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Effects of ocean acidification on copepods

Minghua Wang[a,*], Chang-Bum Jeong[b], Young Hwan Lee[b], Jae-Seong Lee[b,*]

[a] *Key Laboratory of the Coastal and Wetland Ecosystems (Xiamen University), Ministry of Education, Xiamen, 361102, China*
[b] *Department of Biological Science, College of Science, Sungkyunkwan University, Suwon 16419, South Korea*

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ABSTRACT

Ocean acidification (OA) leads to significant changes in seawater carbon chemistry, broadly affects marine organisms, and considered as a global threat to the fitness of marine ecosystems. Due to the crucial role of copepods in marine food webs of transferring energy from primary producers to higher trophic levels, numerous studies have been conducted to examine the impacts of OA on biological traits of copepods such as growth and reproduction. Under OA stress, the copepods demonstrated species-specific and stage-dependent responses. Notably, different populations of the same copepod species demonstrated different sensitivities to the increased pCO_2. In copepods, the deleterious effects of OA are also reinforced by other naturally occurring co-stressors (e.g., thermal stress, food deprivation, and metal pollution). Given that most OA stress studies have focused on the effects of short-term exposure (shorter than a single generation), experiments using adults might have underestimated the damaging effects of OA and the long-term multigenerational exposure to multiple stressors (e.g., increased pCO_2 and food shortage) will be required. Particularly, omics-based technologies (e.g., genomics, proteomics, and metabolomics) will be helpful to better understand the underlying processes behind biological responses (e.g., survival, development, and offspring production) at the mechanistic level which will improve our predictions of the responses of copepods to climate change stressors including OA.

1. Introduction

Due to anthropogenic activities, atmospheric CO_2 has steadily increased from a pre-industrial level of ~280 µatm to a present-day concentration of ~400 µatm (Siegenthaler et al., 2005). As the atmospheric CO_2 increases, significant changes in the seawater carbonate chemistry occur as a result of higher CO_2 absorption by the ocean, ultimately leading to continuous reduction in pH and carbonate concentration (Orr et al., 2005). Moreover, average surface pH of ocean has decreased by 0.1 units (a 26% increase in hydrogen ion concentration) since the industrial revolution (Orr et al., 2005). Also atmospheric CO_2 level will reach 1000 µatm by the end of this century and 1900 µatm in the year 2300, leading to a decline of 0.3–0.4 units and 0.77 units in the atmosphere and the seawater surface pH, respectively (Caldeira and Wickett, 2003). Decrease in seawater pH can be attributed to multiple factors; for example, upwelling of deeper acidified water into continental shelves (Feely et al., 2008), high levels of heterotrophic respiration (Wootton et al., 2008), and CO_2 leakage from sub-seabed carbon capture and storage sources (Shitashima et al., 2013). Organisms inhabiting in these zones will be subjected to lower pH values than those predicted for the global sea surface, which can potentially confer on their acclimatory plasticity and/or adaptive responses to pCO_2 stress. In addition, carbon fixation and storage in sub-seabed geological formations have been proposed as a potential strategy to reduce the marked accumulation of anthropogenic CO_2 in the atmosphere (Reguera et al., 2009).

Ocean acidification (OA) is also known to modulate calcification as a result of reduction in the carbonate ion concentration, which can elicit profound impact on many calcifying organisms (Langdon et al., 2000; Orr et al., 2005). Moreover, organisms exposed to high pCO_2 are vulnerable to hypercapnia and acidosis (Melzner et al., 2009) and tend to endure the adverse outcome of the reduced pH by energy reallocation from fitness-priority traits (growth and reduction) to mobilization of energy-consuming acid-base regulatory processes.

Thus, OA has been reported to perturb a multitude of physiological processes including but not limited to calcification (Langdon et al., 2000), metabolism (Lannig et al., 2010), survival (Talmage and Gobler, 2010), development (Kurihara et al., 2004a), and reproduction (Havenhand et al., 2008) in calcified and non-calcified species and is considered a major global threat to the fitness of marine ecosystems.

Copepods are abundant small crustaceans and often comprise the majority of the biomass of zooplankton in marine ecosystems. They play a crucial role in the marine food web by transferring energy from primary producers to higher trophic levels. As a result, accurate

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[*] Corresponding authors.
E-mail addresses: mhwang45@xmu.edu.cn (M. Wang), jslee2@skku.edu (J.-S. Lee).

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Table 1
Summary of OA effects on growth, development, and reproduction of copepods with or without co-stressors.

