Review

Meta-analysis identifies metabolic sensitivities to ocean acidification

Running title: Ocean acidification impacts metabolic function

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Abstract: Ocean acidification is expected to have wide-ranging and complicated impacts on organismal physiology, notably metabolism. Effects on metabolism may have numerous consequences at the whole-organism level, in particular costs to growth, locomotion, reproductive output, and homeostasis. Negative effects on these metrics may further cascade up to impact ecosystem structure and function, and thus human society. As such, the study of metabolism in response to ocean acidification is a pivotal research avenue within the study of global ocean change. Here, the metabolic responses of marine species to ocean acidification are reviewed and examined through meta-analysis. We reviewed a total of 44 published studies and used a traditional meta-analysis to identify broad-scale trends in the metabolic responses of species to ocean acidification. Results from this study indicate varied metabolic strategies in response to OA, further complicating our predictive power to forecast ecosystem-level consequences of ongoing CO_2 increases. However, strong effects were observed with respect to ontogeny, marine ecosystem, motility, and taxonomic origin, thereby reinforcing the need for a multi-faceted approach to both management of sensitive species and mitigation of future impacts.

Keywords: metabolism; ocean acidification; meta-analysis; oxygen consumption rate; whole-organism; physiology

1. Introduction

Ocean acidification (OA), the reduction of ocean pH through an alteration in the carbonate system is predicted to modify the structure and function of marine ecosystems worldwide [1-4]. The primary driver of the observed shifts in the marine carbonate system is the absorption of anthropogenic atmospheric carbon dioxide—CO_2, by the world's oceans [5,6]. These shifts are derived from an increase in atmospheric CO_2 previously unseen in the geologic record over the course of the last 300 million years [7]. This rise in atmospheric CO_2 translates to roughly a 30% increase in hydrogen ion concentration in the global ocean since pre-industrialization [8]. For marine biota, this may pose a serious environmental challenge [9,10], yet it is unknown to what extent ocean acidification will affect marine organisms and the habitat in which they reside.

Generally, marine species may mitigate the impacts of climate change, and specifically ocean acidification, via several strategies [9]: (1) Migration—Mobile species, and to a lesser degree species with planktonic larvae, may move to habitats that are more physiologically amenable; however, sensitivities to other abiotic variables (increasing or decreasing temperature, for instance) may physiologically constrain the use of this response [10]. (2) Evolution—Although there is some evidence of adaptation to ocean acidification [11,12], it is unlikely that all marine species possess the same ability to rely on standing-stock genetic variation to supply genotypes that are less OA-sensitive. Furthermore, the rates of environmental change will likely outpace the ability of organisms to utilize adaptation as a meaningful strategy to alleviate OA-related stress [13-15]. (3) Phenotypic plasticity—The intra-generational variation in physiological response at the organismal level can be employed so that tolerance windows shift within the lifetime of an organism (via acclimation or acclimatization) [16,17]. Of these responses, it is probable that plasticity will play a very important role in determining the resiliency of species to OA, and, at least for some organisms, may prove substantial enough to overcome the deleterious effects of OA [16]. Already, current genotypes are indisputably experiencing end-of-century pH levels in areas with strong upwelling events [12], and similarly, long-lived species will persist and be forced to face the future OA seascape. As such, investigating the physiological response of marine organisms to future OA scenarios can identify patterns regarding the potential for species resilience and persistence under such conditions.

Historically, physiological measurements have provided an opportunity for understanding how environmental stress affects the phenotypic response and performance of an organism [18-20]. For example, describing the lethal thermal limits of closely related species of porcelain crabs has offered some insight into the mechanism that has driven evolution in this genus [21]. These traditional physiological approaches can also be used to ascertain effects of predicted future climate change impacts. Of these approaches, characterizing the organismal-level metabolic response to a stressor is paramount to identifying the physiological strategy an organism employs to attempt a return to homeostasis. Shifts in metabolic output signal a restructuring of energy allocation [18,22]. For instance, under stressful conditions, ventilation and stress responses are favored over somatic tissue growth and gametogenesis [23]. The most common organismal metric used as a proxy to indirectly measure metabolic demand is the consumption of oxygen, O_2 [24]. Oxidative phosphorylation is the aerobic metabolic pathway predominately used by eukaryotes to harness cellular chemical energy [25]. Because oxygen is the terminal electron receptor in the electron transport chain and it is necessary for the synthesis of adenosine triphosphate (ATP), oxygen consumption rate via respiration can be measured and used to approximate the relative metabolic demand of an organism under varying

conditions [25]. Through either closed- or open-system respirometry, the whole-animal metabolic rate can be calculated (via oxygen consumption rate), and that value divided by some index of body mass of an animal, to estimate the mass-specific metabolic rate. From an organismal perspective, metabolic energy partitioning includes: energy storage, basal or standard metabolism, somatic tissue growth, gametogenesis/reproduction, specific dynamic action, locomotion, and stress response. Modifications in metabolic needs due to ocean acidification can highlight the adjustments in energy allocation that an organism uses to attempt to return to homeostasis.

At the organismal level, there are generally three metabolic responses that can be captured using respirometry techniques. (1) Metabolic increase—MI: Under hypercapnia, this response indicates the initiation of processes that require more cellular energy to attempt a return to, or maintain, homeostasis; i.e., the regulation of intracellular and extracellular acid-base balance, calcification, or apoptosis [26,27]. (2) Metabolic suppression—MS: Considered a short-term strategy for dealing with OA-related stress, MS involves the down-regulation of metabolic processes which can result in a decrease in protein synthesis and somatic tissue growth or maintenance [28,29]. Additionally, previous research suggests that unregulated extracellular pH may also promote MS [4,28,30,31]. (3) No change in metabolism—NC: OA-stress is not sufficient enough to warrant a whole-organism metabolic shift, or, energy allocation has shifted on a cellular level, but the overall organismal metabolic demand has not changed [32].

