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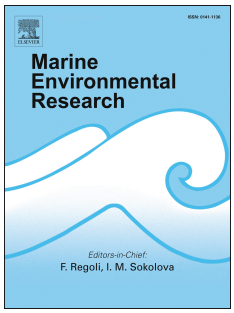

The effects of elevated CO_2 on shell properties and susceptibility to predation in mussels *Mytilus edulis*

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Title: The effects of elevated CO_2 on shell properties and susceptibility to predation in mussels *Mytilus edulis*

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Running Head: Altered shell properties and predation risk in mussels under OA

Abstract:

For many species, ocean acidification (OA) is having negative physiological consequences on their fitness and resilience to environmental change, but less is known about the ecosystem effects of these changes. Here, we assess how OA conditions predicted for 2100 affects the biological functioning of an important habitat-forming species *Mytilus edulis* and its susceptibility to predation by a key predator, the gastropod *Nucella lapillus*. Change in three physiological parameters in *Mytilus* were assessed: (1) shell thickness and cross-sectional surface area, (2) body volume and (3) feeding rate, as well as susceptibility to predation by *N. lapillus*. Shell thickness and cross-section area, body volume and feeding rate of *Mytilus* all reduced under OA conditions indicating compromised fitness. Predation risk increased by ~26% under OA, suggesting increased susceptibility of mussels to predation and/or altered predator foraging behaviour. Notably, predation of large *Mytilus* – that were largely free from predation under control conditions – increased by more than 8x under OA, suggesting that body size was no longer a refuge. Our results suggest OA will impact upon ecosystem structure and functioning and the continued provision of ecosystem services associated with *Mytilus* reefs and the communities associated with them.

Keywords: climate change**,** ecosystem engineer, predation, trophic cascade, environmental change; interaction

Highlights:

- Ocean acidification is a major threat to marine ecosystem structure and functioning
- 2100 pCO_2 scenario reduced *Mytilus* shell thickness & area, body volume and feeding rate

- Predation risk susceptibility changes under future climate scenarios
- Large prey not susceptible to predation today 8x more at risk under OA scenarios
- Significant changes to ecosystem structure and functioning predicted for future

1. INTRODUCTION

Climate change is one of the greatest threats to biodiversity globally (Thomas et al., 2004) altering population and community dynamics (Parmesan and Yohe, 2003) and increasing risks of species extinction (Thomas et al., 2004). There is overwhelming evidence that human activities are driving rates of climate change (Henson et al., 2017); the continued emission of greenhouse gases is a primary driver of increasing global temperatures and ocean acidification (Caldeira and Wickett, 2003). Predictions of global environmental conditions for the end of the century (e.g. RCP8.5 scenario; Stocker et al., 2013) coupled with ever-increasing experimental evidence suggest wide-ranging impacts of future ocean acidification and warming (OAW) scenarios on marine life (Poloczanska et al., 2016).

Climate change may benefit some organisms. A wide-range of taxa including jellyfish, macroalgae, invertebrates and some fish (e.g. Aprahamian et al., 2010; Hall-Spencer and Allen, 2015), especially those with Lusitanian evolutionary origins (Lavergne et al., 2010), are demonstrating increased fitness over wider geographic ranges (e.g. Calosi et al., 2017). But wide-ranging negative effects of OAW have also been shown or are predicted to alter ecology, behaviour and physiology (Gazeau et al., 2013; Hughes, 2000; Lemasson et al., 2017a; Lemasson et al., 2017b). For instance, OA has been shown to alter predator-prey dynamics (Dixson et al., 2010; Harvey and Moore, 2017), intracellular pH, biological functioning (Pörtner et al., 2004), metabolism (Thomsen and Melzner, 2010), and individual energetic needs (Gray et al., 2017; Leung et al., 2017). These changes could change ecosystem structure by amplifying range shifts (Calosi et al., 2017) or cause trophic cascades through lower abundances of key species and reduced trophic transfer (Rossoll et al., 2012). In addition, a decrease in critical ecosystem services (ESs) may also occur (Lemasson et al., 2017a, b).

