

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 $p\text{CO}_2$ 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.

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

27 **1. Introduction**

28 Ocean acidification (OA), changes in seawater carbonate chemistry induced by oceanic
29 uptake of anthropogenic CO₂, poses a major threat to marine biodiversity globally [1], as well as to
30 societies and industries reliant on marine living resources [2]. Studies investigating the ecological
31 effects of OA have increased exponentially over the past decade [3], increasing in complexity to
32 incorporate the highly dynamic nature of carbonate chemistry in many natural systems [4], multi-
33 stressor interactions [5], an ever increasing range of organisms, life history stages, communities,
34 and multiple generations [5]. Whilst this effort has contributed to help better explain species
35 tolerance and increase reliability of future change projections, intraspecies variation in OA responses
36 has received insufficient attention, adding uncertainty to reported responses and their
37 interpretation [6].

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

47 **2. Methods**

48 Following international guidelines, a systematic map protocol (Supplementary Materials,
49 "SM") was used to assess existing evidence (Fig. 1) addressing the research question: *Do OA studies*
50 *consider the impact of sex on organism responses?* Search term strings using Boolean logic were run

51 through Web of Science to collect relevant peer reviewed literature, and subsequently narrowed to
52 target literature published between January 2008 and May 2016, limited to studies on fish,
53 crustaceans, echinoderms and molluscs. This ensured a manageable literature set was reviewed
54 whilst providing a contemporary representation of the OA field. Search results were further refined
55 at three levels to exclude studies irrelevant to our research question (Fig. 1, SM). Study inclusion was
56 determined objectively against a set of inclusion criteria which defined pertinent population, study
57 type, intervention, comparator, and outcomes (SM for details).

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

66 **3. Results and discussion**

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

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

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

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

99 $p\text{CO}_2$ is shown to result in higher mortality in female shrimps (*Palaemon pacificus*) compared to
100 males [19], whilst the median lethal level (LC_{50}) for CO_2 is also lower in female copepods (*Acartia*
101 *tonsa*) compared to males [18]. Sex-specific physiological impacts can result in a 2-fold increase in
102 the respiration rate of male copepods under elevated $p\text{CO}_2$, but respiratory suppression in females
103 [20]. Similarly in molluscs, males and females respond differently to elevated $p\text{CO}_2$ and temperature,
104 with sex-based differences demonstrated in the mussel (*Mytilus edulis*) metabolome [10] and the
105 biochemical composition of limpet (*Nacella concinna*) gonad [21].

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

118 Here we demonstrate that whilst less than 4 % of the OA literature tests for sex-based
119 differences, there is a clear precedent for differential responses to elevated $p\text{CO}_2$ between sexes. If
120 sex-based differences do exist for economically important species, as seems likely, then capturing
121 this variance is crucial for accurately forecasting the future societal and economic repercussions of
122 OA for dependant sectors, such as coastal management, conservation, fisheries and aquaculture [2].
123 Unfortunately, the lack of a sufficiently wide evidence base for sex-specific responses currently limits

124 this ambition. As a starting point towards fully elucidating population-level impacts, stronger efforts
125 are needed to consider the influence of sex throughout an organism's life-cycle, and its contribution
126 to the variability in species level responses.

