatment	Difference from Pooled Estimate	Pooled Estimate	SE	DF (Satt)	Lower 95% CI	Upper 95% CI	t-stat	p-val (Satt)
OA	-0.405	5.153	1.2409	3.78	0.702	9.6041	-2.92	0.0462
OW	-0.902	4.656	1.1408	1.18	0.256	9.0565	-23.46	0.016
OA and OW	-2.059	3.499	1.5656	1.14	-4.992	11.9902	-4.44	0.1178



**Table 5. Moderator analysis of larval treatment/exposure on the transgenerational exposure to OA effect on *S. glomerata* mortality**

Larval Treatment	Pooled Estimate	S.E.	DF (Satt)	Lower 95% CI	Upper 95% CI	t-stat	p-val (Satt)
Ambient Larvae	0.542	0.154	3.02	0.0534	1.03	3.52	0.0386
Elevated Larvae	-0.453	0.359	2.09	-1.8073	0.88	-4.86	0.0364

## DISCUSSION

Overall these meta-analyses used robust procedures to ensure independence,<sup>97–101</sup> the best fit<sup>102,103</sup>, reduce bias,<sup>104</sup> avoid distortion of results<sup>105</sup> and ensure robust variance estimates<sup>106</sup> to enhance model robustness and validity by correction for heterogeneity and varying sample sizes.<sup>107–109</sup>

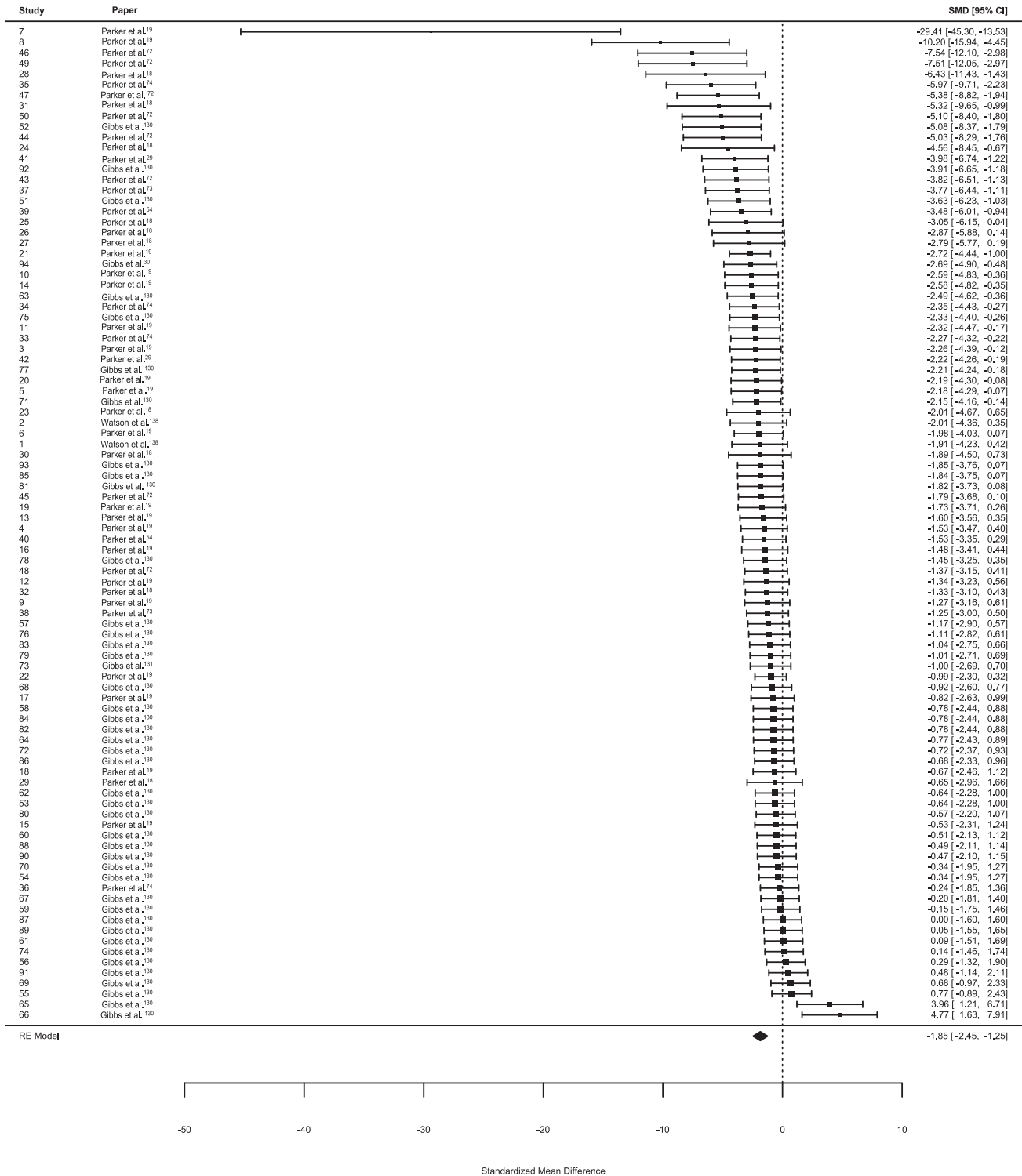
As a consequence of this robust methodology, these meta-analyses confirmed that OA has a highly significant impact on shell size and mortality, and OW has a significant impact on the shell size of the Sydney rock oyster, *S. glomerata*, an ecologically and economically significant oyster in Australia. The high R values (all between 0.69 and 0.97; Summary Table 6) reinforce the significance of the impact of OA and OW on oysters. These meta-analyses also provide evidence that across repeated independent studies OA and OW decreased the shell size of oysters and OA increased mortality. This outcome agrees with that found in multispecies meta-analyses of oyster species and marine bivalves in general.<sup>110</sup> When OA and OW were combined, temperature ameliorated the impacts on mortality in elevated pH conditions. Specifically, for each 1°C increase in temperature there was 0.165 decrease in the mortality of oysters. While other studies have found OW to ameliorate OA when combined<sup>40</sup> there maybe a threshold temperature beyond which OW is ineffective to ameliorate OA.

