Indications of future performance of native and non-native adult oysters under acidification and warming

Anaëlle J. Lemasson[1,2*]; Jason M. Hall-Spencer[1,3]; Stephen Fletcher [2,4,]; Samuel Provstgaard-Morys[1]; Antony M. Knights[1]

[1] Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, Plymouth University, Plymouth, UK

[2] Marine Conservation and Policy Research Centre, School of Biological and Marine Sciences, University of Plymouth, Plymouth, UK

[3] Shimoda Marine Research Centre, Tsukuba University, Japan

[4] UN Environment World Conservation Monitoring Centre, Cambridge, UK

*Corresponding author

E-mail: anaelle.lemasson@plymouth.ac.uk (AJL)

Abstract

Globally, non-native species (NNS) have been introduced and now often entirely replace native species in captive aquaculture; in part, a result of a perceived greater resilience of NSS to climate change and disease. Here, the effects of ocean acidification and warming on metabolic rate, feeding rate, and somatic growth was

assessed using two co-occurring species of oysters – the introduced Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*), and native flat oyster *Ostrea edulis*. Biological responses to increased temperature and pCO_2 combinations were tested, the effects differing between species. Metabolic rates and energetic demands of both species were increased by warming but not by elevated pCO_2. While acidification and warming did not affect the clearance rate of *O. edulis*, *M. gigas* displayed a 40% decrease at ~750 ppm pCO_2. Similarly, the condition index of *O. edulis* was unaffected, but that of *M. gigas* was negatively impacted by warming, likely due to increased energetic demands that were not compensated for by increased feeding. These findings suggest differing stress from anthropogenic CO_2 emissions between species and contrary to expectations, this was higher in introduced *M. gigas* than in the native *O. edulis*. If these laboratory findings hold true for populations in the wild, then continued CO_2 emissions can be expected to adversely affect the functioning and structure of *M. gigas* populations with significant ecological and economic repercussions, especially for aquaculture. Our findings strengthen arguments in favour of investment in *O. edulis* restoration in UK waters.

Keywords: climate change; ecosystem change; exotic species; living resources; oyster; physiology; UK

Introduction

Ocean acidification and warming (OAW) affects the behaviour, metabolism, and performance of a diversity of marine organisms (Barry *et al.*, 2011; Kroeker *et al.*, 2013). Early-life history stages, especially important in population persistence, are shown to be particularly vulnerable (Byrne & Przeslawski, 2013; Kurihara, 2008; Przeslawski *et al.*, 2015), and is raising concerns for the continued provision of important ecosystem services (Lacoue-Labarthe *et al.*, 2016; Lemasson *et al.*, 2017; Sunday *et al.*, 2016; Weatherdon *et al.*, 2016). Calcifying species are especially at risk as they are susceptible to alterations in ocean chemistry (Hofmann *et al.*, 2010; Parker *et al.*, 2013; Pörtner *et al.*, 2014), manifested by increased metabolism, respiration and energy expenditure (Pörtner & Farrell, 2008).

Species most resilient to OAW may well be those best able to enhance their energy assimilation. A common way for marine organisms to balance their energy intake and expenditure is to increase their feeding rate, (Ramajo *et al.*, 2015; Sanders *et al.*, 2013; Thomsen *et al.*, 2012; Towle *et al.*, 2015) or reallocate energy through partitioning and trade-offs between reproduction, somatic growth and calcification (Leung *et al.*, 2017). Species less able to manipulate their feeding activity to offset stress from OAW may show reduced energetic levels and capacity for metabolic maintenance (Houlbrèque *et al.*, 2015; Mackenzie *et al.*, 2014; Vargas *et al.*, 2015). OAW may therefore be an important selection pressure that dictates the distribution of species and functioning of marine ecosystems. Today, there is pressure to understand the effects of OAW on species that provide important ecosystem goods

and services (Osborn *et al.*, 2017) and mitigate negative impacts of OAW to ensure the sustainable delivery of the services derived from those species in to the future.

