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Does sex really matter? Explaining intraspecies variation in ocean acidification responses

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Abstract Ocean acidification (OA) poses a major threat to marine ecosystems globally, having significant ecological and economic importance. The number and complexity of experiments examining the effects of OA has substantially increased over the past decade, in an attempt to address multi-stressor interactions and long-term responses in an increasing range of aquatic organisms. However, differences in the response of males and females to elevated $p\mathrm{CO}_2$ have been investigated in less than 4 % of studies to date, often being precluded by the difficulty of determining sex non-destructively, particularly in early life stages. Here we highlight that sex significantly impacts organism responses to OA, differentially affecting physiology, reproduction, biochemistry and ultimately survival. What's more, these impacts do not always conform to ecological theory based on differential resource allocation towards reproduction, which would predict females to be more sensitive to OA due to the higher production cost of eggs compared to sperm. Therefore, non-sex specific studies may overlook subtle but ecologically significant differences in the responses of males and females to OA, with consequences for forecasting the fate of natural populations in a near-future ocean.

Key Words: Carbon dioxide, climate change, gender, systematic map

1. Introduction

Ocean acidification (OA), changes in seawater carbonate chemistry induced by oceanic uptake of anthropogenic CO_2, poses a major threat to marine biodiversity globally [1], as well as to societies and industries reliant on marine living resources [2]. Studies investigating the ecological effects of OA have increased exponentially over the past decade [3], increasing in complexity to incorporate the highly dynamic nature of carbonate chemistry in many natural systems [4], multi-stressor interactions [5], an ever increasing range of organisms, life history stages, communities, and multiple generations [5]. Whilst this effort has contributed to help better explain species tolerance and increase reliability of future change projections, intraspecies variation in OA responses has received insufficient attention, adding uncertainty to reported responses and their interpretation [6].

Identifying the sources and consequences of variability in biological responses is pivotal to understanding a population's ability to cope with environmental change [7, 8]. However, despite recent evidence that many physiological, behavioural, immunological, molecular and neuro-toxicological functions are influenced by sex-based differences [9, 10], the overarching role of sex in determining response to OA remains understudied [11]. Here, we employ a systematic map approach: a transparent, robust and repeatable method to identify and collect relevant literature to answer the question of how sex is considered within experimental OA research [12]. By critically reviewing existing literature, we highlight evidence for, and discuss potential implications of omitting, sex-based variation in species responses.

2. Methods

Following international guidelines, a systematic map protocol (Supplementary Materials, "SM") was used to assess existing evidence (Fig. 1) addressing the research question: *Do OA studies consider the impact of sex on organism responses?* Search term strings using Boolean logic were run through Web of Science to collect relevant peer reviewed literature, and subsequently narrowed to target literature published between January 2008 and May 2016, limited to studies on fish, crustaceans, echinoderms and molluscs. This ensured a manageable literature set was reviewed whilst providing a contemporary representation of the OA field. Search results were further refined at three levels to exclude studies irrelevant to our research question (Fig. 1, SM). Study inclusion was determined objectively against a set of inclusion criteria which defined pertinent population, study type, intervention, comparator, and outcomes (SM for details).

Upon inclusion, data on experimental subject (organismal group and species) and life-stage (gamete, embryo, larvae, juvenile, adults, as well as transgenerational and reproduction/fertilisation processes) were extracted. Each study was then searched for the inclusion of seven sex-related terms within the main body of text (sex, gender, male, female, imposex, intersex or hermaphrodite), and scored according to one of five categories: 1) Not mentioned; 2) Mentioned but not accounted for; 3) Accounted for but not measured [e.g. only males used]; 4) Measured but not tested statistically; and 5) Tested statistically. For the last, end-points measured and the significance of sex-based differences were extracted.

3. Results and discussion

Despite an exponential increase in experimental OA studies over the past decade (Fig. 1), only 3.9% of these statistically assessed sex-based differences in OA responses (Table 1; Fig. 2a-d). Only 10.5 % of studies account for possible sex effects by assessing males and/or females independently, with over 85 % of studies failing to mention or account for sex (Table 1). Where tested, sex significantly modified the response of aquatic organisms to OA, and thus failure to account for sex-based differences could significantly influence the predicted impact of OA on populations.