These meta-analyses also confirm that transgenerational parental exposure to OA has potential to improve offspring outcomes as found over a decade ago.<sup>74</sup> In this study, both the magnitude of the effect between like-for-like groups (e.g., ambient parents and ambient offspring vs. ambient parents and elevated offspring) and unlike groups (i.e., ambient parents and elevated offspring vs. elevated parents and elevated offspring) were tested. As mentioned, TGP has been considered as a potential rescue for marine organisms from the impacts of OA and OW, allowing sufficient time for genetic adaptation to occur.<sup>31</sup> In this study, meta-analyses of transgenerational experiments revealed that TGP moderated the negative effects of OA on the size of *S. glomerata* and the negative effects of OA (but not OW) on mortality. Interestingly, TGP to OA reduced the mortality of *S. glomerata* when offspring were reared in the same treatment as their parents (i.e., elevated OA parents, elevated OA offspring) but increased mortality when the parent-offspring environment was mismatched (i.e., elevated OA parents, ambient OA offspring). This maladaptive nature of TGP when there is a mismatch between the parent and offspring environment has been reported in several studies,<sup>111–113</sup> as parents prime the phenotype of their offspring for an environment that they subsequently do not encounter. As OA is projected to be a constant stressor in our future, such mismatches in the OA level of the parent-offspring environment may not be encountered in oceans but perhaps more common in estuaries where pH can vary. Importantly, this study found that TGP was not a moderating factor for all traits measured. In this study, we were unable to complete the meta-analyses to test whether parental exposure to OW will increase mortality of offspring. While we predict that parental exposure to OW will cause increased mortality, the experiments to provide evidence for this hypothesis are yet to be done. More research is needed to resolve the impact of parental thermal history, warming, and heat wave stress events on gamete quality and performance of oyster offspring in the context where responses are expected to be influenced by local thermal adaptation.

That OA decreases size and increases mortality of oysters is an old rather than new finding. Several studies from 2009 onward have reported on this, through experiments that have used both short-term acute exposures (days) and longer-term chronic exposures (weeks–months).<sup>18,19,74,114</sup> While individual studies have not always reported decreases in oyster size in response to OA at all life history stages,<sup>21,115</sup> in the analyses undertaken here OA decreased size across life history stages, although there are surprisingly few studies on adult Sydney rock oyster species to be conclusive. It is possible that these negative effects still may have greater implications for larvae than for juveniles or adults. For example, a reduction in shell size may have only mild consequences for juveniles and adults. A reduction in shell size for larvae, however, may mean that larvae spend more time in the water column, increasing their risk of starvation, predation, and disease.<sup>14</sup> Further, stressed larvae that are smaller at settlement have been found to have reduced energy reserves and thus may suffer reduced metamorphosis, settlement, and recruitment into the adult population.<sup>65,116</sup> Still reductions in size of *S. glomerata* juveniles could lead to increased predation, reduced competitive advantage over other cooccurring faster growing species such as the Pacific oyster, *Crassostrea gigas*,<sup>117</sup> and an increased time to reach market size, potentially reducing the profitability of the Sydney rock oyster industry. While TGP has promise to enhance resilience of oysters in the face of changing climate, they live in a multi-stressor world, and it will be important to understand what traits confer resilience, in response to stressors and their combination.

### Limitations of experimental designs

There have been criticisms of the experimental studies done on the impact of OA and OW on early life history stages of Sydney rock oysters for reasons such as they have been based on selected family lines<sup>32</sup> or species that are commercially important.<sup>63</sup> The effects described in these meta-analyses are, however, inclusive of both wild and selected family lines. The use of selected lines may have also increased the robustness of work on *S. glomerata*, ensuring a level of genetic diversity exists in the oysters being tested. For example, studies that rely on wild oysters (or any marine organism) are almost always assuming their test subjects represent genetic diversity. However, the use of selectively bred oysters allows for a greater level of assured genetic diversity, which may represent greater genetic diversity compared to wild oysters.<sup>118</sup>



**Figure 1.** Forest plot showing the standardised mean differences for each study on the effect of OA on shell size. The black diamond represents the pool estimate.

**Table 6. Summary of meta-analyses of OA and OW and TGP to OA, effects on shell size and mortality and their significance as well as direction and magnitude of the effect**

Meta analysis	Variable	Significance	Direction	Magnitude
1. OA	Shell size	✓	↓	-0.95
2. OA	Mortality	✓	↑	0.68
3. OW	Shell size	✓	↓	-0.97
4. TGP to OA	Shell size	✓	↑	0.79
5. TGP to OA	Mortality	✓	↓	-0.31 <sup>a</sup>

Up arrows indicate a “positive impact” e.g., increasing shell size, decrease in mortality.

Down arrows indicate a “negative impact” e.g., decreasing shell size, increase in mortality.

<sup>a</sup>This effect had significant moderators which change the interpretation (caution advised on interpreting overall effect). (i) Moderator analysis showed that larval treatment (larvae exposed to ambient or elevated OA), had different effects between the groups. (ii) Ambient larvae and ambient parents lead to an increase in mortality. (iii) Elevated larvae and elevated parental exposure lead to a decrease in mortality.