Molluscs and other calcifying organisms are particularly prone to environmental change and especially OA (Gazeau et al., 2013; Parker et al., 2013). Increased pCO_2 has been shown to reduce calcification (but see Ries et al., 2009), alter crystalline ultrastructure (Duquette et al., 2017; Fitzer et al., 2016; Leung et al., 2017) and increase dissolution rates in oysters and mussels (Berge et al., 2006; Gazeau et al., 2007; Ries et al., 2009). These changes are predicted to alter the capacity of individuals to maintain their exoskeleton via biomineralisation of calcium carbonate mechanisms; an

effect illustrated by a reduction in shell thickness (e.g. Chen et al., 2015) and strength (Speights et al., 2017; Welladsen et al., 2010) in some bivalves. The effect of these changes may extend beyond the fitness of the individual, affecting the wider ecosystem by changing survivorship and/or increasing susceptibility of prey to predation (Dixson et al., 2010; Freeman and Byers, 2006) with consequences that cascade up the food chain.

Many calcifying organisms are of ecological and economic importance, and provide numerous ecosystem services (MEA, 2005). Often ecosystem engineers (*sensu* Jones et al., 1994) or habitat-forming species, they create habitat for other species and support disproportionately high biodiversity in comparison to other habitats (Gutierrez et al., 2003). Bivalve molluscs, which include the mussel *Mytilus* spp., are especially important. Abundant worldwide, mussels account for 30% of global mollusc aquaculture, and in 2015, global production was ~16.5 million tonnes with a market value of ~$18 billion (FAO, 2015). They also provide a number of other important supporting ecosystem services including nutrient cycling and improving water quality (Asmus and Asmus, 1991; Dame and Dankers, 1988; Pejchar and Mooney, 2009).

Here, we test the effect of future OA scenarios of the functioning of *Mytilus* spp. Firstly, we consider how OA impacts the fitness of individuals, specifically their shell thickness, body volume, and feeding rate. We then test if changes in individual fitness alters trophic interactions strength between *Mytilus* and one of its key predators.

2. MATERIALS AND METHODS

Adult individuals of *M. edulis* were collected from Queen Anne's Battery Marina, Plymouth (50°21'50.8"N, 4°07'53.4"W) in October 2016. Mussels were cleaned of all epibiota and placed in tanks of seawater (temperature ≈ 15°C, Salinity ≈ 34, pH ≈ 8) for 2-wk to acclimatise. All mussels were measured and grouped into one of two arbitrary size classes: small (40 ± 10mm) and large (60 ± 10mm). Mussels were fed three times a week ~3 mL (concentration = 50,000 cells/mL) of mixed shellfish diet (Shell diet 1800, Reed Mariculture, USA).

2.1. Experimental design

After 2-wk acclimation, 30 mussels were randomly selected from each size class and placed in tanks simulating one of two pCO_2 emission scenarios (Stocker et al., 2013) representing current (~400 ppm) and 2100 scenarios (1000 ppm) for 8-wk. Three replicate tanks of each treatment were established for each mussel size class (a total of 12 tanks). Five mussels were placed in each tank,

with each mussel marked with non-toxic nail varnish to identify individuals. Mussels were grouped by size (small and large) as it is predicted that size may: (i) influence metabolism and vulnerability to climate change (Carey et al., 2016), and (ii) predation risk is greater in smaller mussels (Navarrete and Castilla, 2003). Mussels were fed as per the acclimation period.

2.2. OA system

A mesocosm system was used to reach relevant CO_2 concentrations. Full details are described in Lemasson et al. (2017b) to allow for brevity here. For 400 ppm, each tank contained an air stone, and atmospheric air was bubbled gently into each replicate tank. For 1000 ppm, pure CO_2 was slowly released into a Buchner flask mixed with dry air (≈400 ppm pCO_2) using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK). CO_2 levels were monitored using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA). pH was measured three times a week using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK) attached to a pH meter (S400 SevenExcellence; Mettler-Toledo Ltd, Beaymont Leys, UK), calibrated with NIST traceable buffers.

2.3. Carbonate chemistry

Total alkalinity (TA) was measured weekly using a calibrated potentiometric titrator (TitraLab AT1000© series HACH Company, USA). Three 50 mL samples were taken from each experimental tank and tested to calculate TA. Temperature was taken *in situ* using a temperature probe (HH806AU, Omega, U.K.) and salinity was recorded using a handheld refractometer (S/Mill, Atago, Tokyo, Japan). These data were used to calculate calcite and aragonite saturation, and CO_2 concentration in water for acidified and control conditions on a weekly basis using CO_2SYS software (Lewis and Wallace, 1998) using Mehrbach solubility constants (Mehrbach et al., 1973), refitted by Dickson & Millero (1987) (see Supplementary Table 1).

