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Competitive interactions moderate the effects of elevated temperature and atmospheric CO_2 on the health and functioning of oysters

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ABSTRACT: Global increases in sea temperatures and atmospheric concentrations of CO_2 may affect the health of calcifying shellfish. Little is known, however, about how competitive interactions within and between species may influence how species respond to multiple stressors. We experimentally assessed separate and combined effects of temperature (12 or 16°C) and atmospheric CO_2 concentrations (400 and 1000 ppm) on the health and biological functioning of native (*Ostrea edulis*) and invasive (*Crassostrea gigas*) oysters held alone and in intraspecific or interspecific mixtures. We found evidence of reduced phagocytosis under elevated CO_2 and, when combined with increased temperature, a reduction in the number of circulating haemocytes. Generally, *C. gigas* showed lower respiration rates relative to *O. edulis* when the species were in intraspecific or interspecific mixtures. In contrast, *O. edulis* showed a higher respiration rate relative to *C. gigas* when held in an interspecific mixture and exhibited lower clearance rates when held in intraspecific or interspecific mixtures. Overall, clearance rates of *C. gigas* were consistently greater than those of *O. edulis*. Collectively, our findings indicate that a species' ability to adapt metabolic processes to environmental conditions can be modified by biotic context and may make some species (here, *C. gigas*) competitively superior and less vulnerable to future climatic scenarios at local scales. If these conclusions are generic, the relative role of species interactions, and other biotic parameters, in altering the outcomes of climate change will require much greater research emphasis.

KEY WORDS: Warming · Ocean acidification · Intraspecific competition · Interspecific competition · Species interactions · Complementarity

INTRODUCTION

Climate change and the introduction of non-indigenous species are particularly prevalent consequences of human activities that have potential to alter marine biodiversity and ecosystem functioning on a global scale (Rahel & Olden 2008). In particular, it is anticipated that rising temperatures and increasing levels of atmospheric CO_2 (leading to acidification of aquatic habitats; Doney et al. 2012) will occur in tandem (IPCC 2014). It is important, therefore, to gain mechanistic insights by considering both the independent effects of temperature and CO_2 and those of non-additive (antagonistic or synergistic) interactions associated with combined changes in temperature and CO_2 that are hard to predict (Duarte et al. 2014). Such changes in temperature and CO_2 have potential to increase the successful establish-

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ment of opportunistic invasive species by enhancing their dispersal (Kokko & López-Sepulcre 2006), survival (Sorte et al. 2010) or dominance (Smith et al. 2000), or by inhibiting native species (Sorte & White 2013). Some native and invasive species have been found to respond differently to environmental stress in aquatic habitats (Tomanek & Zuzow 2010), yet few experimental studies have explicitly compared their physiological responses to predicted scenarios of climatic change (Sorte & White 2013). Moreover, to compound this uncertainty, these studies are based on a limited number of 'model species', which may not encapsulate the full spectrum of physiological complexity in even closely-related coastal marine species.

Oysters that build reefs create biogenic habitat important for estuarine biodiversity (Lenihan & Peterson 1998, Grabowski et al. 2012), promote benthic–pelagic coupling and fishery production (Barbier et al. 2011), and can also influence local benthic and pelagic community structure (Wheat & Ruesink 2013). The European flat oyster *Ostrea edulis* (Linnaeus 1758) occurs throughout the Atlantic and Mediterranean coasts of Europe (Airoldi & Beck 2007, Lallias et al. 2007) and is native to parts of Europe, including the UK. This species historically has represented one of the most commercially important resources in European waters (Beck et al. 2011), although natural stocks of *O. edulis* have been in decline over the last 50 yr (Edwards 1997, Mackenzie et al. 1997, Laing et al. 2006). In contrast, the Pacific oyster *Crassostrea gigas* has proliferated throughout Europe since its introduction in the mid-1960s (or possibly much earlier, Humphreys et al. 2014). In places, this species has become naturalised (Ruesink et al. 2005); for example, *C. gigas* natural spatfall was recorded in the Oosterschelde estuary (southwest Netherlands) in 1975 (Smaal et al. 2009), and their presence was reported in the East Frisian Wadden Sea, North Sea, between 2003 and 2005 (Brandt et al. 2008). *C. gigas* has been reported in Strangford Lough in Northern Ireland from the 1990s (Guy & Roberts 2010), as well as Scotland (Smith et al. 2015) and as far north as Sweden and Norway (Wrange et al. 2010). Information from experimental studies on *C. gigas* (e.g. Havenhand & Schlegel 2009, Dutertre et al. 2010) indicate, with high probability, further spread of this species across Europe, leading to large-scale biogeographic shifts in coastal marine communities (Thomas et al. 2016) and potential to outcompete the native oyster *O. edulis*.