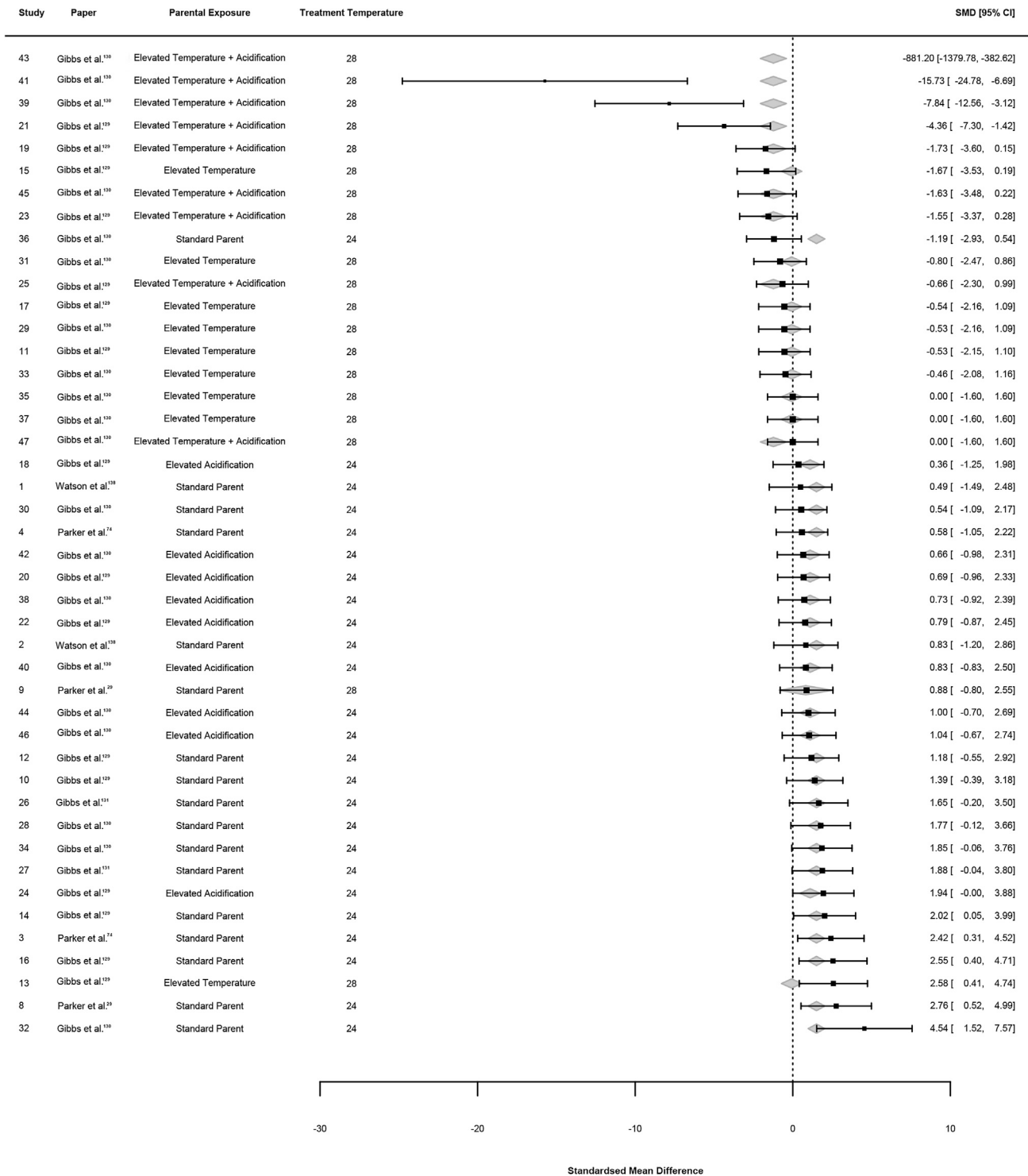
Limitations may also arise from results of laboratory-controlled experiments because they may not reflect the real and variable multi-stressor estuary environment.<sup>11</sup> It is important that future experiments move toward mesocosm studies more representative of the real world of an oyster. Mesocosm studies have also found reduced adult growth in some species of bivalves including *Trichomya hirsuta* but not in *Mytilus galloprovincialis*.<sup>119</sup> Short-term exposures, whether based in the laboratory or mesocosm, have been criticized for potentially not reflecting the real world and simulating the more realistic and gradual changes that will occur with climate change.<sup>120</sup> In some areas of the globe such as Antarctica<sup>121</sup> or coral reefs,<sup>122</sup> free-ocean CO<sub>2</sub> enrichment (FOCE) systems to simulate OA experiments in the field have been successful. It is also important that experimental designs incorporate fluctuations in pH that marine organisms experience on a daily and seasonal basis. Concerningly the estuaries where Sydney rock oysters are located are acidifying, warming and freshening at an order of magnitude greater than that predicted by global oceanic models.<sup>2</sup> Studies done to determine the interactive effect of tidal (air) emersion and OA have found the impacts of OA to potentially be underestimated.<sup>123</sup> A significant limitation of previous studies is that they do not simulate tidal fluctuations and reductions of daily and seasonal pH. Nor do they combine the impact of OA and OW with variation of other stressors such as salinity, tidal emersion, and food availability.