In the UK, the native European flat oyster, *Ostrea edulis*, and the non-native Pacific oyster, *Magallana gigas* (which until recently was named *Crassostrea gigas*) are two valuable commercially-exploited species. They provide relatively similar and numerous ecosystem services (Herbert *et al.*, 2012) including: reef formation, erosion control, improvement of water quality (through cycling and purification), raw material supply, and food provision (through aquaculture and fisheries) (see Coen *et al.*, 2007, for a review of oyster-associated ecosystem services; Herbert *et al.*, 2012). Historically, *O. edulis* was highly abundant and was the basis of a major shellfish fishery in the UK and Europe (Coolen, 2017; Orton, 1937), but today is a protected species in the UK with active restoration efforts underway to counteract ever declining stocks from overharvesting, competition, pests, diseases, and reproductive failures (Laing *et al.*, 2006; Lallias *et al.*, 2010; Woolmer *et al.*, 2011). In contrast, *M. gigas* was introduced to the UK within regulated aquaculture settings in the mid-20th Century in response to the decline of *O. edulis,* and today this species represents over 90% of UK oyster aquaculture production, worth an estimated £10.14 million annually (Humphreys *et al.*, 2014).

Magallana gigas was originally introduced under the assumption that local seawater temperatures would prevent its reproduction and the formation of viable wild populations, nonetheless the species has formed unintended wild populations on UK and Irish shores where it is often considered invasive (Dolmer *et al.*, 2014; Herbert *et al.*, 2016; Kochmann *et al.*, 2013; Troost, 2010). Despite the occurrence of wild

93 populations, the harvest of *M. gigas* is currently mostly limited to regulated
94 aquaculture sites (Herbert *et al.*, 2012). Today, beds comprised of both *M. gigas* and
95 *O. edulis* occur, such as in Ireland (Zwerschke *et al.*, 2017) and at sites along the
96 South-West coast of the UK (pers. observations; Fig 1.a,b). It is often speculated that
97 *M. gigas* and *O. edulis* compete for space and resources, with the presence of
98 *M. gigas* having negative consequences for *O. edulis*, although there is no
99 documented evidence of this. In fact, a recent study suggests no evidence of
100 competition between the two species (Zwerschke *et al.*, 2016). Nevertheless, the
101 negative perception of wild *M. gigas* populations has led to management measures
102 being introduced to prevent its further proliferation, and to promote the recovery of
103 *O. edulis* (Harding *et al.*, 2016; Herbert *et al.*, 2012; Laing *et al.*, 2006; Sawusdee,
104 2015; Woolmer *et al.*, 2011).
105

