Aquatic Toxicology 203 (2018) 51–60

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox

Copper and ocean acidification interact to lower maternal investment, but have little effect on adult physiology of the Sydney rock oyster *Saccostrea glomerata*

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ARTICLE INFO

Keywords:
Copper
Ocean acidification
Mollusc
Oysters
Lipids
Eggs
Transgenerational effects
Resilience
Climate change

ABSTRACT

It remains unknown how molluscs will respond to oceans which are increasingly predicted to be warmer, more acidic, and heavily polluted. Ocean acidification and trace metals will likely interact to increase the energy demands of marine organisms, especially oysters. This study tested the interactive effect of exposure to elevated pCO_2 and copper on the energetic demands of the Sydney rock oyster (*Saccostrea glomerata*) during reproductive conditioning and determined whether there were any positive or negative effects on their offspring. Oysters were exposed to elevated pCO_2 (1000 µatm) and elevated copper (Cu 50 µg L^{-1} [0.787 µM]) in an orthogonal design for eight weeks during reproductive conditioning. After eight weeks, energetic demands on oysters were measured including standard metabolic rate (SMR), nitrogen excretion, molar oxygen to nitrogen (O:N) ratio, and pH_e of adult oysters as well as the size and total lipid content of their eggs. To determine egg viability, the gametes were collected and fertilised from adult oysters, the percentage of embryos that had reached the trochophore stage after 24 h was recorded. Elevated pCO_2 caused a lower extracellular pH and there was a greater O:N ratio in adult oysters exposed to copper. While the two stressors did not interact to cause significant effects on adult physiology, they did interact to reduce the size and lipid content of eggs indicating that energy demand on adult oysters was greater when both elevated pCO_2 and copper were combined. Despite the lower energy, there were no negative effects on early embryonic development. In conclusion, elevated pCO_2 can interact with metals and cause greater energetic demands on oysters; in response oysters may lower maternal investment to offspring.

1. Introduction

The legacy of poor environmental management lingers in estuaries around the world. Anthropogenic rubbish, persistent organic compounds, nutrients and trace metals pollute estuaries adjacent to urban and industrial areas (Nriagu, 1990; Ridgway and Shimmield, 2002; Halpern et al., 2008). Despite their degraded nature, polluted estuaries support a diversity of marine organisms including bivalve oysters. Oysters are integral to functioning estuaries because they filter the water column, cycle nutrients and provide biogenic habitat, but these traits also make oysters vulnerable to toxic pollutants like trace metals (Connell, 1990). Exposure to trace metals can affect cellular function, damage DNA, and ultimately lead to cellular death because oysters exposed to trace metals need to expend energy on protein synthesis to repair and detoxify cells (Cherkasov et al., 2006; Sokolova and Lannig, 2008).

As oceans warm and acidify over the next century (Collins et al., 2013) oysters and other bivalves will be particularly vulnerable (Gazeau et al., 2013). Several studies have shown that ocean acidification alone will alter the physiology of oysters and restrict their capacity to grow and reproduce (Pörtner, 2001; Pörtner et al., 2004; Fabry, 2008; Doney et al., 2009; Parker et al., 2013). Other stressors like low-salinity, air exposure or trace metals have been shown to exacerbate the effects of elevated CO_2 (Ivanina and Sokolova, 2015; Parker et al., 2017a, b; Scanes et al., 2017).

Ivanina and Sokolova (2015) found that elevated partial pressure of CO_2 (pCO_2) and trace metals acted indirectly on a range of metabolic and physiological processes in oysters but it is difficult to identify the mechanisms that underpin such interactions. Lewis et al. (2016) found copper and CO_2 interacted synergistically to reduce extracellular pH (pH_e) in the mussel *Mytilus edulis*. The mussel respired less when exposed to the trace metal copper. Respiring less had no effect on pH_e

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https://doi.org/10.1016/j.aquatox.2018.07.020
Received 15 June 2018; Received in revised form 26 July 2018; Accepted 26 July 2018

when copper was a sole stressor, but when copper was combined with elevated pCO_2 the authors found that the mussel could not ventilate the excess CO_2 which caused internal pCO_2 to increase, and as a result, synergistically lower pH_e (Lewis et al., 2016). Trace metals and elevated pCO_2 also interacted to affect the proteome expression in bivalves (Götze et al., 2014). When exposed to elevated copper and CO_2, the clam *Mercenaria mercenaria* increased its energy use to synthesise new proteins and repair damaged cells (Götze et al., 2014).

Environmental stress can increase the cost of homeostasis and place strong demands on the energy budget of marine invertebrates like oysters (Sokolova, 2013). Investing extra energy into homeostasis often comes at the expense of other functions such as somatic growth, digestion and reproduction. This may begin to explain why trace metals and ocean acidification interact to cause a diverse range of effects on marine bivalves (Sokolova, 2013; Ivanina and Sokolova, 2015). However, our understanding of how trace metals and ocean acidification interact is based on short term, single generation studies, an approach that neglects the real-world scenario where organisms respond over multiple generations.

Exposure to stress can cause carryover effects (both positive and negative) across generations to offspring. The environment that adults experience while they are developing gametes (called reproductive conditioning) is known to significantly influence the response of their offspring. When Parker et al. (2012) exposed oysters to elevated CO_2 throughout reproductive conditioning, it was found that the oyster's offspring were larger, and more resilient to ocean acidification as both larvae and adults (Parker et al., 2015). Parents can influence their offspring by allocating extra energy and resources (called maternal investment). In some cases, mothers that are stressed during conditioning may produce larger eggs, so larvae have more energy (Marshall and Uller, 2007). Alternatively, stress from sources like metals can cause parents to invest less energy resulting in smaller eggs which lead to smaller juveniles (Marshall and Uller, 2007; Alquezar et al., 2006). For example, parents of marine fish have been shown to produce smaller eggs when exposed to the trace metal copper (Alquezar et al., 2006). While stressors like trace metals or ocean acidification can influence offspring when parents are exposed during conditioning, no study has investigated how these stressors may interact to affect oysters and have positive or negative carryover effects on their offspring.