Although logistically complex and difficult, experimental designs that overcome the limitations of current understandings mainly based on laboratory-based experiments are needed. Further, to compare results from different studies and improve reproducibility in experiments, standard protocols are needed for experiments and reporting of carbonate chemistry.<sup>124</sup>

### Adaptive capacity and solutions

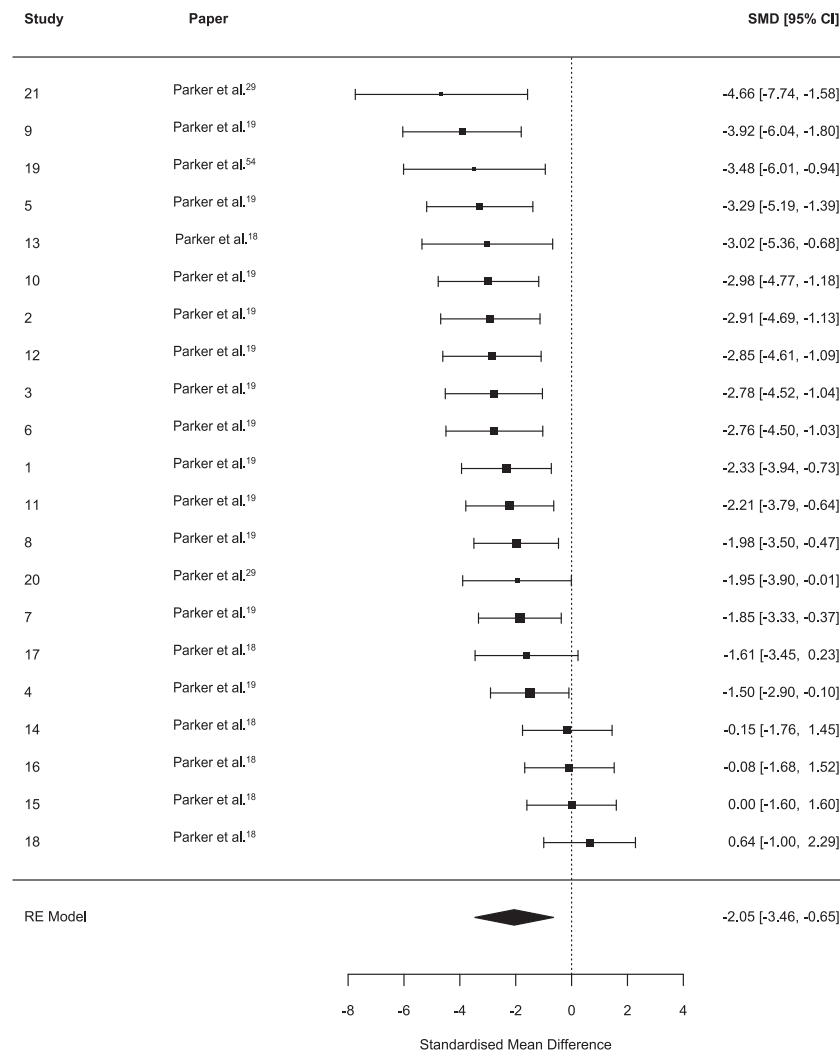
Whether the adaptive capacity of oysters and other marine organisms will rescue species from OA and OW remains largely unknown.<sup>6,31</sup> Although TGP has been identified as a mechanism of acclimation to global change, the weight of evidence indicates that parental conditioning over generations is not a universal panacea to reduce stress sensitivity in offspring.<sup>71</sup> While parental exposure was found in this study to be an important moderating variable that reduced the severity of the impacts of OA on the size and mortality (OA only) of *S. glomerata*, several critical questions relating to TGP in bivalves remain unanswered. These questions include the following: (1) Do TGP effects persist long enough to protect *S. glomerata* across their entire life cycle? (2) What are the implications of TGP for subsequent generations? (3) How does TGP interact with within-generation plasticity? (4) Are there any maladaptive effects of TGP? (5) What is the combined influence of TGP on OA and OW on offspring? (6) What is the influence of TGP on OW? It is clear given the number of questions and as mentioned above, more studies on TGP and especially TGP and OW are required.

To date, studies have focused predominantly on the short-term benefits of TGP for offspring during their early life history stage, with little consideration of later life history stages, particularly adults.<sup>31</sup> This likely stems from the fact that early life history stages have been found to be most vulnerable to OA and OW, coupled with the difficulties in rearing marine organisms with long generation times across their entire life cycle. Potential maladaptive effects of TGP are also rarely considered. A greater understanding of these critical knowledge gaps across a broad range of oyster species is essential to determine with more certainty whether TGP will protect oyster populations against the OA and OW over this century.



**Figure 2. Forest plot showing the standardised mean differences for each study on the effect of OA on mortality**

The gray diamonds represent each studies effect size when adjusted for moderators within the moderator models. Overall pooled estimate is not shown on this figure due to it being misrepresentative of the underlying effects.



**Figure 3. Forest plot showing the standardised mean differences for each study on the effect of OW on shell size**  
The black diamond represents the pool estimate.

Emerging evidence suggests that the impact of climate change and the response of oysters will also be likely to differ depending on the estuary in which oysters are located. An oyster in a highly urbanized estuary is more likely to experience greater stress compared to an oyster in a relatively pristine estuary because of influences and changes in the surrounding catchment. Scanes et al.<sup>2</sup> found that temperature, pH, and salinity of estuaries were dependent on their morphology, estuaries being warmer when catchments were urbanized. An increase in hot-paved areas and a decrease in riparian vegetation that provides shade increases the temperature of water that flows into estuaries. Estuaries where oysters are located are also known for acid-sulfate runoff with strong diel and seasonal metabolic shifts.<sup>125</sup> These stressors can then also interact with pollution from anthropogenic sources.<sup>56</sup> Nature-based solutions such as coculture of oysters with seagrasses and macroalgae have been suggested as solutions to limit the impact of OA and other multiple stressors and may act as refugia for oysters in estuaries.<sup>126,127</sup> Seagrasses can also act as a microbial filter, remove 50% of bacterial pathogens, and reduce disease in oysters.<sup>44</sup> The survival of oysters is likely to depend on how many stressors oysters face at one point in time, and the interaction with disease will make persistence more challenging.<sup>44,45</sup>