Species	pH (pCO$_2$)	Tested combined effects	Summary of the results	References
Acartia bifilosa	8.0 7.6	Temperature variance (17, 20 °C), cyanobacteria	- High temperature caused negative effects on egg viability, development, and oxidative status. - Antioxidant capacity and developmental rate were increased under OA condition, whereas they were decreased together with high temperature. - Co-exposure to cyanobacteria enhanced development which may contribute to alleviation of OA and temperature effects.	Vehmaa et al. (2013)
Acartia sp.	8.1 7.45	Temperature variance (17, 20 °C)	- Egg and nauplii production rates were increased in warmer condition (+3 °C), but the increase was smaller under OA condition. - Transplant experiments have revealed positive maternal effects in response to OA, particularly in warmer condition.	Vehmaa et al. (2012)
Acartia tonsa	385 1000 2000 3000 6000 (μatm)	–	- Nauplii exhibited high mortality whereas the survival of embryo, copepodite, and adult stages were not affected at pCO$_2$ < 3000 μatm. - Reproduction was negatively impacted.	Cripps et al. (2014b)
Acartia tsuensis	380 2380 (μatm)	–	- No significant effects were observed on survival, body size, reproduction, and development.	Kurihara and Ishimatsu (2008)
Acartia tsuensis, A. erythraea	365 2365 10,365 (μatm)	–	- Hatching rate and nauplius mortality were increased under high pCO$_2$ exposure. - Reproduction was decreased in high pCO$_2$ exposure groups. - Little effect on survival rate was shown for adult copepods.	Kurihara et al. (2004a)
Calanus finmarchicus	390 3300 7300 9700 (μatm)	–	- Survival rate was decreased at high pCO$_2$ for 28-day exposure, whereas no significant effects were observed in 3300 μatm. - Size and lipid volume were increased in stage IV copepodites under 3300 μatm exposure and decreased in stage III copepodites exposed to 7300 μatm.	Pedersen et al. (2013)
Calanus finmarchicus	8.23 6.95	–	- Egg production and biomass were not affected by OA in adult female copepods. - Hatching rate of nauplii was highly decreased in OA condition (up to 4%).	Mayor et al. (2007)
Calanus glacialis	8.2 7.6 6.9		- No significant effects on egg production were shown under OA. - Hatching success rate was decreased at pH 6.9.	Weydmann et al. (2012)
Calanus glacialis, C. hyperboreus	390 3000 (μatm)	Temperature variance (0, 5, 10 °C)	- No significant effects were shown in response to OA. - Decrease of body carbon mass was observed under OA at 5 °C	Hildebrandt et al. (2014)
Calanus spp., Oithona similis	370 700 1000 (μatm)	–	- Survival rate was significantly decreased at 700 and 1000 μatm in *O. similis*	Lewis et al. (2013)
Centropages tenuiremis	8.18 7.83	–	- Respiration and feeding rates were increased in response to OA.	Li and Gao, (2012)
Centropages typicus, Temora longicornis	8.04 7.97 7.85 7.78 6.71	–	- Egg production and hatching success were decreased only at the lowest pH in *C. typicus*.	McConville et al. (2013)
Ophiothrix fragilis	8.1 7.9 7.7	–	- Increased mortality was shown in low pH (7.9 and 7.7) exposed copepods for 8 days in contrast to control. - Negative effect was observed on development, growth and skeletogenesis of copepods under OA exposure.	Dupont et al. (2008)
Tigriopus japonicus	400 1000 (μatm)	Mercury	- Mercury-induced decrease of reproduction was alleviated by OA. - Negligible impacts were observed on survival, development, reproduction, and sex ratio during multigenerational OA exposure.	Li et al. (2017b)
Tigriopus japonicus	8.1 7.3	Dietary restriction	- Delays in development and decrease of reproduction under OA condition were more induced by dietary restriction. - SOD activity was increased in response to dietary restriction and enhanced under OA condition.	Li et al. (in press)
Tisbe battagliai	8.06 7.95 7.82 7.67	Copper	- Negative synergic effect was observed on reproduction under combined exposure of OA and copper. - The copper plus OA exposure enhanced growth when compared with the impact of OA alone. - Different alterations in cuticle compositions were observed in response to OA with or without copper exposure.	Fitzer et al. (2013)
Tisbe battagliai	8.06 7.95 7.82 7.67	–	- Nauplilar production was increased under OA exposure, whereas multigenerational modeling predicted the decrease of naupliar production over the next 100 years. - Reduced body length was observed under OA condition - Increased proportion of carbon relative to oxygen in cuticle was observed for OA stress.	Fitzer et al. (2012)

projections of copepod in responses to OA are pivotal to our understanding of how marine ecosystems will respond to climate change stressors.

As one of the unique physical feature, copepods possess an impermeable chitinous integument and have adapted to dynamic habitats with great fluctuations in pCO_2 (e.g., sea bottom, tide pools, and vertical migration). Due to the survivability exhibited against highly fluctuated pCO_2 regions, copepods are perceived as one of the insensitive species to OA. Indeed, a number of studies have reported that copepods are resistant to elevated pCO_2 (6000–10000 μatm) level (Table 1) (Kita et al., 2013; Kurihara et al., 2004b; Mayor et al., 2007; McConville et al., 2013; Pedersen et al., 2013). Nevertheless, the idea that copepods are resilient against OA is primarily based only on short-term (less than one generation) exposure of adult females to elevated pCO_2. In contrast, short-term and long-term (multigenerational) exposures of copepods to artificially generated near-future pCO_2 levels had significantly negative impacts on growth/reproduction (Cripps et al., 2014a; Vehmaa et al., 2016; Fitzer et al., 2012; Thor and Dupont, 2015), making it unclear whether copepods are sensitive or insensitive to OA. In addition, OA-exposed marine organisms tend to increase their energy reallocation into defense/repair processes (i.e. energetic trade-off), while concomitantly demonstrating inferior growth and reproduction (Fig. 1). Interestingly, a recent multigenerational study indicated that transgenerational effects could mitigate the negative effects of OA on fecundity in the ubiquitous planktonic copepod *Pseudocalanus acuspes* (Thor and Dupont, 2015), indicating that copepods are likely developing the physiological acclimation and/or genetic adaptation in response to long-term multigenerational exposure to high pCO_2. Thus, it would not be surprising if copepods are indeed sensitive to increased pCO_2 (i.e. increase in OA). Meanwhile, the response of copepods to OA can also be modulated by their responses to other naturally occurring co-stressors (Table 1) such as temperature (Garzke et al., 2016; Hildebrandt et al., 2014; Vehmaa et al., 2012; Vehmaa et al., 2013), food availability (Li et al., 2017a; Pedersen et al., 2014), and metals (Li et al., 2017b; Pascal et al., 2010; Wang et al., 2017). Taken together, these findings indicate high variability of the OA effect among marine copepods.