2.4. Morphological and physiological parameters

2.4.1. Shell thickness and surface area

After 8-wk, two morphological parameters were measured: shell thickness (mm) and the surface area (mm^2) of each cross-section. Both metrics were measured using images collected using micro computerised tomography (microCT) (Skyscan 1174, Bruker, Germany), which produces a reconstructed 3D image of the individual made up of images taken from 3 planes (x, y, z). Scaled images of cross-sections (black shell profile; Fig. 1) of the right valve from 10 individuals per treatment were imported into ImageJ (Schneider et al., 2012). Shell thickness at three haphazardly-located transverse section points within the lip (TS1), middle (TS2) and umbo (TS3) regions of each

cross-section was then measured, as well as the surface area (mm^2) of each cross-section based on the scaled image mask (surface area of the black shell in Fig. 1). A two-sample t-test using Welch's correction for unequal variance was used to compare shell thickness and cross-sectional surface area between mussels from control and elevated pCO_2 treatments.

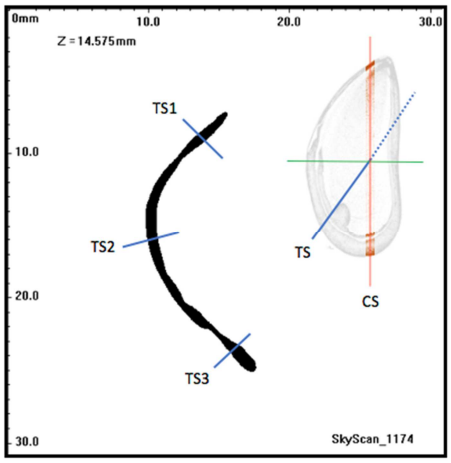

Figure 1. Indicative measurement of shell thickness at three transverse section locations (TS1-3) from a haphazardly chosen cross-section (CS) of the right valve of *Mytilus* spp. Images taken using microCT.

2.4.2. Mussel body volume

The body volume of all mussels (N = 60) was calculated using water displacement (mL). Individual mussels were placed in a 250 mL volumetric cylinder containing 100 mL of sea water (salinity = 35) and the liquid displacement estimated (nearest 1 mL). Individuals were measured at the start of the experiment (t_0) and after 8-wk (t_8) of exposure to control and OA conditions. A 3-factor linear mixed-effects model (*lme*) was used to test for a change in body volume between pCO_2 treatments and mussel size class after 8-wk, incorporating 'mussel' within 'tank' as a nested random factor to take account of the repeated measurement of the same individual. Posthoc pairwise contrasts of significant interactions were made using the *multcomp* package in R.

2.4.3. Feeding rate

Weekly over the 8-wk experiment, 30 mussels were randomly selected and starved for 24 hours. Each individual was then placed in its own beaker with 400 mL of 2µm-filtered seawater (salinity = 35) and a magnetic stirrer (400 rpm) to ensure the water was well mixed. Once all mussels had fully opened, 3 mL of *Tetraselmis* at a density of 10,000 cells/ml was added to each beaker. Three replicate 5mL water samples were taken from haphazard locations throughout the chamber (1) prior

to the addition of food (t_i); (2) immediately after addition of food (t_0) to check the initial algal concentration; and (3) at 10 minute intervals following food addition for a duration of 30 minutes, providing 6 sampling times (i.e. t_i, t_0, t_{10}, t_{20} *and* t_{30}). If the mussel shut its valves, the chronometer was stopped and restarted once the valves re-opened. Counts of algae in all water samples were performed in triplicate using a Coulter Counter (Beckman Coulter Z2). Clearance rates (CR) were calculated using the following equation after Coughlan (1969):

$$CR = \frac{V \times \ln(\frac{C_{n-1}}{C_n})}{t_n - t_{n-1}}$$

where CR is the clearance rate measured during the 10 minute interval between sampling times t_{n-1} and t_n, ($L.h^{-1}$), V is the volume of the chamber in litres, C_{n-1} is the concentration ($cell.L^{-1}$) in the sample taken at time t_{n-1} (h), and C_n is the concentration ($cell.L^{-1}$) in the sample taken at time t_n (h). Results are presented as CR_{max} - the maximum clearance rate observed over the 30-minute feeding period.