Even against a stressful background there may be some capacity of oysters to resist stressors. Responses to OA and OW in *S. glomerata* have been observed to differ among oyster genotypes for several traits, including standard metabolic rate and extracellular pH<sub>e</sub>.<sup>21,22,27,74,128</sup> Exposure of *S. glomerata* adults from 24 genetically distinct pair-mated families to OA and OW for example led to a significant reduction in extracellular pH (pH<sub>e</sub>) in 11 families but not in the remaining 13.<sup>27</sup> Similarly, the microbiome of *S. glomerata* was altered by OA and OW in only three out of 12 selected families.<sup>22</sup> This provides hope that sufficient genetic diversity exists within *S. glomerata* populations to adapt to their rapidly changing environment, or at the very least, that selective breeding may be a tool to minimize the impacts of climate change on this iconic aquaculture species and help with oyster farming and reef restoration efforts into the future. A concern is that we do not know the

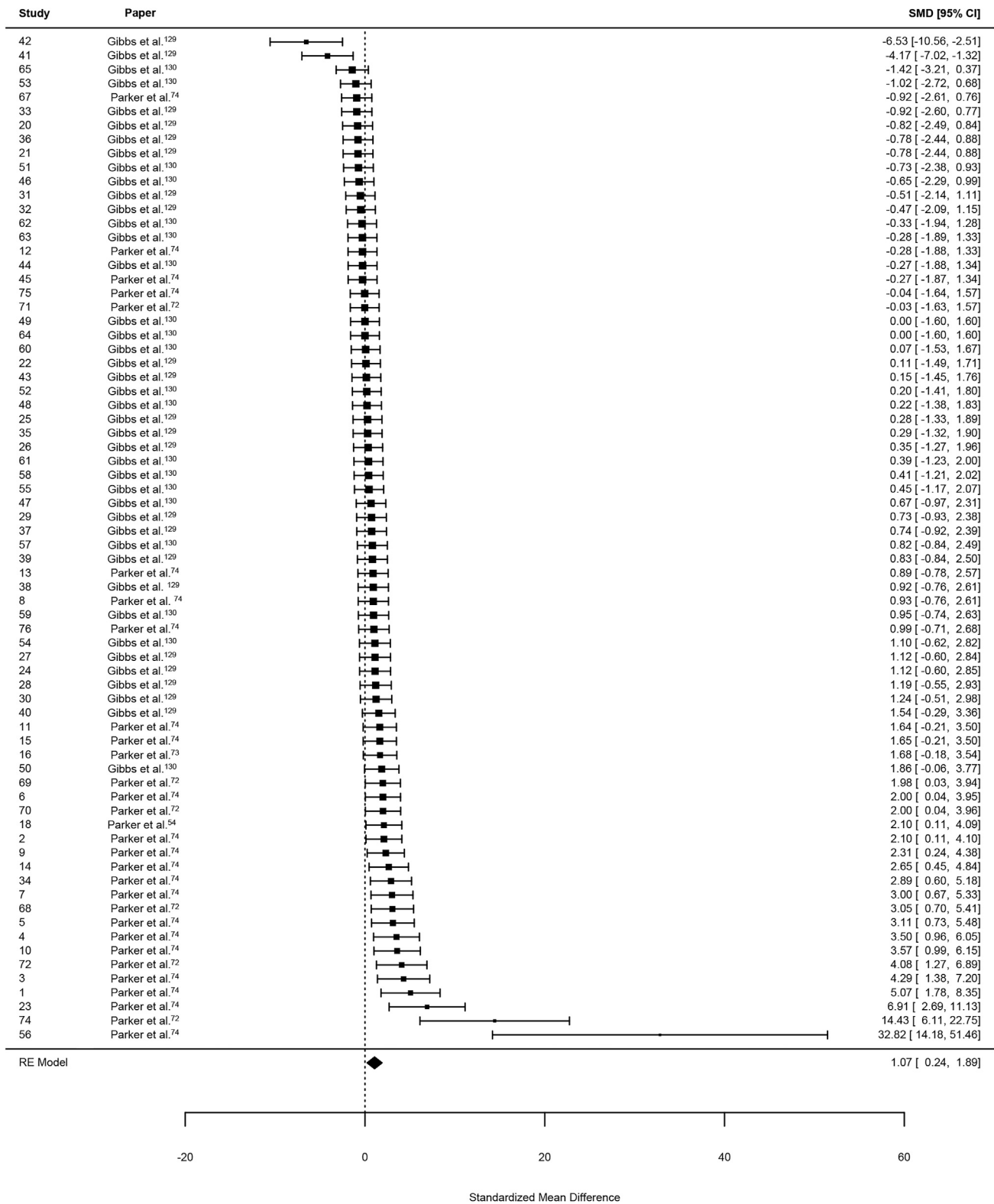
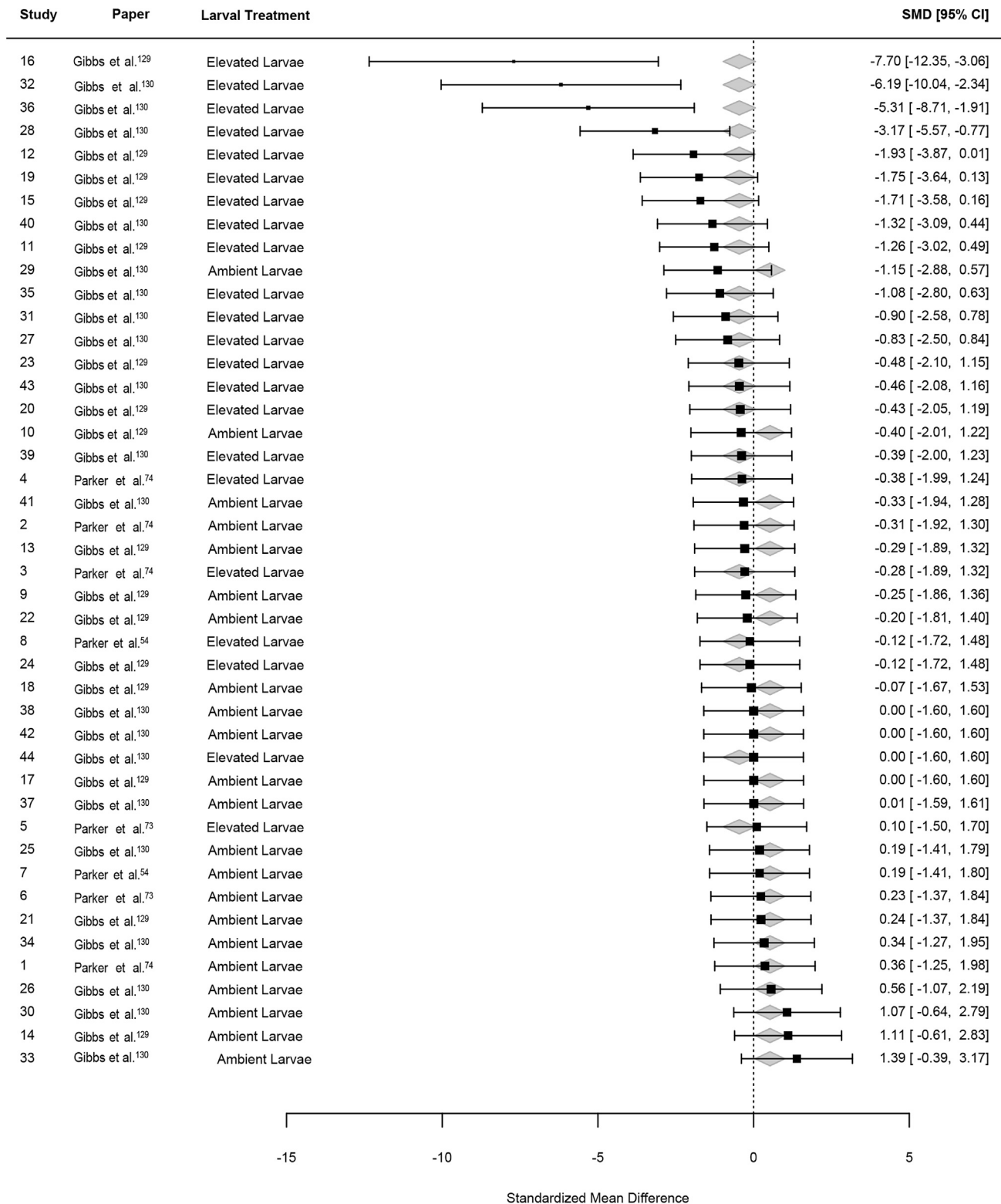


