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# Futuristic Ocean Acidification Levels Reduce Growth and Reproductive Viability in the Pacific Oyster (*Crassostrea gigas*)

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**ABSTRACT:** In this study, we investigated the effects of futuristic pH because of climate change on the growth and reproductive viability of the Pacific oysters. The futuristic pH levels to which adult oysters were exposed are 7.5 and 7.8 (as extreme case) and 8.1 (as moderate case), with pH 8.2 serving as the control. We monitored growth and reproductive viability over a four-week exposure period. The reproductive viability of the oysters exposed to each pH level were assessed based on the sperm motility and egg viability. Throughout the exposure period, the induced acidified nature of each treatment aquaria was maintained. Data obtained from this investigation revealed significant decrease in weight of oysters exposed to pH 7.5 and 7.8 compared to the groups exposed to pH levels 8.1 and 8.2 ($p < 0.05$). Groups of oysters exposed to pH 7.5 recorded as much as 10.49% decrease in weight, with specific growth rate (SGR) of -0.4 %/day. Reproductive viability was significantly compromised in groups exposed to pH 7.5 and 7.8 as evident with reduced sperm motility and percentage of ruptured eggs in these groups of oysters. We therefore postulate that climate change will have significant impact on the recruitment of oysters in coastal waters as growth and reproduction will be impaired at extreme levels of futuristic ocean acidification.

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Global climate change is a phenomenon that cannot be overlooked in the present century. It has made the oceans warmer and more acidic, trends that are projected to continue in the current century. As indicated by Intergovernmental Panel on Climate Change (IPCC, 2014) the occurrence of global climate change, has consequently led to coastal ecosystems being increasingly vulnerable to temperature and carbon dioxide ($CO_2$) increases due to their shallow nature and proximity to terrestrial and anthropogenic carbon loading and these increases may not be seen in open ocean waters for many decades. Additionally, increasing atmospheric $CO_2$ is projected to have cascading negative effects on the world's oceans through ocean acidification (Gazeau et al. 2007). Jiaqi *et al*. (2014) noted that several studies have suggested that a vicissitude in marine ecosystems has been the result of ocean acidification. The study of ocean acidification impacts, and consequences requires transdisciplinary work (Yates et al. 2015), linking chemical, physiological, ecological and socio-economic research to properly assess whole-system effects and potential management responses (Birchenough et al. 2015). Blackford and Gilbert (2007) provided model outputs of ocean acidification assuming a range of $CO_2$ emission scenarios, and hence atmospheric $CO_2$ levels for the time horizons 2050 and 2100. Assuming atmospheric $CO_2$ increases to 500 ppm by 2050 (the median IPCC scenario), a decrease of approximately 0.1 pH units over most of the ocean is projected; however, if atmospheric $CO_2$ rises to 1000 ppm (worst-case IPCC Ocean Acidification 10 scenario) over the next 50 years, a decrease of 0.5 pH units below pre-industrial levels is projected. The Mollusca is a very large and highly diverse phylum of invertebrate animals. Between 50 000 and 120 000 living molluscan species have been described (Gazeau et al. 2013). Among these, 30 000 species are found in marine environments, a figure which accounts for 23% of all marine organisms (Gazeau et al. 2013). Among the six classes of this phylum namely Gastropoda, Bivalvia, Cephalopoda, Polyplacophora, Scaphoda and Monoplacophora, Gastropods and Bivalves are the largest classes accounting for more than 80% of the described living marine molluscan species (Gazeau et al. 2013). *Crassostrea gigas* is an oviparous oyster with a high level of fecundity (Byung et al. 1988). It changes its sex during life, usually spawning first as a male and subsequently as a female (Miossec et al. 2009). Once activated at around 12$^{O}$C, gametogenesis depends directly on the duration of this temperature (degree

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days). A temperature of at least 18 – 20$^{O}$C is necessary for spawning, depending on location (Byung et al. 1988). In the lagoons of the Mediterranean coast, *Crassostrea gigas* ceases to reproduce when summer temperature reaches 24 – 25$^{O}$C and salinity reaches 37 – 38 mg/l (Miossec et al. 2009). *Crassostrea gigas* has high fecundity; a female produces between 20 – 100 million eggs (of diameter 50 – 60 µm) per spawning. Fertilization is external, taking place in the seawater column. The larvae are initially free-swimming and planktonic, developing for 2 – 3 weeks prior to metamorphosis, when they find a suitable clean hard substratum on which to settle. They usually attach to rocks but can settle in muddy or sandy areas (attached to debris, small rocks, shells) or on other oysters, which leads to reef building (Byung, et al. 1988).