A three-factor linear-mixed effects model (lme) was used to compare maximum clearance rate between pCO_2 treatments (400 ppm; 1000 ppm) and mussel size over time (fixed factor; 2, 4, 6 and 8 wk) with 'tank' included as a random factor to account for potential differences in mussel functioning as a result of the experimental tank. Significant differences were compared using post-hoc pairwise comparisons (Tukey HSD).

2.5. Predation risk to mussels under control and OA conditions

Change in predation risk to mussels (measured as mortality) under control and OA conditions was assessed using a common predator of mussels, the dog whelk, *Nucella lapillus*. *Nucella* were collected from the same location as the mussels, returned to the lab and acclimated in either the control or OA conditions (as above) for 2-wk. During acclimation, individuals were provided with mussels for food, but were starved for 48-h prior to being placed in the experimental tanks.

Predation tanks were arranged in the OA mesocosm system as described above. In total, 24 tanks (3 tanks per body size × predator × OA scenario) were established. In each tank, five small or large acclimated mussels were placed with or without a single *Nucella lapillus* for 24 h, after which mortality was measured as percentage of dead mussels. Mussels were considered dead when the mussel shell gaped and would not close despite direct physical disturbance. In tanks without predators, there was no mussel mortality in either the 400ppm or 1000ppm tanks. As such, there

was no need to include 'predator' (levels: present; absent) as a factor in the analysis, which therefore compares mussel mortality in treatments with *Nucella* present only.

A two-factor linear mixed effects model was used to test for differences in proportional mussel mortality between pCO_2 treatments (400 ppm; 1000 ppm) and mussel size class (small; large), with 'tank' included as a random factor to account for potential differences in predation risk as a result of the experimental tank. All statistical analyses were conducted using R (R Development Core Team, 2017)

3. RESULTS

3.1. Morphological parameters

3.1.1. Shell thickness

Shells held under OA conditions were on average, 13-25% thinner and their cross-sectional surface area ~13% less than mussels held under control pCO_2 (400 ppm) after 8-wk. Shells became thinner at all locations of the shell, reducing by ~0.10 mm (TS1), 0.11 mm (TS2) and 0.17 mm (TS3) respectively (Fig. 2). Body size had no effect on size or surface area reductions.

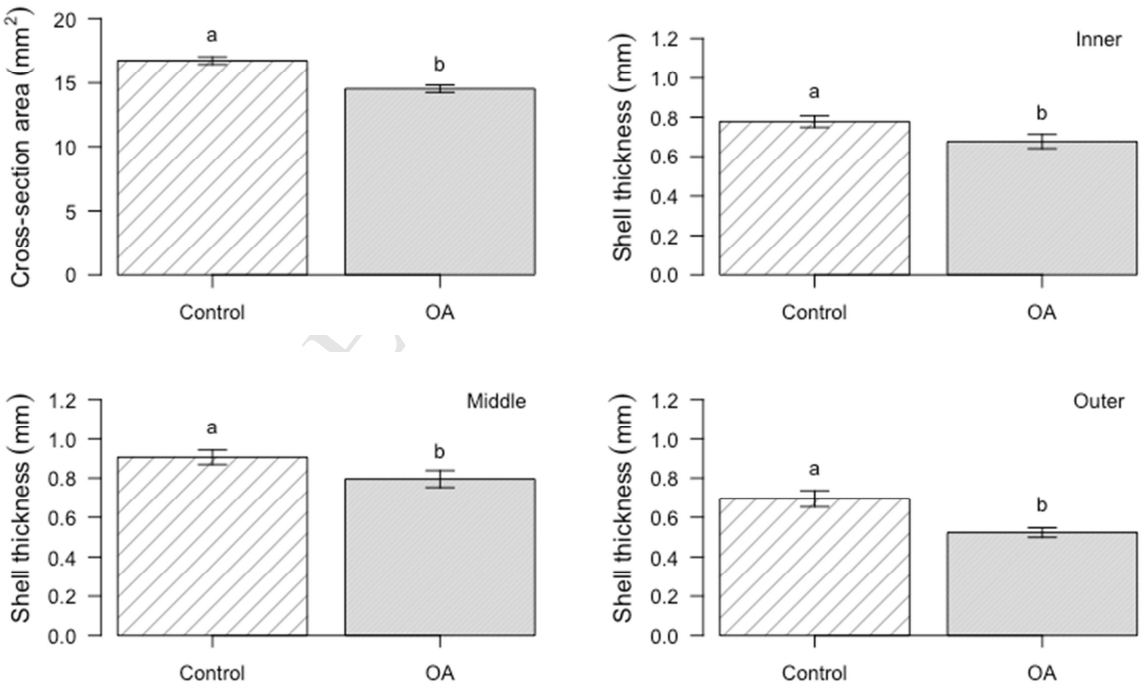

Figure 2. Mean (± S.E) cross-sectional surface area (mm^2), and shell thickness (mm) at the inner (TS1), middle (TS2) and outer edges (TS3) of the right valve of *M. edulis* after 8-wk exposure to

Control (400ppm pCO_2) and OA (1000ppm pCO_2) conditions. Different letters indicate significant differences between groups ($p<0.05$).