The potential for competition between *C. gigas* as a non-indigenous species and native oysters has been the subject of debate. For example, in Australia, a consensus is yet to be reached on whether or not *C. gigas* is likely to outcompete the native rock oyster *Saccostrea glomerata* (Krassoi et al. 2008, Bishop et al. 2010). In Europe, initial observations suggested that the presence of *C. gigas* would not affect *O. edulis* populations, or their settlement and establishment, because the 2 species tend to occupy different niches (Walne & Helm 1979, Reise et al. 1998). *O. edulis* has a more limited tolerance range for salinity than *C. gigas* and is commonly found in subtidal or low intertidal zones (Askew 1972), whilst *C. gigas* usually occupies the mid to low intertidal zone (Askew 1972). Recently, however, it has been recognised that these 2 oyster species are not necessarily spatially separated in both subtidal and intertidal habitats. For example, in Sweden and Norway, *C. gigas* has been regularly observed in deeper waters (1–9 m) where *O. edulis* also occurs, and *O. edulis* has been documented occupying intertidal waters (Bodvin et al. 2010, Dolmer et al. 2014). Furthermore, *C. gigas* has been found in subtidal *O. edulis* beds in Donegal, Ireland (Tully & Clarke 2012, Zwerschke et al. 2016), suggesting that *C. gigas* and *O. edulis* can co-exist.

When *C. gigas* is non-indigenous, it may be more resilient to environmental stress than native species, such as *O. edulis*, because *C. gigas* seems less vulnerable to parasites (Romestand et al. 2002, Comesaña et al. 2012) and generally has a greater tolerance to environmental fluctuations, such as salinity (Miossec et al. 2009) and CO_2 (Havenhand & Schlegel 2009). With regards to temperature, however, the 2 species occupy an overlapping thermal tolerance range, from –1.5°C to ~35°C for *O. edulis* (Reise 1998, Piano et al. 2002) and –5 to 35°C for *C. gigas* (Reise 1998, Nehring 2011). When these 2 species do co-occur, *C. gigas* may compete for food and space with *O. edulis*, but little is known about competitive interactions between these species, or if competition could exacerbate direct, multiple effects of climate change.

Here, we assessed how the separate and combined effects of temperature and atmospheric CO_2 concentrations affect the health and biological functioning of native (*O. edulis*) and invasive (*C. gigas*) oysters held alone and in intraspecific or interspecific mixtures. Specifically, our goal was to (1) determine the separate and interactive effects of elevated temperature and CO_2 on the health and biological functioning of native and non-indigenous oysters and (2) test the prediction that competition within and between these species of oysters will alter the response of individual oysters to future climatic scenarios.

MATERIALS AND METHODS

Experimental design and set-up

The experiment was set up and carried out at the Biodiversity and Ecosystem Futures Facility at the University of Southampton, UK. This facility is designed for manipulating marine environmental variables, and follows European Project on Ocean Acidification (EPOCA: www.epoca-project.eu/) recommendations for manipulating marine carbonate systems. *Crassostrea gigas* oysters were collected from Woolston Shore (50.891° N, 1.384° W) whilst *Ostrea edulis* were collected from Ryde Middle (50.762° N, 1.180° W) near Southampton, UK. The experiment consisted of 3 fixed factors; 'Temperature' (2 levels: 12°C [ambient] and 16°C [elevated]), 'CO_2' (2 levels: 400 ppm [ambient] and 1000 ppm [elevated]) and 'Oyster' (6 levels: 2 individuals of *C. gigas*; 2 individuals of *O. edulis*; 1 *C. gigas* with 1 *O. edulis* (*C. gigas* sampled); 1 *O. edulis* with 1 *C. gigas* (*O. edulis* sampled); a single *C. gigas*; a single *O. edulis*). Since individuals maintained in interspecific mixtures involve the same combination of oysters, only 1 set of mesocosms was used for this treatment and measurements of our response variables were achieved by independently examining both individuals. In total, our experimental design required 120 mesocosms (n = 6) and 192 oysters.