The Pacific oyster is the most cosmopolitan of all oyster species. It was intentionally introduced to dozens of countries for aquaculture purposes, and it now dominates global shellfish aquaculture production. As an ecosystem engineer, the Pacific oyster can dramatically alter its environment in ways that both benefit and harm native species and ecosystems.

Suffice to mention that several recent studies aimed at determining the impacts of ocean acidification on marine and estuarine organisms conclude that the likelihood of severe consequences for calcifying marine and estuarine organisms is high (Parker et al. 2013). Berge et al. 2006) described decreased growth and metabolic rates in the blue mussel (*Mytilus edulis*) at pH levels of 7.4. Similarly, Talmage (2011) reported that in the marine mussel, *Mytilus galloprovincialis* a reduction in growth resulted from ocean acidification. The earliest developmental stages of calcifying shellfish larvae are critical to the population dynamics of the adult populations, as any decline in larval populations can have profound implications for future shell fisheries (Gosselin & Qian, 1997). Similarly, larvae of the Sydney rock oysters, *Saccostrea glomerata*, demonstrated reduced survival and slower growth and development when reared under conditions simulating future oceanic $CO_2$ levels (Schiffman et al. (2012).

Although Namibian waters are pristine enough to promote the growth of Pacific oysters (Iitembu, 2005), *Crassostrea gigas* does not breed successfully in Namibian waters and up until recently much of the spats for culture were imported from Chile and South Africa. However, with the projected climate change, ocean acidification has been recently recognized as an additional potential threat to mariculture (Gazeau et al. 2013).

This research is significant from a physiological perspective in that it seeks to investigate and hence provide an insight into the effects of ocean acidification on the growth of Pacific oyster and its sperms motility and egg viability. The importance of this research is also further amplified in that fact that there have been a few investigations on the effect that ocean acidification has on sperm motility and egg viability of *Crassostrea gigas*.

The main objective of this study is to investigate the impact of futuristic oceanic pH levels. Based on earlier studies, futuristic ocean pH level has been projected to in the range of 7.5 to 8.1 (Blackford and Gilbert (2007).

## MATERIALS AND METHODS

*Sample Collection and Maintenance:* A total of 120 *Crassostrea gigas* adults were collected from a private oyster farm, Tetelestai® Mariculture in Walvis Bay, Namibia. The collected samples were transported in an immersed condition to the University of Namibia's Sam Nujoma mariculture research facilities in Henties Bay, Namibia. Upon arrival, they were cleaned to remove sediments and fouling organisms and thereafter acclimatized to laboratory conditions for two weeks in natural sea water (pH 8.2 and 18$^{O}$C). After the acclimatization, the oysters were thereafter transferred into the experimental aquaria at 10 oysters per aquarium.

*Experimental design:* Each aquarium was supplied with 10 l of filtered and UV sterilized seawater through which $CO_2$ was bubbled to maintain the desired pH levels of 7.5, 7.8 and 8.1 as Treatments 1, 2 and 3 respectively by regulating the flow rates of $CO_2$ with flow meter. Normal seawater served as the controlled experiment (pH 8.2). The pH, water temperature, carbon dioxide and alkalinity of the experimental aquaria were monitored on a daily bases during the exposure period. The pH and water temperature were measured using HANNA® HI9828 water analysis kit, while the carbon dioxide and alkalinity were determined according to APHA standard methods as modified by Khanna and Bhutiani (2013).

Each pH treatment had three replicates and the exposure period lasted for 4 weeks. During the exposure period, the oysters were fed daily with 10 ml mixture of microalgae (Shellfish Diet 1800®). Shellfish Diet 1800 is a unique mix of six marine microalgae which are: *Isochrysis sp.*, *Pavlova sp.*, *Tetraselmis sp.*, *Chaetocerous calcitrans*, *Thalassiosira weissflogii* and *Thalassiosira pseudonana* that have all demonstrated success with a variety of shellfish including oysters, clams, mussels,

and scallops. This mixed diet provides excellent nutrition for bivalve species, from first feeding larvae all the way up through brood stock, increasing both growth rates and survival.

