

2021-04-29

# Differential responses in anti-predation traits of the native oyster *Ostrea edulis* and invasive *Magallana gigas* to ocean acidification and warming

Lemasson, Anaelle

<http://hdl.handle.net/10026.1/17235>

---

10.3354/meps13687

Marine Ecology: Progress Series

Inter Research

---

*All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.*



# Differential responses in anti-predation traits of the native oyster *Ostrea edulis* and invasive *Magallana gigas* to ocean acidification and warming

Anaëlle J. Lemasson\*, Antony M. Knights

Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK

**ABSTRACT:** Ocean acidification and warming (OAW) pose a threat to marine organisms, with particular negative effects on molluscs, and can jeopardize the provision of associated ecosystem services. As predation is an important factor shaping populations in the marine environment, the ability of organisms to retain traits valuable in predation resistance under OAW may be decisive for future population maintenance. We examine how exposure to seawater temperature (control: 16.8°C and warm: 20°C) and atmospheric  $p\text{CO}_2$  (ambient [~400], ~750, and ~1000 ppm) conditions affects traits linked to predation resistance (adductor muscle strength and shell strength) in two ecologically and economically important species of oysters (*Magallana gigas* and *Ostrea edulis*) and relate them to changes in morphometry and fitness (condition index, muscle and shell metrics). We show that *O. edulis* remained unimpacted following exposure to OAW scenarios. In contrast, the adductor muscle of *M. gigas* was 52% stronger under elevated temperature and ~750 ppm  $p\text{CO}_2$ , and its shell was 44% weaker under combined elevated temperature and ~1000 ppm  $p\text{CO}_2$ . This suggests greater resistance to mechanical predation toward the mid-21<sup>st</sup> century, but greater susceptibility toward the end of the century. For both species, individuals with more somatic tissue held an ecological advantage against predators; consequently, smaller oysters may be favoured by predators under OAW. By affecting fitness and predation resistance, OAW may be expected to induce shifts in predator–prey interactions and reshape assemblage structure due to species and size selection, which may consequently modify oyster reef functioning. This could in turn have implications for the provision of associated ecosystem services.

**KEY WORDS:** Predator–prey interactions · Multi-stressors · Climate change · Ecological interactions · Non-native species

Resale or republication not permitted without written consent of the publisher

## 1. INTRODUCTION

Diverse and sustainable populations of marine organisms are key for the continued provision of marine ecosystem services, i.e. the 'direct and indirect benefits people obtain from [marine] ecosystems' (Beaumont et al. 2007, p. 254), and importantly for food biosecurity (Palumbi et al. 2009). However, food biosecurity appears threatened by environmental stress and future climate change (Ekstrom et al.

2015, Lloret et al. 2016, Kibria et al. 2017, Lemasson et al. 2019). Commercially important fisheries such as cod (Koenigstein et al. 2018), scallops (Cooley et al. 2015, Richards et al. 2015), and prawns (Richards et al. 2015) display reduced recruitment success, altered growth, and declines in harvests under ocean acidification and warming (OAW) scenarios. The sustainability of the shellfish industry, and that of molluscs in particular, which constituted in excess of 15% of global aquaculture production

\*Corresponding author: anaelle.lemasson@plymouth.ac.uk

in 2018 (FAO data: [www.fao.org/fishery/statistics/global-aquaculture-production](http://www.fao.org/fishery/statistics/global-aquaculture-production)), are of particular concern under future OAW scenarios (Cooley et al. 2012, Gazeau et al. 2013, Parker et al. 2013, Froehlich et al. 2018).

OAW can alter the fitness of molluscs, including changes in physiology and shell properties, and alter energy budgets (Spalding et al. 2017). Increased respiration rates (Ong et al. 2017, Lemasson et al. 2018), decreased feeding rates (Vargas et al. 2015, Clements & Darrow 2018, Sadler et al. 2018), and reduced condition (Ong et al. 2017, Lemasson et al. 2019) are evident and indicative of environmentally induced stress. Important calcification and mineralogical processes may also undergo crucial changes in functioning (Li et al. 2015, Fitzner et al. 2016, Duquette et al. 2017, Leung et al. 2017a, Knights et al. 2020), and as a consequence, shells can be less dense (Chatzinikolaou et al. 2017), lighter (Lagos et al. 2016), thinner (Lagos et al. 2016), and weaker (e.g. Li et al. 2015, Speights et al. 2017, Meng et al. 2018, Wright et al. 2018, Barclay et al. 2019, Zhao et al. 2020). It is predicted that these individual fitness consequences will have important implications for the stability and resilience of impacted populations as a result of changes in the strength of population-regulating mechanisms and community interactions, particularly in the case of ecosystem engineers (McCann 2000, Gribben et al. 2009, 2013).

Predation is a well-recognised top-down driver of population dynamics in the marine environment (Myers et al. 2007, Peckarsky et al. 2008), the strength of which can change when exposed to multiple environmental stressors such as OAW. The extent to which changes occur, as well as their effects on population and community dynamics, are not well understood, but a number of negative consequences of OAW have been shown. In addition to the alterations in shell properties mentioned above which would alter predator–prey dynamics, these negative consequences include changes in the responses of prey to predator olfactory cues (Jellison et al. 2016, Froehlich & Lord 2020, but see Clements et al. 2021), altered predator consumption rates (Harvey & Moore 2017, Sampaio et al. 2017, Sadler et al. 2018, Wright et al. 2018), the induction of important non-consumptive effects on prey (Lord et al. 2017), or changes in behavioural prey defences (Clements & Comeau 2019). Individually or collectively, these changes point toward significant alterations to community dynamics and highlight a particular concern that assessing the physiological effects of OAW on a target species (e.g. a commercially valuable prey species) may

alone be insufficient to accurately predict sustainability outcomes for the future.

There are a number of predators of commercially important bivalves with a wide range of predation strategies. Mechanical approaches are common: for instance, gastropods drill holes into the shell, starfish pry open the 2 valves and tear the muscles responsible for valve closure (Lavoie 1956, Reimer & Tedengren 1996), crabs and other durophagous predators crush the shell (Menzel & Nichy 1958, Elner 1978; although some species of crabs such as *Carcinus maenas* can also pry the valves open, Sanchez-Salazar et al. 1987), and some birds are known to also target the hinge (Butler & Kirbyson 1979). The ability of individuals to resist predation is crucial for survival and long-term population maintenance (Knights et al. 2012). Bivalve resistance to predation is closely linked to their ability to build robust protective shells (therefore linked to shell metrics such as size, thickness, and strength) as well as strong adductor muscle to control their gaping behaviour, affecting predator handling time (Boulding 1984, Reimer & Tedengren 1996, Beadman et al. 2003, López et al. 2010).

Oysters are among the bivalve molluscs at risk from OAW (Lemasson et al. 2017). Amongst the numerous factors contributing to the decline in oyster populations globally (Beck et al. 2011)—overfishing, disease, cold winter conditions, low recruitment rates, and competitors—predation plays an important role (O'Connor et al. 2008, Knights et al. 2012). Predators of the native European flat oyster *Ostrea edulis* in the UK include molluscs such as *Ocenebra erinacea* and *Urosalpinx cinerea* (Woolmer et al. 2011, Sawusdee 2015), crab species including *Cancer pagurus*, *Carcinus maenas*, and *Necora puber* (Mascaró & Seed 2001, Shelmerdine & Leslie 2009), and starfish (e.g. *Asteria rubens*, *Solaster papposus*) (Hancock 1955, 1958, Woolmer et al. 2011). The introduced Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*) has similar predators to *O. edulis*, with additional potential predation by the whelk *Buccinum undatum* and species of birds (Cadée 2001). Any negative effects of OAW on traits of oysters that reduce predation risk (i.e. adductor muscle strength, shell properties, gaping behaviour) may alter predator–prey interactions and reshape reef structure and functioning.

Here, we tested the effects of OAW on aspects of oyster fitness and morphology linked with predation resistance, comparing a native (*O. edulis*) and a non-native (*M. gigas*) species of oyster. Both species are of high economic value in the UK (Herbert et al. 2012), and the basis of a major UK shellfish fishery valued at

~US \$10.3 million in 2018 ([www.fao.org/fishery/statistics/global-aquaculture-production](http://www.fao.org/fishery/statistics/global-aquaculture-production)). Changes in adductor muscle strength and shell fracture strength were evaluated under simulated warming and acidification scenarios over a 12 wk period, and linked to changes in physiological (condition indices, tissue weight) and morphological (muscle diameter and area, shell density, shell thickness, shell weight) traits. We had 3 hypotheses: (1) OAW scenarios would negatively impact on the fitness and morphological properties of oysters valuable to predation resistance (condition index, muscle diameter and area, tissue weight, shell density and thickness), which in turn would affect the (2) adductor muscle strength and (3) shell fracture strength of oysters, linked to 2 distinct mechanical predation strategies (prying and crushing, respectively).

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

Adult Pacific oysters (length:  $79.5 \pm 5.9$  mm; weight:  $45.1 \pm 8.5$  g;  $n = 48$ ; mean  $\pm$  SD) and European flat oysters (length:  $70.3 \pm 5.5$  mm; weight:  $41.4 \pm 19.5$  g;  $n = 48$ ) were wild-collected from a low-intertidal site in Plymouth Sound ( $50^{\circ} 23' 29.95''$  N,  $4^{\circ} 13' 16.77''$  W), UK, in August and November 2016, respectively. Organisms were brought back to the Marine Biology and Ecology Research Centre at the University of Plymouth within 1 h of collection and kept in large stock tanks (200 l) under ambient laboratory conditions for 2 wk. These conditions consisted of a temperature of  $\sim 16.5^{\circ}\text{C}$ , salinity of 32–33, and atmospheric pressure of  $\sim 400$  ppm  $p\text{CO}_2$ , during which oysters were fed ad libitum with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

### 2.2. Experimental design and set-up, treatments, and measurements of seawater parameters

#### 2.2.1. OAW experimental treatments

Both species of oyster were exposed to 6 OAW scenarios: 3 levels of atmospheric  $p\text{CO}_2$  (ambient [ $\sim 400$ ],  $\sim 750$ ,  $\sim 1000$  ppm) and 2 seawater temperatures (control:  $16.8^{\circ}\text{C}$ , warm:  $20^{\circ}\text{C}$ ) in an orthogonal design. These scenarios, previously used and presented by Lemasson et al. (2018), simulate current and future OAW scenarios predicted for the UK. The 2 temperature treatments selected reflected maximum current

sea surface temperature (SST;  $16.8^{\circ}\text{C}$ ), and predicted SST for the end of the century ( $20^{\circ}\text{C}$ , corresponding to the predicted increase by  $3\text{--}4^{\circ}\text{C}$  along the southwestern coast of the UK; UKCP09 data, Hadley Centre for Climate Prediction and Research 2014). The atmospheric  $p\text{CO}_2$  levels chosen were based on IPCC scenario (representative concentration pathway [RCP] 8.5) for mid- and end-century (IPCC 2013). However, while organisms living in coastal and estuarine habitats experience localized variability in environmental conditions which may be amplified by future OAW, the predictions tested here do not take this into account. The experiment was run sequentially grouped by species due to the capacity of the OAW system to accommodate the number of replicate tanks used. We set up 48 experimental tanks (3 l capacity each,  $n = 8$  per OAW scenario) for each run of the experiment (Fig. A1 in the Appendix). A single oyster was placed in each tank and exposed to the treatment conditions for 12 wk (for each species  $n = 8$  oysters per treatment). Throughout the 12 wk duration of the experiment, oysters were fed daily with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture) to obtain a concentration of approximately  $10^8$  cells  $\text{l}^{-1}$  within the experimental tank. Tanks were gently brushed and siphoned 3 times a week, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater.

