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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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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.

## 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, 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

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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 ± 10 mm) and large (60 ± 10 mm). 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 $CO_2$SYS software (Lewis and Wallace, 1998) using Mehrbach solubility constants (Mehrbach et al., 1973), refitted by Dickson and Millero (1987) (see Supplementary Table 1).

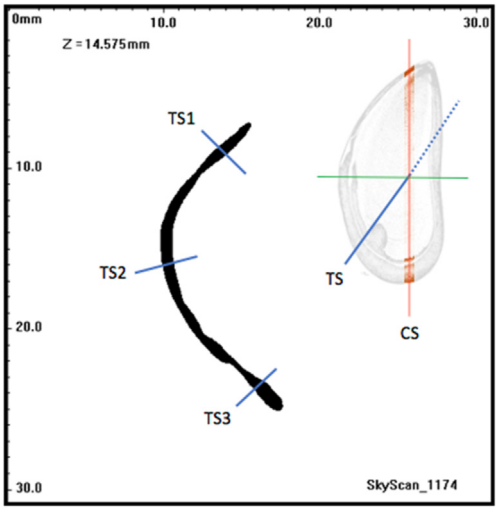


**Fig. 1.** Indicative measurement of shell thickness at three transverse section locations (TS1-3) from a haphazardly chosen cross-section (CS) of the right valve of *Mytilus* spp. Images taken using microCT.

### 2.4. 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.

#### 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