Life history traits such as growth, development, and reproduction are critical for population recruitment and maintenance of copepods. In this paper, we review the impacts of both short- and long-term exposures to OA on these traits. We also discuss possible underlying mechanisms on the effects of OA at the molecular and cellular levels. We highlight future research priorities such as long-term multi-generational exposure to co-stressors (e.g., seawater warming, food shortage, and metal pollution) for OA stress studies in copepods. Overall, our purpose in this review is to provide a better understanding of how marine copepods will respond to OA stress in future oceans.

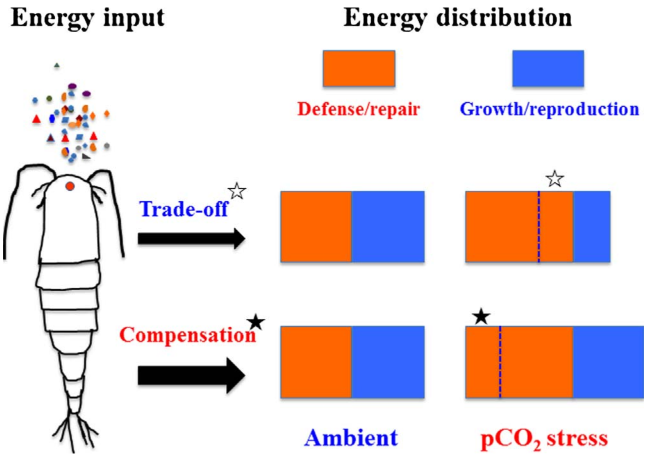

Fig. 1. Energetic trade-off hypothesis showing how compensatory response can potentially offset the negative impacts of pCO_2 stress on copepods as exemplified by increased feeding under excess food provision. Open and closed stars indicate the trade-off for defense/repair and the compensation for growth/reproduction, respectively. Under trade-off situation, the defense/repair takes higher proportion compared to the growth/reproduction energy distribution in a given 100% energy distribution condition. Under compensation situation, the OA (e.g. pCO_2 stress) reinforces organism to take even higher energy distribution (> 100%) to compensate the stress to re-allocate the energy in defense/repair.

2. Versatility of the copepod in response to OA

2.1. *Species-specificity*

Due to the importance of a niche of copepods in marine ecosystems, many research groups have explored the effects of OA in copepods. For example, the projected near-future pCO_2 scenario (1000 μatm) had no influence on copepods (Table 1) (Hildebrandt et al., 2014; Kita et al., 2013; Kurihara and Ishimatsu, 2008; Kurihara et al., 2004a,b; Li et al., 2017a; Mayor et al., 2007; McConville et al., 2013; Pedersen et al., 2013; Watanabe et al., 2006; Weydmann et al., 2012; Zhang et al., 2011); in particular, growth/reproduction were the only affected life traits at extremely elevated pCO_2 concentrations (6000–10000 μatm) (Kita et al., 2013; Kurihara et al., 2004b; Mayor et al., 2007; McConville et al., 2013; Pedersen et al., 2013) but such OA effects were species-specific. Also the effects of increased pCO_2 exposure were examined on the reproduction of two calanoid copepods *Centropages typicus* and *Temora longicornis* (McConville et al., 2013) and found that the projected near-future OA level had insignificant impacts on these two species, whereas a very high CO_2 level (9830 μatm; representative of CO_2 capture and storage scenarios) significantly decreased egg production rate and hatching success of *C. typicus* but not *T. longicornis*. In female *Acartia pacifica*, *Acartia spinicauda*, *Calanus sinicus*, and *Centropages tenuiresis*, pCO_2 exposure (800, 2000, 5000, and 10000 μatm) affected survival rates and egg hatching success by pCO_2 elevation in a species-specific manner (Zhang et al., 2011). Given that copepods have a cuticle (covered by a small calcium carbonate), their responses to OA are primarily determined by an individual's ability to maintain the balance between acid-base under reduced pH/increased pCO_2. The distinctive acid-base regulatory performance between species may account for the observed species-specificity in response to OA stress. In vertically-migrating *Calanus* spp., adults copepod exposed to a pCO_2 change > 140 μatm daily showed remarkable resilience to high pCO_2 levels (700 and 1000 μatm) (Lewis et al., 2013). However, in *Oithona similis* adults dwelling in surface waters exposed to a pCO_2 change < 75 μatm, significant outcomes were demonstrated (Lewis et al., 2013), as calanoid adults actively undergo long-stance vertical migrations. Thus, in *Calanus* spp., acid-base balancing performance might be more robust than that of *O. similis* adults (Metz, 1995), supporting the differences in the sensitivities of these two species to OA exposure.