We used oysters that were adults with an average length of (mean ± SE) 75.48 ± 1.88 mm and 73.80 ± 0.98 mm and an average wet weight of 4.12 ± 0.30 and 4.14 ± 0.22 g for *C. gigas* and *O. edulis*, respectively. *C. gigas* and *O. edulis* oysters did not differ in length (t_{104} = 0.875, p = 0.384) or wet weight (t_{128} = 0.029, p = 0.976). The temperatures (12 and 16°C) and CO_2 levels (400 and 1000 ppm) were selected to represent present-day conditions (based on yearly UK averages for 2014) and a warming scenario of +4°C (IPCC 2014). Individual mesocosms were constructed from plastic buckets filled with 4 l of natural seawater (source: Itchen estuary, Southampton), filtered through 5 µm mesh and UV sterilised. Water was changed every 3 d by replacing 50 % of the volume with fresh, equilibrated seawater. Each mesocosm was provided with 200 ml of live *Isochrysis galbana* and *Tetraselmis* sp. (at $1.51 \times 10^5 \pm 4.11 \times 10^4$ cells ml^{-1}) 3 times wk^{-1}. Mesocosms were maintained at their required temperatures using separate water baths. Elevated CO_2 concentrations were achieved by bubbling air mixed with the appropriate concentration of CO_2 gas into each mesocosm and were constantly monitored using a LiCOR calibration system (Licor LI-840A; LI-COR Biosciences). Alongside the experiment, pH (NBS), temperature and salinity measurements were taken 3 times wk^{-1} using a temperature and salinity probe and a Mettler Toledo™ pH meter. Mean mesocosm water temperature was 12.74 ± 0.15°C for ambient and 16.46 ± 0.10°C for elevated treatments, and pH was 8.063 ± 0.072 for ambient and 7.762 ± 0.038 for elevated CO_2 concentration. Alkalinity was determined by titration (Apollo SciTech Alkalinity Titrator AS-ALK2) using standard protocols at the National Oceanography Centre, Southampton, UK, Carbonate Facility. Bicarbonate ($H_2CO_3^-$), carbonate (CO_3^{2-}) and pCO_2 were calculated from measured pH, total alkalinity, temperature and salinity (Dickson et al. 2007, Dickson 2010) and dissolved inorganic carbon was calculated using CO2calc (Robbins et al. 2010) from pH and alkalinity (Table 1). All mesocosms were exposed to a 12:12 h light:dark cycle. The experiment commenced on 31 January 2014 and ran for 61 d.

Assessment of biological activity of the oysters

Clearance

Clearance rates were measured by placing a single oyster inside a glass chamber, each containing a magnetic stir bar (separated from the oyster by wire mesh) and 300 ml of filtered (0.1 µm) seawater. Once an oyster had opened its valves, *I. galbana* (Coccolithophyceae) were added at a concentration of 25 000 cells ml^{-1}. A 20 ml water sample was taken

Table 1. Salinity, total alkalinity (TA), pCO_2, dissolved inorganic carbon (DIC) and resistance based on aragonite and calcite for sea water at ambient and elevated temperature and pCO_2. Data are mean ± SE

Temp (°C)	CO_2	Salinity	TA (mmol l^{-1})	pCO_2 (µatm)	DIC (µmol kg^{-1})	$\Omega_{aragonite}$	$\Omega_{calcite}$
12	400	32.24 ± 0.13	2.01 ± 0.08	458.36 ± 17.14	1770 ± 61	1.49 ± 0.06	2.35 ± 0.09
	1000	32.24 ± 0.12	2.10 ± 0.09	996.48 ± 40.72	1848 ± 81	0.82 ± 0.04	1.28 ± 0.06
16	400	34.25 ± 0.15	1.82 ± 0.17	429.28 ± 39.25	1614 ± 133	1.64 ± 0.15	2.56 ± 0.24
	1000	34.28 ± 0.25	2.10 ± 0.08	1028.53 ± 39.71	1897 ± 72	0.99 ± 0.03	1.54 ± 0.05

from each chamber immediately, and again after 1 h. Although stir bars were included in each chamber to maintain a homogenised cell suspension, we accounted for passive settlement of the algal cells by following the same procedure in control chambers that did not contain an oyster. *I. galbana* were stained by adding 0.3 ml of Lugol's solution to each subsample, and cells were counted (for each sample in triplicate) using a 0.1 mm deep improved Neubauer haemocytometer viewed under bright field. Clearance rates were calculated based on Coughlan (1969) as:

$$\text{Clearance rate} = V \times ((\log_e(C_1) - \log_e(C_2) / (n \times t)) \quad (1)$$

where clearance rate is in l h^{-1}, V = volume of water (l) in the chamber, C_1 = initial cell density (l^{-1}), C_2 = final cell density (l^{-1}), n = number of oysters in the sampling vessel and t = time interval (h).

