



Ocean acidification does not overlook sex: Review of understudied effects and implications of low pH on marine invertebrate sexual reproduction

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Running Title: OA impact on invertebrate reproduction

Abstract

Sexual reproduction is a fundamental process essential for species persistence, evolution, and diversity. However, unprecedented oceanographic shifts due to climate change and anthropogenic effects can impact physiological processes with important implications for sexual reproduction. This article reviews how ocean acidification can impact sexual reproductive processes in marine invertebrates and highlights current research gaps. We focus on four economically and ecologically important taxonomic groups: cnidarians, crustaceans, echinoderms, and molluscs. We identify trends in reproductive performance in acidified conditions, discuss the spatial and temporal variability of experimental designs in the context of reproductive traits (gametogenesis, fertilization, and reproductive resource allocation), and provide a quantitative analysis of the published literature to assess the effects of low pH on fertilization rates across taxa. Our recommendations for future research will allow for better understanding of how reproduction in invertebrates will be affected in the context of a rapidly changing environment. Since models often consider the reproductive parameter as a “black box” and/or often overestimate it, our findings and suggestions will help to design and develop better tools to project future ecological trends in response to global change. We argue for increased focus on fundamental reproductive biology of marine invertebrates and associated molecular mechanisms that may be vulnerable to shifts in ocean chemistry.

Keywords: sperm, egg, fertilization, gametogenesis, fecundity, brooding, spawning, gonad development, climate change

Introduction

Global climate change impacts physical, biological, and chemical processes in the marine environment (Hoegh-Guldberg & Bruno, 2010; Howes et al., 2015; Shukla et al., 2019). Absorption of excess carbon dioxide by seawater lowers pH, and reduces carbonate ion concentration and aragonite saturation state which are both important for calcification (Doney et al., 2009). These alterations in water chemistry are referred to as ocean acidification (OA) and can critically impact marine organisms at multiple levels of biological organization (Melzner et al., 2019). At the organismal level, changing chemistry can influence physiological processes (e.g. calcification, internal pH control, respiration and nutrient uptake), affecting performance and survival (Howes et al., 2015; Kroeker et al., 2010; Melzner et al., 2019). Acidification could result in decoupling of biological and environmental cues, leading to reproductive failure with significant consequences for population dynamics in marine ecosystems (Olischläger & Wild, 2020; Shlesinger & Loya, 2019). Although there is extensive research on the effects of OA in marine invertebrates, gaps in our understanding of how OA affects sexual reproductive processes constrains predictions of species persistence, evolutionary adaptation, and biodiversity.

Reproduction is a complex process that relies on numerous mechanisms (Figure 1, Box 1); using multiple parameters to evaluate reproductive performance is fundamental to predict how fitness may be impacted by future environmental conditions. OA research has centered around processes during early-life history stages (embryos, larvae, and juveniles), and previous reviews have synthesized trends in organismal responses to OA (Albright, 2011; Byrne & Przeslawski, 2013; Dupont et al., 2010; Foo & Byrne, 2017; Kurihara et al., 2008; Przeslawski et al., 2015; Ross et al., 2011, 2016). While larval or juvenile performance can be indicative of successful reproduction, an appropriate chemical and physical environment for gamete development, spawning, mating behavior, and fertilization success is also crucial.

In this review we synthesize and provide a quantitative analysis of the literature to show how OA impacts reproductive processes of four ecologically, economically, and culturally important marine invertebrate taxa: cnidarians, crustaceans, echinoderms, and molluscs. Our timely synthesis identifies important gaps in knowledge of the physiological mechanisms involved in reproduction in response to OA. In addition to fertilization and brooding, we focus on pre-fertilization processes (Figure 1) that are poorly understood both mechanistically and in the context of climate change. We also examine implications for aquaculture, wild fisheries, restoration, and conservation. Finally, we provide recommendations for future research to better understand how invertebrate sexual reproduction will be affected in the context of a rapidly changing environment.

Methods

To identify empirical studies for this review, we searched the literature databases ProQuest, Web of Science, and Google Scholar for articles published in English-language journals through

the end of 2020 that examined the effects of OA on reproduction in our focal taxa. Specific keywords used to search databases included: climate change, ocean acidification, hypercapnia, reproduction, sperm, egg, fertilization, mating behavior, gametogenesis, brooding, hatch rate, spawning, sex determination, and gonad development. For each study, positive, neutral, or negative effects of OA on reproduction were identified for the following processes: 1) sex determination, differentiation and ratio, 2) gametogenesis and gamete quality, 3) fecundity and reproductive output, 4) timing of reproduction and synchronization, 5) mating behavior, and 6) fertilization (Figure 1). While we acknowledge that these processes are linked, we define specific metrics associated with each reproductive process to categorize each finding (Box 1). If a study examined multiple processes, they were categorized independently. To characterize geographic location of research, study site and collection site were assigned to one of the 12 Marine Realms of the World (Spalding et al., 2007). Collection sites are reported in Figure 2, and study sites (which in some instances differ from collection location) are also included in Supplemental Table 1.

Given the extensive literature that quantified and reported percent fertilization success in varying pH conditions (43 studies total, 17, 19, and 7 in echinoderms, molluscs, and cnidarians, respectively; none in crustaceans), we conducted a meta-analysis to explore general trends of marine invertebrate fertilization by pH. A generalized linear mixed model was developed to estimate fertilization rate by pH across all taxa using a logit-linked binomial distribution. When reported, the following metrics were extracted from publications, then tested as candidate predictor variables for fertilization success with chi-squared statistics: 1) difference between experimental and control pH (ΔpH), 2) taxonomic group, 3) insemination time, 4) sperm concentration, 5) sperm:egg ratio, 6) number of sires, and 7) number of dams. Fertilization data from OA-only treatments in multiple stressor studies (e.g. warming, hypoxia) were included in the analysis. When necessary, fertilization rate estimates were extracted from published figures using WebPlotDigitizer v4.2. In addition to exploring fertilization rates across all pH levels tested (6.0 - 8.28), we also examined the response of fertilization to pH using conditions more relevant to ocean acidification (7.6 - 8.3) (Intergovernmental Panel on Climate Change, 2014). For all models tested, study was included as a random effect. Data were weighted by the inverse of one plus the sampling variance squared ($1 / (1 + \sigma^2)^2$), which was determined from the fertilization mean, error rate (standard deviation), and number of trials conducted at each pH level. The value 1 was added to every sampling variance to prevent overinflation of low variances (between 0-1) in models (Fiorenza et al., 2020). Resultant p -values were considered significant if they were less than $\alpha = 0.05$.

Of the 43 fertilization studies identified in this review, five were omitted (echinoderms: 1, molluscs: 3, cnidarians: 1) due to missing error rates (corresponding authors were contacted). We recognize that the fertilization rate reported by some studies may actually represent fertilization rate plus early embryonic development success. Other reproductive processes included in this review (Figure 1) were not considered for meta-analyses given the inconsistent metrics reported and/or limited number of studies (Figure 2c).

Results

We identified a total of 149 studies that have investigated the effects of OA on reproduction in cnidarians, crustaceans, echinoderms, and molluscs (Supplemental Table 1). Echinoderm research comprises the majority of OA and reproduction studies, followed by research in molluscs (Figure 2A, B). With the exception of one echinoderm study published in 1924, all OA and reproduction research was conducted between 2004 and 2020 (Figure 2b). A majority of studies collected organisms from the Marine Realms (Spalding et al., 2007) of Temperate Northern Atlantic, followed by Temperate Northern Pacific, and Temperate Australasia (Figure 2a). There was one study from Temperate Southern Africa and two from the Arctic, but no studies from Western-Indo Pacific, Tropical Eastern Pacific, or Temperate South America.

Broadly, published research suggests that OA has either negative or neutral impacts on reproduction, with positive effects being rare (Figure 2c). Within each taxa, research focuses on a handful of species. All cnidarian studies examined corals. Similarly, there was only one cephalopod study within molluscan research. Copepods dominated crustacean literature, while urchin species were prominent in echinoderm studies.

Impacts of ocean acidification on sexual reproduction processes

In this section, we summarize ocean acidification studies for cnidarians, crustaceans, echinoderms, and molluscs by reproductive process (Box 1, Supplemental Table 1). See Supplementary Materials for a more comprehensive synthesis of results.

Sex determination, differentiation, and ratio

We identified 4 studies related to sex determination, differentiation, and ratio. All four studies examined impacts of OA on oogenesis and spermatogenesis in oysters, and indicate that acidification during gametogenesis may alter sex ratios by impacting egg and sperm development unequally (Boulais et al., 2017; Parker et al., 2018; Spencer et al., 2020). Sex-specific impacts of ocean acidification on development can reduce the proportion of females (Boulais et al., 2017) or males (Parker et al., 2018; Spencer et al., 2020) capable of breeding in a population. These studies are challenging due to the difficulty of identifying sex without destroying the samples being investigated (Ellis et al. 2017), the lack of sex biomarkers, and the limited understanding of hormonal control on reproductive processes in marine invertebrates.

Gametogenesis and gamete quality

This review highlights 40 studies related to gametogenesis, including metrics such as: gamete size, stage, biocomposition, integrity and developmental rate. In cnidarians, OA had a negative effect on octocoral egg size (Rossin et al., 2019), while hexacoral studies showed no effect of OA on gamete size or maturation rates (Fine & Tchernov, 2007; Gizzi et al., 2017; Caroselli et al., 2019). Crustacean species exposed to OA had delayed male and female oogenesis and smaller spermatophores (Conradi et al., 2019; Meseck et al., 2016; Cripps et al., 2014; Fitzer et

al., 2012), but OA did not affect copepod egg viability (Vehmaa et al., 2013) or barnacle gametogenesis (McDonald et al., 2009; Pansch et al., 2018). OA had negative effects on gonad growth, maturation, egg size, and gamete development in some echinoderm species (Dworjanyan et al., 2018; Kurihara, 2008; Marčeta et al., 2020; Siikavuopio et al., 2007; Stumpp et al., 2012; Suckling et al., 2014; Suckling et al., 2015; Hue et al., 2020; Hu et al., 2018; Verkaik et al., 2016), while other studies reported neutral effects on gamete development and quality (Dell'Acqua et al., 2019; Hue et al., 2020; Wood et al., 2008; Dell'Acqua et al., 2019; Hazan et al., 2014; Uthicke et al., 2013b, 2014, 2020; Wong et al., 2019). It is possible that echinoderm egg size is plastic, and longer exposure to low pH can increase egg size (Suckling et al., 2015). Several molluscan studies indicate that OA reduces gametogenesis, gamete quality, egg size, and maternal investment (Boulais et al., 2017; Scanes et al., 2018; Spady et al., 2019; Spencer et al., 2020; Xu et al., 2016). The integrity of mollusc eggs can also be affected by acidification, as oyster (*Crassostrea gigas*) eggs exposed to low pH had higher rupture rates (Omeregíe et al., 2019). There is some evidence that molluscan gametogenesis and gamete quality may be unaffected by OA (Parker et al., 2017; Scanes et al., 2018; Spencer et al., 2020; Venkataraman et al., 2019). While cnidarians and echinoderms exhibited more neutral effects, overall we found that gametogenesis and gamete quality were negatively affected by OA. These negative impacts on gametogenesis may be a result of hormone and pheromone suppression, or an increased metabolic demand resulting in energy directed away from reproductive processes to sustain basal functions (Sokolova et al., 2012).

Fecundity and reproductive output

In this review, we identified 35 studies that assessed fecundity, reproductive output (number of eggs/egg capsules produced), and hatch rate and success (brooders) in varying OA conditions. Some cnidarian studies report neutral effects of OA on fecundity (i.e. number of spawned egg-sperm bundles and eggs) (Jokiel et al., 2008; Caroselli et al., 2019; Gizzi et al., 2017); however, these studies also report reduced adult growth under OA, suggesting resources may have been allocated towards reproduction over growth. In another study, exposure to OA in cnidarians resulted in reduced number of eggs per polyp (Rossin et al., 2019). In echinoderms, OA negatively affected egg production, egg lipid content, and fecundity (Dupont et al., 2013; Verkaik et al., 2016; Wong et al., 2019). Two molluscan studies assessing fecundity reported negative effects of OA on the number of eggs produced and clutch size (Parker et al., 2018; Spady et al., 2019).

We also considered hatch rate as a proxy for fecundity and reproductive success in brooding organisms. Studies that measure hatch rate were limited to crustaceans, as no brooding species from other taxa reported hatch rate. Brooding crustaceans held at low pH were found to have reduced fecundity, hatch success, and clutch size (Kurihara et al., 2004; McConville et al., 2013; Miller et al., 2016; Vehmaa et al., 2012; Weydmann et al., 2012; Zervoudaki et al., 2013; Zhang et al., 2011; Mayor et al., 2007; Schiffer et al., 2014; Cripps et al., 2014; Thor & Dupont, 2015; Gravinese, 2018; Borges et al., 2018; Lee et al., 2020; Vehmaa et al., 2015). Several crustacean studies, however, showed that fecundity and hatch rate were unaffected by OA (Kurihara et al., 2004; McConville et al., 2013; Vehmaa et al., 2013; Weydmann et al., 2012; Zhang et al., 2011; Mayor et al., 2007; Cripps et al., 2014; Egilisdottir et al., 2009; Millet et al.,

2016). In one study, low pH increased reproductive output, but at the cost of body size and shell integrity (Fitzer et al., 2012). Increased egg production may be a short-term stress response or may indicate adaptive potential to pH change (Engström-Öst et al., 2014).

Timing of reproduction and synchronization

Synchronicity and timing of spawning are important to ensure successful fertilization. In this review, we highlight 4 studies in molluscs and echinoderms that provide insight into reproductive timing under acidified conditions. In molluscs, studies that induced spawning found reductions in spawning success in acidified conditions (Parker et al., 2018; Xu et al., 2016). One study examined impacts of OA on volitional spawning in a molluscan species, and reported no latent effects from prolonged acidification exposure on the timing or magnitude of larval production in oyster *Ostrea lurida* (Spencer et al., 2020). Similarly, a volitional spawning experiment in sea cucumbers (*Cucumaria frondosa*) reported no effect of OA on spawn timing (Verkaik et al., 2016). More studies that incorporate natural spawning conditions are needed to understand ecologically relevant impacts of acidification on spawn timing and synchronicity.

Mating Behavior

Marine species are diverse in their mating behaviors. However, the majority of OA studies to date examine mating behavior focus on broadcast spawning species, limiting the number of observable behavioral metrics. We summarize 2 studies that measure mate selection, courtship display, and mate guarding. Male mate tracking and guarding behavior in amphipod *Gammarus locusta* (Borges et al., 2018) were disrupted under OA conditions, which may have negative consequences for fertilization. While female squid (*Idiosepius pygmaeus*) laid denser egg clutches after low pH exposure, mating pair behavior was unaffected by OA (Spady et al., 2019; Steer et al., 2003). More studies are needed to explore the impacts of OA on invertebrate behavior, particularly in cephalopod and gastropod species with sophisticated mating behaviors (e.g. changing body patterns and textures, parallel swimming, and male-male fighting).