*Determination of growth indices:* The weight of each adult oyster in the various treatment aquaria were obtained at the beginning of the exposure period and thereafter on weekly basis for the exposure duration. Mean percentage weight gain and specific growth rate (SGR) of the oysters exposed to each treatment were computed at the end of the exposure period using the following formulae as described by Brown (1957) and Winberg (1957) respectively.

$$\text{Weight gain (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{SGR (\%/day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t_2 - t_1} \times 100$$

Where $W_2$ and $W_1$ are final weight (g) at time $t_2$ (days) and initial weight (g) at time $t_1$ (days) respectively.

*Determination of reproductive viability:* The reproductive viability of the oysters exposed to each pH level were assessed based on sperm motility and egg viability of each group after the exposure duration. For sperm motility, sperms were extracted from the male oysters exposed to each pH treatment at the end of the exposure period. The sperms were pipetted onto labelled petri dishes, from where sperm from each specific treatment were transferred to slide for microscopic observation movement speed. An inverted compound microscope (20 x objective) was used to view the sperms and the sperm movement was recorded for 10s using a digital camera. The videos were post-processed with the Final Cut Pro® and 1s video clips from each slide (replicate) and motility analysed using CellTrak1.3®. The average sperm motility was determined for each slide and this process was repeated for each replicate in each treatment.

Similarly, eggs were extract from exposed female oysters the same way as the as the sperms were extracted. After the extraction of the eggs, they were viewed and observed under the microscope (20x objective) for three hours consecutively to determine the condition of the eggs and estimate the percentage of eggs that ruptured within the three-hour observation time. This was repeated on each replicate for each of the treatments.

*Data Analysis:* All data collected were presented as mean ± SE and variances between exposure groups were subjected to one-way analysis of variance (ANOVA). The Duncan multiple range test was used to test significant differences of data between various pH treatments. Statistical differences in computed data between pH treatments were considered significant at $p < 0.05$. Data sets were analysed with SPSS®.

## RESULTS AND DISCUSSION

During the four-week exposure, the pH of the experimental tanks was maintained at their desired levels and temperature were within consistent range ($p > 0.05$) (Table 1). The mean values of carbon dioxide and alkalinity levels in the experimental aquaria during the exposure period are presented in Figures 1 and 2 respectively. The concentrations of carbon dioxide were significantly higher in Treatment 1 (0.48 ± 0.02 mg/l), followed by Treatment 2 (0.39 ± 0.05 mg/l), Treatment 3 (0.36 ± 0.01 mg/l) and controlled experiment (0.32 ± 0.05 mg/l) in that order ($p < 0.05$). Significantly higher levels of alkalinity (24.8 ± 1.25 mg/l) was observed in the controlled aquaria compared to all the treatment aquaria ($p < 0.05$). Levels of alkalinity in Treatment 1, Treatment 2 and Treatment aquaria were 16.3 ± 0.56 mg/l, 18.91 ± 0.84 mg/l and 20.8 ± 1.05 mg/l respectively.

**Table 1**: Mean values* of pH and temperature in the experimental aquaria.

| Treatments | pH | Temperature ($^O$C) |
|---|---|---|
| Treatment 1 | 7.5 (0.3) | 20.1 (0.31) |
| Treatment 2 | 7.8 (0.2) | 20.0 (0.25) |
| Treatment 3 | 8.1 (0.2) | 19.9 (0.33) |
| Normal seawater (Control) | 8.2 (0.3) | 20.3 (0.29) |

** Values in parenthesis are standard error (SE) of mean values for three replicates per treatment.*

**Table 2:** Mean values* of growth indices of the Pacific oysters exposed to varying levels of pH under laboratory conditions for 4 weeks.

| | pH 7.5 | pH 7.8 | pH8.1 | pH 8.2 |
|---|---|---|---|---|
| Initial weight (g) | 176.40 (0.25)$^a$ | 175.80 (0.51)$^a$ | 181.20 (0.21)$^a$ | 172.40 (0.31)$^a$ |
| Final weight (g) | 157.90 (0.89)$^a$ | 167.60 (1.02)$^b$ | 190.70 (0.89)$^c$ | 173.20 (1.08)$^d$ |
| Weight gain (g) | - 18.50 (0.01)$^a$ | - 8.20 (0.25)$^b$ | 9.50 (0.38)$^c$ | 0.80 (0.15)$^d$ |
| Weight gain (%) | - 10.49 (0.21)$^a$ | - 4.66 (0.56)$^b$ | 5.24 (0.52)$^c$ | 0.46 (0.15)$^d$ |
| SGR** (%/day) | - 0.40 (0.35)$^a$ | - 0.17 (0.61)$^b$ | 0.18 (0.48)$^c$ | 0.02 (0.10)$^d$ |