The relative energetic investment of males and females towards reproduction, in anisogamous systems, is central to the variability observed in organism response to their environment [11]. Consequently, of the studies that differentiated between males and females, around 30 % did so by measuring reproductive endpoints. In echinoderms, 6.6 % of studies tested for sex-based differences (Fig. 2a), with reproduction and gamete functionality receiving the greatest attention (Fig. 2e). Male sea urchins exposed to elevated $p\mathrm{CO}_2$ and temperature fared worse than females, having significantly lower gonad index and 'spawnability' [13, 14]. This sex-specific response to OA seems to contradict theory based on projected reproductive strategy. However, gonads in echinoderms are often used as an energy storage compartment that can be filled or depleted depending on conditions [15]. Under OA, females that invest more in gonadal development may then have access to more energy to cope with stress (increased cost of acclimation) as compared to males [16]. This outlines the importance of measuring the impacts of OA in both males and females, avoiding overgeneralization and elucidating impact mechanisms by observing organism biology.

A key limitation to investigating male/female differences is the ability to successfully determine sex non-invasively. Sexual dimorphism exists in many adult organisms but in some, including bivalve molluscs, morphological distinction can be unreliable [17], precluding its inclusion experimentally. Consequently, over 96 % of studies on the Mollusca neglect to mention or account for sex, the lowest of the four groups investigated (Fig. 2b), despite Mollusca receiving the greatest attention with respect to OA (Fig. 1). Conversely, in many adult crustaceans it is relatively easy to distinguish sex visually, resulting in this group having the greatest percentage of studies that mention or account for sex (63.5%). However, only 3.5 % of studies on crustaceans used sex as a factor when performing statistical tests, whilst 33.9 % indirectly accounted for sex by using females or males in isolation (Fig. 2c).

By pooling data for males and females, or focusing on the response of a single sex, it is likely that species responses to OA will be inaccurate [18]. For example, in Crustacea, exposure to elevated

pCO_2 is shown to result in higher mortality in female shrimps (*Palaemon pacificus*) compared to males [19], whilst the median lethal level (LC_{50}) for CO_2 is also lower in female copepods (*Acartia tonsa*) compared to males [18]. Sex-specific physiological impacts can result in a 2-fold increase in the respiration rate of male copepods under elevated pCO_2, but respiratory suppression in females [20]. Similarly in molluscs, males and females respond differently to elevated pCO_2 and temperature, with sex-based differences demonstrated in the mussel (*Mytilus edulis*) metabolome [10] and the biochemical composition of limpet (*Nacella concinna*) gonad [21].

Whilst sex has the potential to alter the effect of OA on early-life stages, sex-based differences have largely been restricted to maternal and paternal effects to date, with various protective and inhibitory impacts being shown in transgenerational studies [11]. Inability to non-invasively determine sex in early-life stage individuals has precluded the observation of any sex-based differences in larval OA sensitivity. It is therefore unclear whether sex-based differences are more or less pronounced during early-life stages than in adults [9]. Importantly, any differential mortality, or OA sensitivity, in larval stages could significantly impact the sex-ratio of larval recruits, and thus population dynamics. In fish, only 3.2 % of studies have tested for sex effects (Fig. 2d), likely because the largest proportion of fish studies have investigated larval responses (Fig. 2h). However, with abiotic conditions (e.g. temperature) shown to impact sex differentiation and resulting larval condition in fish [22], elucidating the possible sex-specific impacts of OA during early-life stages is key for understanding future population dynamics.

Here we demonstrate that whilst less than 4 % of the OA literature tests for sex-based differences, there is a clear precedent for differential responses to elevated pCO_2 between sexes. If sex-based differences do exist for economically important species, as seems likely, then capturing this variance is crucial for accurately forecasting the future societal and economic repercussions of OA for dependant sectors, such as coastal management, conservation, fisheries and aquaculture [2]. Unfortunately, the lack of a sufficiently wide evidence base for sex-specific responses currently limits this ambition. As a starting point towards fully elucidating population-level impacts, stronger efforts are needed to consider the influence of sex throughout an organism’s life-cycle, and its contribution to the variability in species level responses.