Even for the same copepod species, different populations can potentially display inconsistency in responses to OA stress. Indeed, this has been supported to some extent by a recent study showing that OA resulted in different energetic responses in Arctic and boreal populations of the copepod *P. acuspes* (Thor and Oliva, 2015). More interestingly, Thor et al. (2017) demonstrated contrasting physiological responses in three populations of the keystone Arctic copepod *Calanus glacialis*, exposed to different conditions of OA (pH 6.4–8.1). For example, metabolic rate increased by 136% and 127% in copepodite stage IV of the Kongsfjord and Billefjord populations, respectively, under reduced pH, but remained unchanged for the Disko Bay population (Thor et al., 2017). Variations in the outcomes of different populations of the same species in response to OA stress can be caused by variations in parental history, maternal investment, and biotic variables (e.g., food provision) but are not restricted to these conditions.

Table 2
Summary of multigenerational effects of OA on growth, development, and reproduction of copepods.

Species	pH (pCO_2)	Exposure periods	Summary of the results	References
Tigriopus japonicus	400 1000 (μatm)	Four generations	- Negligible impacts on survival and sex ratio were shown during multigenerational exposure of OA. - Minor effect was also observed in the development and reproduction under multigenerational exposure.	Li et al. (2017b)
Calanus finmarchicus	380 1080 2080 3080 (μatm)	Two generations	- The pCO_2-dependent negative effects were shown on growth, development, feeding, and metabolic rates. - Significant developmental delay shown in parental generation exposed to 2080 was not observed in the following F1 generation, indicating that copepods have adaptive potential to long-term effects of OA. - Energy budget approach has revealed increased energy expenditure via increased respiration, resulting in growth retardation and reproductive depression.	Pedersen et al. (2014)
Tisbe battagliai	8.06 7.95 7.82 7.67	Two generations	- Naupliar production was decreased in response to OA.	Fitzer et al. (2013)
Pseudocalanus acuspes	400 900 1550 (μatm)	Two generations	- Fecundity of second generation was decreased at 900 μatm CO_2 but with increased metabolic rate. - Reciprocal transplant experiments suggested that transgenerational effects reduced the negative impacts of OA.	Thor and Dupont (2015)
Acartia tsuensis	380 2380 (μatm)	Two generations	- No significant effects were observed in survival, body size, reproduction, and development.	Kurihara and Ishimatsu (2008)

2.2. Developmental stage-dependent responses

Life history stages of copepods are normally classified into eggs, nauplii, copepodites, and mature males and females. Understanding the developmental-stage-specific responses to OA is critical to accurately anticipate responses of copepods to future CO_2 scenarios, as it can reveal at which of the life history stage(s) is at risk to pCO_2 stress. Although OA effects on copepods have been studied intensively, little is known about the responses of different life history stages to OA (Garzke et al., 2016; Hildebrandt et al., 2014; Kita et al., 2013; Kurihara and Ishimatsu, 2008; Kurihara et al., 2004a,b; Li et al., 2017b; Mayor et al., 2007; McConville et al., 2013; Pedersen et al., 2013; Wang et al., 2017; Watanabe et al., 2006), although it is widely known that the early developmental stages of many marine species have been shown to display the greatest sensitivity to OA stress (Dupont and Thorndyke, 2009; Kroeker et al., 2010). To date, few groups have investigated the effects of OA using nauplii staged models (Cripps et al., 2014a; Kurihara et al., 2004a; Lewis et al., 2013). Direct comparisons of size-fractioned stages of mixed copepod assemblages have shown that earlier developmental stages are most susceptible to high pCO_2 levels (700 and 1000 μatm) (Lewis et al., 2013). For example, the survival of *Calanus* spp. nauplii was significantly decreased at 700 μatm but *Calanus* spp. adults demonstrated little effect to elevated pCO_2 concentrations (up to 1000 μatm). When the calanoid copepod *Acartia tonsa* was exposed to different pCO_2 concentrations (1000, 2000, 3000, and 6000 μatm), the eggs, early copepodite stages, and adults (males and females) were not lethally impacted by pCO_2 concentrations under 3000 μatm but the nauplii mortality rate increased threefold at the near-future pCO_2 concentration of 1000 μatm (Cripps et al., 2014a). Based on previous studies, it is suggestive that the earlier developmental stages of copepods, especially the nauplii stage, are most sensitive to OA stress, indicating that this finding is critical ecological implication, as these early ontogenetic stages act as bottlenecks for population recruitment and maintenance; hence, disruption of these stages is likely to disrupt population dynamics. Some marine copepods (e.g., calanoids) may intentionally enter into the diapause stage; that is, embryonic dormancy and postembryonic diapause, typically observed on the onset of unfavorable environments (Baumgartner and Tarrant, 2017). During diapause, the arrested development and low metabolic activity are often observed, but they can resume normal status upon favorable conditions. In addition, copepods will accumulate large amounts of lipids before their migration into deeper waters for postembryonic diapause. The diapause strategy assists the copepods to survive for several months under unfavorable environments, playing a crucial role in population recruitment and maintenance. If the copepods suffered from elevated pCO_2 exposure during this resting state, their energy storage (e.g., lipids) might be depleted at a higher rate than usual. In this context, the diapausing copepods seem more vulnerable to OA stress, since they may not compensate for energy losses under such case. However, this topic is still open which requires our attention.