Oysters are ubiquitous on the Australian coastline and often inhabit polluted locations adjacent to urban and industrial areas. The Sydney rock oyster (*Saccostrea glomerata*) is prevalent on the east coast of Australia and inhabits urbanised estuaries such as Sydney Harbour which is polluted with copper (Mayer-Pinto et al., 2015; Scanes et al., 2016). Copper is a common estuarine pollutant in Australia and worldwide (Nriagu, 1990; Mayer-Pinto et al., 2015). In addition to its prevalence, copper is also known to cause significant biological harm to both aquatic and terrestrial animals at the concentrations often found estuaries (Ridgway and Shimmield, 2002). Sydney rock oysters are recognised as vital to functioning ecosystems on the Australian coast (Cole et al., 2007). This study tested the interactive effect of chronic exposure to elevated pCO_2 and the trace metal copper on the energetic demands of the Sydney rock oyster *S. glomerata*, and determined any carryover effects to offspring. It was predicted that elevated pCO_2 combined with copper would interact to affect energetic demands and adult physiology as well as affect gamete quality and survivorship of offspring.

2. Methods

S. glomerata were exposed in the laboratory to elevated pCO_2 predicted for the end of this century (Collins et al., 2013) and aqueous copper (determined by Edge et al., [2015] in previous experiments to be stressful for *S. glomerata*). Oysters were exposed in an orthogonal combination for eight weeks during reproductive conditioning. To determine the energetic state of adults, their physiology was measured including; standard metabolic rate (SMR), nitrogen excretion, molar O:N ratio, and extracellular pH (pH_e). To determine the impact on maternal energy investment into eggs, reproductive variables such as egg size and lipid content were measured (Yasumasu et al., 1984; Gallager et al., 1986; Moran and McAlister, 2009). To determine the effect of parental exposure on offspring viability, after eight weeks of exposure, gametes were collected, fertilised and larval viability assessed.

2.1. Collection of oysters

No ethics approval was required by New South Wales (NSW) or Australian law for this experiment. Adult *S. glomerata* were collected from a commercial oyster grower in Port Stephens NSW, Australia. Oysters were approximately two years old and had been maintained on oyster leases within Cromarty Bay, Port Stephens (32°43′28.83″S, 152° 3′51.14″E). Oysters were collected in the Austral winter (June) 2016 and were then cleaned of all fouling organisms and placed into two 750 L tanks filled with nominal 1 µm filtered seawater (FSW hereafter) at 17 °C, which was incrementally raised to 22 °C over three days. Oysters were then kept in these tanks for another 5 days (8 total) to acclimate to laboratory conditions and recover from handling. Thirty individuals were opened immediately following collection to determine gonadal condition. Macroscopic observation of the gonad surface and microscopic (Leica 400x) observation of gonad smears confirmed the oysters were in poor reproductive condition (regressive – early ripening, after Dinamani, 1974).

2.2. Experimental treatments for adult parental exposure

Adult oysters were conditioned in experimental treatments for eight weeks during which they were exposed to one of four orthogonal nominal experimental treatments: control (ambient $pCO_{2,}$ 400 µatm; and no copper, Cu 0 µg L^{-1}[0 µM]), elevated pCO_2 and no copper (1000 µatm; Cu 0 µg L^{-1} [0 µM]), ambient pCO_2 and elevated copper (400 µatm, Cu 50 µg L^{-1} [0.787 µM]) or elevated pCO_2 and elevated copper (1000 µatm; Cu 50 µg L^{-1} [0.787 µM]). Each treatment was replicated three times in independent 120 L polyethylene tanks (total 12 tanks; Fig. 1). Within each tank there were 15 oysters suspended in a mesh bag (20 mm aperture).

2.3. CO_2 monitoring

The two pCO_2 levels used in this study (390 µatm, 1000 µatm) were based on the multi-model average projection by the IPCC for 2100 (Collins et al., 2013). This equated to a mean ambient pH_{NBS} of (8.19 ± 0.02) and a mean pH_{NBS} at elevated CO_2 levels of (7.84 ± 0.0035; Table 1). Gaseous CO_2 was added to tanks using a negative feedback system as described in detail by (Parker et al., 2012). Briefly, the elevated pCO_2 level was maintained using a pH negative feedback system (Aqua Medic, Aqacenta Pty Ltd, Kingsgrove, NSW, Australia; accuracy ± 0.01 pH units). To determine the pH level corresponding to pCO_2 levels, total alkalinity (TA) was quantified at each water change using triplicate Gran-titration (Gran, 1952). The pH_{NBS}, total alkalinity, and salinity of each thank were also measured at each water change (Table 1). These measurements were then entered into a CO_2 system calculation program (CO_2SYS; Lewis et al., 1998), using the dissociation constants of (Mehrbach et al., 1973) to calculate the desired pH values corresponding with pCO_2 levels. The pH values of each tank were monitored daily, and the pH electrode of each controlling system was checked daily against another calibrated pH probe (NBS buffers, WTW 3400*i*).

Copper treatments have been pooled within their respective pCO_2 treatments. Total alkalinity (TA), Temperature (°C), pH_{NBS}, and salinity were measured every water change. All other variables were calculated using a CO_2 system calculation program (Lewis et al., 1998), using the