In addition, at the end of the experiment, remaining tissues (after extraction of muscle used for protein analysis, see below) were dried to constant weight at 60°C (dry weight) and then placed into a muffle furnace at 450°C for 6 h to determine shell-free ash-free dry weight (AFDW). Hence, clearance rate is expressed as l h^{-1} g^{-1} AFDW.

Respiration

Respiration was measured according to Warkentin et al. (2007). Briefly, oysters were gently scrubbed with a scourer to remove epibiota before each individual was placed inside a 1000 ml flow-through respirometry chamber that contained a 'sensor spot' (planar oxygen-sensitive foil). A PreSens Fibox 3™ fibre-optic oxygen meter was used to measure concentrations of dissolved oxygen which were analysed using OxyView 3.51 software (PreSens). After a 3 h acclimation period, oxygen concentrations were recorded at three 30 min intervals (i.e. for a total of 90 min). A chamber without oysters was used to account for any microbial respiration in the system. The respiration rates of the oysters were corrected for AFDW to give final respiration rates expressed as mg O_2 h^{-1} g^{-1} AFDW.

Assessment of oyster health

Number of haemocytes and phagocytosis rates

To measure the number of haemocytes, 20 µl of haemolymph was extracted from a pool in the promyal chamber using a hypodermic needle and syringe and fixed with 20 µl of 20% formalin in filtered (0.1 µm) seawater. An additional 25 µl of haemolymph was diluted with 25 µl of saline solution (Schlieper 1972) for use in the phagocytosis assay. Formalin-fixed haemocytes were enumerated using a 0.1 mm deep improved Neubauer haemocytometer viewed under bright field (in triplicate for each sample). The total haemocyte count was subsequently used to determine the required concentration of heat-inactivated yeast (*Saccharomyces cerevisiae*) for the phagocytosis assay, using the method of McCormick-Ray (1987). Briefly, 100 µl of diluted haemolymph were added to a baked glass slide, and 100 µl yeast dilution were added to create a 2:1 yeast:haemocyte ratio. The slide was agitated gently and a cover slip added. To estimate the phagocytic rate, the sample was incubated for 1 h as per temperature treatment (12 or 16°C) before the total number of cells that had incorporated yeast particles was counted.

Protein and glucose concentrations

Concentrations of proteins and glucose were measured from the adductor muscle, which was removed, frozen in liquid nitrogen and stored at −80°C. In order to calculate the total concentration of proteins in the adductor muscles, a bicinchoninic acid assay (BCA) was used following Smith et al. (1985) measured at λ = 562 nm. Megazyme™ D-Glucose-HK kits (Megazyme International Ireland) were used to determine the glucose concentrations of the adductor muscles following the manufacturer's protocol and measured with a spectrophotometer at λ = 340 nm.

Statistical analysis

All data were screened for outliers, normality and homoscedasticity before statistical analysis. Phagocytosis rates were expressed as percentages and arcsine then square root transformed to satisfy the requirement for normality. Normality of the data was assessed visually using q–q plots showing theoretical quantiles versus standardised residuals, and homogeneity of variance was determined after plotting residuals versus fitted values. Linear regression models were constructed to which a generalised least squares (GLS) estimation was applied following Pinheiro & Bates (2000), which permitted the variance structure of the data imposed by the experimental design to be modelled. Linear regression models were developed to test the effects of elevated levels of temperature, carbon dioxide, oyster identity and

their interactions on each response variable. We treated all explanatory variables as nominal factors and, where there was a heterogeneous variance, the data were analysed using a 'varIdent' variance-covariate structure and a GLS estimation procedure to allow the residual spread to vary with individual explanatory variables (based on Table 5.1 in Pinheiro & Bates 2000).

Determining the optimal variance covariate structure involved comparisons based on Akaike's information criterion (AIC) using residual estimated maximum likelihood. When the optimal variance structure was identified, minimal adequate models were determined using a backwards selection procedure described by Diggle et al. (2002) and Zuur et al. (2009), based on maximum likelihood (ML) testing. The minimal adequate linear regression models were checked by plotting residuals versus fitted values. The importance of each factor in the model was determined by testing nested models based on ML estimations; briefly, this involves removing a factor of interest (and any interactions including the factor of interest) from the minimal adequate linear regression model and comparing it to the full minimal adequate linear regression model using ML testing. The likelihood ratio (*L*-ratio) test is assumed to have a chi-squared distribution of which the degrees of freedom are based on the difference between the number of parameters in the full and minimal adequate models. Results are presented according to the highest significant interaction term with the data pooled for that term; summary results of each factor can be found in Supplement 1 at www.int-res.com/articles/suppl/m582p093_supp.pdf. All analyses were done using R v3.2.3 (R Core Team 2015) with the *nlme* package v3.1-128 (Pinheiro et al. 2016).