Fertilization

Many marine invertebrates broadcast spawn and undergo external fertilization, which exposes gametes to the surrounding environment prior to and during fertilization. Acidification may affect fertilization success by directly influencing gamete activation, sperm activity, egg biochemistry and polyspermy defense, and by altering chemosensory and biochemical egg-sperm interactions (Lymbery et al., 2019; Miyazaki, 2006; Mortensen & Mortensen, 1921; Nakajima et al., 2005). The impact of ocean acidification on fertilization and gametes has been reviewed previously (Byrne, 2012; Byrne, 2011; Byrne & Przeslawski, 2013; Foo & Byrne, 2017). Here we provide an updated review of 54 studies that have assayed fertilization, sperm quality, and/or egg biochemical processes across pH conditions, including 39 studies not discussed in prior reviews. Additionally, we conducted a meta-analysis to evaluate general trends across and within echinoderms, molluscs, and cnidarians.

Fertilization rate

Fertilization rate was previously found to be robust in pH above ~7.6 (Byrne, 2012; Byrne, 2011). However, these reviews noted discrepancies among studies of the same species and conspecifics from different habitats, and encouraged researchers to examine other taxa with standardized experimental designs. Since then, an additional 34 studies have assessed fertilization rate in varying pH conditions.

The majority of studies, which predominantly evaluate echinoderms (largely urchins) and bivalve molluscs, conclude that acidification negatively affects fertilization rate when gametes are directly exposed (Frieder, 2014; Gonzales-Bernat et al., 2013; Havenhand et al., 2008; Kurihara & Shirayama, 2004; Moulin et al., 2011; Riba et al., 2016; Schlegel et al., 2012; Smith & Clowes, 1924; Smith et al., 2019; Suckling et al., 2014; Sung et al., 2014; Uthicke et al., 2013a; Barros et al., 2013; Kimura et al., 2011; Parker et al., 2009, 2010; Riba et al., 2016; Scanes et al., 2014; Shi et al., 2017a, 2017b; Świeżak et al., 2018; Van Colen et al., 2012), or parents are exposed prior to fertilization (Graham et al., 2015). The pH level at which fertilization rate becomes compromised varies considerably across taxa, and also depends on other environmental factors such as sperm and egg concentration (Albright, 2011; Albright et al., 2010; Ericson et al., 2010; Frieder, 2014; Gonzales-Bernat et al., 2013), gamete incubation time (Bechmann et al., 2011; Kong et al., 2019), inter-individual variability (Schlegel et al., 2012; Smith et al., 2019; White et al., 2014), and species hybridization (Striewski, 2012). Parental acclimatization to low or highly variable conditions (including pH) can either increase (Kapsenberg et al., 2017; Moulin et al., 2011; Suckling et al., 2014), or decrease fertilization rates in low pH (Graham et al., 2015).

Negative effects of OA were not universally reported (Boulais et al., 2017; Bylenga et al., 2015; Byrne et al., 2013; Byrne et al., 2009; Byrne et al., 2010a; Byrne et al., 2010b; Hue et al., 2020; Iguchi et al., 2015; Martin et al., 2011; Miin Chua et al., 2013; Pitts et al., 2020; Schutter et al., 2015), which indicates that fertilization in some species may be more resilient than others to the effects of acidification. *Mytilus* spp. mussels, for example, are capable of maintaining high fertilization rates across a broad pH range (Bechmann et al., 2011; Eads et al., 2016; Gallo et al., 2020; Riba et al., 2016). Fertilization in some molluscan and echinoderm species can persist at severely low pH (e.g. ~23% of *C. gigas* eggs were successfully fertilized at pH 6.0, Riba et al., 2016).

In rare instances, low pH can positively affect fertilization rate. When *M. galloprovincialis* sperm are exposed to varying pH conditions in the presence of egg-derived chemicals, fertilization rates are higher in low pH. These results could be explained by higher rates of incompatible egg and sperm genotypes successfully fertilizing, which would not typically occur in more alkaline conditions and could ultimately reduce population fitness (Lymbery et al., 2019).

Fertilization rate meta-analysis

Studies that reported fertilization rates across pH levels were leveraged to perform a meta-analysis to reveal broad-scale impacts of pH on fertilization rate in cnidarians, echinoderms, and molluscs. Across taxa and experimental pH levels, ΔpH ($\text{pH}_{\text{experimental}} - \text{pH}_{\text{control}}$) significantly

predicts fertilization success, and the fitted model exhibits a sigmoidal curve (pH 6.0 - 8.3, $\chi^2 = 9.85$, $p = 0.0017$, yellow curve in Figure 3a). The probability of fertilization being successful is estimated by

$$p(\text{fertilization success}) = \frac{e^{(2.04 + 2.63 * \Delta\text{pH})}}{1 + e^{(2.04 + 2.63 * \Delta\text{pH})}}$$

where the ΔpH coefficient estimate = 2.63 and the model intercept = 2.04 (Figure 3). This meta-analysis reveals that fertilization rate is relatively stable at moderate pH changes (80% fertilization occurs at $\Delta\text{pH} -0.25$), but is more severely affected as $\sim\Delta\text{pH} < -0.35$.

Since many studies included pH levels beyond those predicted under RPC 8.5 of the IPCC (2014), we re-constructed the meta-analysis using data from $\text{pH} \geq 7.6$ to examine fertilization under more relevant ocean conditions. In the revised model, ΔpH is less predictive of fertilization rate ($\chi^2 = 2.91$, $p = 0.088$, green curve in Figure 3a). This supports previous conclusions that marine invertebrate fertilization is more robust to pH 7.6 and above (Byrne 2011, 2012). However, many intertidal species periodically experience pH levels below 7.6; therefore, the significance of the full model should not be disregarded.

While phylum did not interact significantly with ΔpH to predict fertilization rate in the all-taxa model, the number of studies was unbalanced among phyla, and there were large differences in ΔpH tested (Figure 3). Therefore, we constructed phylum-specific models of fertilization by pH (Figures 3b-e) for the full pH range (yellow) and $\geq \text{pH} 7.6$ (green) for each group. Echinoderm fertilization appears to be more sensitive than molluscs to pH changes, which could reflect habitat differences in the majority of species studied to date (largely subtidal echinoderms and intertidal molluscs). While data are limited, cnidarian studies suggest a similar sensitivity to that observed in echinoderms.

As noted by Byrne (2011, 2012), there are many factors found to influence fertilization success that vary across studies (e.g. sperm concentration, (Frieder, 2014; Gonzalez-Bernat et al., 2013a; Uthicke et al., 2013a)). Additionally, studies quantify “fertilization” at varying stages or time points after insemination (e.g. presence of fertilization envelope vs. cleavage). We tested other experimental variables (when reported, see Methods and Supplemental Table 2) in the all-taxa model, and found none that significantly predict fertilization as sole variables or covariates with ΔpH .

In summary, our meta-analysis indicates OA will reduce fertilization rates in many marine invertebrates. However substantial effects are only likely to occur during periods of severely acidified conditions.

Gamete mechanisms that influence fertilization rate

Successful fertilization involves a cascade of biochemical processes in both sperm and eggs, many of which include intracellular pH and calcium ion changes (Byrne, 2011; Morisawa & Yoshida, 2005; Nakajima et al., 2005; Sherwood et al., 2012). OA could alter fertilization rates through changes to chemoattractants, surface receptors, jelly coat, polyspermy defense system, and activation processes in eggs, and/or activation, motility, velocity, mitochondrial activity, chemotaxis, and fusion reactions in sperm.

Only a handful of studies examined the impact of acidification on egg fertilization mechanisms. In urchins, polyspermy frequency increases in acidified conditions (Reuter et al., 2011), which may be related to low pH reducing the protective egg jelly coat area (Foo, 2015). Egg intracellular pH adjusts according to the external chemical environment (Bögner et al., 2014; Ciapa & Philippe, 2013), which may hamper egg activation by interfering with calcium signaling and actin dynamics (Limatola et al., 2020). Indeed, Shi et al. (2017b) observed that low pH disrupts the intracellular calcium ion (Ca^{2+}) activity in clam oocytes (*Tegillarca granosa*), which was associated with reduced fertilization rates.

The influence of low pH on sperm activity varies. OA reduces sperm swimming speed, motility, and linearity in some echinoderms (Campbell et al., 2016; Havenhand et al., 2008; Morita et al., 2010; Uthicke et al., 2013a), but has no effect or positively impacts sperm swimming speed in others (Caldwell et al., 2011; Graham et al., 2015; Sung et al., 2014). Similarly, pH negatively affects sperm swimming speed, velocity, motility, and mitochondrial activity in some molluscs (Esposito et al., 2020; Omoregie et al., 2019; Shi et al., 2017a; Shi et al., 2017b; Vihtakari et al., 2013, 2016), but has no effect on sperm activity in others (Havenhand & Schlegel, 2009; Vihtakari et al., 2016). In one population of oysters (*C. gigas*), low pH increased sperm motility (Falkenberg et al., 2019). Two cnidarian (hexacoral) studies report negative effects of pH on sperm activity (Albright, 2011; Morita et al., 2010; Nakamura & Morita, 2012). Albright's (2011) examination of OA impacts on hexacoral sperm activity show no effect, but results from a second experiment suggest a negative effect, necessitating further investigation in cnidarians.

Contrasting impacts on sperm activity could be due to varied sensitivities among genotypes within species (Campbell et al., 2016; Falkenberg et al., 2019; Schlegel et al., 2015; Smith et al., 2019; Vihtakari et al., 2016). Individual sperm characteristics can also respond differently to low pH (Graham et al., 2015; Schlegel et al., 2012). For instance, negative effects were observed on the proportion of motile urchin *H. erythrogramma* sperm, but there was no effect on sperm swimming speed (Schlegel et al., 2012). Impacts on sperm activity can also be influenced by experimental conditions, such as the exposure duration (e.g. Eads et al., 2016) or the method of preparing experimental pH levels (pCO_2 vs. HCl, Shi et al., 2017a).

Conclusions

Implications

Commercial and Restoration Aquaculture

In 2016 nearly 26 million tons of invertebrates were produced for consumption via commercial aquaculture, comprising 32% of global aquaculture and 77% of marine and coastal aquaculture that year (Fao, 2018). Invertebrate aquaculture is increasingly leveraged by restoration groups to enhance wild populations that are struggling to recover naturally (Froehlich et al., 2017; Wasson et al., 2020). Both commercial and restoration aquaculture depend upon reliable sources of viable gametes or larvae, which are either produced in controlled hatchery conditions or collected from the wild (Helm, 2004; Washington Sea Grant, 2015). Aquaculture production could be critically limited if acidification partially or fully inhibits reproductive capacity.

Hatcheries that collect broodstock, gametes, or larvae from the wild may need to adjust production timing (Figure 4). Acidification slows gametogenesis in bivalves, urchins, and crustaceans, so it may take longer for adults to mature sexually, resulting in fewer gravid individuals in a given population or delayed maturation (Boulais et al., 2017; Parker et al., 2018). Hatcheries may need to collect organisms later, or condition them for longer in the hatchery. This is particularly important for restoration hatcheries that are tasked with supplementing wild populations with genetically diverse cohorts. As fewer gravid adults could result in fewer individuals contributing gametes, cohort diversity may decrease. Aquaculture groups that collect wild larvae or juveniles may also observe decreased abundances and ontogenetic or geographic shifts due to altered fertilization and gametogenic rates (Ekstrom et al., 2015; Spencer et al., 2020); however, this has yet to be confirmed in the wild.

For species in which reproduction becomes acutely inhibited by acidification, aquaculture programs will need to rely more heavily on in-hatchery culturing techniques. Some invertebrates will need to be held in modified conditions (e.g. buffered seawater) during gametogenesis. For species with sensitive fertilization mechanisms (e.g. urchin *Strongylocentrotus nudus*, (Sung et al., 2014), spawning will need to occur in buffered seawater. Unequal impacts of acidification on oogenesis and spermatogenesis (e.g. oyster *Crassostrea virginica*, (Boulais et al., 2017) could make it difficult for hatcheries to synchronize sperm and egg release. Holding cultured animals in controlled conditions during sexual development or sex change, or gamete cryopreservation technologies (Gwo, 2000), may help to mitigate the effects of OA on reproduction and ensure the availability of gametes. Should these measures be needed, larger facilities with more complex seawater treatment systems, more resources, and increased handling will be required, thus increasing the overall cost of production.

Adjusting practices to protect adults and gametes from exposure to acidification may, however, be detrimental to long-term species and industry resilience. To build resilient populations and lines, restoration and breeding programs could leverage standing genetic variability to target genotypes that are reproductively viable in acidified conditions (Parker et al., 2011). This may include careful consideration of broodstock collection sites, such as areas with naturally low or

variable pH conditions. Additionally, within- and inter-generational acclimatization, also known as carryover effects, have been observed in some cultured species (Ross et al., 2016). Adult exposure to stressors, including acidification, may result in beneficial carryover effects for offspring (Parker et al., 2012; Spencer et al., 2020). While additional research is needed and there may be limitations to intergenerational acclimatization (Byrne et al., 2019), hatcheries may be able to leverage beneficial carryover effects to improve offspring fitness for production and breeding purposes (Durland et al., 2019; Parker et al., 2011).

Wild Fisheries

In contrast to aquaculture, wild fisheries depend upon an organism's ability to reproduce in the natural environment; any impact of acidification on reproduction will directly influence wild stock available for fisheries. How populations will be affected is not yet clear, as few studies have explored impacts of acidification on invertebrate reproduction in the wild, or in conditions that mimic natural settings (a few exceptions being (Caroselli et al., 2019; Gallo et al., 2020; Gizzi et al., 2017). Predictions from laboratory findings discussed above indicate that acidification will negatively impact fertilization rates, maternal provisioning, and fecundity, and will alter spawning or mating behaviors, which will likely decrease overall productivity and possibly alter community composition (Figure 4).

Acidification may directly reduce invertebrate fishery stocks due to decreased reproductive output caused by increased energetic demands. Organisms exposed to acidification allocate more energy to maintain acid-base equilibrium (Sokolova et al., 2012), which likely reduces the amount of energy available for reproduction and negatively impacts fishery productivity. Reproduction-related mortality may also increase, as acidification can alter immune and stress-response function, increasing vulnerability to pathogens and secondary stressors during and after reproduction (Liu et al., 2016; Mackenzie et al., 2014; Wang et al., 2016).

Acidification may affect invertebrate fisheries indirectly by altering reproductive phenology and magnitude. In many invertebrates, reproductive capacity is closely tied to food availability before or during gametogenesis (Bernard et al., 2011). Changes to prey species dynamics due to acidification, such as altered phytoplankton communities' composition, abundance, and bloom timing (Cheung et al., 2011; Gao et al., 2012), may result in asynchronous invertebrate reproductive processes such that larval production and quality decreases. As individual size correlates positively with fecundity in females of some species (Ramirez Llodra, 2002), reduced prey abundance could impede reproductive capacity by reducing growth rate. These predicted impacts on reproductive success need to be assessed in wild populations, and considered in stock assessment and food-web models (Busch et al., 2013; Marshall et al., 2017), and by fisheries managers when determining catch quotas.

Of the taxa discussed in this review, those that constitute the largest fisheries are among the least studied. Combined, all crustacean taxa represent the largest wild-caught group (6.31 billion tons in 2017), with over half of the fishery comprising shrimp and prawn species (3.60 billion tons combined, FAO, 2019). There are limited data on the effects of acidification on crustacean reproduction, and there is no literature on economically important wild-caught

species like the crab *Portunus trituberculatus* and shrimp *Acetes japonicus*, which are among the largest invertebrate capture species worldwide (alongside the Jumbo flying squid *Dosidicus gigas*) (FAO, 2018). Cephalopods are another overlooked group. Despite 3.77 billion tons of squid, cuttlefish, and octopus harvested globally in 2017 (FAO, 2019), we identified only one cephalopod study in this review (Spady et al., 2019). It will be important for future studies to prioritize impacts of acidification on reproduction in species that support fishing industries and communities.