Within the marine environment, organismal variation in metabolic demand is a product of the evolutionary history of species [33]. Differences in life-history traits can also mediate the ability to respond to ocean acidification as an environmental stressor [34,35]. For instance, animal motility (e.g., active vs sessile) is known to regulate the absolute metabolic scope for activity [33]. Particularly, ion regulating capacity has been causally linked to the overall metabolic rate in marine animals, in that organisms with active lifestyles such as fish, crabs, and cephalopods indubitably have the ability to actively regulate both intracellular and extracellular pH, which may be beneficial when coping with elevated pCO_2. Conversely, sessile marine invertebrates characterized by slower metabolic rates like echinoderms and bivalves lack the active proton extruding machinery of their more active counterparts, and thus may be more sensitive to ocean acidification [36-39]. Developmental stage has also been hypothesized to influence an organism's capacity for coping with OA-related stress [35,40]. Larvae may be more susceptible to elevated pCO_2, given that they have yet to develop ion-regulating capabilities relative to other life history stages [35]. Given that these types of life-history traits may impact metabolic scope and function, the question arises—to what degree does ocean acidification shape organismal-level metabolic response?

To date, few comprehensive studies have attempted to quantify the effect of OA on organismal metabolism across species [35]. However, over the course of the last several years, the number of studies examining the effect of ocean acidification on metabolism has increased exponentially (Figure 1: $R^2 = 0.97$; years 2000–2014), permitting a meta-analysis of the ocean acidification literature.

Therefore, the aims of this review are as follows: (1) to categorically enumerate the metabolic response to OA of studies included herein; (2) to identify broad-scale trends and determine if sensitivity is a product of: life history stage, marine ecosystem (polar, tropical, subtropical, temperate, deep sea), locomotor capacity, taxonomy, and acclimatory capacity; (3) to review broad-scale gene expression studies, i.e., case studies, and identify the regulation of cell signaling pathways associated with ocean acidification stress; (4) to explore how changes in organismal metabolism might affect ecosystem function [4,41,42]; and (5) to review framing of OA studies with a particular emphasis on

future predicted levels vs unrealistic levels of hypercapnia, and address the relevance of current studies and directions for future work.
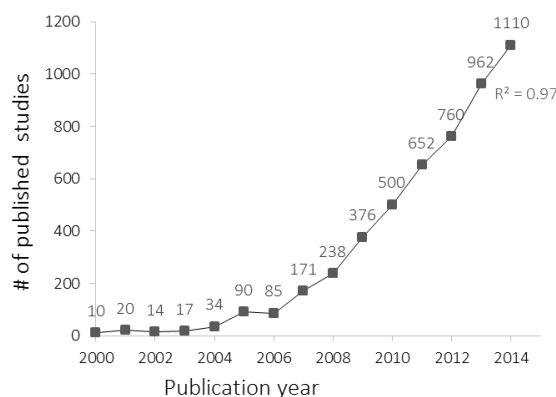

Figure 1. Scatterplot illustrating the number of studies published per year (2000–2014) using the search terms "ocean acidification" + "metabolism" on Google Scholar (exponential regression, $R^2 = 0.97$).

2. Methods

2.1. Data Selection and Extraction

The Google Scholar search engine was used to scan the biological literature using the following relevant key words: CO_2, ocean acidification, carbonate chemistry, hypercapnia, MO_2, respiration, respirometry, oxygen consumption rate, O_2 consumption, and metabolism. Combinations of these search terms were entered, e.g., "ocean acidification, metabolism" without Boolean operators. Further search parameters included limiting each combination of search terms to a single year, starting with 2000. Each study found was then examined, and those studies that included the following information were utilized in this meta-analysis: species name, developmental stage, marine ecosystem, mean oxygen consumption rates, experimental µatm pCO_2, kPa or ppm values (kPa and ppm converted to µatm pCO_2), and exposure period. Only whole-organism oxygen consumption rate measurements were included, i.e., isolated tissue oxygen consumption rate measurements were excluded from the analysis. For articles that included two or more experimental temperatures, only the "control temperature" or "ambient temperature" was used to prevent confounding the effects of pCO_2 and increased temperature. In total, we included 44 studies that reviewed the metabolic response of 54 species to ocean acidification. For studies that did not explicitly list oxygen consumption rates in the text, we used Web Plot Digitizer to extract the numerical values for inclusion in the analysis (http://arohatgi.info/WebPlotDigitizer/). The literature review for this study was concluded on April 24th, 2015.

To permit the use of data that had multiple comparisons between ambient and treatment pCO_2 conditions, each within-study comparison was categorized by treatment level (ambient, low, mid, high) and acclimation days (number of days an organism was exposed to pCO_2 conditions). When a study included several time-sample measurements that compared an ambient and treatment level of pCO_2, i.e., oxygen consumption rate values on day 1, 2, 4, 8 and 16, each day was considered an individual comparison, so that ambient and treatment comparisons included 5 measurements for that

species (Table S1). Similarly, when a species was exposed to multiple, differing µatm pCO_2 levels, e.g., ambient, medium, and high treatments, each treatment was considered an individual comparison. For example, Comeau (2010), [26] measured oxygen consumption rate at an ambient level and treatment levels of "low mid", "high mid", and "high" µatm of pCO_2. Each treatment was compared to the ambient so that this species had 3 comparisons to the ambient treatment (Table S1). Across all studies and species, there were 117 treatment comparisons made to ambient pCO_2 values (Table S1).