The apparent resilience of copepods to OA has largely been determined at particular developmental stages (e.g., adult females) exposed to pCO_2 in one generation (Table 1). Additionally, these copepods were provided food *ad libitum* (Kita et al., 2013; Kurihara et al., 2004b; Mayor et al., 2007; McConville et al., 2013; Pedersen et al., 2013). Excess food can potentially neutralize the negative impacts of high pCO_2 on copepods, as copepods can perform compensatory feeding to enhance their total energy input and thereby reallocate energy into growth and reproduction (Li and Gao, 2012; Wang et al., 2017). If the early life history stages of copepods, especially the nauplii stage, are most susceptible to pCO_2 stress (Cripps et al., 2014a; Kurihara et al., 2004a; Lewis et al., 2013), we might have underestimated the damaging effects of OA in copepods and this topic should be revisited.

2.3. Acclimatization/adaptation

The average pH value of ocean surfaces is predicted to decline by 0.4 units in the year 2100 when CO_2 is released in a “business-as-usual” scenario. These changes will result in drastic changes in carbonate chemistry and consequently have significant large-scale impacts on marine animals (Kroeker et al., 2010). However, marine animals can also potentially acclimatize and/or adapt to OA when they are exposed to increased pCO_2 over multiple generations. In this context, acclimatization confers an individual with plasticity in response to the absence or presence of stressors (Sun et al., 2014). Physiological acclimatization also includes transgenerational effects, e.g., epigenetic changes exemplified by DNA methylation and/or histone modification under OA. In contrast, an adaptive response is due to changes in the genetic structure of a population (i.e. changes in allele frequency) and is likely to be sustained for a long time, regardless of the presence of the stressor (Tsui and Wang, 2005). Thus, physiological acclimatization and genetic adaptation is likely regulating the effects of short-term and long-term exposures of copepods to OA.

Although copepods have been subjected to OA for many generations in marine environments, very few studies have investigated individual responses to long-term multigenerational exposure to increased pCO_2 (Fitzer et al., 2012; Kurihara and Ishimatsu, 2008; Li et al., 2017b; Pedersen et al., 2014; Thor and Dupont, 2015; Wang et al., 2017) (Table 2), which is more realistic scenario for marine animals than short-term exposure. Kurihara and Ishimatsu (2008) studied the effects of OA on the copepod *Acartia tsuensis* over two generations. They found no effects of OA on egg production or hatching success in either generation in response to a pCO_2 exposure of 2380 μatm (Kurihara and Ishimatsu, 2008). Similarly, in the copepod *Tigriopus japonicus*, multigenerational study also showed that a near-future OA level (1000 μatm) had no negative impacts on growth or reproduction of four generations (Li et al., 2017b). Therefore, physiological acclimatization (i.e. phenotypic plasticity) has been involved in explaining copepod robustness to pCO_2 stress (Kurihara and Ishimatsu, 2008; Li et al., 2017b). Proteomic analysis in *T. japonicus* provided further mechanistic insights into the response of individual copepod to OA stress. The OA-exposed copepods appeared to enhance energy production by increasing protein assimilation and proteolysis, thereby negating the negative effects of OA on growth/reproduction (Wang et al., 2017). Also the OA-exposed copepods with dietary source *ad libitum*, their total energy input were increased, neutralizing the damaging impacts of the decreased pH (Wang et al., 2017).

In the planktonic copepod *P. acuspes*, OA effects for two generations were determined on fecundity and metabolism in response to different pCO_2 concentrations (400, 900, and 1550 μatm) (Thor and Dupont, 2015). In this study, all changes between 400–900 μatm were found to be completely reversible due to phenotypic plasticity but transgenerational effects were found to significantly mitigate the decreased fecundity at 1550 μatm at an energetic cost. Thus, the transgenerational effects were result of genetic adaptation, as the high mortality (> 50% per generation) is likely enabled significant selection and consequently genetic adaptation at the highest pCO_2 treatment (1550 μatm) (Thor and Dupont, 2015).

Despite the limited studies, acclimatization/adaptation appears to modify the response of copepods to pCO_2 stress and can partly account for copepod resilience to OA (Table 2). Also differential regulatory processes are likely involved in physiological acclimatization and genetic adaptation, where both involve in energy-reallocative strategies. According to the study of Thor and Dupont (2015), physiological acclimatization plays a role at low pCO_2 stress (900 μatm), while greater pCO_2 exposure (1550 μatm) probably cause involvement in genetic adaptation. Overall, comprehensive knowledge about modulation of acclimatization/adaptation in response to OA in copepods will improve the accuracy of predictions of the consequences of climate change stressors for marine ecosystems.