3.1.2. Mussel body volume changes

There were significant changes in mussel body volume depending on the size of the mussel and pCO_2 conditions ($F_{1,56} = 9.85$, $p < 0.01$; Fig. 3). There was no change in body volume in small mussels irrespective of pCO_2 level, nor in large mussels under control (400 ppm) conditions. In large mussels, however, there was a 17% reduction in volume under 1000 ppm after 8-wk (Fig. 3).

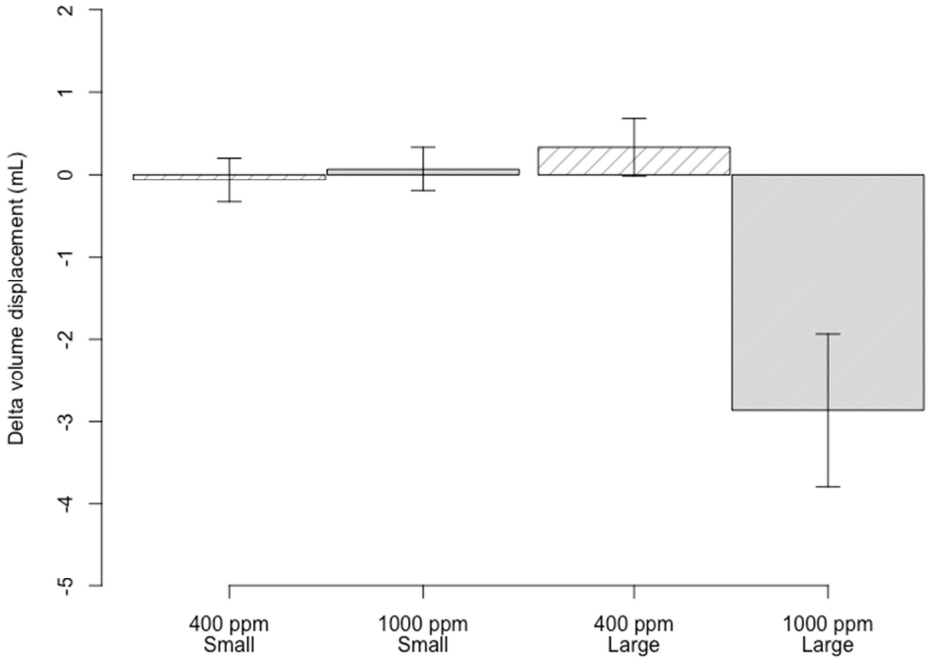

Figure 3. Change in mean body volume of small and large *M. edulis* measured by water displacement (mL ± S.E.) after 8-wk exposure to control (400ppm pCO_2) and OA (1000ppm pCO_2) treatments. Negative values indicate loss of volume.

3.1.3. Feeding rate

Maximum clearance rates ($L.h^{-1}$) were significantly different between mussels from control and OA treatments over time ($F_{3,96} = 6.53$, $p<0.001$) but mussel body size had no effect on these changes. After 2-wk, there was no difference in CR irrespective of mussel size or treatment, but after 4-wk, there was a significant reduction of between ~ 35 - 47% in feeding rate in OA treatments in comparison to the control, where no change in CR was observed (Fig. 4). After wk-4, there was no further change in CR within the OA treatment.