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

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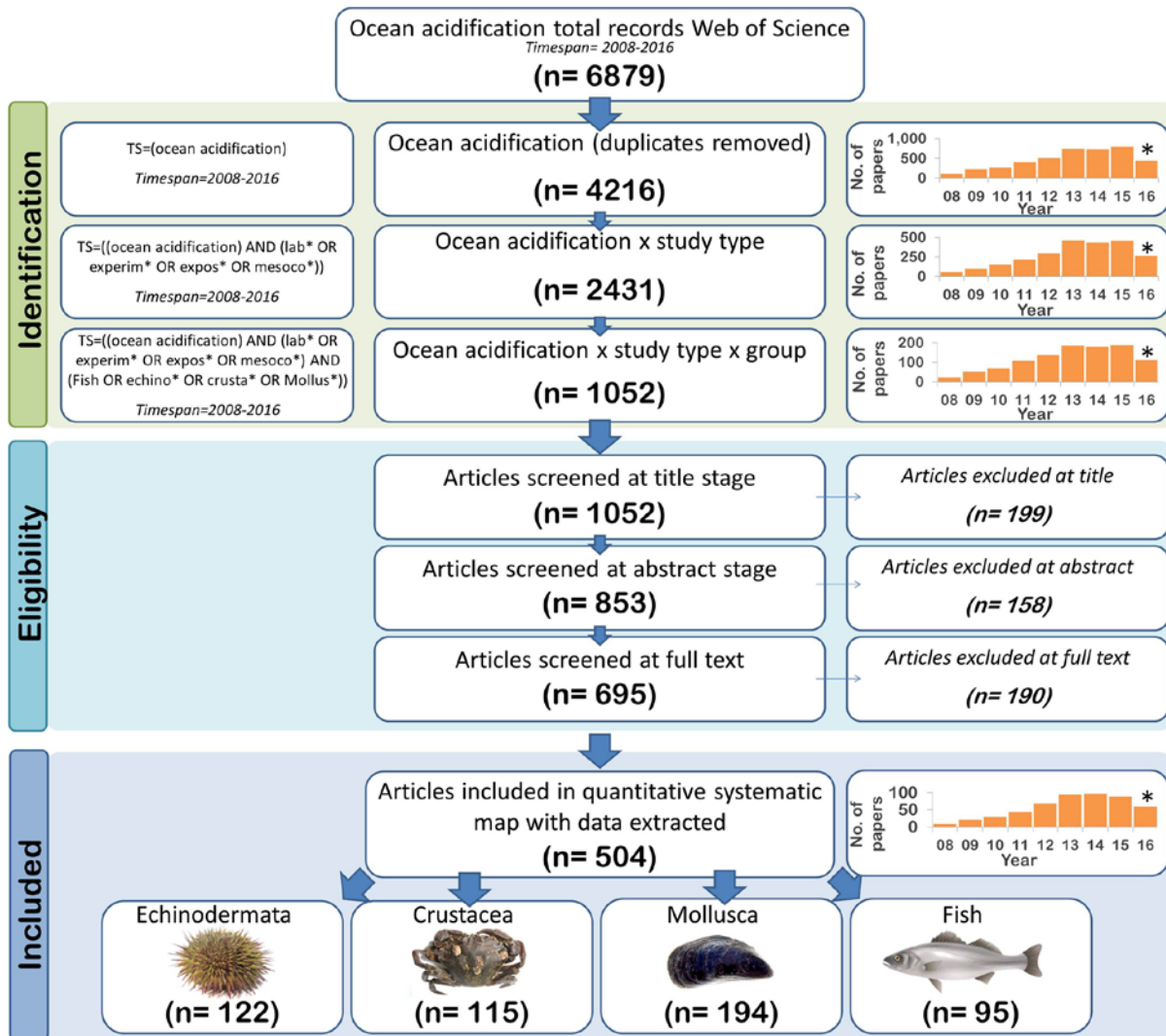
136 **Data** All data are included within supplementary materials.

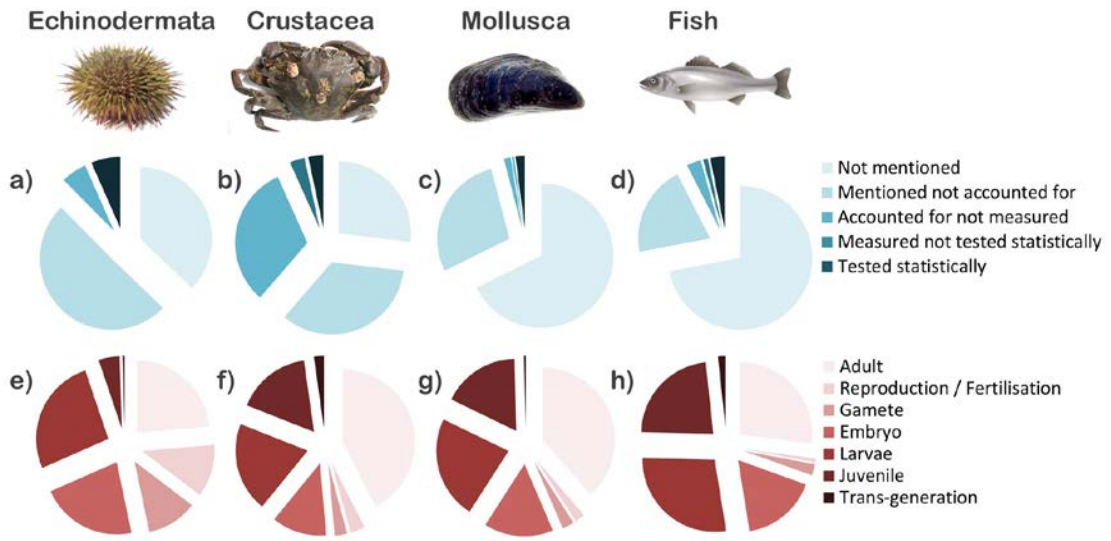
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138 **Figure 1.** Overview of the systematic map process. Values (n = x) are the number of studies at each
139 stage. Asterisk indicates partial record for number of papers published in 2016 as literature sourced
140 on 22/06/2016. Fish image Kovalevska/shutterstock.com.