3. Potential mechanisms on OA effects in copepods

Recently, the increase in applications of omics-based technology (e.g., transcriptomics and proteomics) has been observed in stress ecology; this discipline enabled us to understand the response mechanism in marine organisms in response to environmental stressors including OA. Although many studies have performed transcriptomics/proteomics to provide molecular understanding about the effects of OA on marine animals (de Souza et al., 2014; Dineshram et al., 2015, 2016; Evans et al., 2013; Mukherjee et al., 2013; Todgham and Hofmann, 2009; Wong et al., 2011), to date, only two reports were published using marine copepods (De Wit et al., 2016; Wang et al., 2017). In the larvae of the purple sea urchin *Strongylocentrotus purpuratus*, a transcriptome profiling conducted after exposure to different OA levels, i.e. pH values of 8.01 (control), 7.96, and 7.88 Todgham and Hofmann (2009). They found decrease in the gene expression that are involved in calcification, cellular stress response, metabolism, and apoptosis, suggesting the larval sea urchin is sensitive in response to OA, as molecular toxic events can translate into adverse outcomes at higher levels (e.g., growth/reproduction). In the larvae of the oyster *Crassostrea gigas*, a shotgun-based proteomics showed the decrease of metamorphosis-related proteins via down-regulation of several proteins related to energy production, metabolism, and protein synthesis due to pH reduction (Dineshram et al., 2016). Also, in this study, the *C. gigas* larvae tend to save more metabolic energy via synthesizing fewer proteins at a large scale to ensure their survival under reduced pH, resulting in metamorphosis depression under pCO_2 exposure as a physiological cost (Dineshram et al., 2016). In fact, the conserved energy utilization through a global decrease in protein expression is likely a consequence of phenotypic strategy to tolerate against OA, as shown in several proteomics studies (Dineshram et al., 2016; Mukherjee et al., 2013; Wang et al., 2017; Wong et al., 2011). Thus, energy reallocation is likely responsible for copepods to mitigate OA exposure, giving rise to an individual's resilience to counteract in response to OA. Similarly, De Wit et al. (2016) carried out a *de novo* transcriptome profiling in a reciprocal transplant experiment and reported that RNA transcription were strikingly down-regulated in the copepod populations of *P. acuspes* under pCO_2 treatment. In addition, they further argued that the alteration in the gene expression was possibly modulated by helicase activity, which plays a crucial role in cellular energy distribution. Taken together, omics works in copepods demonstrate that energy allocation into different critical cellular functions determine an individual's response (i.e. “winner” or “loser”) to future OA scenario. However, more omics-base investigations are required to obtain a comprehensive understanding of the underlying process behind the response of copepods against increased pCO_2 exposure.

4. Interaction of OA effects in copepods with other stressors

In addition to OA, marine copepods can be exposed to thermal stress, food deprivation, and metal pollution, all of which have been shown to modulate an individual's response to pCO_2 stress (Hildebrandt et al., 2014; Li et al., 2017a; Li et al., 2017b; Pascal et al., 2010; Pedersen et al., 2014; Vehmaa et al., 2013; Wang et al., 2017). Interactions between multiple stressors in organisms can have additive, synergistic, or antagonistic effects to the individual effects (Ban et al., 2014). A comprehensive understanding of the interactions between OA and other naturally occurring co-stressors will enable more accurate predictions of the response of marine copepods to the increased pCO_2 in future oceans.

4.1. Temperature

In addition to OA, the enhanced CO_2 release into the atmosphere has also driven global warming due to the greenhouse effect. Ocean surface temperature will increase by 3–5 °C by the year 2100 (Caldeira and Wickett, 2003). As a result, copepods are anticipated to experience both OA and thermal stress. Normally, marine animals have an optimal thermal window beyond which an individual's fitness is compromised. This window can be narrowed when the organism is exposed to co-stressors as exemplified by OA. OA can result in hypercapnia/acidosis that will affect in energy-costly reallocation between important biological traits (e.g., growth and reproduction) and defense/repair processes. Also the increased temperatures is likely modulating the response of copepod species to pCO_2 stress (Garzke et al., 2016; Hildebrandt et al., 2014; Vehmaa et al., 2012; Vehmaa et al., 2013). Late copepodites and females of two dominant Arctic copepods *Calanus glacialis* and *Calanus hyperboreus* maintained their developmental stages at 0 °C (close to the *in situ* temperature) during several months of exposure to pCO_2 concentrations of 390 and 3000 μatm (Hildebrandt et al., 2014). Thus, no effects on metabolic rate, body mass, and/or mortality were observed in response to pCO_2 stress. However, incubation of *C. hyperboreus* females at different temperatures (0, 5, and 10 °C) revealed sublethal stress, particularly at the highest thermal treatment (10 °C) and their body carbon content was the lowest at 5 °C and