Conservation and Restoration of Wild Populations

Threatened and endangered marine invertebrates increasingly require human interventions to conserve and restore populations (Elliott et al., 2007). Success of these programs is contingent upon a population's ability to persist naturally and successfully reproduce once interventions cease. Therefore, it is critical that conservation and restoration programs identify whether reproductive processes will be impacted by low pH, and how that will impact their focal species' abundance and distribution.

Conservation and restoration programs may need to update their strategic plans to account for impacts of acidification on reproduction. For instance, natural recovery of wild populations following removal of stressors (e.g., creation of a marine protected area following overharvest) could be impeded by decreased larval supply due to reduced reproductive success. Populations may take longer to recover, requiring programs to allocate more time and resources for longer-term interventions. Conservation and restoration approaches may need to change altogether, such as moving from passive strategies (e.g. removing disturbances and allowing succession to occur naturally), to active strategies (e.g. transplanting or augmenting wild populations) (Figure 4). Coral may be uniquely vulnerable to acidification due to their sexual reproduction strategies (Burke et al., 2011; Richmond & Hunter, 1990). In many coral species, gametes take several months to develop, and spawning occurs only a few nights a year over a few hours (Babcock et al., 1986). If OA interferes with these highly coordinated spawning events (Olischläger & Wild, 2020) sexual reproduction could be severely inhibited in the wild, but this has yet to be tested. Programs may therefore need to incorporate restoration aquaculture and gamete cryopreservation to rebuild coral populations.

Genetic diversity, an important factor for species resilience to environmental stressors (Bernhardt & Leslie, 2013; Timpane-Padgham et al., 2017), could shift as a consequence of acidification-induced reproductive changes. Many invertebrate species are r-strategists with highly fecund females (Ramirez Llodra, 2002). Should acidification reduce the number of individuals capable of reproducing, populations could still be sustained by a few individuals that "win the reproduction lottery," or are selected for based on their ability to reproduce in acidified conditions (Hedgecock & Pudovkin, 2011; Sanford & Kelly, 2011). It may therefore be important for programs to actively facilitate genetic diversity and/or resistance to acidification. Programs may need to design marine protected areas that encompass a variety of current and projected pH conditions, not just buffered areas, to enable selection for low pH-resilient individuals. Programs that supplement wild populations with transplanted or hatchery-produced animals should consider sourcing from a range of pH conditions, or even producing a subset of

organisms under acidified conditions in the hatchery to select for reproductively-resilient individuals. Should the number of individuals contributing gametes become dangerously low, programs may need to begin cryopreserving gametes to preserve allelic diversity in a “seed bank” for future use (Figure 4).

Areas for future research

There is a critical need to know more about the basic biology and natural history of reproduction in marine invertebrate communities (Box 2). Long-term ecological baselines for reproduction are missing for most organisms. This information is essential for understanding the impacts of OA on organismal phenology and the resilience and plasticity of reproductive processes. More ecologically relevant experimental designs are also needed. For instance, knowledge of acidification effects on spawn timing and synchronicity is, to date, largely based on rates of spawning when induced or other metrics that approximate spawn readiness, such as the number of gravid individuals. Moving forward, it will be important to monitor natural spawn rates and timing in variable pH conditions.

The majority of studies identified in this review examine pH as a sole factor. Given observed and projected ocean changes, these studies will be insufficient to accurately predict impacts of climate change on reproduction. Co-occurring environmental stressors may interact to influence reproductive phenotype, as shown by some of the 26 multiple stressor papers identified in this review (see Supplementary Materials). These papers focus primarily on interactive effects of ocean acidification and warming on reproduction, with four studies to date examining other secondary stressors in conjunction with OA: low salinity (Egilsdottir et al., 2009), hypoxia (Graham et al., 2015), and metal contamination (Riba et al., 2016; Scanes et al., 2018). The impact of OA on reproduction varies depending on co-stressors, species, and life history stages, eliciting a range of interactions between stressors: additive, synergistic, antagonistic, or no effect (Todgham & Stillman, 2013). Additional research is necessary to better understand how OA and other stressors interact and affect invertebrate reproduction. The list of studied co-stressors must expand to include variables such as disease and high nutrient loads. Additionally, echinoderm and molluscan species are the most studied taxa in multiple stressor experiments, so research involving multiple stressors should be conducted in other groups.

Arguably, the most pressing need is for increased mechanistic knowledge of reproductive processes, which is crucial for gauging the impact of OA on marine invertebrates (Figure 5). In the remainder of this section, we outline mechanisms that should be explored with future research.

Fertilization mechanisms

Fertilization mechanisms are the most studied reproductive processes in marine invertebrates. Even so, there are still considerable knowledge gaps, particularly in how changes to gamete quality and sperm-egg interactions will affect fertilization (Figure 5). Compared to the numerous echinoderm and molluscan bivalve studies, fertilization data is available for only two gastropod species (Byrne et al., 2010a; Kimura et al., 2011), which found contrasting results), no cephalopod species, and no non-hexacoral cnidarians. More studies are clearly needed for these ecologically and economically important taxa.

Gamete quality depends on maintaining an optimal intracellular pH (pH_i). For example, sperm motility is positively associated with sperm pH_i across taxa (see *Fertilization: Sperm Activity*). Reductions in extracellular pH are thought to affect pH_i , suppress motility, and impact fertilization success (Alavi & Cosson, 2005; Boulais et al., 2018; Christen et al., 1983; Morita et al., 2006). In certain species, an increase in pH may be required for sperm activation (Nakajima et al., 2005). Explicit measurements of sperm pH_i , including those taken in the field, can help uncover the mechanism behind pH_i activation of sperm motility and its vulnerability to environmental pH conditions.

The impact of acidification on spawned eggs likely depends on their composition, structure, and intracellular conditions. Egg size is used as a proxy for well-provisioned eggs, but few studies empirically test this assumption with lipid analyses. As coping with OA may divert resources away from gamete development, more research should be conducted on energy allocation, maternal provisioning, and egg lipid content in low pH conditions. Intracellular egg calcium content can also impact fertilization. Calcium triggers the second meiotic division in eggs, readying it to unite with sperm to complete the fertilization process (Miyazaki, 2006), and is part of the polyspermy defense (Epel, 1978). Since egg calcium content can be influenced by environmental pH (Ciapa & Philippe, 2013), egg calcium dynamics may influence the effect of pH on fertilization.

Other structural components that could be influenced by acidification include the egg jelly coat and gamete recognition proteins. Present in echinoderms and molluscs, the jelly coat protects the egg from intense wave action and prevents polyspermy, and increases the likelihood of fertilization by increasing the size of the egg (Farley & Levitan, 2001; Podolsky, 2004). The dissolution of the jelly coat by low pH conditions observed in echinoderms can reduce percent fertilization. Additionally, the jelly coat is known to contain chemoattractants that activate and guide sperm or prevent polyspermy, and harbor protective chaperone and gamete recognition proteins (Evans & Sherman, 2013). The structure of the jelly coat and corresponding components must be described further to gauge how low pH will affect this important gelatinous layer. Cnidarian eggs do not have jelly coats, and similar structures have not been described in crustaceans. A general survey of gamete accessory structures in marine invertebrates will also be beneficial to determine how low pH will impact eggs.

Mechanisms beyond fertilization

Mechanisms controlling pre-fertilization reproductive processes are understudied generally and in the context of OA. In many marine invertebrates, gametogenesis, spawn timing, and synchronicity is influenced by exogenous signals such as temperature, photoperiod, food, and pheromones from conspecific gametes (Galtsoff, 1938; Lawrence & Soame, 2004), and by endogenous signals such as hormones or neuropeptides (Jouaux et al., 2012; Tanabe et al., 2010). Applying knowledge of how these environmental cues are registered and acted upon by organisms may elucidate how low pH will affect these pathways.

Future studies should examine whether OA impacts biochemical cues involved in gametogenesis, coordinated spawning events, and mating behavior. Since many invertebrates are osmoconformers with open circulatory systems, reproductive hormones that circulate through the hemolymph are likely to be exposed to environmental pH changes (Tarrant, 2007; Treen et al., 2012). For instance, the neuropeptide alanine-proline-glycine-tryptophan-NG2 (APGWamide) regulates bivalve adductor muscle contractions that propel gametes into the water column during spawning (Bernay et al., 2006). Whether acidification alters marine invertebrate reproductive hormones is not yet known. Structural changes have been observed in signaling peptides outside of the reproductive context (Roggatz et al., 2016; Roggatz et al., 2016; Tarrant, 2007). Exposure to acidification, for instance, resulted in protonation of three peptide signalling molecules, which was associated with behavioral impairment in shore crabs (Roggatz et al., 2016). This framework could be extended to invertebrate mating behavior. Pheromones come in direct contact with the environment, and may be important for spawning synchrony, long-distance mate searches, mate guarding, and copulation dances. Female *Sinularia polygactyla* coral colonies have been documented to release estradiol prior to spawning (Slattery et al., 1999). The chemical composition of pheromones, their residence time in acidified conditions, and the ability of invertebrates to produce and detect these compounds may be impacted by low pH. Additionally, a recent hypothesis developed by Olischläger and Wild (2020) suggests that gamete release timing during periods of low water motion is possibly regulated by the carbon concentrating mechanism in corals and/or their symbionts, which may make the synchronized spawning of organisms with photo-symbionts vulnerable to OA. Future work should examine hormones, pheromones, and other factors governing reproductive cues and behavior to identify molecular signals vulnerable to OA.

For a more comprehensive understanding of changes to reproductive resource allocation in acidified conditions, future studies should examine multiple physiological metrics. OA-induced changes in sex-specific growth, tissue regeneration, and energy allocation could impact reproductive resource allocation. Organisms that regulate their internal environment may have increased metabolic demand in low pH conditions, and less energy could be available for reproductive processes (Sokolova et al., 2012). While there are no studies correlating internal pH at the site of gametogenesis and environmental pH, changes to the organism's internal environment have been studied with respect to calcification. Echinoderms are able to buffer coelomic fluid, and molluscs can do the same with extracellular fluid (Collard et al., 2013; Crenshaw, 1972). When exposed to acidified conditions, the intracellular fluid of the crab *Chionoecetes bairdi* did not change in pH. However, there were significantly more dead

hemolymph cells and less granular cells within acidified treatments. This suggests that metabolic demands related to increased cell apoptosis, immune response, and phagocytosis may be diverting energy away from reproduction (Meseck et al., 2016). In corals, tissue damage caused a reduction in coral fecundity (Rinkevich, 1996; Rinkevich & Loya, 1989; Van Veghel & Bak, 1994) and tissue regenerated at a lower rate under OA in various hexacorals (Horwitz & Fine, 2014). If organisms are able to buffer extracellular pH to promote calcification, the diversion of energetic resources to this task may impact reproduction.

Brooding species have fundamentally different reproductive strategies than broadcast spawners, so findings from spawners cannot be universal. While there is a lack of research on OA-effects on brooding behavior and mechanisms in cnidarians, echinoderms, and molluscs, recent studies suggest that brooding could impart tolerance in these taxa. For example, pH conditions in the brood chambers of *Ostrea spp.* oysters are lower than the surrounding water (Gray et al., 2019). Naturally lower pH in brood chambers could make fertilization more resilient to OA (Gray et al., 2019; Lucey et al., 2015), thereby providing a potential evolutionary advantage. Although fertilization and larval development occur internally in brooding and copulating organisms, some species' sperm must spawn into and travel through the water column to make contact with eggs inside the female. In this process, sperm will still be affected by chemical changes in the environment.

Genomic regulation of reproduction in marine invertebrates is a crucial, yet understudied, area. Determining how OA affects baseline genetic processes related to reproduction is imperative for understanding if processes can be phenotypically plastic or evolve to stressors. In the oyster *C. gigas*, genes specific to sex, maturation stage, early gametogenesis, sex determination, and differentiation, have been identified (Cavelier et al., 2017; Dheilly et al., 2012; Yue et al., 2018). Expressed transcriptomes of reproductively active individuals at various time points may elucidate genetic regulation of reproduction in low pH conditions. If organisms experience OA conditions during reproductive conditioning, environmental cues may be integrated in the germline through DNA methylation and other epigenetic modifications (Bell & Hellmann, 2019). In *C. virginica*, OA exposure modified the reproductive tissue methylome (Venkataraman et al., 2020). Changes to DNA methylation due to low pH may impact successful reproduction, or explain intergenerational effects of these conditions on marine invertebrates. Analysis of DNA methylation enzymes during the maternal-to-zygotic transition in coral *M. capitata* embryos exposed to OA suggests that methylome programming occurs within 4 hours of fertilization (Chille et al., 2021), further emphasizing the vulnerability of molecular machinery to OA and its influence on carryover effects. Beyond DNA methylation, chromatin organization may impact reproductive success or carryover effects. Gametes, particularly sperm, are subject to significant chromatin reorganization and packaging that can affect the availability of genes for methylation or expression (Eirin-Lopez & Putnam, 2018). As successful reproduction hinges on the survival of offspring, understanding genetic and epigenetic mechanisms that drive reproduction is important for understanding changes to reproductive processes and offspring performance.