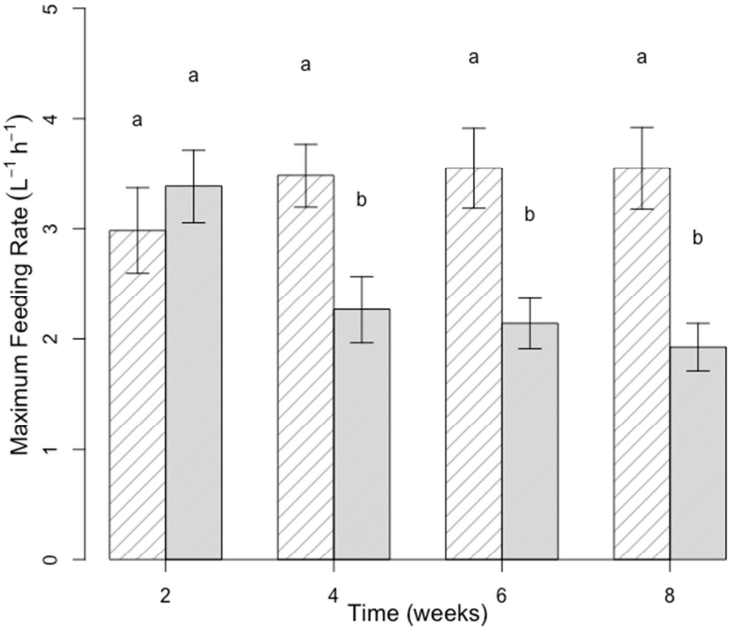

Figure 4. Change in mean (± S.D.) maximum feeding rate (L.h^{-1}) of *M. edulis* (small and large) over 8-wk in control (400 ppm pCO_2; hatched bars) and OA (1000 ppm pCO_2; grey bars) treatments. Different letters indicate significant differences in mean values between groups ($p<0.05$).

3.2. Predation

There was a significant effect of mussel size ($F_{1,8} = 10.7$, $p < 0.01$) and pCO_2 (treatment: $F_{1,8} = 16.7$, $p < 0.01$) on the mortality of *M. edulis* by *N. lapillus* (Fig. 5). Mussel mortality increased by differing amounts depending on mussel size. In small mussels, mortality increased by ~1.7x (proportional mortality of between 0.4-0.68), but in large mussels, mortality increased more than ~8.3x (proportional mortality of between 0.06 – 0.5). Comparing the ratio of mortality of small and large mussels in each OA treatment, mortality risk of small mussels was 80% higher than for large mussels in control treatments. In the OA treatment, however, small mussel mortality was only 26% higher than that of large mussels as a result of marked increases in large mussel susceptibility to predation (Fig. 5). There was no mussel mortality in either the control or OA treatment when predators were absent.

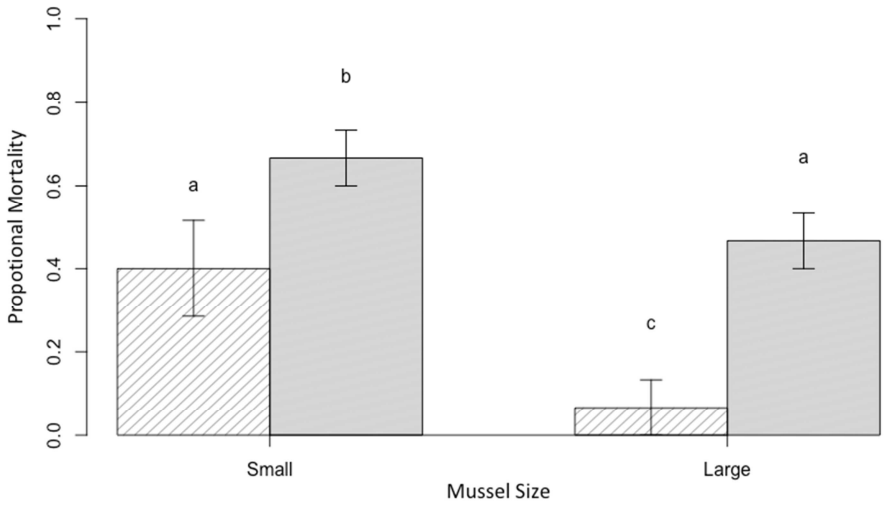

Figure 5. Proportional mortality (mean ± S.E.) of small and large *M. edulis* from *Nucella lapillus* in control (400 ppm pCO_2; hatched bars) and OA (1000 ppm pCO_2; grey bars) treatments. Different letters indicate significant differences between treatment means ($p<0.05$).

4. DISCUSSION

Worldwide, negative consequences of OA on species performance are continuing to be shown (e.g. Gazeau et al., 2013). Here, experiments manipulating atmospheric pCO_2 concentrations to match those predicted for 2100 revealed negative impacts on a number of physiological traits in *Mytilus edulis* including feeding rate, body volume and shell morphometry (shell thickness and surface area), as well as increased predation risk from a key intertidal predator, the dogwhelk *Nucella lapillus*.