3000 µatm, indicating a synergistic interaction between OA and the arising temperature (Hildebrandt et al., 2014). Thus, copepods can endure the increased pCO_2 exposure in future oceans if not coupled with thermal stress but are likely to be susceptible to OA if combined with thermal stress. However, antagonism has also been invoked to explain the combined effects of OA and thermal stress on copepods (Garzke et al., 2016; Vehmaa et al., 2012). A fully-crossed factorial mesocosm study demonstrated that antagonism occurred between OA and warming when several copepod species (*Paracalanus* sp., *Pseudocalanus* sp., *Acartia* sp., *Temora* sp., and *Calanus* sp.) were exposed to increased pCO_2 (560 and 1400 µatm) and temperature (9 and 15 °C), as determined by effects on abundance, body size, and fatty acid composition (Garzke et al., 2016). Although several studies have examined the combined effects of OA and thermal stress, their interactions do not appear to be monotonous (Byrne and Przeslawski, 2013; Harvey et al., 2013; Przeslawski et al., 2015). For example, OA and seawater warming synergistically impacted the marine invertebrate life histories, indicating that additive or antagonistic effects were common (16 of 20 species studies) (Byrne and Przeslawski, 2013). In contrast, a recent meta-analysis on the impacts of multiple stressors on marine embryos and larvae indicated that synergistic interactions (76.1% of 71 individual tests) were more common than additive (15.5%) or antagonistic (8.5%) interactions for pH and temperature. The interaction type was also attributed to stressors, ontogenetic stages, and biological responses (Przeslawski et al., 2015). However, further studies should be conducted to investigate the interaction between increased pCO_2 and the arising temperature in copepods, as this interaction is likely modulating the response of marine animals to future OA scenarios.

4.2. Food availability

Rising ocean temperatures frequently drive ocean stratification, which reduce nutrient supply to photosynthetic organisms on the surface seawater. Regardless of regional variability, an overall decline in primary production is anticipated, which is the ultimate determinant of food availability to marine ecosystems (Bopp et al., 2013; Gröger et al., 2013; Steinacher et al., 2010). Food deprivation is likely to be encountered in future ocean. Adequate food intake is required for an organism to produce sufficient energy to cover their maintenance and repair costs and sustain growth and reproduction. Excess food provision can result in increased energy input and can potentially protect marine copepods against the negative impacts of stressful conditions including OA (Li et al., 2017a; Li and Gao, 2012; Pedersen et al., 2014; Wang et al., 2017). Food deprivation restricts the supply of energy and can potentially exacerbate the damaging effects of pCO_2 stress (Li et al., 2017a; Pedersen et al., 2014). In the copepod *Calanus finmarchicus*, the effects of different OA scenarios (ambient, 1080, 2080, and 3080 µatm) were examined over two generations under food shortage (Pedersen et al., 2014). They observed a 3.2-fold reduction in egg production rate under limited food at the highest pCO_2 level (3080 µatm) but this suppressive effect diminished in the condition of excess food, suggesting that increased energy is required to maintain the balance between acid-base regulation under high pCO_2 exposure. Thus, excess food can minimize the negative impacts of OA on reproductive performance (Pedersen et al., 2014). In the copepod *T. japonicus*, a shotgun-based quantitative proteomic study showed that pCO_2 stress significantly up-regulated several proteins involved in protein assimilation and proteolysis, demonstrating that *ad libitum* treated *T. japonicus* consumed dietary source and concomitantly increased their total energy input, ultimately neutralizing the negative effects of reduced pH (Wang et al., 2017). Particularly, *T. japonicus* were exposed to different pCO_2 levels (i.e. pH values of 8.1 and 7.3) under different food concentrations ($0.5–80.0 \times 10^4$ cells/mL) in order to examine the joint effects of OA and food availability; the development of nauplii and copepodites was delayed at pH 7.3 with a greater effect at lower food concentrations (Li et al., 2017a). Taken together, these findings suggest that food status can alter the response of copepods to future OA, as excess food can potentially increase energy investment in defense/repair response with concurrent neutralization of the corresponding effects on growth and reproduction (Fig. 1).