#### 2.2.2. Experimental design and mesocosm set-up

The OAW mesocosm system used during the experiment is the one presented by Lemasson et al. (2018), which is a modified version of the one described by Calosi et al. (2013). Briefly, each treatment consisted of an 80 l header tank of seawater, supplied from either a  $16.5^{\circ}\text{C}$  or a  $20^{\circ}\text{C}$  sump, and aerated with either the ambient air pipe ( $p\text{CO}_2 \sim 400$  ppm) or one of the 2  $\text{CO}_2$ -enriched air pipes ( $p\text{CO}_2 \sim 750$  or  $\sim 1000$  ppm). A submersible pump (Hydor Koralia Nano 900) was used in all header tanks to achieve sufficient mixing.  $\text{CO}_2$  gas mixes were obtained by slowly releasing  $\text{CO}_2$  into 2 Büchner flasks using multistage  $\text{CO}_2$  regulators (EN ISO 7291; Gas Control Equipment), where it mixed with ambient air, achieving 2 different levels of  $p\text{CO}_2$ . As such, the 3 atmospheric  $\text{CO}_2$  levels varied in a similar manner throughout the experiment following natural variations in  $\text{CO}_2$  in the ambient air, and therefore included natural daily variation, which is considered critical in climate change experimental studies (Reum et al. 2016, Humphreys 2017). A  $\text{CO}_2$  analyser (LI-820; LI-COR) was used to record  $\text{CO}_2$  lev-

els in the 2 CO<sub>2</sub>-enriched pipes as well as in the ambient air pipe to monitor for the control treatments. From the header tanks, seawater was gravity-fed at a constant rate of ~60 ml min<sup>-1</sup> to each of the 8 corresponding replicate tanks (3 l transparent sealed containers). These replicate tanks were held within 4 holding trays (300 l tray<sup>-1</sup>). Each sump supplied seawater to 2 of the holding trays, effectively creating water baths which maintained the replicate tanks at the desired temperature. Each tray held 4 replicates of each CO<sub>2</sub> level. Excess seawater overflowed from the holding trays back into their corresponding sump, where it was filtered, aerated, and recirculated to the corresponding header tanks and trays using a submersible pump (1262; EHEIM). On a daily basis, seawater and/or deionized water was added and replaced in the system to maintain stable salinity levels. In elevated temperature treatments, aquarium heaters (50 W aquarium heater; EHEIM Jager) were placed in header tanks and holding trays to increase the seawater to 20°C.

### 2.2.3. Measurements of seawater parameters

Temperature, salinity, and pH were measured daily in all replicate tanks (Table 1; and see Figs. S1–S4 at [www.int-res.com/articles/suppl/m665p087\\_supp.pdf](http://www.int-res.com/articles/suppl/m665p087_supp.pdf)). A handheld refractometer was used to measure salinity (D&D The Aquarium Solution). A digital thermometer was used to measure temperature (TL; Fisher Scientific). Following calibration with NIST traceable buffers, pH was measured using a micro-electrode (InLab® Expert Pro-ISM; Mettler-Toledo)

coupled to a pH meter (S400 SevenExcellence™; Mettler-Toledo); pH in the header tanks was also monitored (data not shown). Total alkalinity (A<sub>T</sub>) measurements were conducted once weekly on 125 ml water samples in triplicate for each treatment directly from the header tanks. Prior validation was performed to ensure that the replicate tanks had consistently the same A<sub>T</sub> as each other and as the header tank. Water samples were directly analysed for A<sub>T</sub> by automatic Gran titration (Titralab AT1000® Hach Company) within 15 min of being sampled. Partial pressure of carbon dioxide (pCO<sub>2</sub>) in seawater and saturation states of calcite and aragonite (ΩCa and ΩAr) were calculated at the end of the experiment using CO<sub>2</sub> SYS (Pierrot et al. 2006), employing constants from Mehrbach et al. (1973) refitted to the NBS pH scale by Dickson & Millero (1987) and the KSO<sub>4</sub> dissociation constant from Dickson (1990) (Table 1).

### 2.3. Adductor muscle strength, shell strength, and morphometry measurements

#### 2.3.1. Adductor muscle and shell strengths

All oysters survived the 12 wk exposure duration, after which each oyster was removed from its tank, dried, gently scrubbed of epibionts, and a stainless steel hook glued a few centimetres from the edge of the right valve with EPAFD low-viscosity epoxy resin mixed with rapid hardener (Reactive Resins, EP Resins). The left valve was glued with the same epoxy resin/hardener combination onto a 150 × 150 mm metal plate. Adductor muscle strength was deter-

Table 1. Seawater chemistry for *Magallana gigas* and *Ostrea edulis*. Data shown are means ± SD values. ppm: parts per million; ΩCa: saturation state of calcite; ΩAr: saturation state of aragonite; atm: atmosphere; SW: seawater; A<sub>T</sub>: total alkalinity

Treatment (pCO <sub>2</sub> atm × Temperature)	Salinity	Temp. (°C)	A <sub>T</sub> (μmol kg <sup>-1</sup> SW)	pH	pCO <sub>2</sub> sw (μatm)	ΩCa	ΩAr
<b><i>Magallana gigas</i></b>							
Ambient × Control	32.9 ± 2.2	17.0 ± 0.2	2680.4 ± 446.0	7.94 ± 0.08	587.1 ± 116.0	4.0 ± 1.0	2.6 ± 0.6
750 ppm × Control	32.7 ± 2.3	17.0 ± 0.2	2659.7 ± 448.2	7.82 ± 0.05	797.6 ± 157.5	3.1 ± 0.6	2.0 ± 0.4
1000 ppm × Control	32.9 ± 2.2	16.9 ± 0.1	2647.5 ± 466.9	7.68 ± 0.05	1127.1 ± 189.4	2.3 ± 0.6	1.5 ± 0.4
Ambient × Warm	32.7 ± 2.4	20.7 ± 0.2	2848.0 ± 508.9	7.97 ± 0.04	629.7 ± 95.0	4.8 ± 1.2	3.1 ± 0.8
750 ppm × Warm	33.8 ± 2.0	21.8 ± 0.6	2860.0 ± 515.7	7.87 ± 0.03	835.3 ± 140.5	4.1 ± 0.9	2.6 ± 0.6
1000 ppm × Warm	33.9 ± 1.9	20.5 ± 0.1	2850.4 ± 528.1	7.72 ± 0.05	1177.0 ± 130.9	3.0 ± 1.0	1.9 ± 0.6
<b><i>Ostrea edulis</i></b>							
Ambient × Control	33.0 ± 1.8	16.7 ± 0.6	1683.1 ± 144.7	7.96 ± 0.05	492.4 ± 68.7	2.0 ± 0.3	1.3 ± 0.2
750 ppm × Control	33.1 ± 1.6	16.7 ± 0.6	1670.3 ± 150.7	7.86 ± 0.07	626.7 ± 79.3	1.6 ± 0.3	1.0 ± 0.2
1000 ppm × Control	33.3 ± 1.7	16.7 ± 0.6	1665.3 ± 146.7	7.77 ± 0.10	818.5 ± 140.4	1.3 ± 0.4	0.8 ± 0.2
Ambient × Warm	33.6 ± 2.2	19.8 ± 0.8	1978.3 ± 154.7	8.04 ± 0.08	464.8 ± 59.3	3.1 ± 0.6	2.0 ± 0.4
750 ppm × Warm	33.4 ± 2.1	20.3 ± 1.2	1981.4 ± 140.3	7.92 ± 0.06	731.2 ± 84.4	2.3 ± 0.4	1.5 ± 0.3
1000 ppm × Warm	33.5 ± 2.1	19.8 ± 0.9	1979.6 ± 143.3	7.84 ± 0.06	864.3 ± 104.6	1.9 ± 0.3	1.2 ± 0.2

mined by applying a vertical pulling (extensive) force to the right valve, using a force transducer (Instron Testing System) connected to the hook of the oyster (Fig. 1a). The metal plate glued to the lower valve of the oyster was securely held in place onto the base of the force transducer using clamps, to obtain an immobile base and allow accurate measurement of the vertical force exerted. The force profile for each oyster was recorded (Fig. 1b), along with corresponding visual observations. This profile corresponds to the extensive force exerted over time (recorded every 0.1 s; extension rate:  $0.08 \text{ mm s}^{-1}$ ). Three data points were extracted from the force profile: (1) the initial resistance (in  $\text{N s}^{-1}$ ) of the oyster against the pulling pres-

sure, defined as the initial slope of the force curve from onset of the pulling force to the first opening of the valves (Point 1 in Fig. 1b); (2) the force necessary for the valves to start opening (Point 2 in Fig. 1b); (3) the maximum force applied before the onset of muscle rupture (Point 3 in Fig. 1b).