Figure 4. Forest plot showing the standardised mean differences for each study on the effect of transgenerational exposure to OA on shell size





**Figure 5. Forest plot showing the standardised mean differences for each study on the effect of transgenerational exposure to OA on mortality**  
The gray diamonds represent each studies effect size when adjusted for moderators within the moderator models. Overall pooled estimate is not shown on this figure due to it being misrepresentative of the underlying effects.

physiological limits that divert energy away from other energetically demanding processes such as immune responses and reproduction. The consequence of a lowered immunity is that it may permit viral and bacterial infection to result in disease outbreaks and reduced oyster production.<sup>45</sup> The consequences for reproduction include slowed gametogenesis, reduced lipid content of eggs, and/or reduced fecundity,<sup>29</sup> which have the capacity to negatively impact future generations.

The underlying mechanisms that allow some populations of *S. glomerata* to display increased resilience to OA and OW remain largely unknown. Increased resilience via TGP was previously thought to occur via an increase in maternal provisioning, thereby increasing the energy available to offspring to withstand the anticipated suboptimal conditions.<sup>129,130</sup> However, for many organisms, including *S. glomerata*, this is not the case.<sup>29,35,131,132</sup> Studies assessing the size and total lipid content in eggs of *S. glomerata* following parental exposure to OA and/or OW, for example, have found no significant difference between eggs from exposed and non-exposed parents.<sup>54,94–96</sup> Epigenetic mechanisms, such as DNA methylation, histone modification, and non-coding RNA are now believed to be a potential primary driver of beneficial TGP effects to OA and OW.<sup>133</sup> Research into epigenetics and oysters is still largely in its infancy, however, with more research needed to confirm or exclude its role as an underlying mechanism of TGP. Other reasons suggested for resilience to OA and/or OW are an inherited increase in whole-organism standard metabolic rate (SMR).<sup>74</sup> An increased SMR has been suggested to be a feature of resilience to OA in other marine organisms<sup>23,134</sup> and is thought to facilitate higher ion and acid-base regulation, growth, and protein synthesis.<sup>135</sup> Not all studies, however, have reported increased metabolic costs of the major ion regulators Na<sup>+</sup>/K<sup>+</sup>-ATPase, H<sup>+</sup>-ATPase, and Na<sup>+</sup>/H<sup>+</sup>-exchanger in mantle or gill tissues.<sup>136</sup> Instead, they have suggested an increased capacity for metabolic CO<sub>2</sub> removal, via higher, more energy-efficient gill filtration rates. Further research into the underlying mechanisms of resilience of *S. glomerata* to OA and OW is fundamental to our understanding of how this ecologically and economically important species will cope.

## Conclusion

While our view of the potential impacts of OW and OA and their interaction on molluscs, and the Sydney rock oyster in particular, have been fine-tuned over the course of our research, our concerns remain well founded. This study provides confidence about the significant and negative impacts of climate change on this valuable economically and ecologically significant oyster species. Some of this confidence is the result of the hierarchical/multi-level structure approach used in these meta-analyses, which reduced the inherent publication bias and the likelihood of type 1 and 2 errors through robust variance estimation process.<sup>137,138</sup> This study highlights the limitations of experimental designs and research gaps, which include the need for more research on OW, the possible synergistic interactions between OA and OW and other stressors, the need for more long-term mesocosm and field experiments with multiple generational exposures, and finally the need for a greater understanding of whether TGP will be beneficial for the Sydney rock oyster and buy the time needed for genetic adaptation to occur. This study, however, also provides hope through elucidating the adaptive potential of oysters. Our most recent research suggests that there are genetically distinct oyster families that vary in their responses to climate change without a negative trade off and loss of energy as found in our early studies.<sup>27</sup> By addressing the experimental gaps highlighted here, there becomes a clearer pathway forward for the sustainability of this iconic oyster.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- METHODS DETAILS
  - Literature searches
  - Data extraction
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Meta-analytic approach

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2024.110673>.