**Values in parenthesis are standard errors of mean values, values are of 3 replicates for each treatment (n = 30 per treatment). Values with different superscripts in a row are significantly different (p<0.05); **Specific growth rate*

Figure 3 indicate changes in weight of the oysters before and after the exposure to the pH conditions. Oysters in the experimental aquaria at pH 7.5 (Treatment 1) recorded significantly weight decrease (-18.5 g) at the end of the exposure period compared to the other group of oysters in the other treatment aquaria and control aquaria ($p < 0.05$). The group of oysters in experimental aquaria pH 7.8 (Treatment 2) resulted in weight decrease of -8.2 g, while the groups in experimental aquaria at pH 8.1 and the control (pH 8.2) increased in weight after the exposure period at 9.5 and 0.8 g respectively. The percentage weight gain and specific growth rate of each group of oysters exposed to the different pH levels are indicated in Table 2. Figure 4 indicates the mean sperm motility of oyster exposed to each of the experimental aquaria. The highest sperm motility was observed in oysters exposed to pH 8.1 (Treatment 3) with mean sperm speed of 92.4µm/s, followed by the groups exposed to pH 8.2 (control). The difference in the sperm motility of oysters exposed to pH 8.1 and 8.2 was non-significant ($p > 0.05$). Sperm motility in the group exposed to pH 7.5 (Treatment 1) and pH 7.8 (Treatment 2) were 87.2 and 89.5 µm/s respectively, these values were significantly lower than the control groups of oysters ($p < 0.05$).

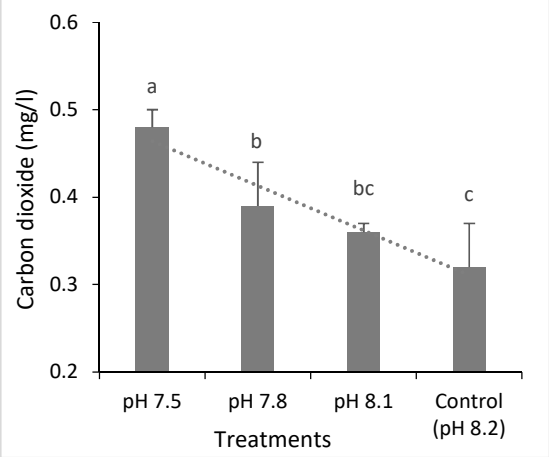


**Fig 1.** Mean levels of carbon dioxide in the experimental aquaria during the four weeks exposure period. Different alphabets along the charts indicate significant difference ($p < 0.05$), values of 3 replicates per treatment.

Figure 5 shows the percentage of ruptured eggs observed on an hourly basis for all the treatment groups. The percentage of ruptured eggs progressed at a faster rate in the eggs extracted from the groups exposed to pH 7.5 and 7.8 compared to the groups exposed to pH 8.1 and 8.2 ($p < 0.05$). It was observed that eggs extracted from oysters exposed to pH 7.5 (Treatment 1) ruptured more quickly, followed by eggs extracted from oysters exposed to pH 7.8 (Treatment 2). Eggs extracted from the groups exposed to pH 8.1 (Treatment 3) and pH 8.2 (Control) had a much slower rupturing time over the three-hour observation period.

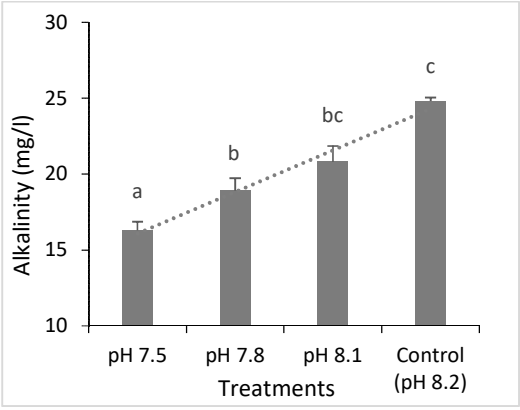


**Fig 2.** Mean levels of alkalinity in the experimental aquaria during the four weeks exposure period. Different alphabets along the charts indicate significant difference ($p < 0.05$), values of 3 replicates per treatment.