The shell strength of the left valve of each individual oyster was determined using a vertical compressive force applied to the shell using a force transducer (Instron Testing System). The left valve of each oyster shell was placed directly underneath the cell load (Fig. 1c) and the force profile for each oyster was recorded, along with corresponding visual observations. This profile corresponds to the compressive

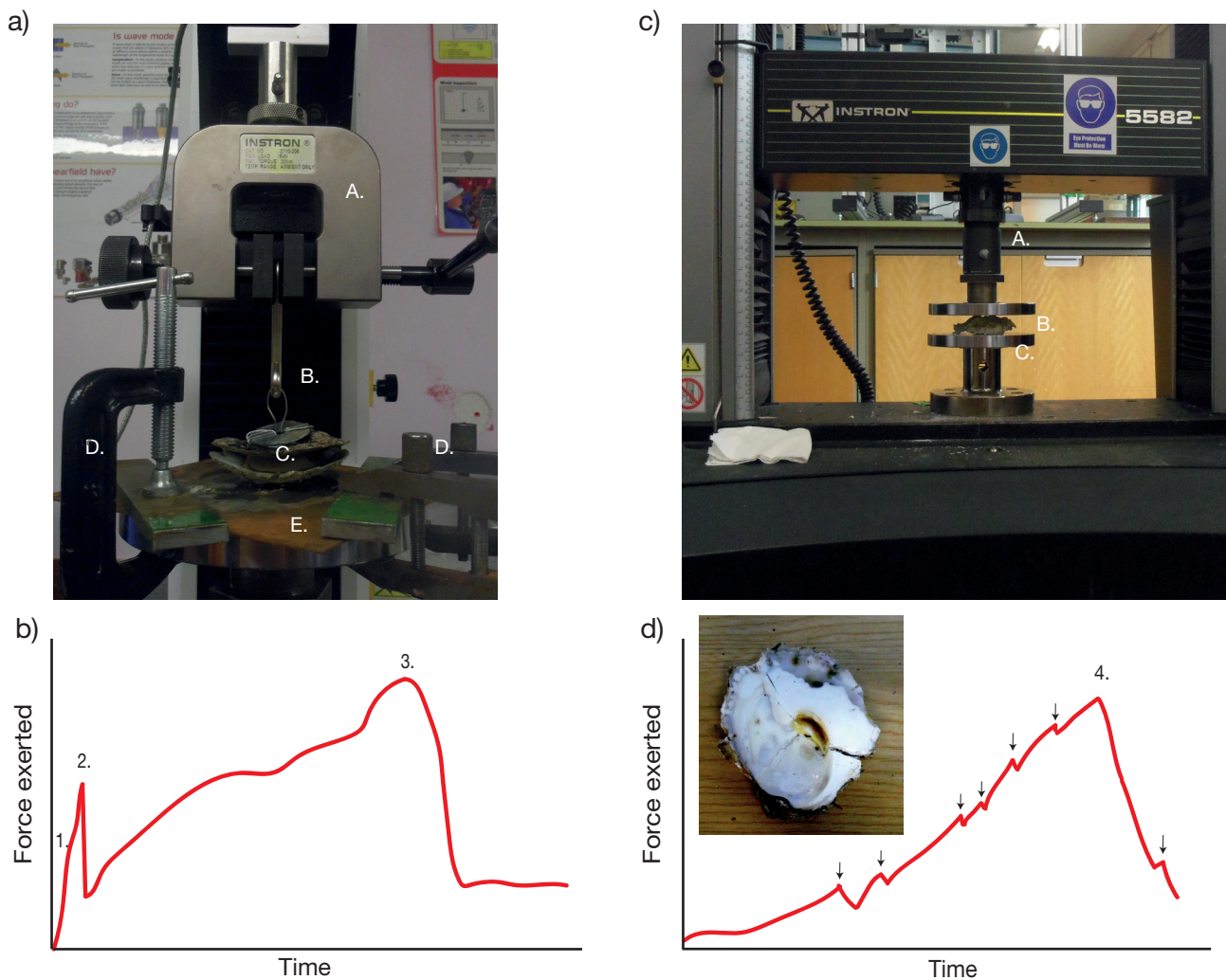


Fig. 1. (a) Experimental set-up for oyster adductor muscle strength assessment. A: Instron cell load; B: hook (glued to C: oyster); D: clamps; E: metal plate. (b) Schematic of a typical curve for the extensive force exerted on the adductor muscle of oysters over time. 1: Initial resistance against the pulling pressure (recorded in  $\text{N s}^{-1}$ , corresponding to the slope of the curve from onset of pulling force to the first opening of the valve [data point 2]). 2: Force necessary for the valves to start opening [data point 2]). 3: Maximum force applied before the onset of muscle rupture. (c) Experimental set-up for oyster shell strength assessment. A: Instron cell load; B: oyster; C: fixed metal base. (d) Schematic of a typical curve for the compressive force exerted on the shell of oysters. Arrows: Minor cracks which did not lead to shell fracture. 4: Major crack which led to shell fracture (shown in inset)

force exerted over time (recorded every 0.1 s; compression rate: 0.08 mm s<sup>-1</sup>). For each oyster, the force required to break the valve in half was recorded (Point 4 in Fig. 1d).

### 2.3.2. Morphometrics

Following the adductor muscle strength tests and before the shell strength tests, the fresh tissue weight and shell length of each oyster was measured using a digital scale (Fisher Scientific Precision series PP5413, precision 0.001 g) and digital callipers (Mitutoya; precision 0.01 mm), respectively. The inside of each valve was then photographed using a digital camera (Pentax OptioLS465) and the diameter and surface area of the adductor muscle were calculated using image analysis software (ImageJ) (Fig. A2). Shell and soft tissue were then dried at 105°C for 24 h or until a constant mass was achieved. The condition index (CI) was then calculated using the following equation after Knights (2012):

$$CI = \frac{\text{dry tissue weight}}{\text{dry shell weight}} \times 100 \quad (1)$$

For each valve (left and right), shell density was quantified using the buoyant weight method described by Denton & Gilpin-Brown (1961), using the following equation:

$$d = w \times \frac{d_{\text{liq}}}{w - y} \quad (2)$$

where  $d$  is the density of the shell valve,  $d_{\text{liq}}$  is the density of the liquid used for immersion,  $w$  is the dry weight of the shell valve in grams, and  $y$  is the buoyant weight of the shell valve in grams.

Following tests of shell strength, the thickness of the left valve of each individual was measured using digital callipers (Mitutoya; precision 0.01 mm) at 3 random points along the fracture line.

## 2.4. Statistical analyses

All data were analysed using the public domain R (R version 4.0.2, R Core Team 2020). Differences were considered significant if  $p < 0.05$ . Due to natural variations in the chemistry of the seawater used during the experiments and the partial pressure of ambient air used, the treatments applied to each species were not consistent (Table 1), and therefore species were not formally compared, and data were analysed separately.

### 2.4.1. Adductor muscle and shell strengths

Data were analysed using 3-factor analysis of covariance (ANCOVA), with 'pCO<sub>2</sub>' and 'Temperature' as fixed factors. The covariate was selected by comparing multiple models, with a different biometric for each model, using residual sum of squares. After determination of which biometric (muscle diameter, muscle surface area, CI, shell thickness, shell length, shell weight, and tissue dry mass) was the best suited predictor for each of the response variables, tissue dry mass (g) was selected as the continuous covariate in the model analysing the adductor muscle strength, and shell weight (g) as the continuous covariate in the model analysing the shell strength. Diagnostics plots were used to visually assess model assumptions that the residuals were unbiased and homoscedastic. Where significant differences were present, post-hoc multiple comparisons ('multcomp' package) were performed to determine which treatment levels differed.

### 2.4.2. Morphometrics

Differences in muscle diameter and area, CI, and shell thickness between treatments were assessed using a linear mixed-effect model (function 'lme' in the 'lme4' package) with 'pCO<sub>2</sub>' and 'Temperature' as fixed factors and including 'individual' as a random factor. Differences in shell density were assessed using 'lme' with the fixed factors 'pCO<sub>2</sub>', 'Temperature', and 'Valve' (left or right) nested in the random variable 'individual'. Data were log-transformed to ensure homogeneity of residual variance. Where significant differences were identified, post-hoc Tukey pairwise comparisons were performed to identify where treatment combinations differed (function 'lsmeans' in the 'emmeans' package). The relationships between muscle diameter and muscle surface area were assessed using linear regressions, and differences in regression parameters between ANOVA (single factor) were used to test the significance of the relationship.

## 3. RESULTS

### 3.1. Muscle strength

#### 3.1.1. Initial resisting force

There was considerable variation between individuals within treatments in their capacity to resist mechanical opening (Fig. 2a,b). This variation led to