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## AUTHOR CONTRIBUTIONS

Conceptualization and funding: P.M.R., L.M.P., W.A.O'C., and M.G.; investigation: P.M.R., L.M.P., W.A.O'C., and M.G.; formal analysis: C.P.; validation: P.M.R., E.S., L.M.P., and M.B.; writing—original draft: P.M.R., C.P., L.M.P., M.B., and E.S.; writing—review & editing: P.M.R., E.S., M.B., and L.M.P.; resources: W.A.O'C.; supervision and administration: P.M.R.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
All Analyzed data and code	This paper	<a href="http://www.doi.org/10.5281/zenodo.12615650">http://www.doi.org/10.5281/zenodo.12615650</a>
Extracted Data from Papers	This paper	<a href="http://www.doi.org/10.5281/zenodo.12615650">http://www.doi.org/10.5281/zenodo.12615650</a>
Software and algorithms		
R Version 4.4.0	R Core Team	<a href="https://www.r-project.org/">https://www.r-project.org/</a>

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Pauline M. Ross ([pauline.ross@sydney.edu.au](mailto:pauline.ross@sydney.edu.au)).

#### Materials availability

This study did not generate new unique materials.

#### Data and code availability

- All raw data files have been deposited at Zenodo Repository 50 and are publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- All original code has been deposited at Zenodo Repository and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- Any additional information required to reanalyse the data reported in this paper is available from the [lead contact](#) upon request.

### METHODS DETAILS

The meta-analysis completed here involved several steps including literature searches, data extraction and the meta-analytic approach as described below. Overall, there were eight separate literature searches each done on.

- (1) OA and size
- (2) OA and mortality
- (3) OW and size
- (4) OW and mortality
- (5) Transgenerational exposure to OA and size
- (6) Transgenerational exposure to OA and mortality
- (7) Transgenerational exposure to OW and size
- (8) Transgenerational exposure to OW and mortality

#### Literature searches

A systematic search strategy was used to identify articles containing relevant information to be included within each meta-analysis topic of interest<sup>83</sup> (Table 1). Appropriate index terms were identified within the first database, and synonyms were identified using a thesaurus.<sup>84</sup> Using these terms, search strings were constructed to investigate the impact of OA and OW on size and mortality. The process involved combining the selected terms with Boolean operators and field codes to identify suitable studies within each database. To ensure high coverage and precise retrieval, these search strings were optimized and iterated until a satisfactory balance was achieved between capturing the maximum number of relevant articles and avoiding excessive non-relevant texts. High coverage of relevant texts was deemed acceptable when search strings could identify all pertinent studies that were also found through non-database methods, such as reference-checking in included studies from initial and pilot searches and author's field expertise.

The search was executed on three databases: Web of Science Core Collection, Scopus, and ProQuest Central<sup>85–87</sup> (Table 1). The choice of databases was based on their coverage: Web of Science and Scopus are known for covering a broad spectrum of high-quality peer-reviewed journals, whereas ProQuest Central was chosen for its amalgamation of peer-reviewed journals and gray literature, a feature deemed essential for this research.<sup>83</sup> Literature searches were conducted from 15<sup>th</sup> to 22<sup>nd</sup> March 2023 for the first four meta-analyses and the 18<sup>th</sup> of April 2024 for the last four meta-analyses.

Upon completion of the searches, duplicate entries were removed. The remaining papers then underwent title and abstract screening to determine their relevance to the defined topic of interest. Studies that cleared this phase then underwent full-text screening for relevance. Those deemed relevant were then evaluated for eligibility for inclusion within each meta-analysis. The eligibility criteria remained consistent across all eight topics of interest. Reports were excluded from the meta-analysis if the data required for calculating standardized mean differences were inaccessible (e.g., missing means and standard deviations), treatment groups were not comparable with respect to the topic of interest, or the data quality and/or experimental design were insufficiently robust to warrant inclusion in the final analysis. Moreover, in the context of meta-analyses on size, studies were disregarded if they used a measure of growth rather than size, as this could introduce confounding factors by blending disparate measures into a single dataset.<sup>88,89</sup>

For the meta-analysis assessing the impact of OA on size, a total of 11 studies were found to be eligible, yielding 94 effect sizes (Tables 1 and 2; Figure S1). The meta-analysis aimed at elucidating the interplay between OA and mortality incorporated eight studies, resulting in a total of 47 effect sizes (Tables 1 and 2; Figure S2).

The meta-analysis investigating the effect of transgenerational exposure to OA on size found six studies, with a total of 76 effect sizes (Table 1; Figure S3), whereas the meta-analysis assessing the effect of transgenerational exposure to OA on mortality incorporated five studies, with a total of 44 effect sizes (Tables 1 and 2; Figure S4).

Literature searches investigating the effect of OW on mortality ( $K = 1$ ) fell short of the minimum study count ( $K = 3-5$ ) considered for a robust meta-analysis (Figure S5–S7)<sup>90–92</sup>; and as such, meta-analyses could not be done. Additionally, literature searches conducted on the effect of transgenerational parental exposure to OW on size ( $K = 0$ ) and mortality ( $K = 0$ ) did not uncover any studies that were eligible for analysis (Tables 1 and 2).