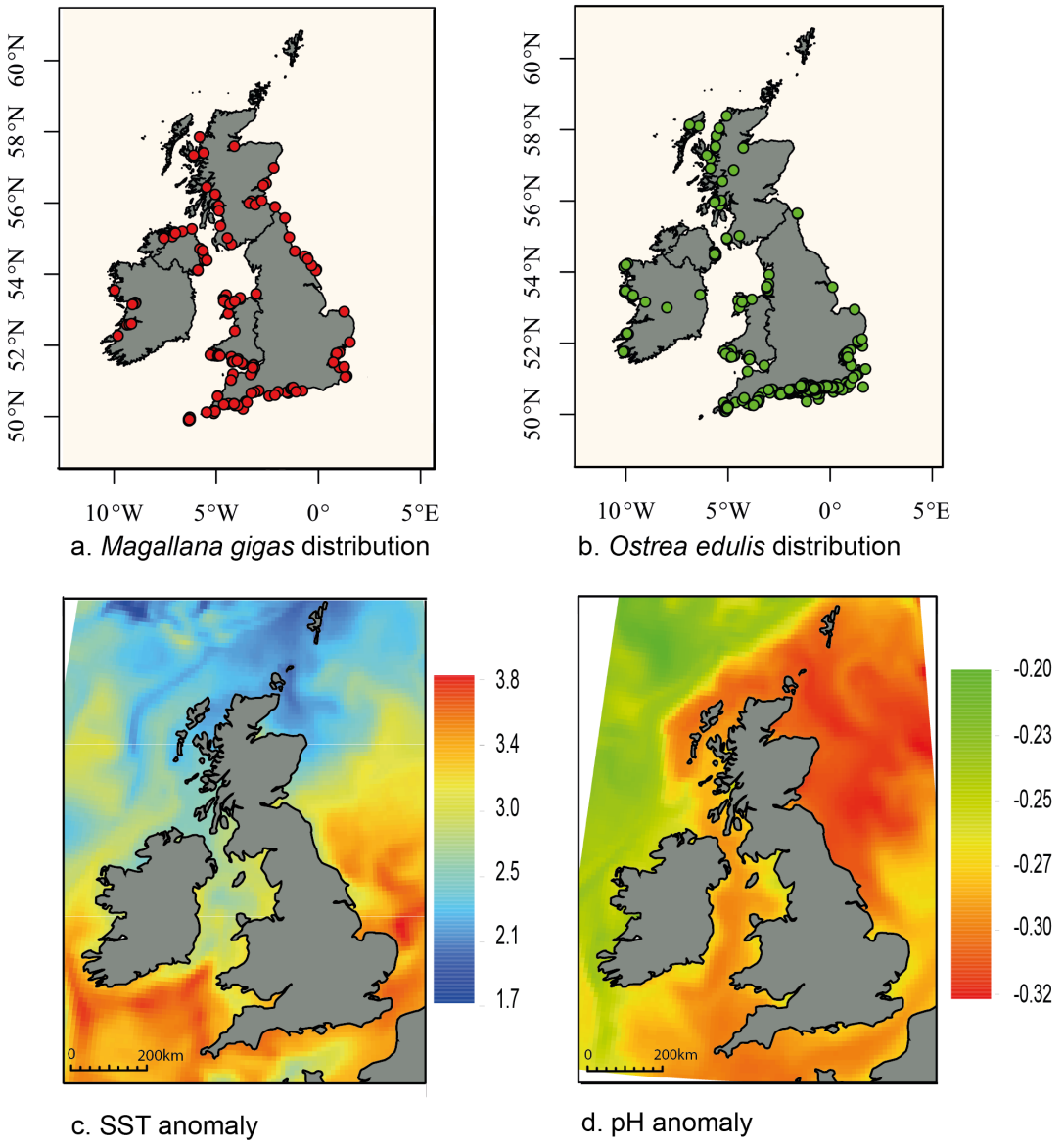


Fig 1. Current UK wild distribution of (a) *Magallana gigas* (red) and (b) *Ostrea edulis* (green) (data obtained from the Global Biodiversity Information Facility (GBIF) database), (c) maximum mean annual sea surface temperature (SST) anomaly (SST, in °C; medium emission scenario IPCC SRES: A1B for 2070-2099, data obtained from UKCP09) and (d) minimum mean annual surface water pH anomaly (scenario for 2080-2099, data obtained from the Marine Ecosystem Evolution in a Changing Environment (MEECE) database).

Since its introduction to Europe, *M. gigas* has been spreading northward across European shores (Shelmerdine *et al.*, 2017) facilitated by increasing average sea surface temperatures (SST) (Angles d'Auriac *et al.*, 2017; Rinde *et al.*, 2016; Thomas *et al.*, 2016; Townhill *et al.*, 2017). In contrast, the extent of *O. edulis* is continuing to decline, and native oyster reefs are considered some of the most endangered coastal habitats in Europe (Airoldi & Beck, 2007; Beck *et al.*, 2011). The success of introduced species is often attributed to their greater tolerance (and physiological plasticity) to fluctuating environmental conditions than their native counterparts (Hall-Spencer & Allen, 2015; Lodge, 1993; Stachowicz *et al.*, 2002). For example, in Australia, early-life stages of *M. gigas* (introduced) were shown to be less sensitive to OAW than the native *Saccrostrea glomerata* (Parker *et al.*, 2010) and in Brazil, introduced *M. gigas* was more resilient to extreme hypercapnic conditions than the native *Crassostrea brasiliana* (Moreira *et al.*, 2018). A similar response has also been shown in other taxa. For example, in Spain, the non-indigenous mussel *Xenstrobus securis* was found more resilient to reduced pH than the native *Mytilus galloprovinciallis* (Gestoso *et al.*, 2016). This precedent would suggest it is not unreasonable to expect *M. gigas* to display similar tolerance in the UK, and be more resilient than its native counterpart *O. edulis* to future change in environmental conditions.