References

- Alavi, S. M. H., & Cosson, J. (2005). Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biology International*, 29(2), 101–110.
<https://doi.org/10.1016/j.cellbi.2004.11.021>
- Albright, R., & Mason, B. (2013). Projected near-future levels of temperature and pCO₂ reduce coral fertilization success. *PloS One*, 8(2), e56468.
<https://doi.org/10.1371/journal.pone.0056468>
- Albright, R. (2011). Effects of ocean acidification on early life history stages of Caribbean scleractinian corals. University of Miami.
- Albright, R., Mason, B., Miller, M., & Langdon, C. (2010). Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *Proceedings of the National Academy of Sciences of the United States of America*, 107(47), 20400–20404.
<https://doi.org/10.1073/pnas.1007273107>
- Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, J. K., Wallace, C. C., & Willis, B. L. (1986). Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, 90(3), 379–394. <https://doi.org/10.1007/BF00428562>
- Barros, P., Sobral, P., Range, P., Chícharo, L., & Matias, D. (2013). Effects of sea-water acidification on fertilization and larval development of the oyster *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology*, 440, 200–206.
<https://doi.org/10.1016/j.jembe.2012.12.014>
- Bechmann, R. K., Taban, I. C., Westerlund, S., Godal, B. F., Arnberg, M., Vingen, S., Ingvarsdottir, A., & Baussant, T. (2011). Effects of ocean acidification on early life stages of shrimp (*Pandalus borealis*) and mussel (*Mytilus edulis*). *Journal of Toxicology and Environmental Health. Part A*, 74(7-9), 424–438.
<https://doi.org/10.1080/15287394.2011.550460>
- Bell, A. M., & Hellmann, J. K. (2019). An Integrative Framework for Understanding the Mechanisms and Multigenerational Consequences of Transgenerational Plasticity. *Annual Review of Ecology, Evolution, and Systematics*, 50(1), 97–118.
<https://doi.org/10.1146/annurev-ecolsys-110218-024613>
- Bernard, I., de Kermoyan, G., & Pouvreau, S. (2011). Effect of phytoplankton and temperature on the reproduction of the Pacific oyster *Crassostrea gigas*: Investigation through DEB theory. *Journal of Sea Research*, 66(4), 349–360.
<https://doi.org/10.1016/j.seares.2011.07.009>
- Bernay, B., Baudy-Floc'h, M., Zanuttini, B., Zatylny, C., Pouvreau, S., & Henry, J. (2006). Ovarian and sperm regulatory peptides regulate ovulation in the oyster *Crassostrea gigas*. *Molecular Reproduction and Development*, 73(5), 607–616.
<https://doi.org/10.1002/mrd.20472>
- Bernhardt, J. R., & Leslie, H. M. (2013). Resilience to climate change in coastal marine ecosystems. *Annual Review of Marine Science*, 5, 371–392.
<https://doi.org/10.1146/annurev-marine-121211-172411>
- Bögner, D., Bickmeyer, U., & Köhler, A. (2014). CO₂-induced fertilization impairment in *Strongylocentrotus droebachiensis* collected in the Arctic. *Helgoland Marine Research*, 68(2), 341–356. <https://doi.org/10.1007/s10152-014-0394-3>

- Borges, F. O., Figueiredo, C., Sampaio, E., Rosa, R., & Grilo, T. F. (2018). Transgenerational deleterious effects of ocean acidification on the reproductive success of a keystone crustacean (*Gammarus locusta*). *Marine Environmental Research*, 138, 55–64. <https://doi.org/10.1016/j.marenvres.2018.04.006>
- Boulais, M., Chenevert, K. J., Demey, A. T., Darrow, E. S., Robison, M. R., Roberts, J. P., & Volety, A. (2017). Oyster reproduction is compromised by acidification experienced seasonally in coastal regions. *Scientific Reports*, 7(1), 13276. <https://doi.org/10.1038/s41598-017-13480-3>
- Boulais, M., Suquet, M., Arsenault-Pernet, E. J., Malo, F., Queau, I., Pignet, P., Ratiskol, D., Le Grand, J., Huber, M., & Cosson, J. (2018). pH controls spermatozoa motility in the Pacific oyster (*Crassostrea gigas*). *Biology Open*, 7(3). <https://doi.org/10.1242/bio.031427>
- Burke, L., Reyta, K., Spalding, M., & Perry, A. (2011). *Reefs at risk revisited*. World Resources Institute. <https://digitalarchive.worldfishcenter.org/handle/20.500.12348/1107>
- Busch, D. S., Harvey, C. J., & McElhany, P. (2013). Potential impacts of ocean acidification on the Puget Sound food web. *ICES Journal of Marine Science*, 70(4), 823–833. <https://doi.org/10.1093/icesjms/fst061>
- Butts, I. A. E., Roustaiian, P., & Litvak, M. K. (2012). Fertilization strategies for winter flounder: effects of spermatozoa density and the duration of gamete receptivity. *Aquatic Biology*, 16(2), 115–124. <https://doi.org/10.3354/ab00439>
- Bylenga, C. H., Cummings, V. J., & Ryan, K. G. (2015). Fertilisation and larval development in an Antarctic bivalve, *Laternula elliptica*, under reduced pH and elevated temperatures. *Marine Ecology Progress Series*, 536, 187–201. <https://doi.org/10.3354/meps11436>
- Byrne, M., Foo, S. A., Ross, P. M., & Putnam, H. M. (2019). Limitations of cross and multigenerational plasticity for marine invertebrates faced with global climate change. *Global Change Biology*. <https://doi.org/10.1111/gcb.14882>
- Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology*, 53(4), 582–596. <https://doi.org/10.1093/icb/ict049>
- Byrne, M., Gonzalez-Bernat, M., Doo, S., Foo, S., Soars, N., & Lamare, M. (2013). Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*. *Marine Ecology Progress Series*, 473, 235–246. <https://doi.org/10.3354/meps10058>
- Byrne, M. (2012). Global change ecotoxicology: Identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches. *Marine Environmental Research*, 76(Supplement C), 3–15. <https://doi.org/10.1016/j.marenvres.2011.10.004>
- Byrne, M. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in changing ocean. *Oceanography and Marine Biology: An Annual Review*, 49, 1–42. <https://doi.org/10.1016/B978-0-12-819570-3.00016-0>
- Byrne, M., Soars, N. A., Ho, M. A., Wong, E., McElroy, D., Selvakumaraswamy, P., Dworjanyn, S. A., & Davis, A. R. (2010a). Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Marine Biology*, 157(9), 2061–2069. <https://doi.org/10.1007/s00227-010-1474-9>

- Byrne, M., Soars, N., Selvakumaraswamy, P., Dworjanyn, S. A., & Davis, A. R. (2010b). Sea urchin fertilization in a warm, acidified and high pCO₂ ocean across a range of sperm densities. *Marine Environmental Research*, 69(4), 234–239. <https://doi.org/10.1016/j.marenvres.2009.10.014>
- Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H. D., Dworjanyn, S. A., & Davis, A. R. (2009). Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proceedings of the Royal Society B*, 276(1663), 1883–1888. <https://doi.org/10.1098/rspb.2008.1935>
- Caldwell, G. S., Fitzner, S., Gillespie, C. S., Pickavance, G., Turnbull, E., & Bentley, M. G. (2011). Ocean acidification takes sperm back in time. *Invertebrate Reproduction & Development*, 55:4, 217–221. <https://doi.org/10.1080/07924259.2011.574842>
- Campbell, A. L., Levitan, D. R., Hosken, D. J., & Lewis, C. (2016). Ocean acidification changes the male fitness landscape. *Scientific Reports*, 6, 31250. <https://doi.org/10.1038/srep31250>
- Caroselli, E., Gizzi, F., Prada, F., Marchini, C., Airi, V., Kaandorp, J., Falini, G., Dubinsky, Z., & Goffredo, S. (2019). Low and variable pH decreases recruitment efficiency in populations of a temperate coral naturally present at a CO₂ vent. *Limnology and Oceanography*, 64: 1059–1069. <https://doi.org/10.1002/lno.11097>
- Cavelier, P., Cau, J., Morin, N., & Delsert, C. (2017). Early gametogenesis in the Pacific oyster: new insights using stem cell and mitotic markers. *The Journal of Experimental Biology*, 220(21), 3988–3996. <https://doi.org/10.1242/jeb.167734>
- Cheung, W. W. L., Dunne, J., Sarmiento, J. L., & Pauly, D. (2011). Integrating ecophysiology and plankton dynamics into projected maximum fisheries catch potential under climate change in the Northeast Atlantic. *ICES Journal of Marine Science*, 68(6), 1008–1018. <https://doi.org/10.1093/icesjms/fsr012>
- Chille, E., Strand, E., Neder, M., Schmidt, V., Sherman, M., Mass, T., & Putnam, H. M. (2021). Developmental series of gene expression clarifies maternal mRNA provisioning and maternal-to-zygotic transition in the reef-building coral *Montipora capitata*. *bioRxiv* (p. 2021.04.14.439692). <https://doi.org/10.1101/2021.04.14.439692>
- Christen, R., Schackmann, R. W., & Shapiro, B. M. (1983). Metabolism of sea urchin sperm. Interrelationships between intracellular pH, ATPase activity, and mitochondrial respiration. *The Journal of Biological Chemistry*, 258(9), 5392–5399. <https://www.ncbi.nlm.nih.gov/pubmed/6222053>
- Ciapa, B., & Philippe, L. (2013). Intracellular and extracellular pH and Ca are bound to control mitosis in the early sea urchin embryo via ERK and MPF activities. *PloS One*, 8(6), e66113. <https://doi.org/10.1371/journal.pone.0066113>
- Collard, M., Laitat, K., Moulin, L., Catarino, A. I., Grosjean, P., & Dubois, P. (2013). Buffer capacity of the coelomic fluid in echinoderms. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 166(1), 199–206. <https://doi.org/10.1016/j.cbpa.2013.06.002>
- Conradi, M., Sánchez-Moyano, J. E., Galotti, A., Jiménez-Gómez, F., Jiménez-Melero, R., Guerrero, F., Parra, G., Bonnail, E., & DelValls, T. Á. (2019). CO₂ leakage simulation: Effects of the decreasing pH to the survival and reproduction of two crustacean species. *Marine Pollution Bulletin*, 143, 33–41. <https://doi.org/10.1016/j.marpolbul.2019.04.020>
- Crenshaw, M. A. (1972). The inorganic composition of molluscan extrapalial fluid. *The Biological*

- Bulletin*, 143(3), 506–512. <https://doi.org/10.2307/1540180>
- Cripps, G., Lindeque, P., & Flynn, K. (2014). Parental exposure to elevated pCO₂ influences the reproductive success of copepods. *Journal of Plankton Research*, 36(5), 1165–1174. <https://doi.org/10.1093/plankt/fbu052>
- Dell'Acqua, O., Ferrando, S., Chiantore, M., & Asnaghi, V. (2019). The impact of ocean acidification on the gonads of three key Antarctic benthic macroinvertebrates. *Aquatic Toxicology*, 210, 19–29. <https://doi.org/10.1016/j.aquatox.2019.02.012>
- Dheilly, N. M., Lelong, C., Huvet, A., Kellner, K., Dubos, M.-P., Riviere, G., Boudry, P., & Favrel, P. (2012). Gametogenesis in the Pacific oyster *Crassostrea gigas*: a microarrays-based analysis identifies sex and stage specific genes. *PloS One*, 7(5), e36353. <https://doi.org/10.1371/journal.pone.0036353>
- Doney, S. C., Fabry, V. J., Feely, R. A., & Kleypas, J. A. (2009). Ocean acidification: the other CO₂ problem. *Annual Review of Marine Science*, 1, 169–192. <https://doi.org/10.1146/annurev.marine.010908.163834>
- Dupont, S., Dorey, N., Stumpp, M., Melzner, F., & Thorndyke, M. (2013). Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology*, 160(8), 1835–1843. <https://doi.org/10.1007/s00227-012-1921-x>
- Dupont, S., Ortega-Martínez, O., & Thorndyke, M. (2010). Impact of near-future ocean acidification on echinoderms. *Ecotoxicology*, 19(3), 449–462. <https://doi.org/10.1007/s10646-010-0463-6>
- Durland, E., Waldbusser, G., & Langdon, C. (2019). Comparison of larval development in domesticated and naturalized stocks of the Pacific oyster *Crassostrea gigas* exposed to high pCO₂ conditions. *Marine Ecology Progress Series*, 621, 107–125. <https://doi.org/10.3354/meps12983>
- [Dworjanyn S. A. & Byrne M. \(2018\). Impacts of ocean acidification on sea urchin growth across the juvenile to mature adult life-stage transition is mitigated by warming. *Proceedings. Biological sciences / The Royal Society*, 285\(1876\). <https://doi.org/10.1098/rspb.2017.2684>](https://doi.org/10.1098/rspb.2017.2684)
- Eads, A. R., Kennington, W. J., & Evans, J. P. (2016). Interactive effects of ocean warming and acidification on sperm motility and fertilization in the mussel *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, 562, 101–111. <https://doi.org/10.3354/meps11944>
- Eckelbarger, K. J., Linley, P. A., & Grassle, J. P. (1984). Role of ovarian follicle cells in vitellogenesis and oocyte resorption in *Capitella* sp. I (Polychaeta). *Marine Biology*, 79(2), 133–144. <https://doi.org/10.1007/bf00951822>
- Egilsdottir, H., Spicer, J. I., & Rundle, S. D. (2009). The effect of CO₂ acidified seawater and reduced salinity on aspects of the embryonic development of the amphipod *Echinogammarus marinus* (Leach). *Marine Pollution Bulletin*, 58(8), 1187–1191. <https://doi.org/10.1016/j.marpolbul.2009.03.017>
- Eirin-Lopez, J. M., & Putnam, H. M. (2018). Marine Environmental Epigenetics. *Annual Review of Marine Science*. <https://doi.org/10.1146/annurev-marine-010318-095114>
- Ekstrom, J. A., Suatoni, L., Cooley, S. R., Pendleton, L. H., Waldbusser, G. G., Cinner, J. E., Ritter, J., Langdon, C., van Hooijdonk, R., Gledhill, D., Wellman, K., Beck, M. W., Brander, L. M., Rittschof, D., Doherty, C., Edwards, P. E. T., & Portela, R. (2015). Vulnerability and adaptation of US shellfisheries to ocean acidification. *Nature Climate Change*, 5(3), 207–

214. <https://doi.org/10.1038/nclimate2508>
- Elliott, M., Burdon, D., Hemingway, K. L., & Apitz, S. E. (2007). Estuarine, coastal and marine ecosystem restoration: confusing management and science—a revision of concepts. *Estuarine, Coastal and Shelf Science*, 74(3), 349–366. <https://doi.org/10.1016/j.ecss.2007.05.034>
- Ellis, R. P., Davison, W., Queirós, A. M., Kroeker, K. J., Calosi, P., Dupont, S., Spicer, J. I., Wilson, R.W., Widdicombe, S. & Urbina, M. A., (2017). Does sex really matter? Explaining intraspecies variation in ocean acidification responses. *Biology letters*, 13(2), 20160761. <http://dx.doi.org/10.1098/rsbl.2016.0761>
- Engström-Öst, J., Holmborn, T., Brutemark, A., Hogfors, H., Vehmaa, A., & Gorokhova, E. (2014). The effects of short-term pH decrease on the reproductive output of the copepod *Acartia bifilosa* – a laboratory study. *Marine and Freshwater Behaviour and Physiology*, 47(3), 173–183. <https://doi.org/10.1080/10236244.2014.919096>
- Epel, D. (1978). Chapter 7 Mechanisms of Activation of Sperm and Egg During Fertilization of Sea Urchin Gametes. *Current Topics in Developmental Biology*, 12, 85–246. [https://doi.org/10.1016/s0070-2153\(08\)60597-9](https://doi.org/10.1016/s0070-2153(08)60597-9)
- Ericson, J. A., Lamare, M. D., Morley, S. A., & Barker, M. F. (2010). The response of two ecologically important Antarctic invertebrates (*Sterechinus neumayeri* and *Parborlasia corrugatus*) to reduced seawater pH: effects on fertilisation and embryonic development. *Marine Biology*, 157(12), 2689–2702. <https://doi.org/10.1007/s00227-010-1529-y>
- Esposito, M. C., Boni, R., Cuccaro, A., Tosti, E., & Gallo, A. (2020). Sperm motility impairment in free spawning invertebrates under near-future level of ocean acidification: uncovering the mechanism. *Frontiers in Marine Science*, 6, 794. <https://doi.org/10.3389/fmars.2019.00794>
- Evans, J. P., & Sherman, C. D. H. (2013). Sexual selection and the evolution of egg-sperm interactions in broadcast-spawning invertebrates. *The Biological Bulletin*, 224(3), 166–183. <https://doi.org/10.1086/BBLv224n3p166>
- Falkenberg, L. J., Styan, C. A., & Havenhand, J. N. (2019). Sperm motility of oysters from distinct populations differs in response to ocean acidification and freshening. *Scientific Reports*, 9(1), 7970. <https://doi.org/10.1038/s41598-019-44321-0>
- Farley, G. S., & Levitan, D. R. (2001). The role of jelly coats in sperm-egg encounters, fertilization success, and selection on egg size in broadcast spawners. *The American Naturalist*, 157(6), 626–636. <https://doi.org/10.1086/320619>
- Fine, M., & Tchernov, D. (2007). Scleractinian coral species survive and recover from decalcification. *Science*, 315(5820), 1811. <https://doi.org/10.1126/science.1137094>
- Fiorenza, E. A., Wendt, C. A., Dobkowski, K. A., King, T. L., Pappaionou, M., Rabinowitz, P., Samhuri, J. F., & Wood, C. L. (2020). It's a wormy world: Meta-analysis reveals several decades of change in the global abundance of the parasitic nematodes *Anisakis spp.* and *Pseudoterranova spp.* in marine fishes and invertebrates. *Global Change Biology*, 26(5), 2854–2866. <https://doi.org/10.1111/gcb.15048>
- Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C., & Bentley, M. G. (2012). Ocean acidification induces multi-generational decline in copepod naupliar production with possible conflict for reproductive resource allocation. *Journal of Experimental Marine Biology and Ecology*, 418–419, 30–36. <https://doi.org/10.1016/j.jembe.2012.03.009>