2.2. Data Categorization

In order to assess the effect of ocean acidification across numerous ecological groups, the organism in each study was classified by listing the relative taxonomy, marine ecosystem habitat type, motility-mobility mode, developmental stage and the number of pCO_2 acclimation days. Thus, for each species in a study we had categorical groupings with which we could perform statistical analyses [43]. For the experimental pCO_2 values, each was assigned a category, "ambient", "low mid", "mid", "high mid", or "high" based on the number of pCO_2 comparisons to the ambient condition in each study. In total, 5 major categories were examined: ontogeny, marine ecosystem habitat type, motility, taxonomy, and pCO_2 acclimation period.

2.3. Enumeration of Ocean Acidification Physiological Impacts (within-Study) on Ecological Groups

Each comparison (ambient vs treatment) was grouped so that the within-study significance could be pooled and compared with each ecological category: ontogeny, marine ecosystem habitat type, motility, and taxonomy. For instance, of all the adults examined pooled across all comparisons, roughly 75% had no significant response to OA, while 13% significantly up-regulated their metabolism and 12% significantly down-regulated their metabolism. The significance recorded was statistically determined within each study examined.

2.4. Meta-analysis: Effect Size

A traditional meta-analysis approach was used to calculate the relative sensitivities of the different ecological categories [44,45]. The effect of OA on metabolism was calculated for each comparison using the following equation:

$$ES=(\text{oxygen consumption }_{treatment}) - (\text{oxygen consumption}_{ambient}) \div \text{mean oxygen consumption} \qquad (1)$$

Where ES is the difference between the oxygen consumption rate at the elevated µatm pCO_2 level and ambient µatm pCO_2 level divided by the mean of the treatment and ambient oxygen consumption rates. The ES statistical calculation allowed us to standardize across experimental treatment pCO_2 levels so that metabolic responses of different organisms could be compared across studies. We pooled ES values for each subgroup within each ecological category, e.g., adult, larvae, etc. Using these pooled values, the bootstrap 95% confidence intervals were generated for each category using the Minitab 17 bootstrap macro (based on 999 permutations, α = 0.05) [45]. The metabolic response to ocean acidification of a subgroup was considered significant when the mean and 95% confidence intervals of a group did not transect zero. Many of the studies included in this

review did not include the sample size or standard error, which precluded the use of natural log-transformed weighted meta-analytical approach which has been used in other similar studies [1].

2.5. The Effect of pCO_2 Acclimation on Metabolic Response

Studies that included multiple respirometry measurements over the course of an experiment were grouped by taxa, and the in-study significance was identified. Through this approach, we sought to identify the potential for acclimation to OA conditions, i.e., organisms that returned to "ambient level" oxygen consumption rates over time.
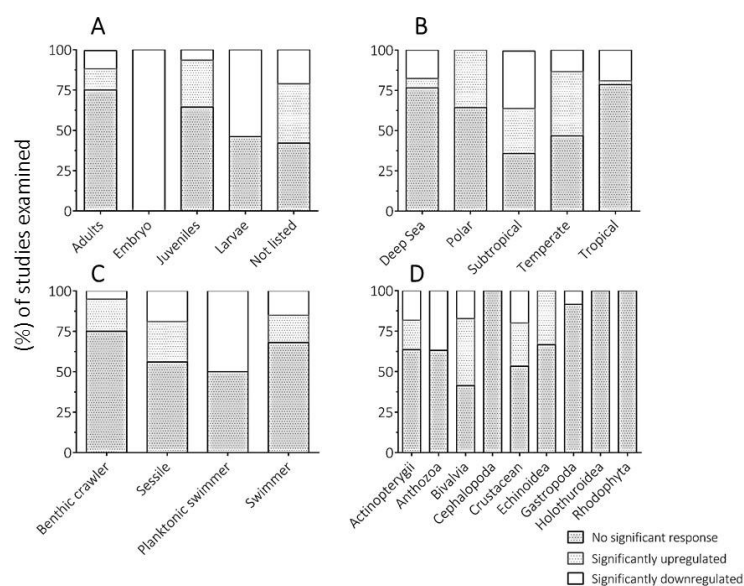

Figure 2. Enumeration of metabolic responses to ocean acidification, within-study, based on percentage of each group in each ecological category (i.e., of all adults reviewed, 75% showed no within-study significant difference compared to ambient conditions). A—ontogeny; B—marine ecosystem; C—motility; D—taxonomy. Significance responses (up- or down-regulation of metabolism) were determined individually by each study and then pooled based on category.

3. Results

3.1. Enumeration of Ocean Acidification Physiological Impacts (Within-Study) on Ecological Groups

The impact of OA on organismal metabolism was significant for 2 developmental stages, including the subgroups "embryos" and "larvae". These two groups exhibited >50% significant response of metabolic increase or decrease (Figure 2; Table 1). Of adults examined, the majority, 75%, did not have a significant metabolic response to OA when compared to within-study controls (ambient conditions). Within the marine ecosystem category, subtropical organisms significantly altered their metabolism—28% MI and 36% MS in response to ocean acidification. Similarly, more than 50% of temperate species significantly augmented oxygen consumption rates under hypercapnia

conditions. Fifty percent of planktonic swimmers significantly down-regulated their metabolism when faced with OA conditions. Of all the taxonomic groups, Bivalvia exhibited the most significant response to OA, with 41% exhibiting MI and 17% MS (Figure 2; Table 1).