Reduced growth under OA has been shown in a number of bivalve species and other taxa (Gazeau et al., 2013; Kroeker et al., 2013; Milano et al., 2016; Waldbusser et al., 2013), especially in those with the capacity to divert energy from growth to other physiological processes (Michaelidis et al., 2005; Wood et al., 2008). Energy diversion is an adaptive strategy, with mechanisms such as metabolic upregulation used to ameliorate the negative effects of OA on processes such as shell dissolution (Leung et al., 2017) and biomineralisation (Fitzer et al., 2016). The energetic costs of diversion mechanisms have, in some taxa, been compensable by increased feeding (Lardies et al., 2017; Ramajo et al., 2016; Thomsen et al., 2013), although no evidence of a response (Sanders et al., 2013) or reduced feeding has also been shown in a number of studies (Navarro et al. 2016). Here, after 2-wk exposure to future OA conditions, *Mytilus edulis* demonstrated a reduction in feeding (clearance rate) of up to 47% that continued for the duration of the study. It is well documented that reduced feeding rate can reduce scope for growth (Widdows and Johnson, 1988), and this was manifested as reductions in shell thickness and shell surface area over the 8-wk period. Volume also reduced, but only in large individuals. This suggests that over time, OA conditions sufficiently hampered key

physiological pathways (Hüning et al., 2013; Weiner and Addadi, 2011) important for shell and somatic tissue maintenance, and that adult *Mytilus edulis* - unlike juvenile conspecifics (Thomsen et al., 2013) - have limited capacity to mitigate the effects of OA by increasing feeding. Reduced feeding appears especially problematic for larger individuals who are likely to have greater basal energetic requirements than smaller individuals (Lefort et al., 2015), resulting in significant reductions in mussel volume (~17%) over relatively short time scales, with potential consequences for reproductive output and population stability for the future (Knights, 2012).

Shell maintenance is a key function for many taxa, especially calcifying species including molluscs, echinoderms and corals (Bibby et al., 2007; Gazeau et al., 2013; Maier et al., 2012; Milano et al., 2016; Riebesell et al., 2000) who use their shells for buoyancy and protection (e.g. Sherker et al., 2017). Numerous studies have shown the potential of OA to reduce an organism's capacity to obtain sufficient calcium carbonate to maintain their shell (e.g. Kroeker et al., 2014; Waldbusser et al., 2013), which over time, can lead to increased susceptibility to predation (Fitzer et al., 2015). Here, after just 8-wk exposure to 1000 ppm pCO_2, reductions in shell thickness and surface area were observed across the whole shell; the outer edge (where the shell is typically thinnest) exhibited greatest reductions (up to 25%). Molluscan shells are usually composed of two species of calcium carbonate; aragonite and calcite. In the case of *M. edulis*, while it uses a combination of aragonite (nacreous layer) and calcite (prismatic layer), it is predominantly (~83%) made up of aragonite (Hubbard et al., 1981), which is more susceptible to dissolution by carbonic acid than calcite (Feely et al., 2004). From this experiment, it is not clear which of the surfaces have eroded due to OA; we can only state that there were reductions in shell thickness and surface area. Irrespective of this, thinner, more brittle shells can be expected over relatively short-term exposures to OA. Over time, the biomineralisation process may change to compensate for this. Individuals have been shown to switch between the carbonate polymorphs used during biomineralisation, which may lead to more OA resilient shells in the future (e.g. Leung et al., 2017), but at this stage, negative consequences for shell maintenance processes are predicted.

OA conditions are likely to have wider ecosystem effects beyond changes in individual responses (i.e. changes in biological functioning). For example, a number of studies have shown that predator-prey interactions can be influenced by increased pCO_2 (Bibby et al., 2007; Harvey and Moore, 2017; Lord et al., 2017; Xu et al., 2017). Differentiating between impacts on the functioning of the prey, predator or both can be difficult. In instances where the predator and prey are both likely to be under stress due to the environment (i.e. both the predator(s) and prey are calcifying species), then

non-linear or interactive responses are possible, with both trophic levels potentially implementing mitigation measures to counteract the stress associated with the environmental conditions. This may explain some of the inconsistencies in response to OA reported in the literature. For example, under OA, Sanford et al. (2014) found predation of *Crassostrea virginica* by predatory snails increased by 20-48%, while Harvey and Moore (2017) found *Nucella lapillus* reduced feeding to the point of starvation, releasing prey from predation. Schalkhausser et al. (2013) observed impaired clapping ability in *Pecten maximus* under OA, resulting in reduced escape performance due to diverted energetics.