- Food and Agriculture Organization of the United Nations. (2019). *FAO Yearbook. Fishery and Aquaculture Statistics 2017/FAO annuaire. Statistiques des pêches et de l'aquaculture 2017/FAO anuario. Estadísticas de pesca y acuicultura 2017*. Food & Agriculture Org.
- Food and Agriculture Organization of the United Nations. (2018). *The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals*. FAO Rome, Italy.
- Foo, S. A. (2015). Acclimatisation and adaptive capacity of sea urchins in a changing ocean: Effects of ocean warming and acidification on early development and the potential to persist. University of Sydney. <http://ses.library.usyd.edu.au/handle/2123/14988>
- Foo, S. A., & Byrne, M. (2017). Marine gametes in a changing ocean: Impacts of climate change stressors on fecundity and the egg. *Marine Environmental Research*, 128, 12–24. <https://doi.org/10.1016/j.marenvres.2017.02.004>
- Forconi, M., Canapa, A., Barucca, M., Biscotti, M. A., Capriglione, T., Buonocore, F., Fausto, A. M., Makapedua, D. M., Pallavicini, A., Gerdol, M., De Moro, G., Scapigliati, G., Olmo, E., & Scharl, M. (2013). Characterization of Sex Determination and Sex Differentiation Genes in *Latimeria*. *PLoS ONE*, 8(4), e56006. <https://doi.org/10.1371/journal.pone.0056006>
- Frieder, C. A. (2014). Present-day nearshore pH differentially depresses fertilization in congeneric sea urchins. *The Biological Bulletin*, 226(1), 1–7. <https://doi.org/10.1086/BBLv226n1p1>
- Froehlich, H. E., Gentry, R. R., & Halpern, B. S. (2017). Conservation aquaculture: Shifting the narrative and paradigm of aquaculture's role in resource management. *Biological Conservation*, 215, 162–168. <https://doi.org/10.1016/j.biocon.2017.09.012>
- Gallo, A., Esposito, M. C., Cuccaro, A., Buia, M. C., Tarallo, A., Monfrecola, V., Tosti, E., & Boni, R. (2020). Adult exposure to acidified seawater influences sperm physiology in *Mytilus galloprovincialis*: Laboratory and in situ transplant experiments. *Environmental Pollution*, 265, 115063. <https://doi.org/10.1016/j.envpol.2020.115063>
- Galtsoff, P. S. (1938). Physiology of reproduction of *Ostrea virginica*. *The Biological Bulletin*, 75(2), 286–307. <https://doi.org/10.2307/1537736>
- Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., Wang, L., Zheng, Y., Jin, P., Cai, X., Häder, D.-P., Li, W., Xu, K., Liu, N., & Riebesell, U. (2012). Rising CO₂ and increased light exposure synergistically reduce marine primary productivity. *Nature Climate Change*, 2(7), 519–523. <https://doi.org/10.1038/nclimate1507>
- Gizzi, F., de Mas, L., Airi, V., Caroselli, E., Prada, F., Falini, G., Dubinsky, Z., & Goffredo, S. (2017). Reproduction of an azooxanthellate coral is unaffected by ocean acidification. *Scientific Reports*, 7(1), 13049. <https://doi.org/10.1038/s41598-017-13393-1>
- Gonzalez-Bernat, M. J., Lamare, M., & Barker, M. (2013a). Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biology*, 36(2), 235–247. <https://doi.org/10.1007/s00300-012-1255-7>
- Gonzales-Bernat, M. J., Lamare, M., Uthicke, S., & Byrne, M. (2013b). Fertilization, embryogenesis and larval development in the tropical intertidal sand dollar *Arachnoides placenta* in response to reduced seawater pH. *Marine Biology*, 160, 1927–1194. <https://doi.org/10.1007/s00227-012-2034-2>
- Graham, H., Rastrick, S. P. S., Findlay, H. S., Bentley, M. G., Widdicombe, S., Clare, A. S., & Caldwell, G. S. (2015). Sperm motility and fertilisation success in an acidified and hypoxic

- environment. *ICES Journal of Marine Science*, 73(3), 783–790.
<https://doi.org/10.1093/icesjms/fsv171>
- Gravinese, P. M. (2018). Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*. *Journal of Experimental Marine Biology and Ecology*, 500, 140–146. <https://doi.org/10.1016/j.jembe.2017.09.001>
- Gray, M. W., Chaparro, O., Huebert, K. B., O'Neill, S. P., Couture, T., Moreira, A., & Brady, D. C. (2019). Life History Traits Conferring Larval Resistance against Ocean Acidification: The Case of Brooding Oysters of the Genus *Ostrea*. *Journal of Shellfish Research*, 38(3), 751. <https://doi.org/10.2983/035.038.0326>
- Gwo, J.-C. (2000). Cryopreservation of aquatic invertebrate semen: a review. *Aquaculture Research*, 31(3), 259–271. <https://doi.org/10.1046/j.1365-2109.2000.00462.x>
- Havenhand, J. N., Buttler, F.-R., Thorndyke, M. C., & Williamson, J. E. (2008). Near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Current Biology*, 18(15), R651–R652. <https://doi.org/10.1016/j.cub.2008.06.015>
- Havenhand, J. N., & Schlegel, P. (2009). Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics in the oyster *Crassostrea gigas*. *Biogeosciences*, 6(12), 3009–3015. <https://doi.org/10.5194/bg-6-3009-2009>
- Hazan, Y., Wangensteen, O. S., & Fine, M. (2014). Tough as a rock-boring urchin: adult *Echinometra* sp. EE from the Red Sea show high resistance to ocean acidification over long-term exposures. *Marine Biology*, 161(11), 2531–2545. <https://doi.org/10.1007/s00227-014-2525-4>
- Hedgecock, D., & Pudovkin, A. I. (2011). Sweepstakes Reproductive Success in Highly Fecund Marine Fish and Shellfish: A Review and Commentary. *Bulletin of Marine Science*, 87(4), 971–1002.
- Helm, M. M. (2004). *Hatchery culture of bivalves: a practical manual*. FAO.
- Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, 328(5985), 1523–1528. <https://doi.org/10.1126/science.1189930>
- Horwitz, R., & Fine, M. (2014). High CO₂ detrimentally affects tissue regeneration of Red Sea corals. *Coral Reefs*, 33(3), 819–829. <https://doi.org/10.1007/s00338-014-1150-5>
- Howes, E., Joos, F., Eakin, M., & Gattuso, J.P. (2015). An updated synthesis of the observed and projected impacts of climate change on the chemical, physical and biological processes in the oceans. *Frontiers in Marine Science*, 2, 36. <https://doi.org/10.3389/fmars.2015.00036>
- Hue, T., Chateau, O., Lecellier, G., Kayal, M., Lanos, N., Gossuin, H., Adjeroud, M., & Dumas, P. (2020). Temperature affects the reproductive outputs of coral-eating starfish *Acanthaster* spp. after adult exposure to near-future ocean warming and acidification. *Marine Environmental Research*, 162, 105164. <https://doi.org/10.1016/j.marenvres.2020.105164>
- Hu, M. Y., Lein, E., Bleich, M., Melzner, F., & Stumpp, M. (2018). Trans-life cycle acclimation to experimental ocean acidification affects gastric pH homeostasis and larval recruitment in the sea star *Asterias rubens*. *Acta Physiologica*, 224(2), e13075. <https://doi.org/10.1111/apha.13075>
- Iguchi A., Suzuki A., Sakai K. & Nojiri Y. (2015). Comparison of the effects of thermal stress and CO₂-driven acidified seawater on fertilization in coral *Acropora digitifera*. *Zygote*, 23(4), 631–634. <https://doi.org/10.1017/S0967199414000185>

- IPCC (Intergovernmental Panel on Climate Change) (2014) Climate change 2014: impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. In: Field CB, Barros VR, Dokken DJ, Mach KJ and others (eds) Contribution of Working Group II to the 5th assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Jokiel, P. L., Rodgers, K. S., Kuffner, I. B., Andersson, A. J., Cox, E. F., & Mackenzie, F. T. (2008). Ocean acidification and calcifying reef organisms: a mesocosm investigation. *Coral Reefs*, 27(3), 473–483. <https://doi.org/10.1007/s00338-008-0380-9>
- Jouaux, A., Franco, A., Heude-Berthelin, C., Sourdain, P., Blin, J. L., Mathieu, M., & Kellner, K. (2012). Identification of Ras, Pten and p70S6K homologs in the Pacific oyster *Crassostrea gigas* and diet control of insulin pathway. *General and Comparative Endocrinology*, 176(1), 28–38. <https://doi.org/10.1016/j.ygcen.2011.12.008>
- Kapsenberg, L., Okamoto, D. K., Dutton, J. M., & Hofmann, G. E. (2017). Sensitivity of sea urchin fertilization to pH varies across a natural pH mosaic. *Ecology and Evolution*, 7(6), 1737–1750. <https://doi.org/10.1002/ece3.2776>
- Kimura, R., Takami, H., Ono, T., Onitsuka, T., & Nojiri, Y. (2011). Effects of elevated pCO₂ on the early development of the commercially important gastropod, Ezo abalone *Haliotis discus hannai*: Effects of high pCO₂ on larval Ezo abalone. *Fisheries Oceanography*, 20(5), 357–366. <https://doi.org/10.1111/j.1365-2419.2011.00589.x>
- Kong, H., Jiang, X., Clements, J. C., Wang, T., Huang, X., Shang, Y., Chen, J., Hu, M., & Wang, Y. (2019). Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. *Marine Environmental Research*, 145, 73–80. <https://doi.org/10.1016/j.marenvres.2019.02.011>
- Kroeker, K. J., Kordas, R. L., Crim, R. N., & Singh, G. G. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, 13(11), 1419–1434. <https://doi.org/10.1111/j.1461-0248.2010.01518.x>
- Kurihara, H. (2008). Effects of CO₂-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series*, 373, 275–284. <https://doi.org/10.3354/meps07802>
- Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M., & Ishimatsu, A. (2008). Long-term effects of predicted future seawater CO₂ conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *Journal of Experimental Marine Biology and Ecology*, 367(1), 41–46. <https://doi.org/10.1016/j.jembe.2008.08.016>
- Kurihara, H., Shimode, S., & Shirayama, Y. (2004). Effects of raised CO₂ concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). *Marine Pollution Bulletin*, 49(9-10), 721–727. <https://doi.org/10.1016/j.marpolbul.2004.05.005>
- Kurihara, H., & Shirayama, Y. (2004). Effects of increased atmospheric CO₂ on sea urchin early development. *Marine Ecology Progress Series*, 274, 161–169. <https://doi.org/10.3354/meps274161>
- Lawrence, A. J., & Soame, J. M. (2004). The effects of climate change on the reproduction of coastal invertebrates. *The Ibis*, 146, 29–39. <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1474-919X.2004.00325.x>
- Lee, E. H., Choi, S. Y., Seo, M. H., Lee, S. J., & Soh, H. Y. (2020). Effects of temperature and

- pH on the egg production and hatching success of a common Korean copepod. *Diversity*, 12(10), 372. <https://doi.org/10.3390/d12100372>
- Limatola, N., Chun, J. T., & Santella, L. (2020). Effects of salinity and pH of seawater on the reproduction of the sea urchin *Paracentrotus lividus*. *The Biological Bulletin*, 239(1), 13–23. <https://doi.org/10.1086/710126>
- Liu, S., Shi, W., Guo, C., Zhao, X., Han, Y., Peng, C., Chai, X., & Liu, G. (2016). Ocean acidification weakens the immune response of blood clam through hampering the NF- κ B and toll-like receptor pathways. *Fish & Shellfish Immunology*, 54, 322–327. <https://doi.org/10.1016/j.fsi.2016.04.030>
- Lucey, N. M., Lombardi, C., DeMarchi, L., Schulze, A., Gambi, M. C., & Calosi, P. (2015). To brood or not to brood: Are marine invertebrates that protect their offspring more resilient to ocean acidification? *Scientific Reports*, 5, 12009. <https://doi.org/10.1038/srep12009>
- Lymbery, R. A., Kennington, W. J., Cornwall, C. E., & Evans, J. P. (2019). Ocean acidification during prefertilization chemical communication affects sperm success. *Ecology and Evolution*, 9, 1. <https://doi.org/10.1002/ece3.5720>
- Mackenzie, C. L., Lynch, S. A., Culloty, S. C., & Malham, S. K. (2014). Future oceanic warming and acidification alter immune response and disease status in a commercial shellfish species, *Mytilus edulis* L. *PloS One*, 9(6), e99712. <https://doi.org/10.1371/journal.pone.0099712>
- Marčeta, T., Matozzo, V., Alban, S., Badocco, D., Pastore, P., & Marin, M. G. (2020). Do males and females respond differently to ocean acidification? An experimental study with the sea urchin *Paracentrotus lividus*. *Environmental Science and Pollution Research International*. <https://doi.org/10.1007/s11356-020-10040-7>
- Marshall, K. N., Kaplan, I. C., Hodgson, E. E., Hermann, A., Busch, D. S., McElhany, P., Essington, T. E., Harvey, C. J., & Fulton, E. A. (2017). Risks of ocean acidification in the California Current food web and fisheries: ecosystem model projections. *Global Change Biology*, 23(4), 1525–1539. <https://doi.org/10.1111/gcb.13594>
- Martin, S., Richier, S., Pedrotti, M.-L., Dupont, S., Castejon, C., Gerakis, Y., Kerros, M.E., Oberhänsli, F., Teyssié, J.-L., Jeffree, R., & Gattuso, J.-P. (2011). Early development and molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO₂-driven acidification. *The Journal of Experimental Biology*, 214(Pt 8), 1357–1368. <https://doi.org/10.1242/jeb.051169>
- Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F., & Hay, S. (2007). CO₂-induced acidification affects hatching success in *Calanus finmarchicus*. *Marine Ecology Progress Series*, 350, 91–97. <https://doi.org/10.3354/meps07142>
- McConville, K., Halsband, C., Fileman, E. S., Somerfield, P. J., Findlay, H. S., & Spicer, J. I. (2013). Effects of elevated CO₂ on the reproduction of two calanoid copepods. *Marine Pollution Bulletin*, 73(2), 428–434. <https://doi.org/10.1016/j.marpolbul.2013.02.010>
- McDonald, M. R., McClintock, J. B., Amsler, C. D., Rittschof, D., Angus, R. A., Orihuela, B., & Lutostanski, K. (2009). Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. *Marine Ecology Progress Series*, 385, 179–187. <https://doi.org/10.3354/meps08099>
- Melzner, F., Mark, F. C., Seibel, B. A., & Tomanek, L. (2019). Ocean Acidification and Coastal Marine Invertebrates: Tracking CO₂ effects from seawater to the cell. *Annual Review of*