Table 1. Percent of each subgroup within-study (ontogeny, marine ecosystem, motility, taxonomy) metabolic response to OA.

Ecological category	No significant response (%)	Significantly upregulated (%)	Significantly downregulated (%)
Adults	75	13	12
Embryo	0	0	100
Juveniles	65	29	6
Larvae	46	0	54
Not listed	42	37	21
Deep Sea	76	6	18
Polar	64	36	0
Subtropical	36	28	36
Temperate	47	40	13
Tropical	79	2	19
Benthic crawler	75	20	5
Sessile	56	25	19
Planktonic swimmer	50	0	50
Swimmer	68	17	15
Actinopterygii	64	18	18
Anthozoa	63	0	37
Bivalvia	41	42	17
Cephalopoda	100	0	0
Crustacean	53	27	20
Echinoidea	67	33	0
Gastropoda	92	0	8
Holothuroidea	100	0	0
Rhodophyta	100	0	0

3.2. Meta-analysis: Effect Size

In each ecological category, at least one group elicited a significant effect size response to OA. Larvae were most affected by OA, with a 95% confidence interval—CI of −0.55 to −0.23—suggesting OA had a profoundly negative effect on this life history stage across taxa and ecosystems (Figure 3, Table 2). Polar organisms produced an effect size that was significantly positive, with the 95% CI ranging from 0.21 to 0.54, indicating this group up-regulated metabolic function as a result of exposure to acidified conditions. Tropical species, on the other hand, had a significantly negative ES response to OA (Figure 3, Table 2). Planktonic swimmers, which included mainly larval invertebrates and holoplanktonic gastropods, showed a significant ES response by down-regulating their metabolism as a consequence of hypercapnia. Finally, three taxonomic groups significantly up-

regulated their metabolism, highlighted by a positive ES: Actinopterygii, Echinoidea and Rhodophyta (Figure 3, Table 2).
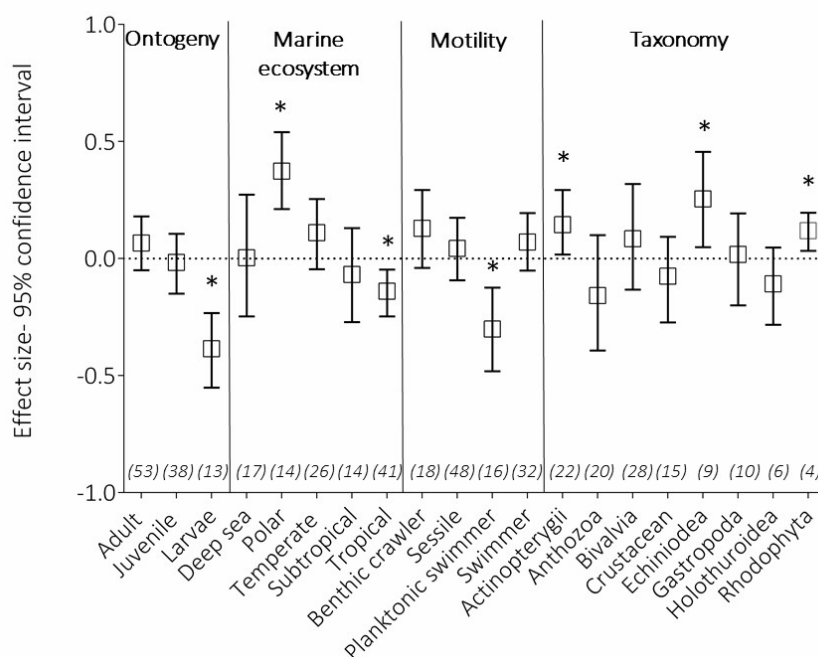

Figure 3. Metabolic performance responses effect size by category: ontogeny (developmental life stage); marine ecosystem (distinct assemblages of natural communities); motility (locomotor capacity); and taxonomy (clade assignment). Error bars represent 95% bootstrap intervals. Asterisk (*) indicates groups that are significantly different (95% confidence intervals do not cross dotted line) within a category, e.g., larval response to OA in the ontogeny category. Effect size mean and 95% confidence intervals below zero (dotted line) are groups that had a significant response to OA via metabolic suppression (MS), and those groups above zero had a significant response to OA by metabolic increase (MI). Italicized values represent the sample size from which the 95% bootstrap confidence intervals were generated. Cephalopoda was omitted due to a small sample size (n = 2) for generating 95% bootstrap confidence intervals.