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

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150 **Table 1.** Overview of the systematic mapping of evidence. Data is pooled across organismal groups (N = 504 articles, Fig.1). Asterisk indicates partial record
 151 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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153 **References**

- 154 [1] Dupont, S. & Pörtner, H. 2013 Marine science: Get ready for ocean acidification. *Nature* **498**, 429-
155 429.
- 156 [2] Queirós, A.M., Huebert, K.B., Keyl, F., Fernandes, J.A., Stolte, W., Maar, M., Kay, S., Jones, M.C.,
157 Hamon, K.G. & Hendriksen, G. 2016 Solutions for ecosystem-level protection of ocean systems under
158 climate change. *Global Change Biology*.
- 159 [3] Riebesell, U. & Gattuso, J.-P. 2015 Lessons learned from ocean acidification research. *Nature*
160 *Climate Change* **5**, 12-14.
- 161 [4] Boyd, P.W., Cornwall, C.E., Davison, A., Doney, S.C., Fourquez, M., Hurd, C.L., Lima, I.D. &
162 McMinn, A. 2016 Biological responses to environmental heterogeneity under future ocean
163 conditions. *Global Change Biology*.
- 164 [5] Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M. & Gattuso,
165 J.-P. 2013 Impacts of ocean acidification on marine organisms: quantifying sensitivities and
166 interaction with warming. *Global Change Biology* **19**, 1884-1896.
- 167 [6] Calosi, P., Turner, L.M., Hawkins, M., Bertolini, C., Nightingale, G., Truebano, M. & Spicer, J.I. 2013
168 Multiple Physiological Responses to Multiple Environmental Challenges: An Individual Approach.
169 *Integrative and Comparative Biology* **53**, 660-670. (doi:10.1093/icb/ict041).
- 170 [7] Bennett, A.F. 1987 Interindividual variability: an underutilized resource. *New directions in*
171 *ecological physiology* **15**, 147-169.
- 172 [8] Dillon, M.E. & Woods, H.A. 2016 Introduction to the symposium: Beyond the mean: Biological
173 impacts of changing patterns of temperature variation. *Integrative and comparative biology*, icw020.
- 174 [9] McClellan-Green, P., Romano, J. & Oberdörster, E. 2007 Does gender really matter in
175 contaminant exposure? A case study using invertebrate models. *Environmental Research* **104**, 183-
176 191.
- 177 [10] Ellis, R.P., Spicer, J.I., Byrne, J., Sommer, U., Viant, M.R., White, D. & Widdicombe, S. 2014 ¹H
178 NMR metabolomics reveals contrasting response by male and female mussels exposed to reduced

179 seawater pH, increased temperature and a pathogen. *Environmental Science & Technology* **48**, 7044-
180 7052.

181 [11] Lane, A., Campanati, C., Dupont, S. & Thiyagarajan, V. 2015 Trans-generational responses to low
182 pH depend on parental gender in a calcifying tubeworm. *Scientific Reports* **5**, 10847.

183 [12] Collaboration for Environmental Evidence. 2013 Guidelines for Systematic Review and Evidence
184 Synthesis in Environmental Management. Version 4.2. Environmental Evidence:
185 www.environmentalevidence.org/Documents/Guidelines/Guidelines4.2.pdf.

186 [13] Uthicke, S., Soars, N., Foo, S. & Byrne, M. 2013 Effects of elevated pCO₂ and the effect of parent
187 acclimation on development in the tropical Pacific sea urchin *Echinometra mathaei*. *Marine Biology*
188 **160**, 1913-1926.

189 [14] Uthicke, S., Liddy, M., Nguyen, H. & Byrne, M. 2014 Interactive effects of near-future
190 temperature increase and ocean acidification on physiology and gonad development in adult Pacific
191 sea urchin, *Echinometra* sp. A. *Coral Reefs* **33**, 831-845.

192 [15] Russell, M.P. 1998 Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic
193 changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). *Journal of Experimental*
194 *Marine Biology and Ecology* **220**, 1-14.

195 [16] Dupont, S., Dorey, N., Stumpp, M., Melzner, F. & Thorndyke, M. 2013 Long-term and trans-life-
196 cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus*
197 *droebachiensis*. *Marine Biology* **160**, 1835-1843.

198 [17] Yusa, Y. 2007 Causes of variation in sex ratio and modes of sex determination in the Mollusca—
199 an overview. *American Malacological Bulletin* **23**, 89-98.

200 [18] Cripps, G., Lindeque, P. & Flynn, K.J. 2014 Have we been underestimating the effects of Ocean
201 Acidification in zooplankton? *Global Change Biology* **20**, 3377-3385.

202 [19] Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M. & Ishimatsu, A. 2008 Long-term effects of
203 predicted future seawater CO₂ conditions on the survival and growth of the marine shrimp
204 *Palaemon pacificus*. *Journal of Experimental Marine Biology and Ecology* **367**, 41-46.

205 [20] Cripps, G., Flynn, K.J. & Lindeque, P.K. 2016 Ocean Acidification affects the phyto-zoo plankton
206 trophic transfer efficiency. *PLoS One* **11**, e0151739.

207 [21] Schram, J.B., Schoenrock, K.M., McClintock, J.B., Amsler, C.D. & Angus, R.A. 2016 Testing
208 Antarctic resilience: the effects of elevated seawater temperature and decreased pH on two
209 gastropod species. *ICES Journal of Marine Science* **73**, 739-752.

210 [22] Sfakianakis, D.G., Papadakis, I.E., Papadaki, M., Sigelaki, I. & Mylonas, C.C. 2013 Influence of
211 rearing temperature during early life on sex differentiation, haemal lordosis and subsequent growth
212 during the whole production cycle in European sea bass *Dicentrarchus labrax*. *Aquaculture* **412**, 179-
213 185.

214