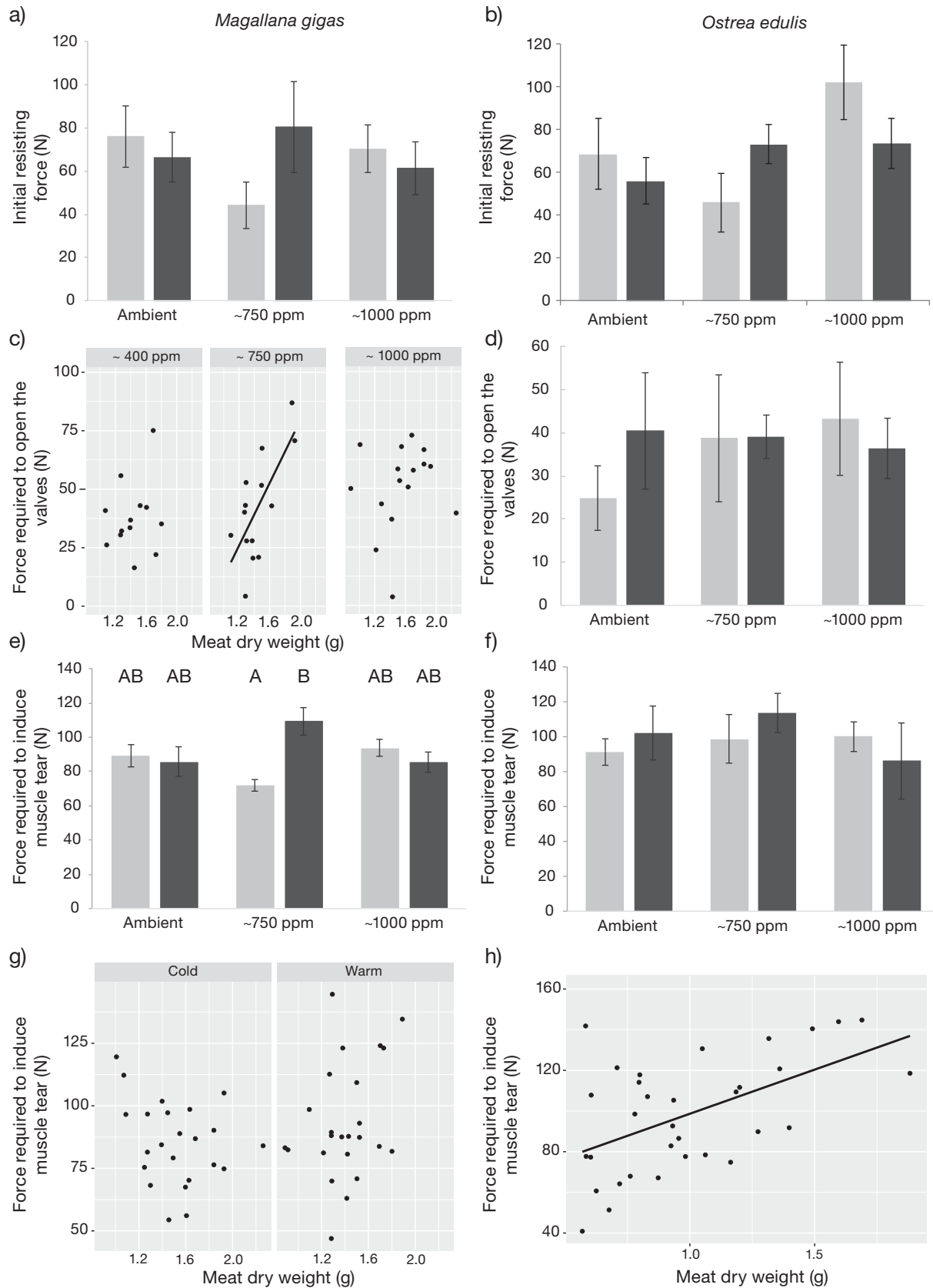


Fig. 2. Changes in 3 adductor muscle traits of oysters exposed to 6 temperature and  $p\text{CO}_2$  scenarios. Values are means  $\pm$  SE. (a,b) Initial resistance to the extensive force. (c,d) Extensive force required to open the valves. (e-h) Extensive force required to induce muscle tear. Significant regressions are shown: (c) *M. gigas* at 750 ppm:  $y = 67.1x - 54.7$ ,  $R^2 = 0.46$ ,  $p < 0.01$ ; (h) *O. edulis*  $y = 43.4x + 55.3$ ;  $R^2 = 0.27$ ;  $p < 0.01$ . In panels a, b, d, e and f, light grey bars: control temperature (16.8°C), dark grey bars: warm temperature (20°C). Treatments that do not share a letter are significantly different



no statistically significant differences between treatments, yet there were clear and marked differences in the mean initial resistance depending on temperature and  $p\text{CO}_2$  (Fig. 2a,b, Table S1). In both oyster species, under ambient and 1000 ppm  $p\text{CO}_2$ , initial resistance reduced under elevated temperatures by 8.6–28.3% ( $9\text{--}29\text{ N s}^{-1}$ ) compared to controlled temperature, and the Newton forces were largely similar within temperature between these  $p\text{CO}_2$  treatments. In contrast, under 750 ppm, elevated temperatures led to a marked increase of 59.2–81.4% ( $27\text{--}36\text{ N s}^{-1}$ ) in the mean initial resistance (Fig. 2a,b).

### 3.1.2. Force necessary to open the valves

There were marked differences in the force required to open the valves depending on species. In *Magallana gigas*,  $p\text{CO}_2$  and tissue dry weight, but not temperature, affected the force required to open the valves (Fig. 2c, Table S1). An 11–35% increase in force was required to open oysters exposed to elevated  $p\text{CO}_2$  treatments, increasing from  $\sim 37.8 \pm 4.2\text{ N}$  (mean  $\pm$  SE) at ambient  $p\text{CO}_2$ , to  $\sim 42.0 \pm 6.0$  and  $\sim 50.8 \pm 4.5\text{ N}$  at  $\sim 750$  and  $\sim 1000$  ppm, respectively. ANCOVA results indicated a positive relationship between biomass and opening force, but only under  $\sim 750$  ppm. In *Ostrea edulis*, there was no statistically significant effect of temperature,  $p\text{CO}_2$ , or biomass on the required opening force (all individuals opened under  $\sim 36.4 \pm 3.9\text{ N}$  extensive force), although, as in *M. gigas*, results were highly variable between individuals (Fig. 2d). Nevertheless, some patterns are apparent. *O. edulis* reared under ambient  $p\text{CO}_2$  and control temperature conditions required less force ( $\sim 16\text{ N}$ ) to open the valves than those reared under all other scenarios. Increasing  $p\text{CO}_2$  also appeared to increase the force necessary to open their valves, rising from  $24.8 \pm 7.5\text{ N}$  under ambient  $p\text{CO}_2$  to  $\sim 38.7 \pm 14.8$  and  $\sim 43.3 \pm 13.1\text{ N}$  at  $\sim 750$  and  $\sim 1000$  ppm, respectively.

### 3.1.3. Force required to induce muscle tear

Temperature and  $p\text{CO}_2$  affected the force required to induce muscle tear in *M. gigas* but not *O. edulis*. Post-hoc tests revealed that under  $\sim 750$  ppm  $p\text{CO}_2$ , the force required to induce muscle tear was 18.8% ( $\sim 17\text{ N}$ ) lower under control temperature and 23.5% higher ( $\sim 21\text{ N}$ ) in warm temperatures in comparison to all other  $p\text{CO}_2$  treatments that were not different from one another. In *O. edulis*, but not *M. gigas*, there was a positive relationship between the force required to tear

muscle fibres and tissue dry weight, which increased by  $43.4\text{ N g}^{-1}$  of tissue dry weight (Fig. 2e–h, Table S1).

## 3.2. Shell strength

A combination of temperature and  $p\text{CO}_2$  affected the shell strength of *M. gigas* (Table S2), with a marked reduction in shell strength with elevated  $p\text{CO}_2$  at the control temperature. Shells reared under ambient  $p\text{CO}_2$  withstood a compressive force of  $\sim 619\text{ N}$ , but this strength was reduced by between 36 and 44% after exposure to  $\sim 750$  ppm ( $\sim 390\text{ N}$ ) and  $\sim 1000$  ppm ( $\sim 342\text{ N}$ )  $p\text{CO}_2$ , respectively (Fig. 3a). Rearing at warm temperatures reduced the mean strength of *M. gigas* shells by over 40% (from  $\sim 619$  to  $\sim 367\text{ N}$ ), but changes in  $p\text{CO}_2$  had no effect. Under ambient  $p\text{CO}_2$ , increased shell mass led to increased shell strength, but this relationship was negated under elevated  $p\text{CO}_2$  conditions (Fig. 3b). In contrast, change in temperature and  $p\text{CO}_2$  had no effect on the strength of *O. edulis* shells, although heavier shells exhibited increased shell strength, increasing by  $15.8\text{ N g}^{-1}$  of shell dry weight (Table S2, Fig. 3c,d).

Despite not being formally compared, there were notable differences in the strength of *O. edulis* shells compared to those of *M. gigas*. On average, *O. edulis* shells were  $\sim 69.1\%$  stronger ( $\sim 300.5\text{ N}$  stronger) than those of *M. gigas*.

## 3.3. Morphometrics

For both *M. gigas* and *O. edulis*, muscle diameter and area were positively correlated (Fig. A3e,f), but neither were affected by Temperature,  $p\text{CO}_2$ , or their interaction (Table S3, Fig. A3a–d).

A combination of temperature and  $p\text{CO}_2$  statistically significantly affected the CI of *M. gigas* ( $p < 0.05$ ; Table S3, Fig. 4a). Statistically, post-hoc tests were unable to differentiate between treatment combinations due to natural variations, yet clear trends were apparent. Under control temperatures, a positive trend between CI and  $p\text{CO}_2$  was apparent; this trend appeared to reverse under warm conditions. Within  $p\text{CO}_2$ , at ambient 400 ppm, increased temperature led to a 19.5% increase in condition, whereas under 750 and 1000 ppm, elevated temperatures led to a reduction in condition by 11.6 and 18.1%, respectively. In *O. edulis*, there was no effect of any OAW scenarios on condition (Table S3, Fig. 4b).

Shell density was dependent on which valve was examined in both species (Table S3). Left valves were

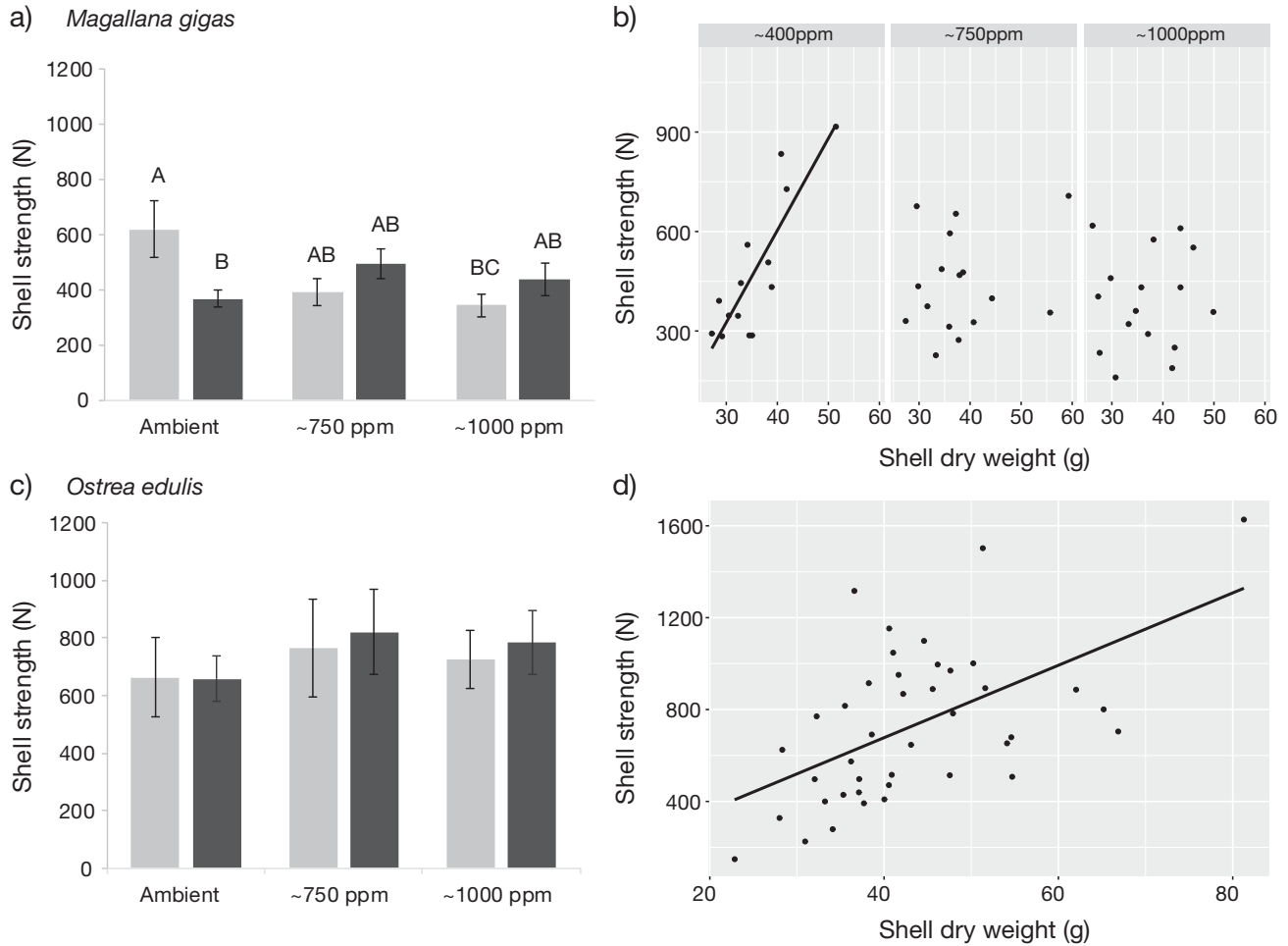


Fig. 3. Strength (left-hand panels: mean  $\pm$  SE) of the left valve of (a) *Magallana gigas* and (c) *Ostrea edulis* shells under temperature and  $p\text{CO}_2$  treatments. Significant regressions (right-hand panels) are shown: (b) *M. gigas*  $y = 27.8x - 507.3$ ;  $R^2 = 0.70$ ;  $p < 0.001$ ; (d) *O. edulis*  $y = 15.8x + 47.3$ ;  $R^2 = 0.28$ ;  $p < 0.001$ . In panels a and c, light grey bars: control temperature (16.8°C), dark grey bars: warm temperature (20°C). Treatments that do not share a letter are significantly different

approximately 8.5 and 9.7% more dense than the right valves in both *M. gigas* and *O. edulis*, respectively (Fig. 4c,d).