RESULTS

Effects of elevated temperature and CO_2 on biological activity

There were no significant effects of elevated temperature or CO_2 concentration on clearance rates for either *Crassostrea gigas* or *Ostrea edulis*, but clearance rate did depend on the composition of each species mixture (Fig. 1, Fig. S1 and Table S1a in Supplement 2; Oyster: *L*-ratio = 34.47, df = 5, p < 0.001). Overall, *O. edulis* had lower clearance rates compared with *C. gigas*, and the clearance rates of *O. edulis* were lower when in intraspecific (single vs. intra, $t = -2.27$, p = 0.024) or interspecific (single vs. inter, $t = -2.25$, p = 0.026) mixtures (Table S1b) relative to when individuals were alone. Clearance rates of *C. gigas* did not differ between intra- or interspecific mixtures (Table S1b).

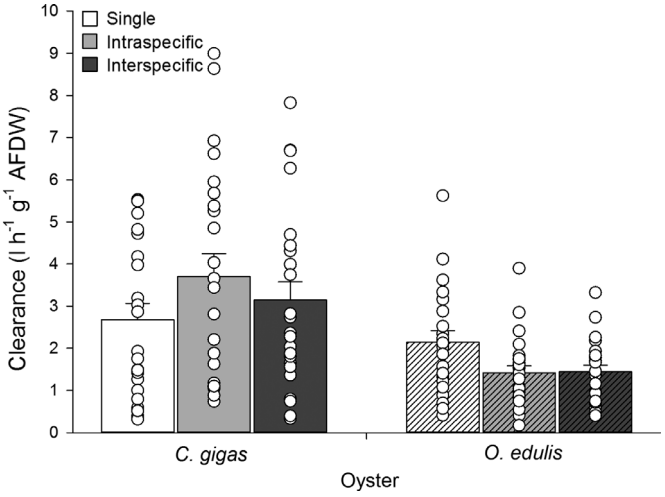

Fig. 1. Independent effect of oysters on clearance rate of *Crassostrea gigas* and *Ostrea edulis* when alone or in intra- or interspecific mixtures. Bars are means ± SE, dots represent individual observations. AFDW: ash-free dry weight

Similarly, regardless of levels of temperature or CO_2 concentration, *C. gigas* and *O. edulis* showed different respiration rates depending on the composition of each species mixture (Fig. 2, Fig. S2 and Table S2a in Supplement 3, Oyster: *L*-ratio = 21.44, df = 5, p < 0.001). Specifically, *O. edulis* had greater respiration rates whilst in interspecific mixture than when alone (Table S2b; single vs. inter, $t = 2.17$, p < 0.032). In contrast, *C. gigas* respired less when in

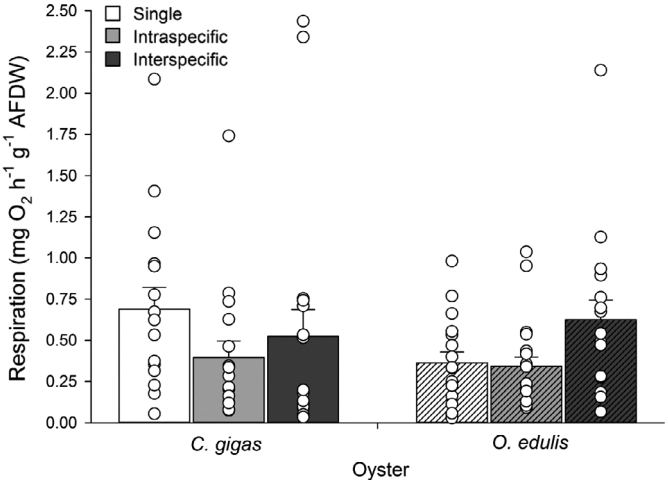

Fig. 2. Independent effect of oysters on the respiration of *Crassostrea gigas* and *Ostrea edulis* when alone or in intra- or interspecific mixtures. Bars are means ± SE, dots represent individual observations