3.3. The Effect of pCO$_2$ Acclimation on Metabolic Response

Laboratory acclimation in hypercapnic conditions spanned from 0 days to one year. For some groups, like Antarctic fishes, the ability to acclimate to conditions of OA permitted a return to control levels of oxygen consumption, suggesting that these groups are capable of dealing with OA stress when acclimated, showing signs of physiological plasticity (Figure 4). Bivalvia taxa, on the contrary, were not able to physiologically compensate their metabolic function; thus, continued a significant MI, regardless of acclimation time (Figure 4). Two of the three crustaceans, *Pandalus borealis* (shrimp) and *Pagurus tanneri* (hermit crab), were able to metabolically compensate over time and return to oxygen consumption rates that did not significantly differ from control levels (Figure 4). These results highlight the complex responses of organisms across taxa, further complicating our ability to predict how species will respond to future ocean change.
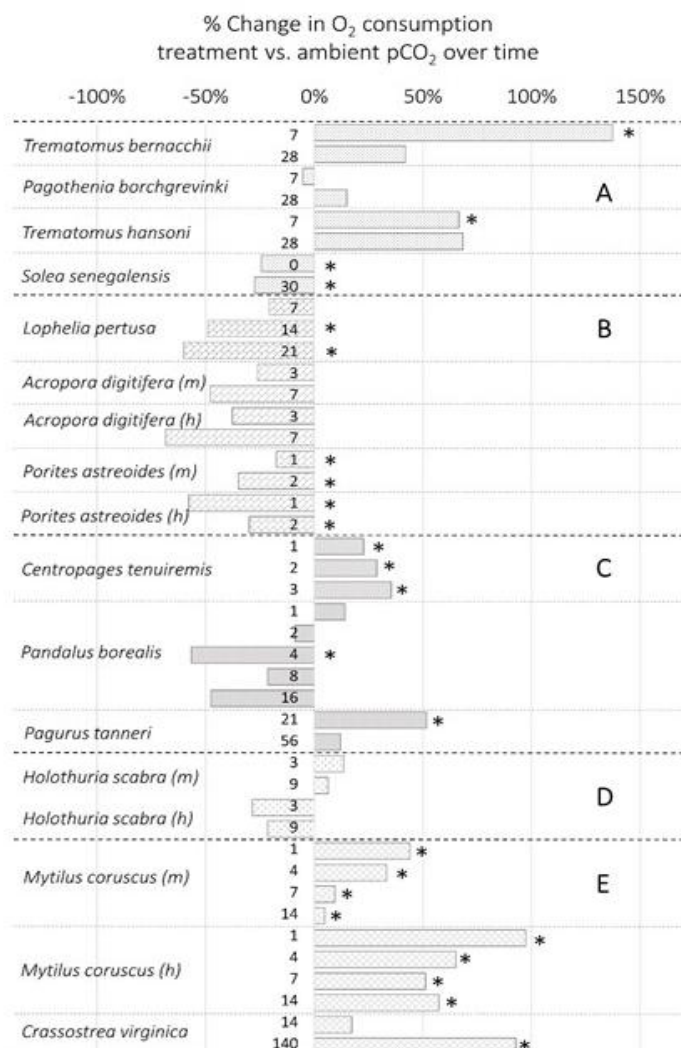
Figure 4. Selected studies that recorded O_2 consumption measurements over time (days). Asterisk (*) denotes a significant difference from the within-study control, $p < 0.05$. (A) Teleostei (B) Anthozoa (C) Crustacea (D) Holothuroidea (E) Bivalvia.

3.4. Experimental OA Parameters: How Relevant are Current Studies

Studies quantifying the impact of OA have employed a wide range of pCO_2 values to capture organismal metabolic rates. Studies varied widely in terms of what was considered "ambient", "low mid", "mid", "high mid", and "high" pCO_2 levels. For "ambient" values, the mean and median were 496 and 436 µatm pCO_2 respectively, with the range of values spanning 258 to 1379 µatm pCO_2 (Figure 5). "Mid" treatments ranged from 491 to 2047 µatm pCO_2. "Low mid" and "high mid" had mean values of 584 and 929 µatm pCO_2, while "mid" treatments had a mean of 1404 µatm pCO_2, higher than the "low mid" and "high mid" means (Figure 5). Medium treatments ranged from 491 to 2047 µatm pCO_2. "High" pCO_2 values ranged from 633 to 9085 µatm pCO_2, while mean and median values were 2186 and 1161 µatm pCO_2, respectively. Table 3 lists the labeled treatment categories listed in the reviewed studies.

Table 2. Mean, upper, and lower 95% confidence intervals of effect size.

| Ecological group	Mean	Lower 95% CI of mean	Upper 95% CI of mean
Adult	0.066	−0.051	0.18
Juvenile	−0.017	−0.15	0.11
Larvae	−0.39	−0.55	−0.23
Deep sea	0.0032	−0.25	0.27
Polar	0.37	0.21	0.54
Temperate	0.11	−0.046	0.25
Subtropical	−0.068	−0.27	0.13
Tropical	−0.14	−0.25	−0.048
Benthic crawler	0.13	−0.041	0.29
Sessile	0.043	−0.093	0.17
Planktonic	−0.301	−0.481	−0.124
Swimmer	0.0705	−0.052	0.194
Actinopterygii	0.14	0.016	0.29
Anthozoa	−0.16	−0.39	0.099
Bivalvia	0.084	−0.13	0.32
Crustacea	−0.076	−0.27	0.093
Echinoidea	0.25	0.048	0.45
Gastropoda	0.018	−0.2	0.19
Holothuroidea	−0.11	−0.28	0.047
Rhodophyta	0.12	0.032	0.2
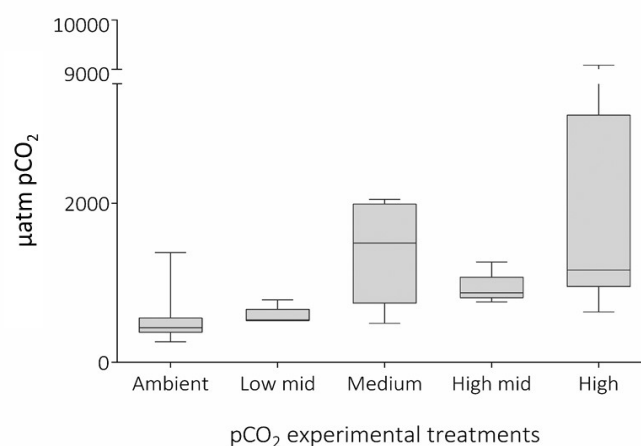
Figure 5. pCO_2 values (µatm) collected from all of the studies used in the meta-analysis. Groupings ("ambient", "low mid", etc.) were based on the within-study pCO_2 experimental treatment designation.