- Marine Science*, 12, 499–523. <https://doi.org/10.1146/annurev-marine-010419-010658>
- Meseck, S. L., Alix, J. H., Swiney, K. M., Long, W. C., Wikfors, G. H., & Foy, R. J. (2016). Ocean acidification affects hemocyte physiology in the Tanner crab (*Chionoecetes bairdi*). *PloS One*, 11(2), e0148477. <https://doi.org/10.1371/journal.pone.0148477>
- Miin Chua, C., Leggat, W., Moya, A., & Baird, A. H. (2013). Temperature affects the early life history stages of corals more than near future ocean acidification. *Marine Ecology Progress Series*, 475, 85–92. <https://doi.org/10.3354/meps10077>
- Miller, J. J., Maher, M., Bohaboy, E., Friedman, C. S., & McElhany, P. (2016). Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biology*, 163(5). <https://doi.org/10.1007/s00227-016-2883-1>
- Miyazaki, S. (2006). Thirty years of calcium signals at fertilization. *Seminars in Cell & Developmental Biology*, 17(2), 233–243. <https://doi.org/10.1016/j.semcdb.2006.02.007>
- Morisawa, M., & Yoshida, M. (2005). Activation of motility and chemotaxis in the spermatozoa: From invertebrates to humans. *Reproductive Medicine and Biology*, 4(2), 101–114. <https://doi.org/10.1111/j.1447-0578.2005.00099.x>
- Morita, M., Nishikawa, A., Nakajima, A., Iguchi, A., Sakai, K., Takemura, A., & Okuno, M. (2006). Eggs regulate sperm flagellar motility initiation, chemotaxis and inhibition in the coral *Acropora digitifera*, *A. gemmifera* and *A. tenuis*. *The Journal of Experimental Biology*, 209(22), 4574–4579. <https://doi.org/10.1242/jeb.02500>
- Morita, M., Suwa, R., Iguchi, A., Nakamura, M., Shimada, K., Sakai, K., & Suzuki, A. (2010). Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. *Zygote*, 18(2), 103–107. <https://doi.org/10.1017/S0967199409990177>
- Mortensen, T., & Mortensen, T. (1921). *Studies of the development and larval forms of echinoderms*. GEC Gad.
- Moulin, L., Catarino, A. I., Claessens, T., & Dubois, P. (2011). Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). *Marine Pollution Bulletin*, 62(1), 48–54. <https://doi.org/10.1016/j.marpolbul.2010.09.012>
- Nakajima, A., Morita, M., Takemura, A., Kamimura, S., & Okuno, M. (2005). Increase in intracellular pH induces phosphorylation of axonemal proteins for activation of flagellar motility in starfish sperm. *The Journal of Experimental Biology*, 208(23), 4411–4418. <https://doi.org/10.1242/jeb.01906>
- Nakamura, M., & Morita, M. (2012). Sperm motility of the scleractinian coral *Acropora digitifera* under preindustrial, current, and predicted ocean acidification regimes. *Aquatic Biology*, 15(3), 299–302. <https://doi.org/10.3354/ab00436>
- Olischläger, M., & Wild, C. (2020). How Does the sexual reproduction of marine life respond to ocean acidification? *Diversity*, 12(6), 241. <https://doi.org/10.3390/d12060241>
- Omeregíe, E., Mwatilifange, N. S. I., & Liswaniso, G. (2019). Futuristic ocean acidification levels reduce growth and reproductive viability in the Pacific oyster (*Crassostrea gigas*). *Journal of Applied Sciences & Environmental Management*, 23(9), 1747–1754. <https://www.ajol.info/index.php/jasem/article/view/190483>
- Pansch, C., Hattich, G. S. I., Heinrichs, M. E., Pansch, A., Zagrodzka, Z., & Havenhand, J. N. (2018). Long-term exposure to acidification disrupts reproduction in a marine invertebrate. *PloS One*, 13(2), e0192036. <https://doi.org/10.1371/journal.pone.0192036>
- Parker, L. M., O'Connor, W. A., Byrne, M., Dove, M., Coleman, R. A., Pörtner, H.O.,

- Scanes, E., Virtue, P., Gibbs, M., & Ross, P. M. (2018). Ocean acidification but not warming alters sex determination in the Sydney rock oyster, *Saccostrea glomerata*. *Proceedings of the Royal Society B*, 285(1872), 20172869. <https://doi.org/10.1098/rspb.2017.2869>
- Parker, L. M., O'Connor, W. A., Byrne, M., Coleman, R. A., Virtue, P., Dove, M., Gibbs, M., Spohr, L., Scanes, E., & Ross, P. M. (2017). Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of multiple stressors. *Biology Letters*, 13(2). <https://doi.org/10.1098/rsbl.2016.0798>
- Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., & Pörtner, H.-O. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, 18(1), 82–92. <https://doi.org/10.1111/j.1365-2486.2011.02520.x>
- Parker, L. M., Ross, P. M., & O'Connor, W. A. (2011). Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. *Marine Biology*, 158(3), 689–697. <https://doi.org/10.1007/s00227-010-1592-4>
- Parker, L. M., Ross, P. M., & O'Connor, W. A. (2010). Comparing the effect of elevated pCO₂ and temperature on the fertilization and early development of two species of oysters. *Marine Biology*, 157(11), 2435–2452. <https://doi.org/10.1007/s00227-010-1508-3>
- Parker, L. M., Ross, P. M., & O'Connor, W. A. (2009). The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Global Change Biology*, 15(9), 2123–2136. <https://doi.org/10.1111/j.1365-2486.2009.01895.x>
- Parker, K. L., Schedl, A., & Schimmer, B. P. (1999). Gene interactions in gonadal development. *Annual Review of Physiology*, 61, 417–433. <https://doi.org/10.1146/annurev.physiol.61.1.417>
- Podolsky, R. D. (2004). Life-history consequences of investment in free-spawned eggs and their accessory coats. *The American Naturalist*, 163(5), 735–753. <https://doi.org/10.1086/382791>
- Pitts K. A., Campbell J. E., Figueiredo J. & Fogarty N. D. (2020). Ocean acidification partially mitigates the negative effects of warming on the recruitment of the coral, *Orbicella faveolata*. *Coral reefs*, 39(2), 281–292. <https://doi.org/10.1007/s00338-019-01888-4>
- Przeslawski, R., Byrne, M., & Mellin, C. (2015). A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology*, 21(6), 2122–2140. <https://doi.org/10.1111/gcb.12833>
- Ramirez Llodra, E. (2002). Fecundity and life-history strategies in marine invertebrates. *Advances in Marine Biology*, 43, 87–170. [https://doi.org/10.1016/s0065-2881\(02\)43004-0](https://doi.org/10.1016/s0065-2881(02)43004-0)
- Reuter, K. E., Lotterhos, K. E., Crim, R. N., Thompson, C. A., & Harley, C. D. G. (2011). Elevated pCO₂ increases sperm limitation and risk of polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. *Global Change Biology*, 17(1), 163–171. <https://doi.org/10.1111/j.1365-2486.2010.02216.x>
- Riba, I., Gabrielyan, B., Khosrovyan, A., Luque, A., & Del Valls, T. A. (2016). The influence of pH and waterborne metals on egg fertilization of the blue mussel (*Mytilus edulis*), the oyster (*Crassostrea gigas*) and the sea urchin (*Paracentrotus lividus*). *Environmental Science and Pollution Research International*, 23(14), 14580–14588. <https://doi.org/10.1007/s11356-016-6611-7>
- Richmond, R. H., & Hunter, C. L. (1990). Reproduction and recruitment of corals: comparisons

- among the Caribbean, the tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*. Oldendorf. <http://www.int-res.com/articles/meps/60/m060p185.pdf>
- Rinkevich, B. (1996). Do reproduction and regeneration in damaged corals compete for energy allocation? *Marine Ecology Progress Series*, 143, 297–302. <https://www.int-res.com/abstracts/meps/v143/p297-302/>
- Rinkevich, B., & Loya, Y. (1989). Reproduction in regenerating colonies of the coral *Stylophora pistillata*. *Environmental Quality and Ecosystem Stability*, 4, 257–265.
- Roggatz, C. C., Lorch, M., Hardege, J. D., & Benoit, D. M. (2016). Ocean acidification affects marine chemical communication by changing structure and function of peptide signalling molecules. *Global Change Biology*, 22(12), 3914–3926. <https://doi.org/10.1111/gcb.13354>
- Rossin, A. M., Waller, R. G., & Stone, R. P. (2019). The effects of in-vitro pH decrease on the gametogenesis of the red tree coral, *Primnoa pacifica*. *PloS One*, 14(4), e0203976. <https://doi.org/10.1371/journal.pone.0203976>
- Ross, P. M., Parker, L., & Byrne, M. (2016). Transgenerational responses of molluscs and echinoderms to changing ocean conditions. *ICES Journal of Marine Science*, 73(3), 537–549. <https://doi.org/10.1093/icesjms/fsv254>
- Ross, P. M., Parker, L., O'Connor, W. A., & Bailey, E. A. (2011). The impact of ocean acidification on reproduction, early development and settlement of marine organisms. *Water*, 3(4), 1005–1030. <https://doi.org/10.3390/w3041005>
- Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science*, 3, 509–535. <https://doi.org/10.1146/annurev-marine-120709-142756>
- Scanes, E., Parker, L. M., O'Connor, W. A., Gibbs, M. C., & Ross, P. M. (2018). Copper and ocean acidification interact to lower maternal investment, but have little effect on adult physiology of the Sydney rock oyster *Saccostrea glomerata*. *Aquatic Toxicology*, 203, 51–60. <https://doi.org/10.1016/j.aquatox.2018.07.020>
- Scanes, E., Parker, L. M., O'Connor, W. A., & Ross, P. M. (2014). Mixed effects of elevated pCO₂ on fertilisation, larval and juvenile development and adult responses in the mobile subtidal scallop *Mimachlamys asperima* (Lamarck, 1819). *PloS One*, 9(4), e93649. <https://doi.org/10.1371/journal.pone.0093649>
- Schiffer, M., Harms, L., Pörtner, H. O., Mark, F. C., & Storch, D. (2014). Pre-hatching seawater pCO₂ affects development and survival of zoea stages of Arctic spider crab *Hyas araneus*. *Marine Ecology Progress Series*, 501, 127–139. <https://doi.org/10.3354/meps10687>
- Schlegel, P., Binet, M. T., Havenhand, J. N., Doyle, C. J., & Williamson, J. E. (2015). Ocean acidification impacts on sperm mitochondrial membrane potential bring sperm swimming behaviour near its tipping point. *The Journal of Experimental Biology*, 218(7), 1084–1090. <https://doi.org/10.1242/jeb.114900>
- Schlegel, P., Havenhand, J. N., Gillings, M. R., & Williamson, J. E. (2012). Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. *PloS One*, 7(12), e53118. <https://doi.org/10.1371/journal.pone.0053118>
- Schutter M., Nozawa Y. & Kurihara H. (2015). The effect of elevated CO₂ and increased temperature on in vitro fertilization success and initial embryonic development of single male: female crosses of broad-cast spawning corals at mid-and high-latitude locations. *Journal of Marine Science and Engineering*, 3(2), 216–239.

- <https://doi.org/10.3390/jmse3020216>
- Sherwood, L., Klandorf, H., & Yancey, P. (2012). *Animal Physiology: From Genes to Organisms*. Cengage Learning.
- Shi, W., Zhao, X., Han, Y., Guo, C., Liu, S., Su, W., Wang, Y., Zha, S., Chai, X., Fu, W., Yang, H., & Liu, G. (2017a). Effects of reduced pH and elevated pCO₂ on sperm motility and fertilisation success in blood clam, *Tegillarca granosa*. *New Zealand Journal of Marine and Freshwater Research*, 51(4), 543–554. <https://doi.org/10.1080/00288330.2017.1296006>
- Shi, W., Han, Y., Guo, C., Zhao, X., Liu, S., Su, W., Wang, Y., Zha, S., Chai, X., & Liu, G. (2017b). Ocean acidification hampers sperm-egg collisions, gamete fusion, and generation of Ca²⁺ oscillations of a broadcast spawning bivalve, *Tegillarca granosa*. *Marine Environmental Research*, 130, 106–112. <https://doi.org/10.1016/j.marenvres.2017.07.016>
- Shlesinger, T., & Loya, Y. (2019). Breakdown in spawning synchrony: A silent threat to coral persistence. *Science*, 365(6457), 1002–1007. <https://doi.org/10.1126/science.aax0110>
- Shukla, P. R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H. O., Roberts, D. C., Zhai, P., Slade, R., Connors, S., & Van Diemen, R. (2019). *IPCC, 2019: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Intergovernmental Panel on Climate Change (IPCC).
- Siikavuopio, S. I., Mortensen, A., Dale, T., & Foss, A. (2007). Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, 266(1), 97–101. <https://doi.org/10.1016/j.aquaculture.2007.02.044>
- Slattery, M., Hines, G. A., Starmer, J., & Paul, V. J. (1999). Chemical signals in gametogenesis, spawning, and larval settlement and defense of the soft coral *Sinularia polydactyla*. *Coral Reefs*, 18(1), 75–84. <https://doi.org/10.1007/s003380050158>
- Smith, H. W., & Clowes, G. H. A. (1924). The influence of hydrogen ion concentration on the fertilization process in *Arbacia*, *Asterias* and *Chætopterus* Eggs. *The Biological Bulletin*, 47(6), 333–344. <https://doi.org/10.2307/1536693>
- Smith, K. E., Byrne, M., Deaker, D., Hird, C. M., Nielson, C., Wilson-McNeal, A., & Lewis, C. (2019). Sea urchin reproductive performance in a changing ocean: poor males improve while good males worsen in response to ocean acidification. *Proceedings of the Royal Society B*, 286(1907), 20190785. <https://doi.org/10.1098/rspb.2019.0785>
- Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, 79, 1–15. <https://doi.org/10.1016/j.marenvres.2012.04.003>
- Spady, B. L., Munday, P. L., & Watson, S.-A. (2019). Elevated seawater pCO₂ affects reproduction and embryonic development in the pygmy squid, *Idiosepius pygmaeus*. *Marine Environmental Research*, 104812. <https://doi.org/10.1016/j.marenvres.2019.104812>
- Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *Bioscience*, 57(7), 573–583. <https://doi.org/10.1641/B570707>
- Spencer, L. H., Venkataraman, Y. R., Crim, R., Ryan, S., Horwith, M. J., & Roberts, S. B.