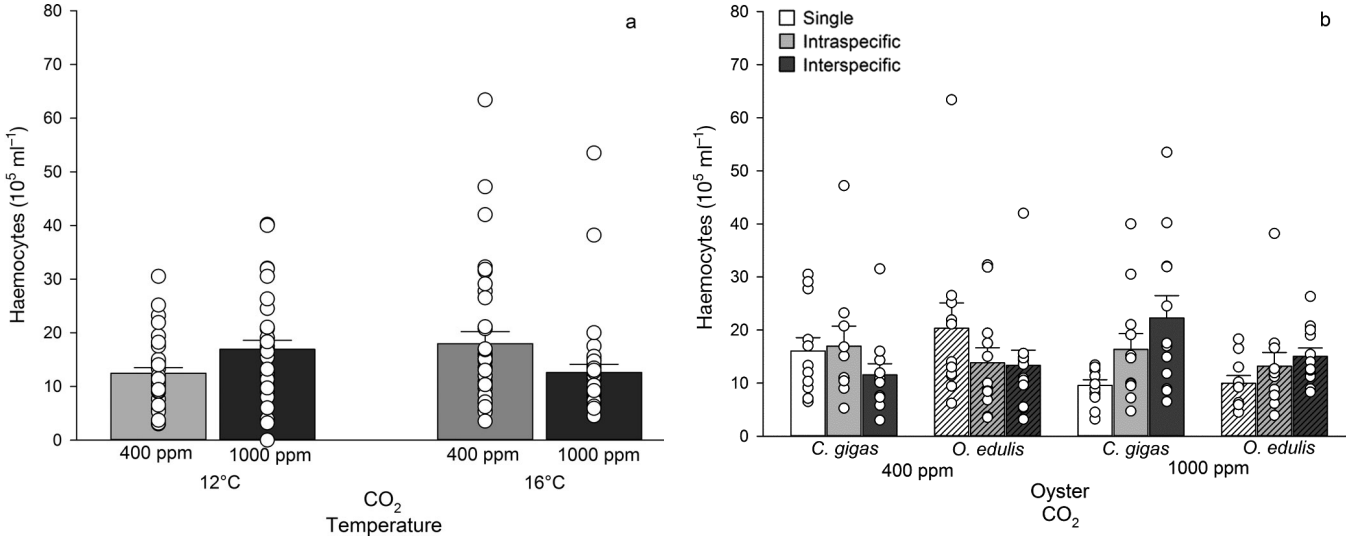

Fig. 3. Interactive effects of (a) temperature × CO_2 and (b) CO_2 × Oyster on the density of haemocytes in haemolymph of *Crassostrea gigas* and *Ostrea edulis*. In (b), individuals of each species were maintained alone or in intra- or interspecific mixtures. Bars are means ± SE, dots represent individual observations

intraspecific (single vs. intra, $t = -3.64$, $p < 0.001$) or interspecific (single vs. inter, $t = -3.16$, $p = 0.002$) mixtures relative to when alone.

Effects of elevated temperature and CO_2 on health

The combined effect of elevated temperature and CO_2, regardless of the composition of each species mixture (Temp × CO_2: *L*-ratio = 14.22, df = 5, p = 0.014), resulted in fewer haemocytes in the haemolymph of oysters (Fig. 3a, Table S3a,b in Supplement 4, 16°C 400 ppm vs. 16°C 1000 ppm: $t = 3.35$, $p = 0.001$). The number of haemocytes, however, depended on whether individuals were in inter- or intraspecific mixtures and on CO_2 concentration (Fig. 3b, Fig. S3, Table S3a,c; CO_2 × Oyster: *L*-ratio = 9.04, df =12, p = 0.003). Specifically, at elevated CO_2 concentration and whilst in intra- (single vs. intra, $t = 2.11$, $p = 0.037$) or interspecific (single vs. inter, $t = 2.94$, $p = 0.004$) mixture, *C. gigas* had more haemocytes than lone individuals (Fig. 3b, Fig. S3, Table S3c). Similarly, at the elevated CO_2 concentration, *O. edulis* in interspecific mixtures had more haemocytes than lone individuals ($t = 2.36$, $p = 0.020$).

Phagocytic rates were similar for *C. gigas* and *O. edulis*, regardless of species composition and temperature, but were affected by CO_2 concentrations (Fig. 4, Fig. S4 & Table S4 in Supplement 5, CO_2: *L*-ratio = 6.07, df = 1, p = 0.014), with greater rates observed at 400 ppm than at 1000 ppm ($t = 2.45$, $p = 0.016$).

Protein concentrations in the adductor muscle were affected by the interaction between temperature and oyster (Fig. 5, Fig. S5 & Table S5a in Supplement 6, Temp × Oyster: *L*-ratio = 12.77, df = 5, p = 0.025), but no further effects could be determined for either species of oyster (Table S5b).

Glucose concentrations were extremely variable for each species of oyster, and were not affected by our explanatory variables (Fig. S6 in Supplement 7).