Climate change is rapidly causing alteration of the environment including the marine ecosystem. Its effects can, aside from resulting in direct environmental degradation, indirectly lead to changes in the physiology and distribution of many species. Some of the direct impacts on the marine environment as documented by the International Panel on Climate Change (IPCC 2014) include decrease in pH leading to gradual ocean acidification. The implications of a changing marine ecosystem are vast and intricate, many of these changes are observable and quantifiable for a variety of species, across multiple phyla. As climate change progresses, it is predicted that waters in many coastal regions will become acidified, these effects may impose a biological strain on the local fauna, including farmed Pacific oyster (*Crassostrea gigas*). The culture of the Pacific oysters in the waters of the south western coast of Africa is rapidly expanding due to the vast available export market for this species. Understanding the Pacific oyster's response to acidified waters will provide a better understanding of the future of this species in coastal waters of south western Africa. One measure to test the likelihood of a species surviving in a changing coastal environment due to climate change is to quantify its growth and reproductive viability in such an environment which have been artificially induced in the laboratory. Oyster growth and reproduction are affected by environmental parameters, of which

changing pH is among the major factors (Evans and Langdon 2006 and Swan et al. 2007).

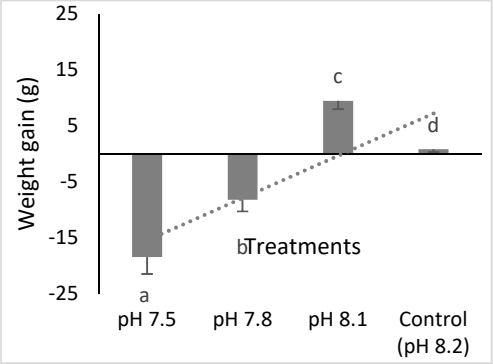


**Fig 3.** Mean weight gain of oysters in the experimental aquaria during the four weeks exposure period. Different alphabets along the charts indicate significant difference ($p < 0.05$), values are of 3 replicates for each treatment ($n = 30$ per treatment).

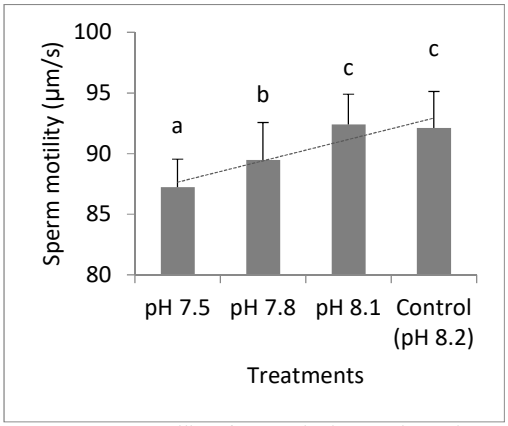


**Fig 4.** Mean sperm motility of oysters in the experimental aquaria during the four weeks exposure period. Different alphabets along the charts indicate significant difference ($p < 0.05$), values are of 3 replicates for each treatment ($n = 30$ per treatment).

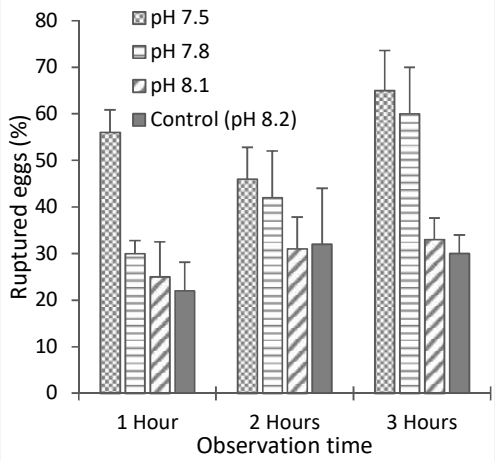


**Fig 5.** Mean percentage of ruptured eggs extracted from the Pacific oysters after exposure to varying levels of laboratory induced futuristic acidified ocean.