- (2020). Carryover effects of temperature and pCO₂ across multiple Olympia oyster populations. *Ecological Applications*, 30(3), e02060. <https://doi.org/10.1002/eap.2060>
- Steer, M. A., Moltschaniwskyj, N. A., & Jordan, A. R. (2003). Embryonic development of southern calamary (*Sepioteuthis australis*) within the constraints of an aggregated egg mass. *Marine and Freshwater Research*, 54(3), 217–226. <https://doi.org/10.1071/mf02107>
- Striewski, S. (2012). *Impact of ocean acidification on the reproduction, recruitment and growth of scleractinian corals*. University Bochum.
- Stumpp, M., Trübenbach, K., Brennecke, D., Hu, M. Y., & Melzner, F. (2012). Resource allocation and extracellular acid–base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO₂ induced seawater acidification. *Aquatic Toxicology*, 110–111, 194–207. <https://doi.org/10.1016/j.aquatox.2011.12.020>
- [Suckling, C. C., Clark, M. S., Richard, J., Morley, S. A., Thorne, M. A. S., Harper, E. M., & Peck, L. S. \(2015\). Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. *Journal of Animal Ecology*, 84\(3\), 773–784. <https://doi.org/10.1111/1365-2656.12316>](#)
- Suckling, C. C., Clark, M. S., Beveridge, C., Brunner, L., Hughes, A. D., Harper, E. M., Cook, E. J., Davies, A. J., & Peck, L. S. (2014). Experimental influence of pH on the early life-stages of sea urchins II: increasing parental exposure times gives rise to different responses. *Invertebrate Reproduction & Development*, 58(3), 161–175. <https://doi.org/10.1080/07924259.2013.875951>
- Sung, C. G., Kim, T. W., Park, Y. G., Kang, S. G., Inaba, K., Shiba, K., Choi, T. S., Moon, S. D., Litvin, S., Lee, K. T., & Lee, J. S. (2014). Species and gamete-specific fertilization success of two sea urchins under near future levels of pCO₂. *Journal of Marine Systems*, 137, 67–73. <https://doi.org/10.1016/j.jmarsys.2014.04.013>
- Świeżak, J., Borrero-Santiago, A. R., Sokołowski, A., & Olsen, A. J. (2018). Impact of environmental hypercapnia on fertilization success rate and the early embryonic development of the clam *Limecola balthica* (Bivalvia, Tellinidae) from the southern Baltic Sea - A potential CO₂ leakage case study. *Marine Pollution Bulletin*, 136, 201–211. <https://doi.org/10.1016/j.marpolbul.2018.09.007>
- Tanabe, T., Yuan, Y., Nakamura, S., Itoh, N., Takahashi, K. G., & Osada, M. (2010). The role in spawning of a putative serotonin receptor isolated from the germ and ciliary cells of the gonoduct in the gonad of the Japanese scallop, *Patinopecten yessoensis*. *General and Comparative Endocrinology*, 166(3), 620–627. <https://doi.org/10.1016/j.ygcen.2010.01.014>
- Tarrant, A. M. (2007). Hormonal signaling in cnidarians: do we understand the pathways well enough to know whether they are being disrupted? *Ecotoxicology*, 16(1), 5–13. <https://doi.org/10.1007/s10646-006-0121-1>
- Thiele. (2017). *The Role of Neuropeptides in Addiction and Disorders of Excessive Consumption*. Academic Press.
- Thor, P., & Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology*, 21(6), 2261–2271. <https://doi.org/10.1111/gcb.12815>
- Timpane-Padgham, B. L., Beechie, T., & Klinger, T. (2017). A systematic review of ecological attributes that confer resilience to climate change in environmental restoration. *PloS One*, 12(3), e0173812. <https://doi.org/10.1371/journal.pone.0173812>

- Todgham, A. E., & Stillman, J. H. (2013). Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integrative and Comparative Biology*, 53(4), 539–544. <https://doi.org/10.1093/icb/ict086>
- Treen, N., Itoh, N., Miura, H., Kikuchi, I., Ueda, T., Takahashi, K. G., Ubuka, T., Yamamoto, K., Sharp, P. J., Tsutsui, K., & Osada, M. (2012). Mollusc gonadotropin-releasing hormone directly regulates gonadal functions: a primitive endocrine system controlling reproduction. *General and Comparative Endocrinology*, 176(2), 167–172. <https://doi.org/10.1016/j.ygcen.2012.01.008>
- Uthicke, S., Patel, F., Karelitz, S., Luter, H. M., Webster, N. S., & Lamare, M. (2020). Key biological responses over two generations of the sea urchin *Echinometra* sp. A under future ocean conditions. *Marine Ecology Progress Series*, 637, 87–101. <https://doi.org/10.3354/meps13236>
- Uthicke, S., Liddy, M., Nguyen, H. D., & Byrne, M. (2014). Interactive effects of near-future temperature increase and ocean acidification on physiology and gonad development in adult Pacific sea urchin, *Echinometra* sp. A. *Coral Reefs*, 33(3), 831–845. <https://doi.org/10.1007/s00338-014-1165-y>
- Uthicke, S., Pecorino, D., Albright, R., Negri, A. P., Cantin, N., Liddy, M., Dworjanyn, S., Kamya, P., Byrne, M., & Lamare, M. (2013a). Impacts of ocean acidification on early life-history stages and settlement of the coral-eating sea star *Acanthaster planci*. *PloS One*, 8(12), e82938. <https://doi.org/10.1371/journal.pone.0082938>
- Uthicke, S., Pecorino, D., Albright, R., Negri, A. P., Cantin, N., Liddy, M., Dworjanyn, S., Kamya, P., Byrne, M., & Lamare, M. (2013b). Impacts of ocean acidification on early life-history stages and settlement of the coral-eating sea star *Acanthaster planci*. *PloS One*, 8(12), e82938. <https://doi.org/10.1371/journal.pone.0082938>
- Van Colen, C., Debusschere, E., Braeckman, U., Van Gansbeke, D., & Vincx, M. (2012). The early life history of the clam *Macoma balthica* in a high CO₂ world. *PloS One*, 7(9), e44655. <https://doi.org/10.1371/journal.pone.0044655>
- Van Veghel, M. L. J., & Bak, R. P. M. (1994). Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis*. III. Reproduction in damaged and regenerating colonies. *Marine Ecology Progress Series*, 109(2/3), 229–233. <http://www.jstor.org/stable/24846188>
- Vehmaa, A., Almén, A.-K., Brutemark, A., Paul, A., Riebesell, U., Furuhausen, S., & Engström-Öst, J. (2015). Ocean acidification challenges copepod reproductive plasticity. *Biogeosciences*, 13. <https://doi.org/10.5194/bgd-12-18541-2015>
- Vehmaa, A., Hogfors, H., Gorokhova, E., Brutemark, A., Holmborn, T., & Engström-Öst, J. (2013). Projected marine climate change: effects on copepod oxidative status and reproduction. *Ecology and Evolution*, 3(13), 4548–4557. <https://doi.org/10.1002/ece3.839>
- Vehmaa, A., Brutemark, A., & Engström-Öst, J. (2012). Maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes. *PloS One*, 7(10), e48538. <https://doi.org/10.1371/journal.pone.0048538>
- Venkataraman, Y. R., Downey-Wall, A. M., Ries, J., Westfield, I., White, S. J., Roberts, S. B., & Lotterhos, K. E. (2020). General DNA methylation patterns and environmentally-induced differential methylation in the eastern oyster (*Crassostrea virginica*). *Frontiers in Marine Science*, 7, 225. <https://doi.org/10.3389/fmars.2020.00225>

- Venkataraman, Y. R., Spencer, L. H., & Roberts, S. B. (2019). Adult low pH exposure influences larval abundance in Pacific oysters (*Crassostrea gigas*). *Journal of Shellfish Research*, 38(3): 743–750. <https://doi.org/10.2983/035.038.0325>.
- Verkaik, K., Hamel, J. F., & Mercier, A. (2016). Carry-over effects of ocean acidification in a cold-water lecithotrophic holothuroid. *Marine Ecology Progress Series*, 557, 189–206. <https://doi.org/10.3354/meps11868>
- Vihtakari, M., Havenhand, J., Renaud, P. E., & Hendriks, I. E. (2016). Variable individual- and population- level responses to ocean acidification. *Frontiers in Marine Science*, 3, 51. <https://doi.org/10.3389/fmars.2016.00051>
- Vihtakari, M., Hendriks, I. E., Holding, J., Renaud, P. E., Duarte, C. M., & Havenhand, J. N. (2013). Effects of ocean acidification and warming on sperm activity and early life stages of the Mediterranean mussel (*Mytilus galloprovincialis*). *Water*, 5(4), 1890–1915. <https://doi.org/10.3390/w5041890>
- Wang, Q., Cao, R., Ning, X., You, L., Mu, C., Wang, C., Wei, L., Cong, M., Wu, H., & Zhao, J. (2016). Effects of ocean acidification on immune responses of the Pacific oyster *Crassostrea gigas*. *Fish & Shellfish Immunology*, 49, 24–33. <https://doi.org/10.1016/j.fsi.2015.12.025>
- Washington Sea Grant. (2015). Shellfish aquaculture in Washington State. *Final Report to the Washington State Legislature*, 84.
- Wasson, K., Gossard, D. J., Gardner, L., Hain, P. R., Zabin, C. J., Fork, S., Ridlon, A. D., Bible, J. M., Deck, A. K., & Hughes, B. B. (2020). A scientific framework for conservation aquaculture: A case study of oyster restoration in central California. *Biological Conservation*, 250, 108745. <https://doi.org/10.1016/j.biocon.2020.108745>
- Watanabe, W. O., Smith, T. I. J., Berlinsky, D. L., Woolridge, C. A., Stuart, K. R., Copeland, K. A., & Denson, M. R. (2003). Volitional spawning of black sea bass *Centropristis striata* induced with pelleted luteinizing hormone releasing hormone-analogue. *Journal of the World Aquaculture Society*, 34(3), 319–331. <https://doi.org/10.1111/j.1749-7345.2003.tb00070.x>
- Weydmann, A., Søreide, J. E., Kwasniewski, S., & Widdicombe, S. (2012). Influence of CO₂-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis*. *Journal of Experimental Marine Biology and Ecology*, 428, 39–42. <https://doi.org/10.1016/j.jembe.2012.06.002>
- White, M. M., Mullineaux, L. S., McCorkle, D. C., & Cohen, A. L. (2014). Elevated pCO₂ exposure during fertilization of the bay scallop *Argopecten irradians* reduces larval survival but not subsequent shell size. *Marine Ecology Progress Series*, 498, 173–186.
- Wong J. M., Kozal L. C., Leach T. S., Hoshijima U. & Hofmann G. E. (2019). Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling conditions alters maternal provisioning and progeny phenotype. *Journal of experimental marine biology and ecology*, 517, 65–77. <https://doi.org/10.1016/j.jembe.2019.04.006>
- Wood, H. L., Spicer, J. I., & Widdicombe, S. (2008). Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B: Biological Sciences*, 275(1644), 1767–1773. <https://doi.org/10.1098/rspb.2008.0343>
- Xu, X., Yang, F., Zhao, L., & Yan, X. (2016). Seawater acidification affects the physiological energetics and spawning capacity of the Manila clam *Ruditapes philippinarum* during

- gonadal maturation. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, 196, 20–29. <https://doi.org/10.1016/j.cbpa.2016.02.014>
- Yue, C., Li, Q., & Yu, H. (2018). Gonad Transcriptome Analysis of the Pacific Oyster *Crassostrea gigas* Identifies Potential Genes Regulating the Sex Determination and Differentiation Process. *Marine Biotechnology*, 20(2), 206–219. <https://doi.org/10.1007/s10126-018-9798-4>
- Zervoudaki, S., Frangoulis, C., Giannoudi, L., & Krasakopoulou, E. (2013). Effects of low pH and raised temperature on egg production, hatching and metabolic rates of a Mediterranean copepod species (*Acartia clausi*) under oligotrophic conditions. *Mediterranean Marine Science*, 15(1), 74. <https://doi.org/10.12681/mms.553>
- Zhang, D., Li, S., Wang, G., & Guo, D. (2011). Impacts of CO₂-driven seawater acidification on survival, egg production rate and hatching success of four marine copepods. *Acta Oceanologica Sinica*, 30(6), 86–94. <https://doi.org/10.1007/s13131-011-0165-9>

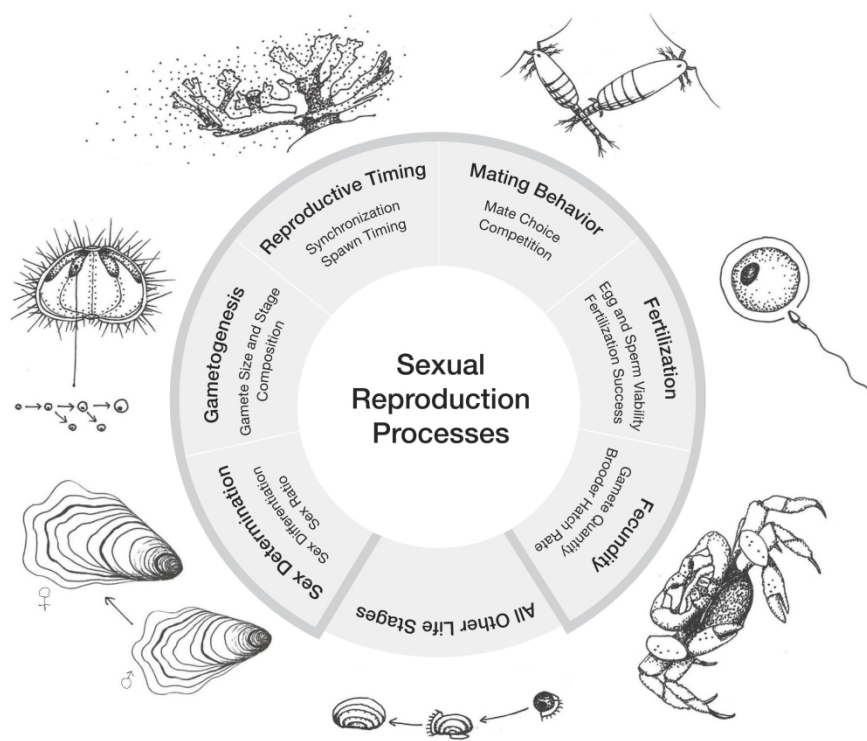


Figure 1. Sexual reproduction processes in marine invertebrates discussed in this review.

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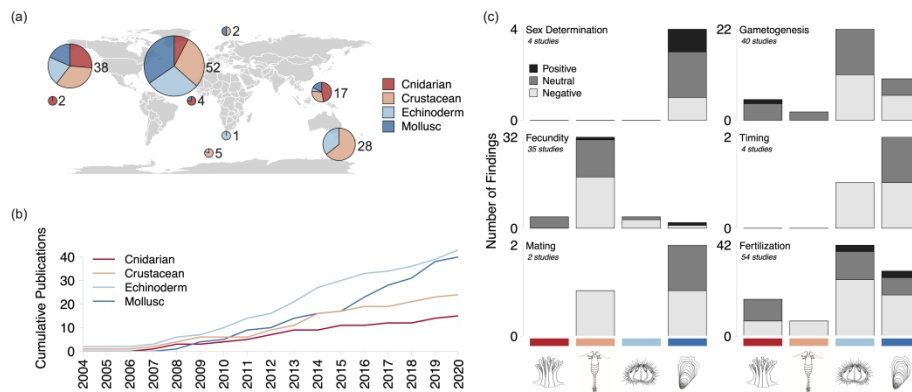


Figure 2. Graphical description of studies used in this review. a) Geographic study locations (from left to right): Eastern Indo-Pacific, Temperate Northern Pacific, Temperate Northern Atlantic, Tropical Atlantic, Southern Ocean, Temperate Southern Africa, Arctic, Central Indo-Pacific, and Temperate Australasia. If organisms were collected from a different marine realm than where the experiment took place, only the collection location was considered. i.e. charts represent the proportion of taxa-specific studies conducted in each region, and numbers underneath the pies represent the total number of studies in that region. b) Cumulative number of papers published for each taxa from 2004 to 2020. One echinoderm study was published in 1924 but was not included in the figure. c) Number of positive (black), neutral (dark grey), or negative (light grey) OA effects for various reproductive processes by taxa (from left to right: cnidarians, crustaceans, echinoderms, molluscs). The total number of studies for a reproductive process are included in each panel.