4. Discussion

Our results indicate that there were divergent metabolic responses after exposure to OA, with at least one group in each category having 50% of the study organisms significantly up- or down-

regulating their metabolism (Figure 2). The meta-analysis identified a number of strong trends with respect to the metabolic response to ocean acidification, with significant effects related to ontogeny, marine ecosystem, motility, and taxonomic origin (Figure 3). For some species, acclimation to OA was sufficient enough to warrant a return to control level metabolic rates (Figure 4).

Table 3. Summary statistics of the range of pCO_2 (μatm) experimental treatments of surveyed literature.

	Ambient	Low mid	Medium	High mid	High
Number of values (n)	78	5	19	5	80
Minimum	259	523	491	760	633
25% Percentile	380	526	744	813	954
Median	436	534	1500	874	1161
75% Percentile	558	668	1989	1072	3108
Maximum	1379	785	2047	1261	9085
Mean	496	584	1404	929	2186
Std. Deviation	196	113	590	192	2126
Std. Error of Mean	22	50	135	86	238
Lower 95% CI of mean	452	444	1119	690	1713
Upper 95% CI of mean	540	724	1688	1168	2659

4.1. Enumeration of Ocean Acidification Physiological Impacts (within-Study) on Ecological Groups

Assessing the responses of different ecological groups to projected future OA allows for general patterns to be detected. While this is not the first time early life history stages have been identified as particularly vulnerable to OA [40,46], this work provides consensus regarding the response of larval and embryonic life stages' sensitivities with more than 50% of the larvae and 100% of the embryos surveyed significantly down-regulating their metabolism (Figure 2, Table 1). For marine environment categories, both polar and tropical groups had similar percentages of organisms with no significant effects of OA on metabolism—64% and 79%, respectively (Figure 2, Table 1); however, polar species that did significantly alter their metabolism did so via up-regulation, while nearly all tropical species that were significantly impacted by OA down-regulated metabolic function (discussed further below).

4.2. Meta-analysis: Effect Size

4.2.1. Ontogenetic metabolic response to OA

Of the developmental life stages surveyed, larval organisms clearly elicited a negative response to OA relative to the other ontogenetic groups (Figure 3, Table 2). Indeed, individual studies of larval species have exhibited MS resulting from rearing under hypercapnic conditions and our results support this trend [47-50]. The significant negative ES of metabolic response shown here includes three diverse taxonomic groups, including Anthozoa (corals), Teleostei (fish) and Crustacea (shrimp) species, highlighting the notion that the identified MS was not restricted largely to any one taxonomic group. Furthermore, MS was found in larvae from all marine ecosystems except polar habitats.

Larval MS can induce a reshuffling of metabolic priorities leading to deleterious outcomes [51]. As such, MS is considered to be a short-term strategy to cope with environmental stress [29]. Long-term organismal MS can lead to reductions in ATP production, protein synthesis, growth, and aerobic scope, affecting the physiological performance and persistence of larvae [52,53]. From a mechanistic standpoint, larvae may be more susceptible to elevated pCO_2, given that they lack the necessary ion regulating epithelia when compared to adults [35]. In the early embryonic and larval stages, while animals are only several cell layers thick, the lack of an extracellular regulation of acid-base equivalents coupled with the reduced volume of the extracellular milieu as a potential buffering source may represent an environmental stress strong enough to induce MS [35]. The relative performance of larval forms is fundamentally important for determining organismal distribution, abundance, the maintenance and growth of populations, and subsequently community structure [54,55].

4.2.2.   Marine ecosystem groups response to OA

From the marine ecosystem perspective, significant but contrasting effect sizes were observed in both the polar and tropical environments. Polar organisms exhibited significant ES with a MI due to OA stress, suggesting enhanced energetic requirements to maintain acid-base balance, homeostasis, and somatic tissue growth. This trend includes vertebrate, invertebrate, embryonic, and adult polar organisms. In contrast, tropical organisms as a whole exhibited significant ES characterized by MS, perhaps owing to a short-term strategy for coping with OA by decreasing protein synthesis and cell maintenance. The pattern of ES MS elicited by tropical organisms was detected in both vertebrates and invertebrates, from nearly all ontological phases, including larval, juvenile, and adult life forms. The overall trends observed here may be rooted in generalized metabolic strategies that have evolved over time.

Our results here suggest that organisms native to polar and tropical marine ecosystems are particularly sensitive to OA, compared with other environments. Relative to the other marine ecosystems examined, both the polar and tropical marine ecosystems have comparatively stable thermal and pH regimes [56], although recently published data suggest some Antarctic species experience a yearly alkalinization event [57]. Under these fairly stable conditions, it is feasible that the resident species evolved under narrow pH conditions and thus elicit strong metabolic responses with varying conditions. Natural environmental variability has been shown to favor selection for OA-tolerant genotypes [12,58], suggesting that organisms within marine ecosystems characterized by invariant environments may be more sensitive to subtle changes in abiotic factors such as pH and temperature. It has been suggested that organisms adapted to warmer environments may be energetically limited in terms of mounting a robust response to further heat stress or other abiotic stressors [21,59]. This may in part explain the significant negative ES response that tropical organisms in this study demonstrated in response to hypercapnia compared to organisms from different habitat types.