4.3. Metal pollution

In addition to the increase in global atmospheric CO_2 level, anthropogenic activities have resulted in metal pollution of marine environments, particularly coastal zones. Copper (Cu) is essential cofactor in many biochemical processes, while cadmium (Cd) and mercury (Hg) have no known biological functions in marine animals. However, all metals are toxic at high levels and capable of disrupting energy and macromolecule metabolism, cellular homeostasis, resulting in cellular/oxidative damage. Metal pollution has been reported to regulate the effects of OA on marine animals (Campbell et al., 2014; Fitzer et al., 2013; Lewis et al., 2016; Li et al., 2017b; Pascal et al., 2010; Roberts et al., 2013; Wang et al., 2017). OA can significantly change metal solubility, speciation, and distribution in seawater and sediments, (i.e., bioavailability) (Millero et al., 2009; Stockdale et al., 2016), and potentially also modify metal toxicity in marine organisms. As an example, one consequence of OA is a remarkable decrease in concentration of hydroxyl and carbonate ions, which form strong complexes with metals in seawater. Metals such as Cd and Hg can form strong complexes with chloride. Therefore, speciation of these two metals is unlikely to be influenced by OA, as chloride formation is independent of pH (Millero et al., 2009; Stockdale et al., 2016). Other metal species (e.g., Cu) is strongly affected by OA, as free Cu ion can form strong complexes with carbonate. Indeed, increase in free Cu ion concentration will occur by approximately 150% in seawaters by the year 2100 due to OA (Stockdale et al., 2016; Millero et al., 2009; Stockdale et al., 2016), which could translate to synergistically enhanced bio-toxicity of Cu in several marine animals including copepods (Campbell et al., 2014; Fitzer et al., 2013; Lewis et al., 2016; Roberts et al., 2013). To date, only a few studies have examined on the interactions between OA and metals (i.e. Cu, Cd, and Hg) in marine copepods (Fitzer et al., 2013; Li et al., 2017b; Pascal et al., 2010; Wang et al., 2017). The combined effects of OA (pH 8.06, and 7.67–7.95) and copper (0 and 20 µg/L) were investigated in the benthic copepod *Tisbe battagliai* across two generations. Nauplius production was reduced with an addition of copper at lower pH values than pH only, indicating a synergistic interaction (Fitzer et al., 2013). However, in the coastal meiobenthic copepod *Amphiascoides atopus*, antagonistic toxicities have been observed between OA (pH values of 8.20 and 6.07–6.36) and Cd, Cu, and free Cu ions during 96 h due to metal-proton competition for binding sites (Pascal et al., 2010). In the copepod *T. japonicus*, the impacts of OA (400 and 1000 µatm) and Hg pollution (1 µg/L) on growth and reproduction during four generations revealed that OA strikingly reduced Hg accumulation and subsequently alleviated the adverse effects of metal toxicity on reproductive performance in each generation. In addition, significant negative interaction was also observed between these two stressors (Li et al., 2017b). Moreover, a shotgun-based proteomics study indicated that copepods exposed to both metals and OA displayed the enhanced lysosome-autophagy (supported by increased energy production mainly due to up-regulated carbohydrate metabolism) to remove accumulated abnormal proteins/enzymes, accounting for the ability of OA to mitigate Hg toxicity (Wang et al., 2017). Taken together, the interaction between OA and metals in copepods appears to be more complex than expected and cannot be directly predicted from OA-induced changes in metal bioavailability. Perhaps, other factors such as metal type and species, exposure conditions (e.g., pH value and metal dose), and physiological functions (e.g., acid-base regulation and energy budget) could be involved and further studies are required to understand these issues.

Table 3
Future priority issues on OA stress studies.

Categories	Detailed objectives and perspectives
Long-term OA effects	- Multigeneration-exposure experiments to identify vulnerable key species and/or sensitive life stages - Multiple generation tests to find out when and how acclimatization/adaptation will work
OA effects coupled with co-stressors	- Incorporation of accompanied environmental changes in OA studies (e.g., hypoxia, ocean warming, and food shortage) - Investigation about combined effects of OA with additional environmental pollutants as exemplified by metal pollution
In-situ OA effects	- Field experiments to assess community and/or ecosystem responses against OA at a local scale (e.g., mesocosm system, CO_2 vents, and upwelling zones)
Molecular study	- Identification of omics-based molecular biomarkers (e.g., genome, transcriptome, proteome, and metabolome) linking molecular alterations to population-level responses - Epigenetic approaches to understand acclimatization/adaptation mechanisms - Understanding molecular mechanisms related to physiological alterations at an individual level

5. Implications

In summary, the effects of OA on copepods are both species- and stage-specific, and different populations of the same species can show different sensitivities to OA stress. The effects of OA in copepods could be modulated by acclimatization/adaptation, which could further be influenced by the interaction of OA with other co-stressors such as thermal stress, food-deprivation, and metal pollution (Tables 1 and 2). Thus, OA effects in copepods are more complex than originally anticipated. Most OA stress studies in copepods have been based on short-term exposure experiments of particular life stages (mostly adults) (Table 1). Furthermore, the copepods are normally provided with excess food, which has been reported to neutralize the negative effects of OA. Lack of consideration of interactive effects of OA and other co-stressors such as temperature, food limitation, and metal pollution can be resulted in underestimation of the damaging effects of OA in copepods. Also, more studies are required to identify vulnerable keystone copepod species to evaluate the combined effects of stressors such as seawater temperature, food shortage, and metal pollution. Multiple life stages are necessary to determine which stage is most vulnerable to OA stress. Furthermore, multiple generation tests in response to OA are important to examine when and how acclimatization/adaptation works to modulate an individual's response to increased pCO_2 stress. Co-stressors such as thermal stress, food limitation, and metal pollution are also considered to be integrated in further OA stress studies. Mesocosm studies are warranted to obtain a more comprehensive and realistic understanding of OA effects in copepods. Taken together, more omics-based experiments (e.g., genomics, proteomics, and metabolomics) should be conducted to determine at the mechanistic level the processes behind biological responses (e.g., growth, development and reproduction) in response to increased pCO_2 exposure, regardless of the energy allocation for maintaining different critical cellular functions via modulating OA effects on marine animals. This will contribute to more accurate projections of copepod responses to abiotic stressors, including OA and climate changes (Table 3).

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