As calcifiers, both oyster species can be expected to be negatively impacted by ocean acidification. The risks that ocean acidification pose to oysters were first highlighted in 2007 when hatcheries in the Pacific North-West region of the US suffered mass mortalities of Pacific oyster larvae. Upwelling of acidified water with

low aragonite saturation (a principle biomineral used in shell maintainance) caused an 80% reduction in hatchery production and significant financial losses (Barton *et al.*, 2015; Cooley *et al.*, 2017). Since then, studies into the effects of OAW on oysters and other commercially important bivalves have rapidly increased in number. Extensive work has been done on early life stages, demonstrating sensitivity to OAW, but also other environmental stressors (Cole *et al.*, 2016; Parker *et al.*, 2017a). Responses include slower calcification (Waldbusser *et al.*, 2016), delayed growth, and delayed or abnormal development (Gray *et al.*, 2017; Parker *et al.*, 2010; Waldbusser *et al.*, 2015).

Less work has been undertaken on juveniles and adults, although impacts on early life stages has been shown to "carry-over" into these life-history stages (Hettinger *et al.*, 2013b; Hettinger *et al.*, 2012). Both juveniles and adults have shown altered immune response (Liu *et al.*, 2016; Wang *et al.*, 2016), reduced calcification and shell growth (Beniash *et al.*, 2010; Waldbusser *et al.*, 2011b; Wright *et al.*, 2014), increased shell dissolution (Waldbusser *et al.*, 2011a), and reductions in shell strength (Dickinson *et al.*, 2012; Mackenzie *et al.*, 2014; Welladsen *et al.*, 2010). Crucial metabolic activities, such as respiration and feeding, can also be impacted (Comeau *et al.*, 2008; Dove & Sammut, 2007; Scanes *et al.*, 2017), the resulting stress likely leading to mortality and reduced population resilience, impaired biological functioning, and reduced ecosystem service provision (Lemasson *et al.*, 2017).

Temperature is considered a major determinant of species and ecosystem structure and functioning. For *M. gigas*, its thermal range is reported as 1.8-35°C (see FAO

164 factsheet; Fig 1.a). While the thermal optima is not known for this species (and may
165 vary as a result of local adaptation, see Sanford & Kelly, 2011), given its evolutionary
166 origins, it is argued that in the UK, increasing average SST that is associated with
167 climate change allows increased metabolic performance, individual growth, and
168 range expansion. For *O. edulis*, the thermal range is less well defined and where
169 data are available, the evidence is contradictory (Shelmerdine & Leslie, 2009). In
170 one instance, temperatures higher than 20°C have been shown to be suboptimal,
171 negatively affecting growth, metabolism and filtration activity in juvenile *O. edulis*
172 (Buxton *et al.*, 1981), but conversely, cold has also been shown to limit larval
173 production, recruitment, and growth below temperatures of 17.5°C (Beiras *et al.*,
174 1995; Davis & Calabrese, 1969; Orton, 1940; Robert *et al.*, 2017; Walne, 1958).
175 Differences in response may be related to dispersal capacity. *Magallana gigas*
176 generate solely planktotrophic larvae, whereas *O. edulis* first brood (larviparous)
177 before generating shorter planktonic duration planktotrophic larvae, which arguably
178 limits dispersal capacity and promotes a greater likelihood of local adaptation
179 (Bertness & Gaines, 1993) in *O. edulis* over *M. gigas* making developmental
180 performance thresholds less clear.
181
182 It is therefore unclear how continued CO_2 emissions and associated increases in
183 ocean acidification and warming will affect wild and harvested populations of
184 *M. gigas* and *O. edulis* in the UK, nor what the consequences for ecological
185 functioning and provisioning of ecosystem services will be. Substitution of one
186 species for another, either partially or entirely, can produce significant ecological
187 impacts (Krassoi *et al.*, 2008), but since *M. gigas* is, in theory, able to provide similar
188 ecological functions and ecosystem services as *O. edulis* (Herbert *et al.*, 2016;