4.2.3.   Motility groups metabolic response to OA

Of the four motility strategies examined, only planktonic swimmers exhibited a significant, negative ES to increased pCO_2. This group includes larval forms of the stony corals *Acropora digitifera* [47] and *Porites astreoides* [60], as well as thecosomatous pteropods. It is well established that larvae are generally more susceptible to ocean acidification and thermal stress [61]. Furthermore,

thecosomatous pteropods must balance the energetic costs of locomotion through the water column with the expense of secreting calcium carbonate shells. However, organisms within this group may have differential sensitivity based on their environmental histories. Maas et al. (2012) [62] tested the effects of elevated pCO$_2$ on five species of thecosomatous pteropods and found that pteropods that vertically migrate through naturally hypoxic (and thus elevated CO$_2$) waters did not significantly alter their metabolism in response to ocean acidification. Notably, the only species tested that does not vertically migrate through hypoxic water exhibited a negative metabolic response to elevated CO$_2$. Due to the importance of planktonic organisms to marine food webs [4], future work should consider the metabolic sensitivity of other planktonic species (e.g., foraminifera, coccolithophores, diatoms) to ongoing ocean acidification.

4.2.4. Taxonomic groups response to OA

It is hypothesized that teleost fish, cephalopods, and crustaceans (brachyurans) have a high ion-regulatory capacity [63], suggesting a potential for high pCO$_2$ tolerance. Due in part to their low metabolic rate, echinoderms and bivalves have decreased ion-regulatory ability, therefore suggesting CO$_2$ sensitivity [39]. The fish group in our study (Actinopterygii, Figure 3) did indeed elicit significant, positive ES response, such that an increase in pCO$_2$ instigated an increase in metabolic demand, counter to the above hypothesis. However, in accordance with the above hypothesis, the echinoderm group did produce a significant ES response, ramping up their metabolism in response to OA. As seen in other meta-analyses, the relative taxonomic category an organism fell under influenced its physiological response to conditions of future hypercapnia [43]. Such studies have identified distinct physiological differences in response to OA, where calcifying organisms have been found to be more sensitive to OA than non-calcifiers due to the increase in metabolic energy associated with the generation and maintenance of biocalcified structures [43,64,65]. Interestingly, only two clades of calcifying species exhibited a significant ES response, Echinodermata and Rhodophyta, while other clades including corals, bivalves, crustaceans, gastropods, and holothuroids, did not exhibit a significant ES response (Figure 3, Table 2). This may be an artifact of short-term OA exposure in these studies, where most exposures were less than one month long. Contrary to this trend, *Crassostrea virginica* did not significantly alter its metabolic rate after a 2-week exposure to elevated pCO$_2$, but did significantly increase organismal metabolism after 140 days in elevated pCO$_2$ conditions (Figure 4). This example illustrates that for bivalves, the exposure time may not have been long enough to detect an impact on organismal metabolism and thus could provide a cause for the lack of significant ES response in calcifiers. Alternatively, this could also be due to considerable interspecific variability within these clades in their response to ocean acidification.

4.3. The Effect of pCO$_2$ Acclimation on Metabolic Response

Several trends were observed while testing the effect of pCO$_2$ acclimation over time. First, the Antarctic fishes (*Trematomus bernacchii, Pagothenia borchgrevinki, Trematomus hansoni*) appeared to recover over time. Following 28 days of acclimation, metabolic rate did not significantly differ between ambient and future OA treatment conditions in these 3 species (Figure 4). Recent work has also demonstrated that embryonic Antarctic Dragonfish (*Gymnodraco acuticeps*) did not significantly adjust their metabolism when exposed to 650 (moderate) and 1000 µatm pCO$_2$ (high)

concentrations after a 3-week acclimation period [66]. Conversely, two of the three coral species examined did not recover over time, significantly down-regulating their metabolism relative to ambient conditions. Finally, all bivalve species examined in the studies herein maintained a significant MI regardless of the duration of the acclimation period (Figure 4, Table 3).

4.4. Linking Cellular Processes to Organismal Metabolic Responses

Linking whole-organism responses to cellular level processes is necessary to resolve the physiological mechanisms used to cope with ocean acidification [32]. Given the advances in high throughput sequencing, characterizing the effects of OA on gene expression is becoming increasingly more common [11,67,68]. Evans and Watson-Flynn (2014) [68] conducted a meta-analysis that reviewed nine gene expression studies examining the response of larval sea urchins to ocean acidification. The top three gene ontologies affected by OA were metabolism, ion transport, and biomineralization. Of all genes that were differentially expressed (both up- and down-regulated), 42% (251 out of 602) were involved in the regulation of cellular metabolic processes. These genes included those involved in carbohydrate, protein, nucleic acid, lipid, and amino acid metabolism. The majority of down-regulated genes were also involved in cellular metabolic processes and composed 40% (194 out of 483) of all down-regulated genes. Conversely, the majority of genes that were differentially up-regulated were also grouped in the metabolic process ontology (65 of 134 genes). Larval urchins appear to be particularly susceptible to OA, which can have long-term consequences that move beyond species-level impacts [54,55].

