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Ocean acidification and warming impacts the nutritional properties of the predatory whelk, *Dicathais orbita*

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ABSTRACT

Ocean warming and acidification have the potential to impact the quality of seafood with flow on effects for future food security and ecosystem stability. Here, we used a 35-day experiment to evaluate how ocean warming and acidification may impact the nutritional qualities and physiological health of *Dicathais orbita*, a predatory muricid whelk common on the east coast of Australia, and discuss the broader ecological implications. Using an orthogonal experimental design with four treatments (current conditions [~23 °C and ~380 ppm of pCO_2], ocean warming treatment [~25 and ~380 ppm of pCO_2], ocean acidification treatment [CO_2 ~23 °C and ~750 ppm of pCO_2], and ocean warming and acidification treatment [CO_2, ~25 °C and ~750 ppm of pCO_2]), we showed that changes in moisture and protein content were driven by significant interactions between ocean warming and acidification. Elevated ocean temperature significantly decreased protein in the whelk flesh and resulted in concurrent increases in moisture. Lipid, glycogen, potassium, sulfur, and phosphorus content also decreased under elevated temperature conditions, whereas sodium, boron and copper increased. Furthermore, elevated pCO_2 significantly decreased lipid, protein and lead content. Whelks from control conditions had levels of lead in excess of that considered safe for human consumption, although lead uptake appears to be lowered under future ocean conditions and will be site specific. In conclusion, while *D. orbita* has received research attention as a potential food product with nutritious value, ocean climate change may compromise its nutritional qualities and reduce sustainable harvests in the future. Furthermore, ocean climate change may have deleterious impacts on the longevity and reproductive potential of this important rocky shore predator.

1. Introduction

The combination of changing ecological landscapes and disturbance regimes associated with ocean warming and acidification, as well as other stressors (e.g. pollution, fishing pressure, species invasions) creates an uncertain future for nearshore marine environments and the important fisheries that they support (Cooley et al., 2012). Ocean climate change is likely to influence fisheries productivity and value, with potentially negative consequences for food security (Cooley et al., 2012; Lloret et al., 2015; Tirado et al., 2010) and ecosystem integrity (Ferrari et al., 2011; Kroeker et al., 2013; Manriquez et al., 2016). While the potential impacts of ocean climate change on fisheries production have been well studied, almost no work has been done investigating the influence of ocean warming and acidification on seafood quality (but see Anacleto et al., 2014 and Valles-Regino et al., 2015). The future production of high protein seafood is important to support ever growing human populations with reduced agricultural capacity (Cooley et al., 2012; Doney et al., 2009).

Proximate variables provide an overview of an animal's energetic resources (Berthelin et al., 2000; Pérez-Camacho et al., 2003) and nutritional value for human consumption, breaking down edible flesh into its primary constituents; moisture, ash, protein, and lipid (Begum et al., 2012; Silva and Chamul, 2000; Soufia et al., 2014). Changes in the composition of these proximate variables may alter the reproductive potential (Marshall et al., 1999) and nutritive value of commercially important fisheries species (Spitz et al., 2010). Thus, an assessment of proximate composition under future ocean conditions provides an opportunity to analyse the potential impacts on seafood quality and population sustainability. A previous study demonstrated that future ocean conditions can lead to a significant decrease in total lipids and omega 3 and 6 polyunsaturated fatty acids in the edible flesh of predatory marine whelks (Valles-Regino et al., 2015). In two species of bivalves, Anacleto et al. (2014) found that elevated temperature significantly decreased the relative proportion of some fatty acids, but not protein. Seafood quality and safety can also depend on accumulation of trace elements, including essential minerals and heavy metals in the flesh

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(Ab Lah et al., 2016), and environmental factors such a temperature and pH can influence the uptake of certain metal ions (Baines et al., 2006; López et al., 2010).

Molluscs have been identified as one of the most sensitive phyla to ocean climate change (Byrne et al., 2013; Kroeker et al., 2013; Parker et al., 2013). Meanwhile, fisheries are diversifying and expanding the breadth of targeted species (Kasperski and Holland, 2013; McClenachan et al., 2014; Witkin et al., 2015). While invertebrate fisheries research focus over the past few decades has generally favoured typical bivalve molluscs, like oysters (Guo et al., 2016), some researchers are exploring underexploited gastropod species that provide potential new sources of protein (Ab Lah et al., 2016; Appukuttan and Philip, 1994; Noble et al., 2013; Woodcock and Benkendorff, 2008; Zarai et al., 2011). For example, gastropods in the family Muricidae represent an emerging fisheries resource and comprise approximately 39% of the global gastropod harvest (FAO, 2015). However, the majority of Muricidae production is based on *Rapana venosa* in China (Castell, 2012), so muricid whelks are still considered to be under-represented in fisheries and aquaculture on a global scale (FAO, 2014).

Dicathais orbita (Muricidae) is a marine predatory whelk species (Neogastropoda) common in the temperate waters off the central and southern coasts of Australia and New Zealand (Woodcock and Benkendorff, 2008). This species has functional food potential, with high quality polyunsaturated fatty acids (Valles-Regino et al., 2015) and anticancer compounds (Benkendorff, 2013; Benkendorff et al., 2015) and have been investigated for their aquaculture potential (Noble et al., 2013; Woodcock and Benkendorff, 2008). Here, we evaluated the influence of ocean warming and acidification on the nutritional properties and physiological health of *D. orbita*. We measured proximate response variables (lipid, moisture, ash and protein content), a stress indicator (glycogen content), trace elements, change in shell length and animal weight as well as feeding rate to establish the biochemical responses of *D. orbita* to future ocean change. It was hypothesised that *D. orbita* nutritional quality and physiological health would deteriorate under induced warming and acidification stress.