While there were only four studies that were identified on the effect of OW on shell size, which was below the ideal number of studies for a robust meta-analysis ( $K = 5$ ), the meta-analysis was still done because of the significant number of effect sizes extracted from this study (Tables 1 and 2; Figure S8). Nonetheless, the results derived from the analysis warrant cautious interpretation because they are more likely to type 1 error due to the low number of publications.

In summary, the five meta-analyses completed were as follows:

- (1) OA and size
- (2) OA and mortality
- (3) OW and size
- (4) Transgenerational exposure to OA and size
- (5) Transgenerational exposure to OA and mortality

## Data extraction

Upon identifying pertinent articles, key metrics such as means, standard deviation, standard error, sample size, and study meta-data (Tables 2 and 3), were meticulously extracted through a detailed examination of the respective study and its associated raw data, when accessible. In instances where raw data were unavailable, means, standard deviations, and standard errors were digitized from the corresponding plots/graphs in the paper, using the r-package *metaDigitise*.<sup>97</sup> Due to different pH scales used throughout the literature, all pHs were converted to the NBS pH scale to ensure consistency throughout the meta-data.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Meta-analytic approach

#### Calculation of effect sizes

Standardized mean differences (Cohen's  $d$ ) were computed for each topic of interest by contrasting the control groups with the treatment groups (e.g., pH 8.2 and pH 7.8). Confounding influences from other experimental variables within each study were mitigated by ensuring comparisons were made only between control and treatment groups that shared identical experimental variables.

In cases where a single control group was utilized to calculate multiple effect sizes within a study, the sample size of the control group was evenly distributed among all comparison groups. This approach was adopted to circumvent unit-of-analysis errors, which are likely to occur when effect sizes are correlated.<sup>88,89</sup> Dividing the sample size of the shared group was chosen over the more optimal approach of group combination, primarily due to the marked dissimilarity among the groups.<sup>88,89</sup> Given the low sample size in each of the studies ( $n = 3$ ), a small-sample correction was applied to the standardized mean differences to generate Hedges'  $g$ . This adjustment is necessary since when the sample size is equal to or less than 20, an upward bias in the results is introduced.<sup>98</sup>

#### Meta-analytic modeling

A three-level correlated and hierarchical effects (CHE) meta-analytic model was selected for all four topics of interest, with each topic of interest being modeled separately. This class of models was chosen to account for the dataset's unique structure, which contains multiple effect sizes derived from a single study.<sup>92,99,100</sup> This approach is given that traditional meta-analytic methods inherently assume effect sizes to be independent.<sup>92,99,100</sup> In contrast, the CHE models acknowledge that effect sizes within clusters (i.e., studies) are inherently correlated and non-independent.<sup>99–101</sup>

To determine the optimal assumed correlation value between within-study effect sizes for the CHE models, several sensitivity analyses were conducted. This entailed operating each model with correlation values ranging from 0 to 1 in increments of 0.01. The best-fitting CHE model was then identified by considering the Akaike information criterion (AIC), Bayesian information criterion (BIC), and log likelihood values.<sup>102,103</sup>

All models were computed using restricted maximum likelihood (REML) estimation testing to provide an unbiased estimation of variance and covariance parameters. This approach was favored over maximum likelihood estimation due to its propensity for downward bias.<sup>90,104</sup>

### Model evaluation

The variance of the meta-analytic models was evaluated by looking at the level-2 and level-3  $I^2$  variance.  $I^2_{\text{level-2}}$  represents the amount of between-study variance, whereas  $I^2_{\text{level-3}}$  represents the amount of variance within studies and is an important tool when evaluating three-level meta-analytic models, as it provides a breakdown of the percentage of variation associated with each level.<sup>99</sup>

### Influence analysis

An influence analysis was carried out on each model upon completion to identify studies that could potentially distort the overall pooled effect of the meta-analysis.<sup>105</sup> This involved the computation of Cook's distance for each data point within the model, with cases deemed influential when their Cook's D value met or exceeded four times the mean of Cook's D.<sup>105</sup>

Once influential cases were identified, they were subsequently removed from the dataset, and the three-level meta-analytic models were re-run. The  $I^2$  variance values of the models, excluding influential cases, were juxtaposed against those of the full models. The dataset exhibiting the lowest  $I^2$  value was selected for analysis and subsequent moderator analysis.

### Moderator analysis

Initially, a meta-analysis was done, followed by the fitting of an additional meta-analytic moderator model to assess the impact of moderators on the treatment effects (Tables 4 and 5). Moderators were incorporated in a stepwise manner into the original model. The Akaike information criterion (AIC), Bayesian information criterion (BIC), and log likelihood of each model were evaluated to identify the model that exhibited the best fit to the data.<sup>102,103</sup>

When pH was included as a moderator, only studies that had a control pH of  $\sim 8.2$  and an elevated treatment of pH 8, 7.94, 7.93, 7.91, 7.9, and 7.84 were included within the analysis. This was because the sample size of effects containing other levels of pH was not large enough for robust analysis. The influence of salinity on mortality was not explored for the same reason.

### Robust variance estimation

A robust variance estimation (RVE) procedure using the R package "ClubSandwich" was conducted on all the models prior to the calculation of coefficients and  $p$  values.<sup>92,106</sup> This procedure was used to account for the dependency of effect sizes within the dataset and enhance the model's robustness against potential misspecification.<sup>91,92</sup> The coefficients of the final model were examined, and  $p$  values were calculated using the "coef\_test()" function in ClubSandwich, allowing identification of moderators that significantly influenced the effect size.<sup>106</sup> Satterthwaite bias-reduced linearization adjustment was applied to the coefficients to precisely unbiased the variance-covariance estimator when the model is user-specified.<sup>101,107</sup> Further, a Satterthwaite correction was applied to the  $p$  values to accommodate varying variances and sample sizes across studies and to account for heterogeneity within the meta-analytic models.<sup>108,109</sup>