Several studies on *Acropora* spp. provide consensus regarding the cellular response of this genus to OA. Kaniewska et al. (2012) [69] conducted a gene expression analysis of *Acropora millepora* and found that in general, there was a concerted down-regulation of genes associated with the electron transport chain and the tricarboxylic acid cycle after a 28 day acclimation period under high CO_2 conditions ranging from 1010 to 1350 μatm pCO_2. In accordance with a down-regulation of cellular metabolism, whole organism oxygen consumption rates were also suppressed, illustrating that cellular-level responses are in agreement with the organismal-level metabolic response [69]. Similarly, in *Acropora aspera*, Ogawa et al. (2013) [70] found a cellular metabolic depression highlighted by a down-regulation of genes involved in the mitochondrial electron transport chain and the tricarboxylic acid cycle that was accompanied by a concomitant suppression of metabolic rate after a 7 day exposure to an elevated level of pCO_2.

Recent work has uncovered a disparate relationship between organismal metabolic output and the cellular-level processes in larvae of the sea urchin *Strongylocentrotus purpuratus* [32]. Under conditions of moderate acidification—roughly 800 μatm pCO_2—metabolic rates, body size, biochemical content, and gene expression were similar to larvae reared under ambient conditions [32]. Although body size did not change between larvae exposed to ambient and elevated levels of pCO_2, there was a 50% increase in the rates of ion transport and protein synthesis largely driven by a shift between a decrease in protein depositional efficiency and protein synthesis [32]. Furthermore, gene expression of the enzyme Na^+/K^+-ATPase did not reflect the overall increase in ion transport or ATP demand that was detected in larvae held in hypercapnic conditions [32]. This work emphasizes the need to identify compensatory physiological mechanisms that organisms utilize to provide some level of resilience to OA aside from relying on traditional methods of physiological performance, including whole-organism respiration rates.

4.5. How Organismal Metabolic Response May Influence Aspects of Ecosystem Function

A fundamental goal of environmental physiology is to integrate individual organismal responses with broad-scale ecological functioning and biogeography [21,22]. As metabolic rate determines the rate of almost all biological processes, it serves as a critical metric toward the broader development of ecological theory [71]. Scaling up from the individual organism, metabolic rate has the potential to influence higher levels of organization such as population dynamics and species interactions. Despite the fact that this remains an active area of research in current biology [71], the onset of anthropogenic climate change has exposed new challenges for organismal biologists integrating individual responses with higher-order processes [72]. Among these existing questions is how small changes in the metabolic functioning of individuals can shape fitness, recruitment, population dynamics, and species interactions, among others [4].

One of the major disadvantages associated with the negative effects of ocean acidification on metabolism is a decrease in the amount of available energy that may be devoted to reproduction and overall fitness of a species. Although the biological consequences of ocean acidification have mostly been identified as sub-lethal, they have dire implications when scaling up to population structure and ecosystem function. First, by increasing the energetic demand to maintain homeostasis, other processes such as reproduction may be compromised [73]. Similar to our findings, several studies have documented metabolic suppression in embryonic [74], larval [48,60], and juvenile stages [75] under acidified conditions. Coupled with decreased settlement rates and changes in growth of invertebrate species, metabolic suppression in larvae may potentially limit recruitment under acidification, leading to changes in population sizes of marine communities. In adult organisms, reproductive output may be affected in an acidifying ocean through impacts on fecundity, maturation, and spawning. Changes in these reproductive traits may also impact populations within marine ecosystems. Nonetheless, explicit links between metabolic costs to reproductive success have yet to be tested in an ocean acidification context.

Beyond impacts at the individual level, metabolic changes induced by ocean acidification may also potentially impact on certain ecological interactions. Several recent studies highlight increased sensitivity in both predatory behaviors and prey responses [76-78], suggesting the potential for altered population dynamics in a high-CO_2 ocean. Here, the mechanism is hypothesized to be rooted in metabolic costs that may compromise activity level, habitat choice, and predator avoidance [79]. Yet, the exact linking of species-level physiological tolerance to ecological predictions may be compounded by inherent system-level complexity, thus necessitating integration across systems [80].

4.6. Experimental OA Parameters: How Relevant are Current Studies

A general unifying goal of the studies described herein is to broadly understand how organismal responses to environmental change will shape the ecology and health of the future ocean and to inform how management practices can ameliorate human impacts on the ocean. At the same time, successful management practices rely on bridging a rapidly expanding body of experimental knowledge with realistic practices that promote conservation and responsible use of marine systems [81]. To this effect, the global change research community has a responsibility to design and conduct experiments that are "ecologically relevant" and reflect both current and predicted levels of change in environmental variables. The results of the present study underscore the discordant yet classical approach taken

when parameterizing ocean acidification experiments, with all treatment categories generally falling far outside of the range of what may be considered ecologically relevant [82]. While studies that use ranges of environmental variables that exceed an organisms' natural range can be incredibly useful in identifying potential tipping points, this approach may complicate management directives and incidentally propel an "overcompensation" response. Future studies should, to the best of their logistical ability, build off of recent efforts to parameterize study environments followed by framing of experimental objectives in order to better predict species' responses to environmental change and to inform conservation efforts.

5. Conclusion

This work is an important first-step towards increasing our predictive capacity in identifying impacts of OA that move beyond single species' responses to OA. Future predictions should be rooted in studies that incorporate multiple stressors that also include variability in these stressors to better mimic the dynamic future ocean that marine species will experience. The logical next step to build off of this work is to conduct meta-analytical studies that include multiple abiotic stressors so that the metabolic responses can be determined, whether additive, synergistic, or antagonistic [83]. These characterizations will be incredibly useful as the research community moves forward, as they represent a more realistic parameterization of metabolic sensitivities—and therefore species' responses—to ocean change.

Acknowledgements

Conflict of interest

The authors declare there is no conflict of interest.

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