DISCUSSION

Oysters are in decline globally, with a ~85% loss over the past century due to over harvesting (Beck et al. 2011). By removing phytoplankton, suspended solids and organic particles from the water column, filter-feeding bivalve molluscs play a key role in nutrient cycling and the control of coastal eutrophi-

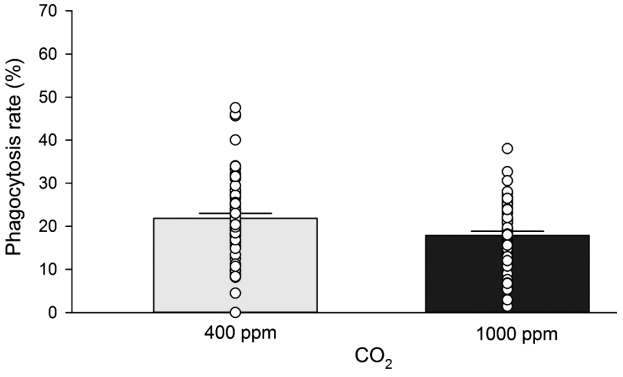

Fig. 4. Independent effect of CO_2 on the phagocytosis rate of oysters. Bars are means ± SE, dots represent individual observations

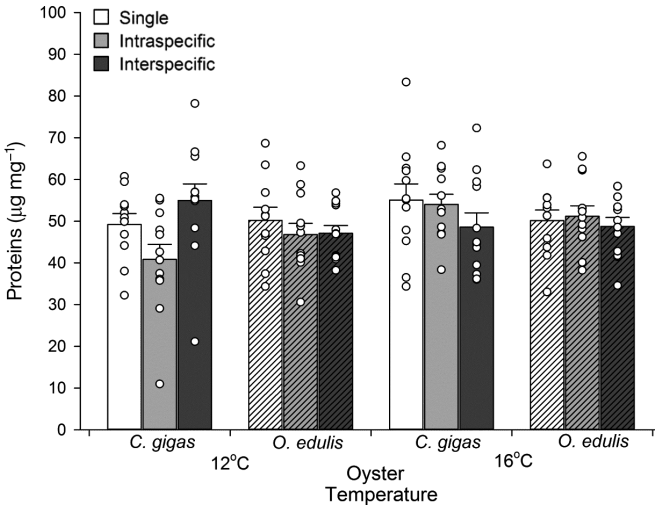

Fig. 5. Interactive effect of temperature × Oyster on the protein concentration in *Crassostrea gigas* and *Ostrea edulis*. Individuals of each species were maintained alone or in intra- or interspecific mixtures. Bars are means ± SE, dots represent individual observations

cation (Newell & Jordan 1983, Ward & Shumway 2004, Fulford et al. 2010). Consequently, further reduction of oyster reefs as a result of climate change could have severe socio-economic impacts (Ekstrom et al. 2015). We have demonstrated that the health, but not the biological activity, of *Crassostrea gigas* and *Ostrea edulis* are altered by future environmental conditions and that the magnitude of this response is dependent on the presence and type of competitive interaction. Effects of exposure to elevated CO_2 are likely due to disruption of the acid–base balance leading to physiological changes (Lannig et al. 2010). Here, decreasing pH negatively affected both species of oyster by reducing the rate of phagocytosis. Although an increase in phagocytosis rate can also indicate stress in bivalves, (e.g. in response to heavy metals, Parry & Pipe 2004; or rising temperatures, Gagnaire et al. 2006), decreases in phagocytosis rates are commonly reported in bivalves following periods of environmental stress (Pipe & Coles 1995, Gagnaire et al. 2007, Hooper et al. 2007, Bouchard et al. 2009). At elevated temperature and CO_2, both species of oyster had fewer haemocytes overall. A reduction in the total number of haemocytes has been found in other bivalves in response to stressors such as heavy metal contamination (Pipe & Coles 1995) and starvation (Husmann et al. 2011). The number of total haemocytes, however, increased when oysters were in mixture, indicating a modifying effect of competitive interactions at increased CO_2. When exposed to elevated CO_2 and interspecific mixtures, both species of oyster had a greater number of circulating haemocytes. Haemocytes are a primary line of defence against contaminants and infections; they are directly linked with immune function (Fisher et al. 1987), and departures from baseline counts (whether an increase or a decrease) can therefore indicate stress (Gagnaire et al. 2006). Elevated haemocyte counts in invertebrates can also be a symptom of other health problems and can be associated with decreased fitness (Auld et al. 2012). A compromised immune status of oysters in response to elevated atmospheric CO_2 could lead to an increased susceptibility to parasites or disease. Even intraspecific competition altered the immune response of *C. gigas* to elevated CO_2. It is therefore likely that the density of oyster beds could affect how individuals respond to climate change; densities of *C. gigas* forming reefs in the wild, or stocked for aquaculture, can be orders of magnitude greater than the densities (~40 m^{-2}) used here (Mitchell 2006). This has implications for determining appropriate stocking densities for oyster management and/or restoration initiatives.