In this study, OA led to marked increases in the predation risk for mussels. Mussel mortality in OA treatments increased over control conditions to differing degrees depending on mussel size. In small mussels, proportional mortality nearly doubled (1.7x), but in large mussels, proportional mortality increased >8x from being relatively 'predation risk free' under control conditions. Size-selectivity is important in many predator-prey interactions (Thompson, 1975; Ward, 1991) and previously, *N. lapillus* has been shown to demonstrate strong size selectivity towards smaller mussels, in order to reduce handling time (Thompson, 1975). Increased mortality due to reduced shell thickness has been shown in other taxa (Sanford et al., 2014) and thickening of the shell is a recognised defence mechanism to limit mechanical predation (Leonard et al., 1999). Thinned shells may therefore reduce predator handling time increasing the risk of predation in larger individuals (Coleman et al., 2014; Fitzer et al., 2015). Alternatively, a change in predator foraging behaviour (Burrows and Hughes, 1989; Etter, 1996) by *Nucella* in an attempt to counter the negative effects of OA by increasing its own feeding and/or adapting its foraging strategy selecting prey of higher nutritional quality (Kohl et al., 2015) could account for the increased mortality observed. Such a mechanism may account for the differential response of *Nucella* to prey under OA seen here in comparison to the response seen in Harvey and Moore (2017) who used barnacles as a prey item, which may be less nutritious on a per individual basis. While it is not possible to differentiate between the mechanisms in this instance, the switch in foraging and significant increase in predation risk to *Mytilus* (especially to larger individuals) suggests considerable change in ecosystem structure and functioning under OA may occur (Schmitz and Trussell, 2016).

Our results demonstrate the potential for OA to impact upon the biological functioning of individual organisms and alter predator-prey dynamics. These findings point toward considerable impacts of OA on the continued provision of ecosystem services by *Mytilus*. Beyond the biological responses shown here, our results suggest a range of ecosystem services including provisioning and regulating

services (MEA, 2005) may be impacted. For example, reduced clearance rates become quickly apparent, and may lead to rapid changes in nutrient load and water quality (Asmus and Asmus, 1991; Broszeit et al., 2016; Dame and Dankers, 1988). Changes in shell thickness, body volume and predation risk may affect the long-term sustainability of populations across multiple trophic levels and opportunities for their exploitation for seafood. It is clear that the performance of individuals is being impacted by the ocean conditions predicted for the end of this century, and increasingly, the effect of these changes on community structure is being realised. As recently advocated by several authors (Knights et al., 2017; Runting et al., 2017), the next challenge is to combine our understanding of how individual species respond to and potentially ameliorate the effects of environmental change into a holistic ecosystem assessment in order to better understand wider ecosystem change.

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Supplemental Material

CO_2 system parameters

Characteristics of the seawater in which *M. edulis* was kept is summarised in Table 1. Salinity and temperature remained similar in both OA and control conditions. Conversely as pCO_2 increased from 400 ppm (control) to 1000 ppm (OA), there was a decrease in total alkalinity, pH, dissolved carbonate and aragonite concentrations.

Table 1. Seawater chemistry in the experimental tanks (mean ± S.D.).

CO_2 system parameters	**Control (400 ppm)**	**OA (1000 ppm)**
pH	8.065 ± 0.02	7.619 ± 0.02
Salinity (psu)	36.16 ± 0.40	36.01 ± 0.63
Temperature (°C)	15.50 ± 0.27	15.58 ± 0.18
TA ($\mu mol.kg^{-1}$)	2078.83± 105.60	2059.83 ± 123.40
pCO_2 (µatm)	411.23 ± 25.96	1074.17 ± 68.02
CO_3^{2-} ($\mu mol.kg^{-1}$)	154.03 ± 4.36	63.61 ± 7.60
Ω_{ca}	3.50 ± 0.21	1.39 ± 0.19
Ω_{ar}	2.20 ± 0.19	0.87 ± 0.15

TA, total alkalinity; CO_3^{2-}, carbonate ion concentration; Ω_{ca}, saturation state of calcite; Ω_{ar}, saturation state of aragonite