The shells of *O. edulis* were ~53% thicker (~1.2 mm thicker) than those of *M. gigas* (Fig. 4e,f). Higher temperature increased the thickness of *O. edulis* shells by ~20% (~0.63 mm thicker) in comparison to ambient temperatures, but this effect was not statistically significant ( $F_{1,39} = 3.359$ ,  $p = 0.075$ ; Table S3), an effect not manifested in the shells of *M. gigas*. Surprisingly, elevated  $p\text{CO}_2$  had no effect on shell thickness in either species.

#### 4. DISCUSSION

Ongoing climate change causing OAW poses a threat to oyster reefs, with a probable decline in the

provision of ecosystem services predicted for the future (Lemasson et al. 2017). As predation is an important factor shaping oyster populations (O'Connor et al. 2008, Knights et al. 2012), the ability of oysters to retain traits valuable in predation resistance in the future may be decisive for population maintenance. Responses to OAW were highly variable within the same species; nevertheless, clear differences in response are shown, in which functional traits of *Ostrea edulis* were relatively unimpacted by the OAW scenarios despite the more challenging seawater chemistry (low  $A_T$ ,  $\Omega\text{Ca}$ , and  $\Omega\text{Ar}$ ; Table 1), whereas several of the same traits in *Magallana gigas* were negatively impacted by exposure to OAW.

Being able to control valve closing has an important survival value for bivalves as a means of protection against harmful environmental stressors and against predators (Reimer & Tedengren 1996). It is

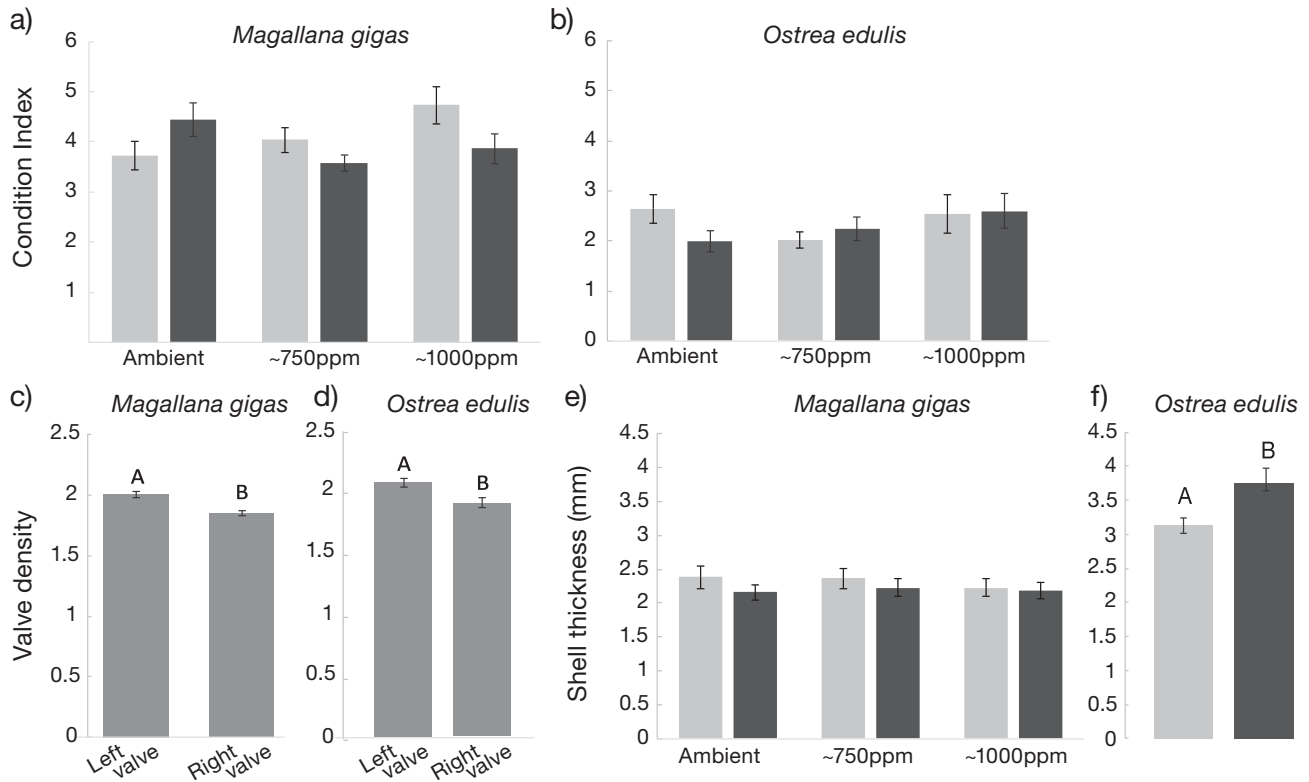


Fig. 4. (a,b) Oyster condition index under temperature and  $p\text{CO}_2$  scenarios. (c,d) Density of lower and upper shell valves of oysters. (e,f) Thickness of the lower valve of *Magallana gigas* and *Ostrea edulis* under 2 temperature treatments. Values are given as means  $\pm$  SE. In panels a, b, e and f, light grey bars: control temperature (16.8°C), dark grey bars: warm temperature (20°C). Treatments that do not share a letter are significantly different

also essential for vital functions such as feeding, respiring, and eliminating waste (Robson et al. 2007). The natural state of oysters is open and gaping, due to the mechanical spring-like effect of the ligament connecting the 2 valves (Kurita et al. 2016), although oysters (and other bivalves) can control the opening and closing of the valves by contracting their adductor muscle (Galtsoff 1964, Kurita et al. 2016). If the valves are forced apart by predators, the muscle fibres will eventually tear from the centre (Galtsoff 1964), leading to loss of a key protective function.

To our knowledge, no studies to date have looked into the effects of OAW on the strength of the adductor muscle in bivalves. Because of the emerging evidence that the physiology of organisms, particularly calcifiers, can be impacted by OAW, we predicted that OAW would affect muscle fitness traits (pry resistance; opening force and muscle tear) and this was evident in *M. gigas*. Under elevated  $p\text{CO}_2$ , individuals required an 11 to 35% increase in force to be opened irrespective of temperature, and a 23.5% increase in the force required for their muscle to tear (at 750 ppm) when combined with elevated temperature. In marked contrast, the amount of force re-

quired to open the valves and tear the muscle of *O. edulis* was unaffected by OAW, likely because its muscle integrity (diameter and CI) was not affected by the conditions, either positively or negatively. For both species, our results appear in contrast with that of Zhao et al. (2020) for the thick shell mussel *Mytilus coruscus* under ocean acidification (OA) conditions alone, where they reported reduced adductor muscle strength at pH 7.8 and pH 7.4. It should be noted, however, that the authors did not directly measure muscle strength, but rather correlated it to adductor muscle diameter. Their results should therefore be interpreted with caution, as we show here for *M. gigas* that muscle strength is not always directly related to muscle diameter.

For both *M. gigas* and *O. edulis*, bigger individuals (i.e. those with higher tissue weight) were able to sustain stronger extensive force on their adductor muscle, likely by having more robust muscle fibres. The action of the adductor muscle is predominantly fuelled by soft-tissue glycogen in oysters (Galtsoff 1964); therefore, bigger individuals would possess more glycogen to allocate to muscle build-up. *M. gigas* has a higher glycogen (carbohydrate) content

than *O. edulis*, and here OAW may be enhancing the use of this biochemical in *M. gigas* into building a stronger (but not necessarily bigger) adductor muscle. Possessing the ability to build a stronger muscle, able to resist more important pulling forces from predators, may confer an ecological advantage to *M. gigas* over *O. edulis* in the future.

Shells constitute the first line of defence of oysters to external threats, and therefore to possess a strong shell is to hold a fitness advantage (Currey & Hughes 1982). The production of shell material is biologically controlled and energetically expensive, but modulated by changes in environmental conditions (Gazeau et al. 2013). The burgeoning evidence from OA studies have shown that molluscan shell calcification is particularly vulnerable to increases in  $p\text{CO}_2$  and decreases in seawater pH (Gazeau et al. 2013, Byrne & Fitzer 2019, but see Knights et al. 2020). Previously observed changes to the mineralogy and structural integrity of shells (Fitzer et al. 2016, Duquette et al. 2017, Knights et al. 2020), and reductions in shell metrics (Lagos et al. 2016, Chatzinikolaou et al. 2017, Wright et al. 2018, Zhao et al. 2020), are likely to negatively affect their protective function.

We predicted reduced shell strength under OAW; this was apparent in *M. gigas* but not *O. edulis*. The literature is similarly conflicted, reporting mixed or no effect of OAW on shell strength of molluscs in some instances (Duquette et al. 2017, Babarro et al. 2018, Clements et al. 2018), and marked reductions in others (Welladsen et al. 2010, Li et al. 2015, Speights et al. 2017, Wright et al. 2018, Barclay et al. 2019, Zhao et al. 2020). Differences between species may be attributed to differences in the biomineralisation process and use of different calcium carbonate polymorphs (Gazeau et al. 2013). Recent studies have shown that the crystal matrices and the orientation of individual crystals that make up those matrices can be modified under OAW (e.g. Li et al. 2015, Fitzer et al. 2016, Knights et al. 2020), which could hold implications for the mechanical integrity of the shell and its protective function (Li et al. 2015). For instance, changes in shell crystallography as well as mineral plasticity may also affect rates of shell dissolution and shell strength (Duquette et al. 2017, Leung et al. 2017a, Barclay et al. 2019, 2020, Chadwick et al. 2019). Changes in crystallographic structure were not assessed here, and although no changes in shell thickness and density were recorded, it is likely that *M. gigas* allocated energy towards tissue production and maintenance of its adductor muscle, at the detriment of calcification. Similarly to *M. gigas* in this study, *Mytilus edulis* was shown to reallocate energy

from shell strength to maintenance under OAW (Mackenzie et al. 2014). For both *O. edulis* and *M. gigas*, individuals with heavier shells were more resistant to crushing forces, but this advantage disappeared for *M. gigas* under elevated  $p\text{CO}_2$ , suggesting that building a heavier shell may not confer an ecological advantage to *M. gigas* in the future. Note that scrubbing off epibionts from shells is a common practice, and it was assumed in this study that this process did not significantly affect the shell integrity or strength.