189 Zwerschke *et al.*, 2016) and is currently present in higher abundances, efforts to
190 eradicate it may be unwise if it becomes increasingly prevalent under climate change.
191
192 In this study, we test the effects of OAW on the physiological responses of a native
193 and a non-native species of UK oyster to determine the potential respective
194 ecosystem service contribution of these species both today and in the future.
195 Individual measures of fitness were assessed using Standard Metabolic Rate (SMR),
196 Clearance Rate (CR), and Condition Index (CI) under simulated warming and
197 acidification scenarios over a 12 week period. SMR was used as a proxy for
198 metabolic costs and energetic requirements, while CR informed us of energy uptake.
199 CI was used to assess overall health and quality and the availability of energy
200 reserves within somatic tissues. Our hypotheses were that future OAW conditions
201 would induce metabolic costs for both species of oysters, along with compensatory
202 increases in energy acquisition through enhanced feeding. Additionally, we
203 hypothesised that *M. gigas* would show evidence of higher tolerance to warming and
204 acidification than *O. edulis*.
205

206 Methods

207 Organism collection and acclimation

208 Adult Pacific oysters (*M. gigas;* 112.4 ± 6.9 mm in length and weighing 285.9 ± 13.4
209 g), and European flat oysters (*O. edulis*; 79.4 ± 5.7 mm in length and weighing 92.8
210 ± 15.1 g) were hand-collected from a wild population at a low-intertidal fully marine
211 site in Plymouth Sound, UK (50°23’29.95”N, 004°13’16.77”W), in July 2015 and
212 January 2016, respectively. Oysters were cleaned of epibionts and allowed to

acclimatise in a recirculating system to ambient laboratory conditions of ~16.5°C and atmospheric pressure of 400ppm at the University of Plymouth (UK). Over an acclimation period of 14 days, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

Experimental design

Following acclimation to laboratory conditions, 24 oysters were placed in their own 3 L experimental tank (four tanks per OAW scenario) and exposed to the treatment conditions. Three levels of pCO_2 (ambient 400 ppm, intermediate 750 ppm, elevated 1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C), were tested in an orthogonal experimental design to simulate current and future OAW scenarios. These six scenarios are in line with warming and acidification conditions predicted for the UK (Fig 1c,d). As such, temperature scenarios reflected maximum current SST (16.8°C), and predicted SST for the end of the century (20°C, corresponding to the predicted increase by 3-4°C in average SST along the South-West of the UK). However, it should be noted that such predictions do not taken into account localized variability in environmental conditions often experienced by organisms in coastal and estuarine habitats, and which may be amplified by future OAW. Due to capacity limitations of our mesocosm system, the experiment ran for 12 weeks between September and November 2015 for *M. gigas*, then repeated with *O. edulis* following the same procedures between January and March 2016. As such, the environmental conditions experienced by each species were inherently different due to natural seasonal variations in seawater properties driven by differences in atmospheric conditions (e.g. barometric pressure). The resulting pH conditions were therefore

237 different between experiments (Fig 2, S1 Table), but the effect size (magnitude of
238 difference in pH between experimental treatments) were comparable.
239