In this study, we investigated the effects of futuristic pH due climate change on the growth and reproductive viability of the Pacific oysters. The futuristic pH levels to which adult oysters were exposed are 7.5, 7.8, 8.1. We monitored growth, sperm motility and egg viability over a four-week exposure period. Throughout the exposure period, the induced acidified nature of each treatment aquaria was maintained. Similarly, temperatures values were statistically the same in all the treatment aquaria. The significant changes in alkalinity and carbon dioxide observed in the various treatment aquaria were as a result of the acidification inducement. Meng et al. (2018) observed similar trends in water chemistry infused with carbon dioxide under laboratory conditions. Changing of marine water chemistry is therefore expected with increased acidification and the reduction in alkalinity will be a major treat to shelled marine organisms. Thomsen and Melzner (2010) observed significant linear decreases in both shell mass and shell length for blue mussels exposed to low pH conditions. This they attributed to the dynamics in alkalinity resulting from low pH levels. Omoregie et al. (2016) observed changes in structural integrity of oyster's shell of the Pacific oyster under low pH conditions which they attributed to reduced calcification rates which is directly related to reduced alkalinity. Gazeau et al. (2013) discussed the extensive impacts of ocean acidification on marine shelled molluscs.

Data from this investigation revealed reduced oyster's growth in laboratory induced futuristic oceanic pH of 7.5 and 7.8 levels. Oysters are sensitive to harsh condition especially when exposed to low pH. This exposure to low pH tends to affect the feeding behaviour, thus resulting in exposed oysters not feeding efficiently which eventually lead to weight loss.

This study has demonstrated that futuristic oceanic pH levels significantly decrease growth of the Pacific oyster. Calcifying organisms' ability to synthesize $CaCO_3$ shells depends on the availability of carbonate ions. High levels of $CO_2$ decrease the concentrations of carbonate ions which in turn reduces ability of shellfish to precipitate shells which according to Dickinson et al. (2013) can cause metabolic depression that reduces growth. Similarly, a previous study by Haruko et al. (2007) attributed such reduced growth to the reduction in metabolic rate and weakening of the oyster's shells. Data obtained from this study is in consistence with earlier studies on effects of futuristic

oceanic pH on calcifying organisms such as, the Eastern oyster, clams, scallops, coral reefs, abalone and mussels (Talmage and Gobler, 2009). In tandem with this study is the fact that marine calcifiers such as the Pacific oysters are especially at risk to ocean acidification as it has been shown that they will inevitably experience difficulties in generating calcium carbonate in acidified waters and since these calcifiers have a crucial role to play at the marine food web, a shift in the energy structure is likely to occur in acidified marine ecosystems (Jiaqi et al. 2014).

Sperm motility is a key determinant factor for fertilization success (Fitzpatrick et al. 2012). Recent studies have found that early life history stages of estuarine and marine organisms, including gametes, are generally more sensitive to elevated carbon dioxide stress (Havenhand et al. 2008 and Parker et al. 2009). Ocean acidification impacts on sperm swimming behaviour have been investigated for a wide range of broadcast spawning marine invertebrates including oysters (Havenhand and Schlegel, 2009). In this investigation, it was observed that sperm motility was significantly reduced when the oysters were exposed to futuristic pH levels. This study revealed that at pH level of 7.5 and 7.8 success of oyster reproduction will be greatly reduced. Kurihara and Shirayama (2004) reported similar correlation between sea urchin's sperm motility and pH levels. Kapsenberg et al. (2017) reported that where pH effects were significant, lower pH increased concentrations of sperm required to achieve a given fertilization rate when they investigated pH sensitivity of fertilization in sea urchins from different coastal ocean pH variability regimes. Results from this present investigation on the reduced sperm motility will be one of the factors that account for why increased sperm concentration will be needed to achieve fertilization as observed in Kapsenberg et al. (2017) investigation.

Results from this investigation revealed that futuristic marine environment pH levels will have negative effect on viability of eggs which will also affect recruitment of the Pacific oyster in acidified coastal waters. At pH levels of 7.5 and 7.8, there was significant rupturing of eggs extracted from adult oysters after the exposure period. The rupturing of the eggs will make them unviable to undergo fertilization. Not only has it been suggested that the impact of ocean acidification will be more significant for larvae than adults (Dupont, 2010), this study has indicated that increased ocean acidification resulting from climate change will be most significant for the earlier sensitive life history stages; including egg and sperm production, fertilisation, cleavage, than the later life history stages of larval development and dispersal, settlement and post-settlement survival (Ross et al. 2011).

*Conclusion:* We therefore postulate that climate change will have significant effect on the recruitment of oysters in coastal waters as reproduction will be compromised at lowered pH levels of 7.8 and below. As global ocean change progresses, assessing the adaptive capacity of marine species is of increasing interest to researchers and coastal ocean management groups. Protecting breeding populations diverse in the pH sensitivities of their functional traits may become an important management approach, especially if such populations are sources to others.

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