Weakened shells represent a reduction in fitness that may put oysters at greater risk of predation, for example, by shortening the prey-handling time from current predators or allowing new species to crush shells that were previously stronger than they could manage (Beadman et al. 2003). For instance, a potential predator with crushing force limited to 400 N would not have the capacity to crush the shells of *M. gigas* or *O. edulis* under control temperature and ambient  $p\text{CO}_2$  conditions, but under future OAW conditions, this predator has in theory sufficient crushing force to predate on *M. gigas*. Such alteration in predator-prey interactions could then induce important community and ecosystem-level destabilization (Kroeker et al. 2014).

Despite differences in muscle and shell strengths, there were no changes in the CI, muscle diameter, shell thickness, or density, i.e. traits indicative of an organism's physiological status and overall health, in either *M. gigas* or *O. edulis* under OAW scenarios. Several studies have shown negative effects of OAW on traits such as CI, especially in molluscs that are known to be effective at reallocating energetic investment between biological processes (e.g. somatic growth, calcification; Mackenzie et al. 2014, Ong et al. 2017, Lemasson et al. 2018, 2019). The absence of changes in these traits over the course of this experiment suggests that both oyster species had sufficient energetic resources available to maintain the range of biological functions (reproductive development and metabolism) under future OAW conditions. Assessment of shell growth under single stressor conditions (OA or ocean warming [OW]), however, revealed thickening of *O. edulis* shells under OW, indicating increased investment in shell calcification driven by higher temperatures, but counteracted by increased shell dissolution as a result of OA. Molluscs are well known to be capable of rapid energy reallocation to different functions in response to changing environmental conditions (e.g. Reimer & Tedengren 1996, Leonard et al. 1999, Beadman et al. 2003, Lagos et al. 2016, Chatzinikolaou et al. 2017), and the results

here suggest differential approaches to energy allocation to biological functions in these 2 species. In contrast, the increased growth of *O. edulis* suggests a 'decision' to increase calcification rates under OW, thereby likely enhancing protection, but the capacity to do so is curtailed under OA.

## 5. CONCLUSION

The 2 oysters had contrasting responses in terms of muscle strength and shell strength. There were no physiological and morphological effects of OAW on *Ostrea edulis*, with the exception of thicker shells, and consequently its adductor muscle and shell strength remained unimpacted. Despite a lack of physiological and morphological effects, the adductor muscle of *Magallana gigas* appeared stronger and more difficult to tear, particularly for bigger individuals, but the shell was weaker. As a consequence, it is likely that in the future *M. gigas* may see its susceptibility to predators change, becoming more resistant to valve-pulling predators, such as starfish, but more vulnerable to durophagous (shell-crushing) predators, such as crabs. Unlike *M. gigas*, *O. edulis* might retain the same level of predation resistance as it currently possesses. Reduction in predation pressure on *O. edulis* could potentially occur indirectly through *M. gigas* selection by predators. However, considering that *M. gigas* is an exotic species in the UK, whether natural enemies would target it is uncertain (enemy release hypothesis: Keane & Crawley 2002, Colautti et al. 2004). For both species, oysters with more somatic tissue hold an ecological advantage when it comes to predator resistance. Under OAW, smaller oysters with a weaker adductor muscle may be favoured by predators due to a reduced handling time enabling optimised foraging and maximised energy usage. However, the findings of this study do not take into account the likely effects of OAW on predators (Landes & Zimmer 2012, Kroeker et al. 2014, Manríquez et al. 2020), which may also affect the level of predation pressure exerted on oysters (Wright et al. 2018).

These changes in fitness have the potential to induce shifts in predator–prey interactions (Harvey & Moore 2017, Sadler et al. 2018, Wright et al. 2018), reshape assemblage structure due to species and size selection (Gooding & Harley 2015, Leung et al. 2017b, Lord et al. 2017, Babarro et al. 2018), and consequently may induce important cascading modifications in the functioning of oyster reefs and the delivery of associated marine ecosystem services. In particular, to

secure viable future production, the oyster aquaculture sector might need to adapt its culture and harvest practices, as well as species selection, according to predicted susceptibility in order to lower predation risks and optimise oyster survival. It is apparent that responses to OAW are highly species-specific, and further studies investigating alterations to predator–prey and community-level interactions are necessary to better understand the ecosystem-level implications, and inform aquaculture practices.

*Acknowledgements.* We thank the technical staff at the Marine Biology and Ecology Research Centre at the University of Plymouth for their laboratory assistance during this experiment, as well as student volunteer Lucy 'Skirt' Jupe for her help with organism collection. We are grateful to the National Trust, the Carew Pole Garden Charitable Trust, and Brian Langley for granting us access to the collection site. Additionally, we thank Dr. Zoltan Gombos for his assistance with the Instron equipment. This research was supported by a grant awarded to A.M.K. by the School of Biological and Marine Science, University of Plymouth, as part of the PhD research of A.J.L.

## LITERATURE CITED

- Babarro JMF, Abad MJ, Gestoso I, Silva E, Olabarria C (2018) Susceptibility of two co-existing mytilid species to simulated predation under projected climate change conditions. *Hydrobiologia* 807:247–261
- ✦ Barclay KM, Gaylord B, Jellison BM, Shukla P, Sanford E, Leighton LR (2019) Variation in the effects of ocean acidification on shell growth and strength in two intertidal gastropods. *Mar Ecol Prog Ser* 626:109–121
- ✦ Barclay KM, Gingras MK, Packer ST, Leighton LR (2020) The role of gastropod shell composition and microstructure in resisting dissolution caused by ocean acidification. *Mar Environ Res* 162:105105
- ✦ Beadman H, Caldow R, Kaiser M, Willows R (2003) How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. *Mar Biol* 142: 487–494
- Beaumont NJ, Austen MC, Atkins JP, Burdon D and others (2007) Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. *Mar Pollut Bull* 54:253–265
- Beck MW, Brumbaugh RD, Airoidi L, Carranza A and others (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience* 61: 107–116
- ✦ Boulding EG (1984) Crab-resistant features of shells of burrowing bivalves: decreasing vulnerability by increasing handling time. *J Exp Mar Biol Ecol* 76:201–223
- ✦ Butler RW, Kirbyson JW (1979) Oyster predation by the black oystercatcher in British Columbia. *The Condor* 81(4):433–435
- ✦ Byrne M, Fitzer S (2019) The impact of environmental acidification on the microstructure and mechanical integrity of marine invertebrate skeletons. *Conserv Physiol* 7:coz062
- Cadée GC (2001) Herring gulls learn to feed on a recent invader in the Dutch Wadden Sea, the Pacific oyster