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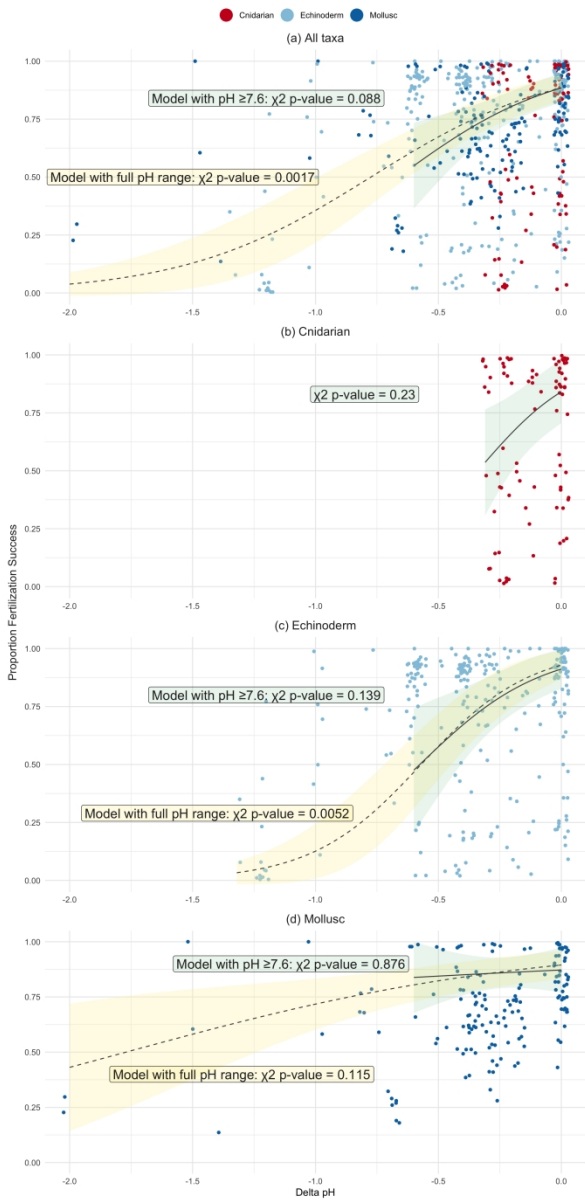


Figure 3: a: Fertilization success by Δ pH (the difference between experimental pH and control) across all taxa. Meta-analysis was performed using a binomial regression model using all experimental data (yellow curve) and pH values ≥ 7.6 (green curve). The general trend indicates that fertilization success decreases with pH when considering all taxa; however, the effect of pH on fertilization rate is only significant across the full pH range (yellow curve). b-d: Taxa-specific binomial regression models for cnidarians (b, six studies), echinoderms (c, 16 studies), and molluscs (d, 16 studies). Δ pH predicts echinoderm fertilization only when data from all experimental pH is included, and does not significantly predict fertilization rates in cnidarians or molluscs. In all figures, each point reflects the average percent fertilization reported by one study at Δ pH. No fertilization studies were found for crustaceans. Ribbons indicate 95% confidence intervals.

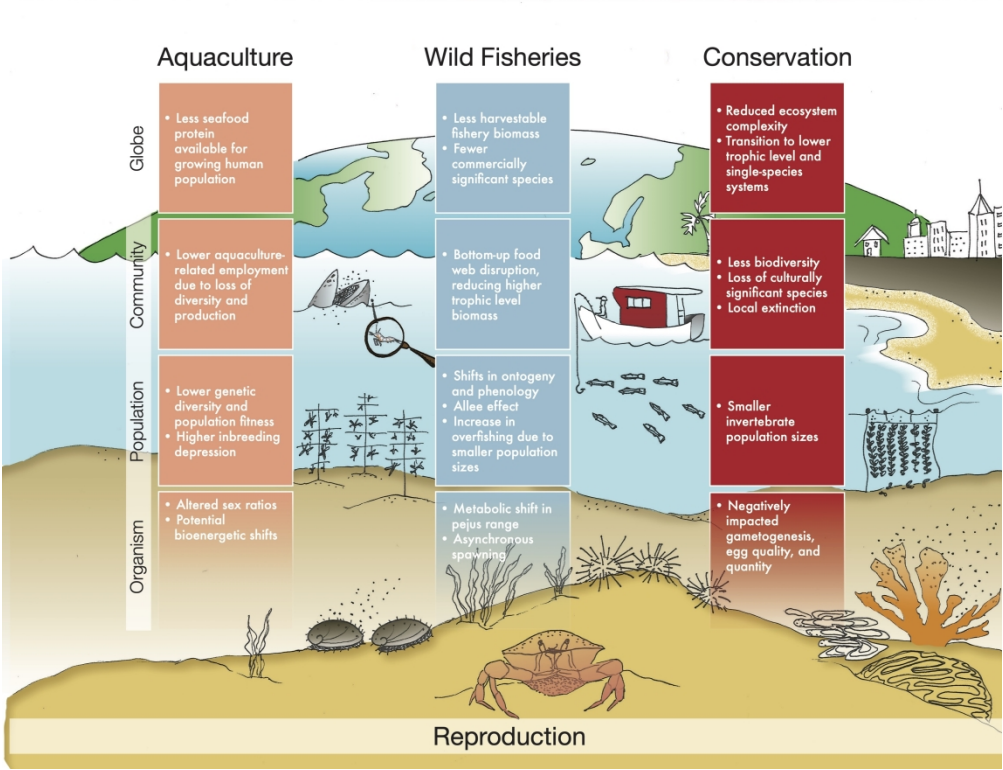


Figure 4. Organism, population, community, and global-level implications of OA impacts on reproduction for commercial and restoration aquaculture, wild fisheries, and conservation and restoration of wild populations. Implications mentioned are not necessarily exclusive to one category.

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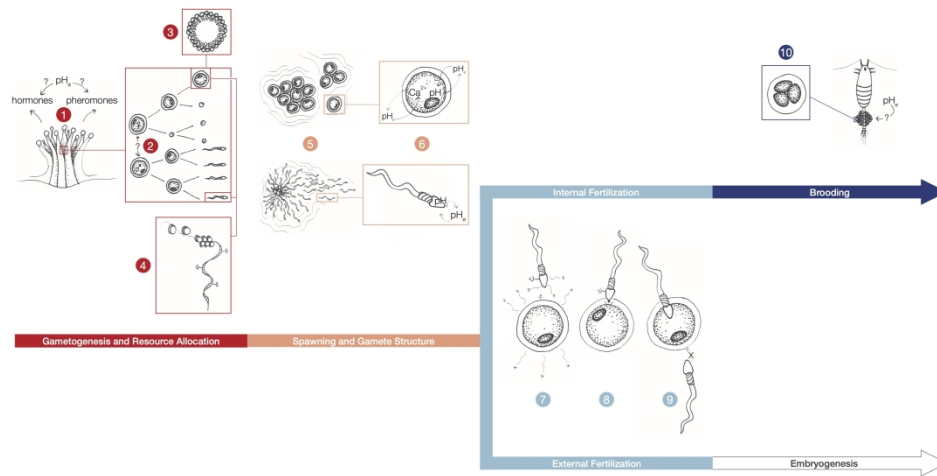


Figure 5. Sexual reproduction mechanisms that may be compromised by OA. Changes in the chemical environment can impact the release of hormones and pheromones that trigger gametogenesis, or gamete release in broadcast spawning species (1). Sex determination and differentiation processes (2), gametogenesis timing, and energy allocation (specifically maternal provisioning) (3) can be impacted by low pH. Gametes undergo substantial chromatin reorganization and DNA methylation (4) that can impact how gametes and subsequent offspring respond to acidified conditions. Spawn timing may be affected by OA (5).

Gamete bundles undergo breakage, further exposing gamete intracellular conditions to changes in extracellular pH (pHe) (6). To begin the fertilization process internally or externally, chemoattractants are released from eggs and sperm (7). A single sperm binds to the egg with the aid of gamete recognition proteins (8). Successful fertilization triggers calcium release or an egg jelly coat to prevent polyspermy (9).

After internal fertilization, brooding may shield embryo development from changes in pHe or expose embryos to more extreme conditions (10).

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BOX 1 | Glossary of key reproduction terms

<p>Egg fertilization mechanisms: molecular processes in eggs involved in successful fertilization, including release of egg-derived chemicals, egg-sperm fusion, egg activation and polyspermy prevention</p> <p>Egg receptivity: the length of time an egg is able to be fertilized by a sperm cell (Butts et al., 2012)</p> <p>Exogenous vs. endogenous reproductive cues: external environmental cues vs. cues originating within the organism</p> <p>F1: first filial generation comprised of offspring resulting from a cross between two individuals from parental generation.</p> <p>Fecundity: number of mature eggs (or embryos in other papers) per unit body volume. For colonial organisms, the number of eggs and/or sperm per module (i.e. polyp), or the proportion of modules that are reproductive within the colony</p> <p>Gamete quality: size, composition, and developmental stage of gametes</p> <p>Gametogenesis: process by which gametes are produced through meiosis and cell differentiation. Formation of ova occurs through oogenesis and spermatozoa through spermatogenesis</p> <p>Gonad condition: Assessment of gonad development via histological examination (Uthicke et al., 2014)</p> <p>Gonad Index (GI) or Gonadosomatic Index (GSI): gonad mass as a proportion of the total body mass [(gonad weight /total tissue weight) X 100]</p> <p>Gravid: carrying eggs or young</p>	<p>Hatching success: percentage of eggs which produce viable offspring</p> <p>Hemocyte cell: phagocytic cells with a role in invertebrate immunity</p> <p>Indeterminate vs. determinate sex: ability to change sex during an organism's lifetime vs having a defined sex at birth</p> <p>Mating behavior: social interaction that prepares for, or increases the success of, copulation and fertilization</p> <p>Mass spawning: synchronous release of gametes by many species or the majority of a mating aggregation</p> <p>Maximum fertilization: asymptotic maximum in fertilization success based on a typical bell-shaped fertilization curve (Albright and Mason, 2013)</p> <p>Naupliar larvae: first planktonic stage of many crustacean larvae, characterized by a single eye</p> <p>Neuropeptides: short sequences of amino acids coexpressed with neurotransmitters and are widely expressed throughout the central nervous system (Thiele, 2017)</p> <p>Oogonia: female gametocyte that produces eggs</p> <p>Oosorption (atresia): process of resorbing vitellogenic eggs under stress to reuse lipids for other physiological processes (Eckelbarger et al., 1994)</p> <p>Phenology: study of periodic events in biological life cycles and how they are influenced by local, seasonal, and interannual environmental variation</p>	<p>Polyspermy: occurs when an egg is fertilized by more than one sperm</p> <p>Reproductively inactive vs. sexually immature: sexually mature individual not presently breeding vs. one not old or big enough to undergo gametogenesis</p> <p>Reproductive output: number of reproductive elements (sperm or egg) per unit body volume. Can also be measured by counting embryos per unit body volume. In colonial organisms, reproductive output is the total amount of gametes released by the colony. In some cases, fecundity and reproductive output are used interchangeably.</p> <p>Resource allocation: proportion of an organism's energy budget allocated to reproduction</p> <p>Sex determination: initial event before sex differentiation that determines whether gonads will develop as male or female (Forconi et al. 2013)</p> <p>Sex differentiation: events after sex determination that ultimately produce either the male or female sexual phenotype</p> <p>Signaling cascade: series of chemical reactions that occur within a biological cell when initiated by a stimulus</p> <p>Sire and dam: father and mother of a genetic line</p> <p>Spawned bundle: sperm and/or egg clusters released to the water column for external fertilization</p> <p>Sperm activity: sperm swimming behavior, including motility, velocity or speed, and path linearity</p>	<p>Sperm linearity: how straight the sperm is swimming, as calculated by the ratio of average velocity on a straight line from start to endpoint of the sperm's path to the the curvilinear velocity along the sperm's path (Eads et al., 2016a)</p> <p>Sperm velocity: defined by (1) curvilinear velocity along the sperm's path, (2) smoothed average sperm path velocity, (3) average velocity on a straight line from start to endpoint of the sperm's path (Eads et al., 2016b)</p> <p>Spermatocyte: male gametocyte from which spermatozoa develop</p> <p>Standardized Gonad Index (SGI): reproductive cycle indicator based on the differences between the observed and expected weights of the gonads for an individual of a given size, takes into account allometric gonadal growth</p> <p>Synchronization: coordination of reproductive events to increase mating potential, fertilization, and offspring success</p> <p>Timing of reproduction: milestones that rely on environmental and chronological cues</p> <p>Vitelli: the proteinous yolk found in an egg</p> <p>Volitional vs. strip or induced spawning: release of gametes via natural or hormonal means vs artificially releasing gametes through anthropogenic means (Watanabe et al., 2003)</p>
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Box 1

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BOX 2 | Experimental considerations for future OA and reproduction studies**Improve field research capability**

- Spawning timing, duration, and intensity in natural settings are not easily predictable, and we lack baseline reproduction knowledge for most marine species. Additionally, physiological and reproductive responses of laboratory-raised organisms may be compromised by stress associated with captivity.
- Research initiatives should collect baseline knowledge. Technologies should be developed and integrated with biophysical models to track gametes and larvae in the natural environment.

Measure multiple reproductive traits simultaneously

- Reproduction is a complex process. It is important to examine multiple reproductive traits simultaneously to get a better understanding of overall reproductive performance.
- Reporting measures of reproductive success that integrate multiple traits will improve the predictability of demographic models (ex. fecundity and survival of mother, survival of offspring).

Incorporate diversity in test subjects and experimental design

- Studies should maximize genetic variation, as organism origin (ie. field site, hatchery) and genotype can affect sperm and egg compatibility and confound findings.
- Future work should incorporate taxonomic diversity and multiple stressors to expand the breadth of species and conditions studied

Account for length of gametogenic cycles

- Gametogenic cycles vary between species. Many studies only expose organisms to OA when gametes are almost fully developed.
- Accounting for gametogenic cycle length improves understanding of OA effects on gamete development and parental effects.

Test multiple sperm concentrations

- The probability of detecting a treatment effect may be dependent upon sperm concentrations. “Optimal” sperm concentration may be higher than ecologically relevant concentrations. Low sperm concentrations can exacerbate OA effects.
- Experiments should use multiple sperm concentrations and gamete incubation times, as these factors may alter the probability of fertilization and/or polyspermy.

Understand limitations of intergenerational studies

- Artificially-induced fertilization methods (ie. strip spawning, chemical/hormonal injection, etc.) used in intergenerational studies may cause organisms to release gametes before complete gametogenesis.
- Many intergenerational OA studies inherently include adult exposure during reproduction, so those studies formally measure and report reproduction metrics.
- Future research should differentiate between within-generation carryover effects, cross-generational impacts (F0-F1), and multigenerational plasticity (F1-F2+), and determine how reproductive conditioning may impact each facet of plasticity.

See previous reviews for additional challenges in the design and execution of ocean acidification experiments (Albright et al. 2011, Grazer and Martin 2012, Przeslawski et al. 2017).

Box 2

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