Authors' contributions R.P.E. and A.M.Q.: produced systematic map protocol and conceptualised figure 1; R.P.E., M.A.U. and W.D.: implemented search, screening and data extraction; All authors contributed to manuscript, approved its final version and agreed to be held accountable for the content therein.

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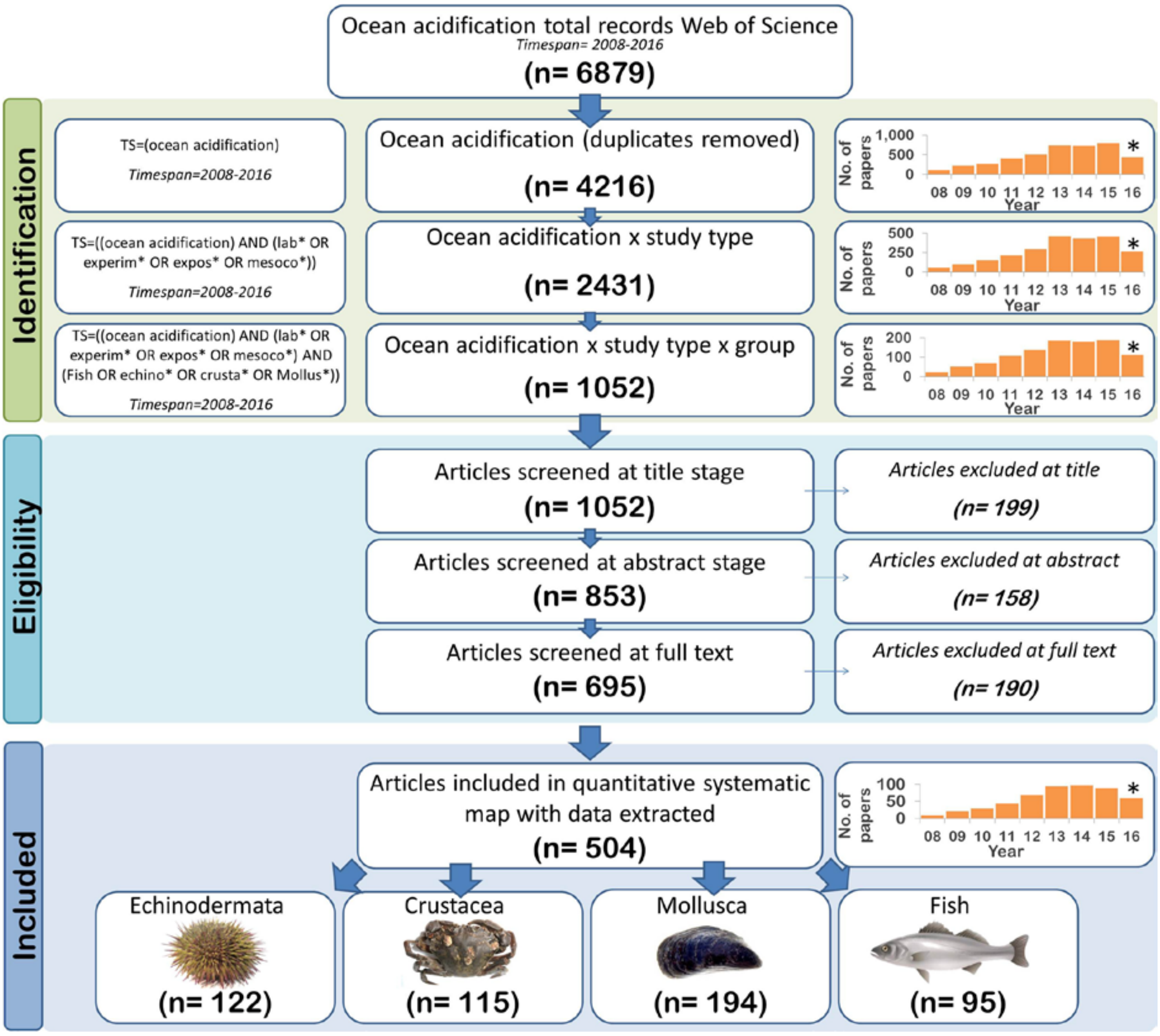
Data All data are included within supplementary materials.

Figure 1. Overview of the systematic map process. Values (n = x) are the number of studies at each stage. Asterisk indicates partial record for number of papers published in 2016 as literature sourced on 22/06/2016. Fish image Kovalevska/shutterstock.com.