- Crassostrea gigas*. *Basteria* 65:33–42
- Calosi P, Turner LM, Hawkins M, Bertolini C, Nightingale G, Truebano M, Spicer JI (2013) Multiple physiological responses to multiple environmental challenges: an individual approach. *Integr Comp Biol* 53:660–670
- Chadwick M, Harper EM, Lemasson AJ, Spicer JI, Peck LS (2019) Quantifying susceptibility of marine invertebrate biocomposites to dissolution in reduced pH. *R Soc Open Sci* 6:190252
- Chatzinikolaou E, Grigoriou P, Keklikoglou K, Faulwetter S, Papageorgiou N (2017) The combined effects of reduced pH and elevated temperature on the shell density of two gastropod species measured using micro-CT imaging. *ICES J Mar Sci* 74:1135–1149
- Clements JC, Comeau LA (2019) Behavioral defenses of shellfish prey under ocean acidification. *J Shellfish Res* 38:725–742
- Clements JC, Darrow ES (2018) Eating in an acidifying ocean: a quantitative review of elevated CO<sub>2</sub> effects on the feeding rates of calcifying marine invertebrates. *Hydrobiologia* 820:1–21
- Clements JC, Coffin MR, Lavaud R, Guyonnet T, Comeau L (2018) Ocean acidification and molluscan shell taphonomy: Can elevated seawater pCO<sub>2</sub> influence taphonomy in a naticid predator–prey system? *Palaeogeogr Palaeoclimatol Palaeoecol* 507:145–154
- Clements JC, Ramesh K, Nysveen J, Dupont S, Jutfelt F (2021) Animal size and seawater temperature, but not pH, influence a repeatable startle response behaviour in a wide-ranging marine mollusc. *Anim Behav* 173:191–205
- Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? *Ecol Lett* 7:721–733
- Cooley SR, Lucey N, Kite-Powell H, Doney SC (2012) Nutrition and income from molluscs today imply vulnerability to ocean acidification tomorrow. *Fish Fish* 13:182–215
- Cooley SR, Rheuban JE, Hart DR, Luu V, Glover DM, Hare JA, Doney SC (2015) An integrated assessment model for helping the United States sea scallop (*Placopecten magellanicus*) fishery plan ahead for ocean acidification and warming. *PLOS ONE* 10:e0124145
- Currey JD, Hughes RN (1982) Strength of the dogwhelk *Nucella lapillus* and the winkle *Littorina littorea* from different habitats. *J Anim Ecol* 51:47–56
- Denton E, Gilpin-Brown J (1961) The buoyancy of the cuttlefish, *Sepia officinalis* (L.). *J Mar Biol Assoc UK* 41:319–342
- Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. *Deep Sea Res A Oceanogr Res Pap* 37:755–766
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res A Oceanogr Res Pap* 34:1733–1743
- Duquette A, McClintock JB, Amsler CD, Perez-Huerta A, Milazzo M, Hall-Spencer JM (2017) Effects of ocean acidification on the shells of four Mediterranean gastropod species near a CO<sub>2</sub> seep. *Mar Pollut Bull* 124:917–928
- Ekstrom JA, Suatoni L, Cooley SR, Pendleton LH and others (2015) Vulnerability and adaptation of US shellfisheries to ocean acidification. *Nat Clim Change* 5:207–214
- Elnor RW (1978) The mechanics of predation by the shore crab, *Carcinus maenas* (L.), on the edible mussel, *Mytilus edulis* (L.). *Oecologia* 36:333–344
- Fitzer SC, Chung P, Maccherozzi F, Dhési SS, Kamenos NA, Phoenix VR, Cusack M (2016) Biomineral shell formation under ocean acidification: a shift from order to chaos. *Sci Rep* 6:21076
- Froehlich HE, Gentry RR, Halpern BS (2018) Global change in marine aquaculture production potential under climate change. *Nat Ecol Evol* 2:1745–1750
- Froehlich KR, Lord JP (2020) Can ocean acidification interfere with the ability of mud snails (*Tritia obsoleta*) to sense predators? *J Exp Mar Biol Ecol* 526:151355
- Galtsoff PS (1964) The American oyster *Crassostrea virginica* Gmelin. *Fish Bull Fish Wildl Serv* 64. US Government Printing Office, Washington, DC
- Gazeau F, Parker LM, Comeau S, Gattuso JP and others (2013) Impacts of ocean acidification on marine shelled molluscs. *Mar Biol* 160:2207–2245
- Gooding RA, Harley CDG (2015) Quantifying the effects of predator and prey body size on sea star feeding behaviors. *Biol Bull* 228:192–200
- Gribben PE, Byers JE, Clements M, McKenzie LA, Steinberg PD, Wright JT (2009) Behavioural interactions between ecosystem engineers control community species richness. *Ecol Lett* 12:1127–1136
- Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. *Oikos* 122:816–824
- Hadley Centre for Climate Prediction and Research (2014) UKCP09: Met Office HadRM3-PPE UK model runs. NCAS British Atmospheric Data Centre, <https://catalogue.ceda.ac.uk/uuid/465ecd8a305ffb9df2bd8b54cada669f>
- Hancock D (1955) The feeding behaviour of starfish on Essex oyster beds. *J Mar Biol Assoc UK* 34:313–331
- Hancock DA (1958) Notes on starfish on an Essex oyster bed. *J Mar Biol Assoc UK* 37:565–589
- Harvey BP, Moore PJ (2017) Ocean warming and acidification prevent compensatory response in a predator to reduced prey quality. *Mar Ecol Prog Ser* 563:111–122
- Herbert RJH, Robert C, Humphreys J, Fletcher S (2012) The Pacific oyster (*Crassostrea gigas*) in the UK: economic, legal and environmental issues associated with its cultivation, wild establishment and exploitation. Report for the Shellfish Association of Great Britain. Available at [http://www.shellfish.org.uk/files/PDF/73434Pacific%20Oysters%20Issue%20Paper\\_final\\_241012.pdf](http://www.shellfish.org.uk/files/PDF/73434Pacific%20Oysters%20Issue%20Paper_final_241012.pdf)
- Humphreys MP (2017) Climate sensitivity and the rate of ocean acidification: future impacts, and implications for experimental design. *ICES J Mar Sci* 74:934–940
- IPCC (2013) Summary for policymakers. In: Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
- Jellison BM, Ninokawa AT, Hill TM, Sanford E, Gaylord B (2016) Ocean acidification alters the response of intertidal snails to a key sea star predator. *Proc R Soc B* 283: 20160890
- Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol* 17:164–170
- Kibria G, Haroon YAK, Nugegoda D (2017) Climate change impacts on tropical and temperate fisheries, aquaculture, and seafood security and implications — a review. *Livest Res Rural Dev* 29:1–29
- Knights AM (2012) Spatial variation in body size and reproductive condition of subtidal mussels: considerations for sustainable management. *Fish Res* 113:45–54
- Knights AM, Firth LB, Walters K (2012) Interactions between multiple recruitment drivers: post-settlement predation mortality and flow-mediated recruitment. *PLOS ONE* 7: e35096
- Knights AM, Norton MJ, Lemasson AJ, Stephen N (2020)

- Ocean acidification mitigates the negative effects of increased sea temperatures on the biomineralisation and crystalline ultrastructure of *Mytilus*. *Front Mar Sci* 7: 567228
- ✦ Koenigstein S, Dahlke FT, Stiasny MH, Storch D, Clemmensen C, Pörtner HO (2018) Forecasting future recruitment success for Atlantic cod in the warming and acidifying Barents Sea. *Glob Change Biol* 24:526–535
- ✦ Kroeker KJ, Sanford E, Jellison BM, Gaylord B (2014) Predicting the effects of ocean acidification on predator–prey interactions: a conceptual framework based on coastal molluscs. *Biol Bull* 226:211–222
- ✦ Kurita Y, Hashimoto N, Wada H (2016) Evolution of the molluscan body plan: the case of the anterior adductor muscle of bivalves. *Biol J Linn Soc* 119:420–429
- ✦ Lagos NA, Benítez S, Duarte C, Lardies MA and others (2016) Effects of temperature and ocean acidification on shell characteristics of *Argopecten purpuratus*: implications for scallop aquaculture in an upwelling-influenced area. *Aquacult Environ Interact* 8:357–370
- ✦ Landes A, Zimmer M (2012) Acidification and warming affect both a calcifying predator and prey, but not their interaction. *Mar Ecol Prog Ser* 450:1–10
- ✦ Lavoie ME (1956) How sea stars open bivalves. *Biol Bull* 111:114–122
- ✦ Lemasson AJ, Fletcher S, Hall-Spencer JM, Knights AM (2017) Linking the biological impacts of ocean acidification on oysters to changes in ecosystem services: a review. *J Exp Mar Biol Ecol* 492:49–62
- ✦ Lemasson AJ, Hall-Spencer JM, Fletcher S, Provstgaard-Morys S, Knights AM (2018) Indications of future performance of native and non-native adult oysters under acidification and warming. *Mar Environ Res* 142:178–189
- ✦ Lemasson AJ, Hall-Spencer JM, Kuri V, Knights AM (2019) Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming. *Mar Environ Res* 143:82–92
- ✦ Leonard GH, Bertness MD, Yund PO (1999) Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. *Ecology* 80:1–14
- ✦ Leung JY, Russell BD, Connell SD (2017a) Mineralogical plasticity acts as a compensatory mechanism to the impacts of ocean acidification. *Environ Sci Technol* 51: 2652–2659
- ✦ Leung JYS, Connell SD, Nagelkerken I, Russell BD (2017b) Impacts of near-future ocean acidification and warming on the shell mechanical and geochemical properties of gastropods from intertidal to subtidal zones. *Environ Sci Technol* 51:12097–12103
- ✦ Li S, Liu C, Huang J, Liu Y, Zheng G, Xie L, Zhang R (2015) Interactive effects of seawater acidification and elevated temperature on biomineralization and amino acid metabolism in the mussel *Mytilus edulis*. *J Exp Biol* 218: 3623–3631
- ✦ Lloret J, Rätz HJ, Lleonart J, Demestre M (2016) Challenging the links between seafood and human health in the context of global change. *J Mar Biol Assoc UK* 96: 29–42
- ✦ López MS, Coutinho R, Ferreira CEL, Rilov G (2010) Predator–prey interactions in a bioinvasion scenario: differential predation by native predators on two exotic rocky intertidal bivalves. *Mar Ecol Prog Ser* 403:101–112
- ✦ Lord JP, Barry JP, Graves D (2017) Impact of climate change on direct and indirect species interactions. *Mar Ecol Prog Ser* 571:1–11
- ✦ Mackenzie CL, Ormondroyd GA, Curling SF, Ball RJ, Whiteley NM, Malham SK (2014) Ocean warming, more than acidification, reduces shell strength in a commercial shellfish species during food limitation. *PLOS ONE* 9: e86764
- ✦ Manríquez PH, González CP, Seguel M, Garcia-Huidobro Moreno MR and others (2020) The combined effects of ocean acidification and warming on a habitat-forming shell-crushing predatory crab. *Sci Total Environ* 758: 143587
- ✦ Mascaró M, Seed R (2001) Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus* (L.). *Mar Biol* 139:1135–1145
- ✦ McCann KS (2000) The diversity–stability debate. *Nature* 405:228
- ✦ Mehrbach C, Culbertson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18:897–907
- ✦ Meng Y, Guo Z, Fitzer SC, Upadhyay A and others (2018) Ocean acidification reduces hardness and stiffness of the Portuguese oyster shell with impaired microstructure: a hierarchical analysis. *Biogeosciences* 15:6833–6846
- ✦ Menzel WR, Nichy FE (1958) Studies of the distribution and feeding habits of some oyster predators in Alligator Harbor, Florida. *Bull Mar Sci* 8:125–145
- ✦ Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science* 315:1846–1850
- ✦ O'Connor NE, Grabowski JH, Ladwig LM, Bruno JF (2008) Simulated predator extinctions: predator identity affects survival and recruitment of oysters. *Ecology* 89:428–438
- ✦ Ong EZ, Briffa M, Moens T, Van Colen C (2017) Physiological responses to ocean acidification and warming synergistically reduce condition of the common cockle *Cerastoderma edule*. *Mar Environ Res* 130:38–47
- ✦ Palumbi SR, Sandifer PA, Allan JD, Beck MW and others (2009) Managing for ocean biodiversity to sustain marine ecosystem services. *Front Ecol Environ* 7:204–211
- ✦ Parker LM, Ross PM, O'Connor WA, Pörtner HO, Scanes E, Wright JM (2013) Predicting the response of molluscs to the impact of ocean acidification. *Biology* 2:651–692
- ✦ Peckarsky BL, Abrams PA, Bolnick DI, Dill LM and others (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator–prey interactions. *Ecology* 89:2416–2425
- ✦ Pierrot D, Lewis E, DWR W (2006) MS Excel program developed for CO<sub>2</sub> system calculations, ORNL/CDIAC-105. Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN
- ✦ Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. [www.r-project.org](http://www.r-project.org)
- ✦ Reimer O, Tedengren M (1996) Phenotypical improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to the predator *Asterias rubens*. *Oikos* 75:383–390
- ✦ Reum JCP, Alin SR, Harvey CJ, Bednaršek N and others (2016) Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. *ICES J Mar Sci* 73:582–595
- ✦ Richards RG, Davidson AT, Meynecke JO, Beattie K, Herniman V, Lynam T, van Putten IE (2015) Effects and mitigations of ocean acidification on wild and aquaculture scallop and prawn fisheries in Queensland, Australia. *Fish Res* 161:42–56
- ✦ Robson A, Wilson R, Garcia de Leaniz C (2007) Mussels flexing their muscles: a new method for quantifying bivalve