2. Materials and method

2.1. Experimental set up

To test the hypotheses that both ocean warming and acidification will reduce the physiological health and nutritional value of *Dicathais orbita*, 144 adult whelks were divided into control and experimentally induced warming and acidification treatments for 35 days at the National Marine Science Centre (NMSC) in Coffs Harbour, Australia (30° 16′3.70″S, 153° 8′15.31″E). The experiment utilised *D. orbita* (51–79 mm shell length) from rock platforms around the Coffs Harbour region and had a two-factor design with four treatments, each with three replicate trays (n = 3, current conditions representing our control, mean ± SD = 22.9 ± 0.5 °C and 378.6 ± 35.6 ppm; elevated temperature, 25.2 ± 0.6 °C and 382.2 ± 35.5 ppm; elevated CO_2, 22.9 ± 0.5 °C and 749.9 ± 80.6 ppm and increased temperature and elevated CO_2, 25.3 ± 0.6 °C and 763.0 ± 104.6 ppm), adapted from Provost et al. (2016). Current sea surface temperatures were based on average sea surface temperature (±SD) for the Coffs Harbour region during the time of the experiment (21.3 ± 1 °C, Navy Metoc (2015)). Current pH during the experimental period was expected to be ~8.1 (Poore et al., 2013). Future temperature and pH were based on the IPCC (2013) predictions for future oceanic conditions under climate change scenario RCP8.5; approximately +3 °C in water temperature and −0.3 pH units.

The temperature and pH of seawater was controlled in twelve, 1100 L circular, fibreglass, outdoor header tanks (1.35 m diameter × 0.9 m high). Sea water was pumped from the adjacent ocean into the NMSC flow-through aquarium system and filtered at 50 μm prior to entering header tanks at 3 $L \cdot min^{-1}$. Water temperature in the header tanks was controlled using heater chiller units (Aquahort Ltd., Omana Beach, New Zealand). Water pH was manipulated by bubbling CO_2-enriched air via a gas mixer (PEGAS 4000MF) through the CO_2-enriched header tanks, while the water in the current treatment header tanks was bubbled with ambient air.

Each of the 12 header tanks supplied temperature and pH controlled water at 3 $L \cdot min^{-1}$ to a tray (860 × 650 × 96 mm) that housed twelve *D. orbita*. Animals were weighed (pan top balance to 0.01 g precision) and their maximum shell length was measured using callipers (1 mm precision) at the start and end of the experiment (analysed as percentage change). Whelks were acclimated in experimental conditions for one week before feeding trials commenced. Whelks were fed Sydney rock oysters (*Saccostrea glomerata*), which are a common prey item on local intertidal reefs. The whelks were initially fed a consistent number of oysters ranging 30–50 mm, with new oysters of similar size added daily to ensure whelk feeding rates were not influenced by food scarcity. Feeding rate was calculated as the mean number of oysters eaten per day per tray. Water pH, alkalinity, temperature and salinity readings were measured daily and used to calculate the partial pressure of CO_2 (pCO_2) using the CO2SYS program (Pierrot et al., 2006) with constants from Mehrbach et al. (1973) as adjusted by Dickson and Millero (1987). For the remainder of this publication, low pH conditions will be referred to as having elevated pCO_2.

2.2. Sample preparation and proximate analyses of foot meat

Whelks were dissected for proximate analysis according to Ab Lah et al. (2016). This was done to establish the percentage of whelk foot meat attributable to moisture (weight loss after drying at 40 °C), protein (Biuret protein assay), lipid (1:1 chloroform: methanol extract) and ash (incinerated in a muffle furnace). One sample of foot meat (~0.35 g) was taken from three whelks per tray at random to create three physically homogenized replicates for each proximate response variable. All samples were frozen (−80 °C) for later use and were defrosted immediately prior to analysis.

2.3. Physiological health indicators from gills

Gills were removed for glycogen analysis. These were dissected from three randomly selected whelks per tray, as above. Samples of gill tissue were frozen in liquid nitrogen to slow enzymatic activity and tissue degradation, and subsequently hand ground in 10 mL of 0.06 M perchloric acid (PCA) within a falcon tube using a Teflon masher for 3 min. Adjusting the method described in Krisman (1962), the homogenized samples were centrifuged at 4200 rpm for 2.5 min and 1 mL of the resultant supernatant was transferred into smaller vials for glycogen analysis. Glycogen was analysed following Krisman (1962) as described by Roberts et al. (2009). Glycogen concentration in the gill samples was then estimated from the linear regression equation of a standard curve using oyster glycogen Type II (Sigma-Aldrich) and adjusted for dilution to obtain the final amount of glycogen as a percent of fresh weight.

2.4. Trace element analysis of foot meat

For trace element analysis, 1 g samples of whelk foot tissue were submitted to the Environmental Analysis Laboratory (EAL), Southern Cross University (NATA Accreditation Number 14960). Fresh samples of *D. orbita* foot tissues were dissolved in a mixture of HNO_3 (25%) and HCl (75%) (1:3, v/v) and subjected to hot-block (Hot-Block; Environmental Express, South Carolina, U.S.A) according to an acid digestion procedure (APHA, 2012). Elemental concentrations were analysed by inductively-coupled plasma mass spectrometry (ICPMS) using a NexION 300 D series ICP spectrometer with an ESI SC-FAST Auto Sampler (Perkin Elmer, Waltham, Massachusetts, U.S.A.).