Competition for resources (e.g. space, food) between species may be altered by climatic change; for example, competitive dynamics between fleshy and calcareous species of seaweed amplify the physiological responses of calcareous algae to acidification, with the calcareous species eventually being overgrown by fleshy morphological forms under acidified conditions, even though recruitment was similar (Kroeker et al. 2013). Here, competitive effects resulted in alterations to the biological functioning (clearance and respiration rates) of the oysters. Notably, regardless of the climatic scenario, *C. gigas* filtered at a greater rate than *O. edulis* and, when in intra- or inter- specific mixtures, *C. gigas* filtered at a greater rate than when alone. In contrast, *O. edulis* had lower clearance rates when in the presence of other oysters (either inter- or intraspecific mixtures). A recent experiment found that *C. gigas* and *O. edulis* filter and retain at least 5 of the same species of algae (Nielsen et al. 2017). It is therefore possible that where the 2 species co-occur and resources are limited, *O. edulis* may be outcompeted by *C. gigas*. However, *C. gigas* appears to exhibit a greater ability to regulate its metabolism relative to *O. edulis*. For example, respiration rates were lower in *C. gigas* exposed to intra- or interspecific competition than in those kept alone, whilst *O. edulis* increased its respiration rates when in interspecific mixture. Alterations to respiration rates can also indicate stress in bivalves (Vernberg & Vernberg 1975), with increases in respiration sometimes associated with stress due to

poor environmental conditions (Lannig et al. 2006). In some cases, however, a suppression of respiration, as found here for *C. gigas*, can be a strategy to conserve energy and increase probability of survival (Guppy & Withers 1999).

O. edulis seems to be more sensitive than *C. gigas* to some other perturbations, including pathogenic infections (e.g. the parasitic protist *Bonamia ostreae*; Culloty et al. 1999, Comesaña et al. 2012) and heavy metal contamination (Helm et al. 2004). Conversely, *C. gigas* is susceptible to infection with ostreid herpes virus-1 (OsHV-1), whereas there are no known occurrences of infection by this virus in *O. edulis*. Clearly, the future status of both oysters in natural systems remains a complex function of the environmental tolerance of both species, in competition and in isolation, combined with the tolerance of existing and emergent protozoan, viral and bacterial pathogens, which also varies as a function of environmental condition (e.g. see de Kantzow et al. 2016).

Given the long-term processes involved with a changing climate, longer exposure periods than attempted here may reveal differences in responses over time as species interactions develop and individuals acclimate to increased concentrations of CO_2 and temperature (Godbold & Solan 2013). Whilst the physiological responses of bivalves to scenarios of climatic change are highly variable and complex (Matozzo et al. 2012), our findings indicate that these responses are further complicated by intra- and interspecific competitive interactions that are sufficient to alter species contributions to functioning (Godbold et al. 2017). Despite the complexity of the results, where the oysters co-occur, it is possible that *C. gigas* may outcompete *O. edulis* for food due to their greater clearance rate. Continued efforts to protect *O. edulis* beds from *C. gigas* invasion are therefore prudent. In the current study, closed chambers were used to estimate clearance rates. Although different methods are likely to produce similar results once standardised (Widdows 1985, 2001), future work using flow-through chambers will help to strengthen understanding of competitive interactions between native and invasive oysters. The 2 species, however, have different reproductive mechanisms (*C. gigas*, broadcast spawning; *O. edulis*, brooding), and it is possible that larval development could be differentially affected by climate change. Indeed, a similar species of brooding oyster, *O. lurida*, builds shells more slowly than *C. gigas,* potentially reducing the energetic burden of acidification at early life stages (Waldbusser et al. 2016). It is possible that brooding species may have inadvertently evolved (exaptation) to cope with high CO_2 environments due to the CO_2-enriched environment of the brood chamber (Waldbusser et al 2016). Whilst the present study has not considered the reproductive potential of either species in isolation or in competition, it is already known that multiple climatic stressors are particularly important for reproduction and during early developmental stages (Byrne 2011). Hence, it is clear that the response of species to climatic change can be moderated in the short term by species' interactions with each other and their environment and by cumulative physiological responses that may accrue over the longer term as species acclimate to novel circumstances.

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