- behaviour. *Mar Biol* 151:1195–1204
- ✦ Sadler DE, Lemasson AJ, Knights AM (2018) Ocean acidification effects on the biological functioning of *Mytilus edulis*. *Mar Environ Res* 139:162–168
  - ✦ Sampaio E, Rodil IF, Vaz-Pinto F, Fernandez A, Arenas F (2017) Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients. *Mar Environ Res* 125:25–33
  - ✦ Sanchez-Salazar ME, Griffiths CL, Seed R (1987) The effect of size and temperature on the predation of cockles *Cerastoderma edule* (L.) by the shore crab *Carcinus maenas* (L.). *J Exp Mar Biol Ecol* 111:181–193
  - Sawusdee A (2015) Restoration of the European flat oyster *Ostrea edulis* using elevated broodstock reefs. PhD thesis, University of Southampton
  - Shelmerdine RL, Leslie B (2009) Restocking of the native oyster, *Ostrea edulis*, in Shetland: habitat identification study. Scottish Natural Heritage Commissioned Report No. 396
  - ✦ Spalding C, Finnegan S, Fischer WW (2017) Energetic costs of calcification under ocean acidification. *Global Biogeochem Cycles* 31:866–877
  - ✦ Speights CJ, Silliman BR, McCoy MW (2017) The effects of elevated temperature and dissolved  $p\text{CO}_2$  on a marine foundation species. *Ecol Evol* 7:3808–3814
  - ✦ Vargas CA, Aguilera VM, San Martín V, Manríquez PH and others (2015)  $\text{CO}_2$ -driven ocean acidification disrupts the filter feeding behavior in Chilean gastropod and bivalve species from different geographic localities. *Estuaries Coasts* 38:1163–1177
  - Welladsen HM, Southgate PC, Heimann K (2010) The effects of exposure to near-future levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia: Pteriidae). *Molluscan Res* 30:125–130
  - Woolmer AP, Syvret M, FitzGerald A (2011) Restoration of native oyster, *Ostrea edulis*, in South Wales: options and approaches. CCW Contract Science Report No. 960, Countryside Council for Wales
  - ✦ Wright JM, Parker LM, O'Connor WA, Scanes E, Ross PM (2018) Ocean acidification affects both the predator and prey to alter interactions between the oyster *Crassostrea gigas* (Thunberg, 1793) and the whelk *Tenguelia marginalba* (Blainville, 1832). *Mar Biol* 165:46
  - ✦ Zhao X, Han Y, Chen B, Xia B, Qu K, Liu G (2020)  $\text{CO}_2$ -driven ocean acidification weakens mussel shell defense capacity and induces global molecular compensatory responses. *Chemosphere* 243:125415

## Appendix. Additional data

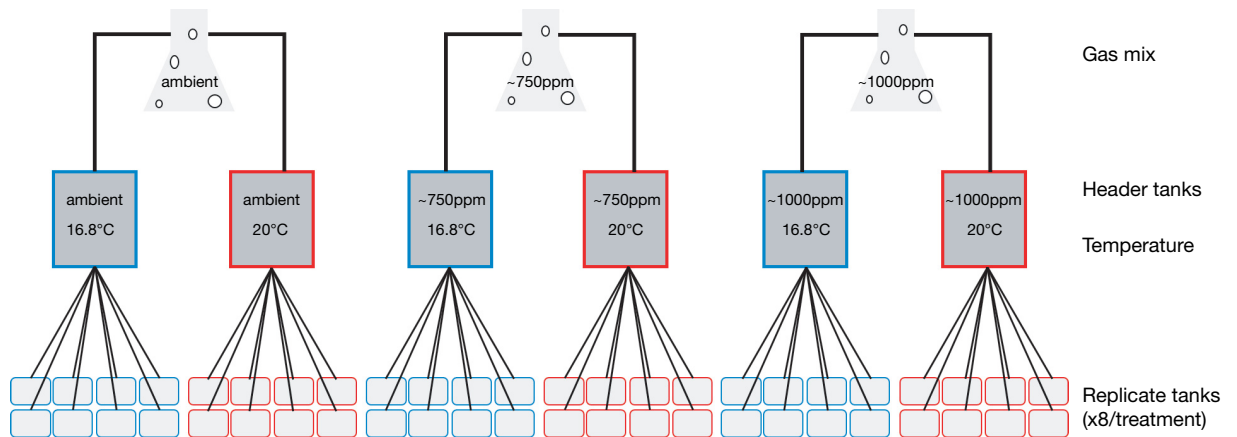


Fig. A1. Experimental design used to maintain adult oysters under ocean acidification and warming (OAW) scenarios. Eight replicate tanks were used per OAW scenario, each containing an individual oyster of the same species ( $n = 48$  oysters per species). The experiment was run twice, sequentially for each species

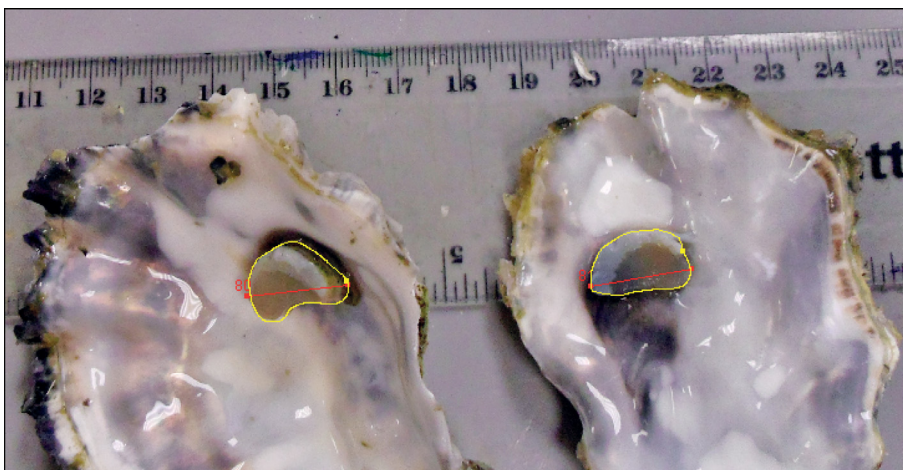


Fig. A2. Diameter (red) and area (yellow) of the adductor muscle of *Magallana gigas* measured using ImageJ software



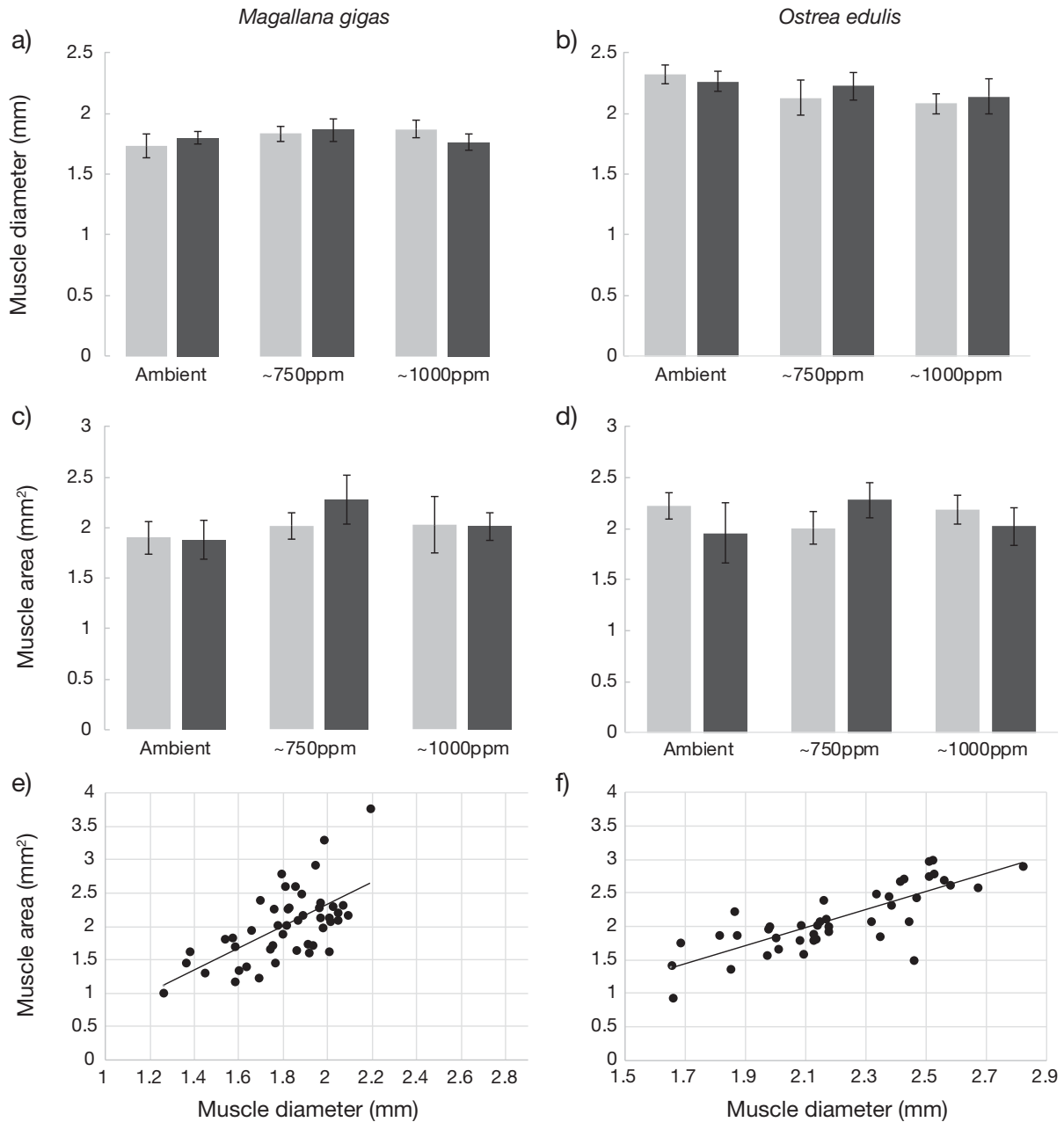


Fig. A3. Variations (mean  $\pm$  SE) in the (a,b) diameter and (c,d) area of the adductor muscle of oysters under temperature and pCO<sub>2</sub> scenarios. Light grey: control temperature (16.8°C); dark grey: warm temperature (20°C). (e,f) For both oyster species, muscle diameter and area were positively correlated. *Magallana gigas*:  $y = 1.6x - 0.9$ ,  $R^2 = 0.38$ ,  $p < 0.001$ ; *Ostrea edulis*:  $y = 1.3x - 0.8$ ,  $R^2 = 0.62$ ,  $p < 0.001$

Editorial responsibility: Emily Carrington,  
Friday Harbor, Washington, USA  
Reviewed by: J. Clements and 2 anonymous referees

Submitted: October 26, 2020  
Accepted: March 4, 2021  
Proofs received from author(s): April 22, 2021