Figure 2. Systematic map results. Proportion of studies based on the inclusion of sex as a factor in a) Echinodermata, b) Crustacea, c) Mollusca and d) Fish. Proportion of studies based on life stage investigated in e) Echinodermata, f) Crustacea, g) Mollusca and h) Fish. Fish image Kovalevska/shutterstock.com.

Figure 1


Ocean acidification total records Web of Science
Timespan= 2008-2016
(n= 6879)
Identification
TS=(ocean acidification)
Timespan=2008-2016
Ocean acidification (duplicates removed)
(n= 4216)
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1,000
500
0
08 09 10 11 12 13 14 15 16
Year
*
TS=((ocean acidification) AND (lab* OR experim* OR expos* OR mesoco*))
Timespan=2008-2016
Ocean acidification x study type
(n= 2431)
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08 09 10 11 12 13 14 15 16
Year
*
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Year
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Eligibility
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Articles screened at full text
(n= 695)
Articles excluded at full text
(n= 190)
Included
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No. of papers
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50
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08 09 10 11 12 13 14 15 16
Year
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Crustacea
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Mollusca
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Fish
(n= 95)

148 **Figure 2**

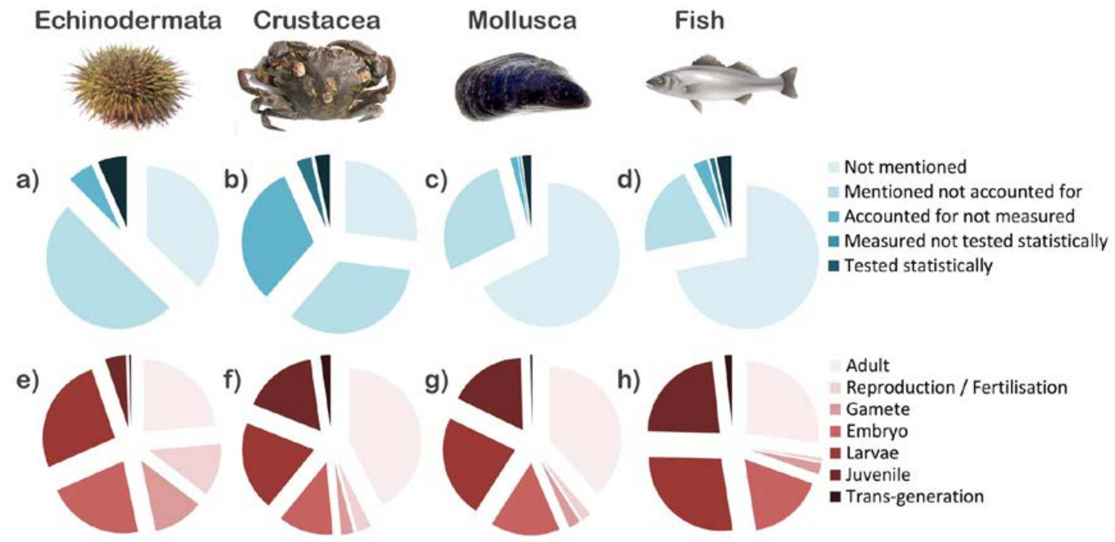

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Table 1. Overview of the systematic mapping of evidence. Data is pooled across organismal groups (N = 504 articles, Fig.1). Asterisk indicates partial record for number of papers published in 2016, as literature sourced on 22/06/2016.

Classification of sex	*No.*	*%*	**Life stage investigated**	*No.*	*%*	**Publication year**	*No.*	*%*
Not mentioned	265	52.58	Adult	245	48.61	2008	8	1.59
Mentioned not accounted for	168	33.33	Reproduction / Fertilisation	36	7.14	2009	20	3.97
Accounted for not measured	53	10.52	Gamete	38	7.54	2010	29	5.75
Measured not tested statistically	6	1.19	Embryo	127	25.20	2011	44	8.73
Tested statistically	19	3.77	Larvae	185	36.71	2012	68	13.49
			Juvenile	116	23.02	2013	94	18.65
			Trans-generation	8	1.59	2014	94	18.65
						2015	88	17.46
						2016 *	58	11.51

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