240 ## Mesocosm set-up

241 The ocean acidification and warming mesocosm system used during the experiment
242 is a modified version of the one described by Calosi *et al.,* (2013). Briefly, each
243 treatment consisted of a header tank (volume=80 L) of seawater, supplied from one
244 of two sumps (16.5 °C and 20 °C), and aerated with either the ambient air pipe
245 (pCO_2 400 ppm) or one of the two CO_2- enriched air pipes (pCO_2 750 ppm, pCO_2
246 1000 ppm). Ambient air consisted of laboratory air subjected to diurnal variability.
247 Mixing in all header tanks was achieved using a submersible pump (Hydor Koralia
248 Nano 900, Italy). CO_2 gas mix were obtained by slowly releasing CO_2 into two
249 Buchner flasks where it mixed with ambient air, achieving two different levels of
250 pCO_2, using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK). As such,
251 throughout the experiment the three CO_2 levels varied in a similar manner following
252 natural variations in CO_2 in the ambient air. The treatments thus took account of
253 natural daily variability, which has been suggested as a critical consideration for
254 climate change experimental studies (Humphreys, 2016; Reum *et al.*, 2015). CO_2
255 levels in the two CO_2-enriched pipes were recorded using a CO_2 analyser (LI-820;
256 LI-COR, Lincoln, NE, USA) and adjusted manually to the desired level twice daily.
257 CO_2 levels in the ambient air pipe were also recorded to monitor the levels of the
258 control treatments. Seawater was gravity-fed from the header tanks to each of the
259 corresponding replicate tanks (3 L transparent sealed containers) at a constant rate
260 of ~60 mL/min. The replicate tanks were held within four larger 300 L holding trays,
261 each sump supplying seawater to two of the holding trays, effectively creating water

baths maintaining the replicate tanks at the desired temperature (two water baths at 16.5°C, two water baths at 20°C). Each tray held two replicates of each CO_2 levels (four replicates per temperature and CO_2 treatment). Excess seawater was allowed to overflow from the trays to their corresponding sump, where it was filtered, aerated, and recirculated to the corresponding header tanks and trays using a submersible pump (1262; EHEIM GmbH and Co. KG, Deizisau, Germany). Seawater in the system originated from Plymouth Sound (UK) and, following mechanical filtering and UV sterilization, was added and replaced on a daily basis to account for evaporation, Deionized water was added as needed to maintain stable salinity levels. In elevated temperature treatments, seawater was increased to 20°C using aquarium heaters (50 W aquarium heater; EHEIM Jager GmbH and Co. KG, Stuttgart, Germany) placed in header tanks and holding trays.

Measurements of seawater parameters

Temperature, salinity, and pH were measured daily in all replicate tanks (Fig 2. see also S1 Table and S1 Fig. for details of temperature and pH data). Salinity was measured using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK) and temperature measured using a digital thermometer (TL; Fisher Scientific, Loughborough, UK). pH was measured using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK) coupled to a pH meter (S400 SevenExcellence™; Mettler-Toledo Ltd, Beaumont Leys, UK), following calibration with NIST traceable buffers. pH in the header tanks was also monitored (data not shown). Total Alkalinity (A_T) was measured once a week in each of the replicate tanks. 125 mL water samples were transferred to borosilicate bottle with Teflon caps and poisoned with 30 μL of saturated $HgCl_2$ solution (0.02 % sample volume) before

being kept in the dark until measurement by automatic Gran titration (Titralab AT1000 © Hach Company). Partial pressure of carbon dioxide (pCO_2) and saturation states of calcite and aragonite (Ωcalcite and Ωaragonite), were calculated at the end of the experiment using CO2 SYS (Pierrot *et al.*, 2006), employing constants from Mehrbach *et al*. (1973) refitted to the NBS pH scale by Dickson and Millero (1987) and the KSO4 dissociation constant from Dickson (1990) (Fig 2., see also S1 Table).

Throughout the duration of the experiment, oysters were fed daily with 20 mL of a live algae (mixed diet of *Isochrysis galbana* and *Tetraselmis* sp.) to obtain a concentration of approximately 10^8 cell.L^{-1} within the experimental tank. Three times a week, tanks were gently brushed and siphoned to remove faeces and excess food, thereby insuring acceptable water quality, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater.

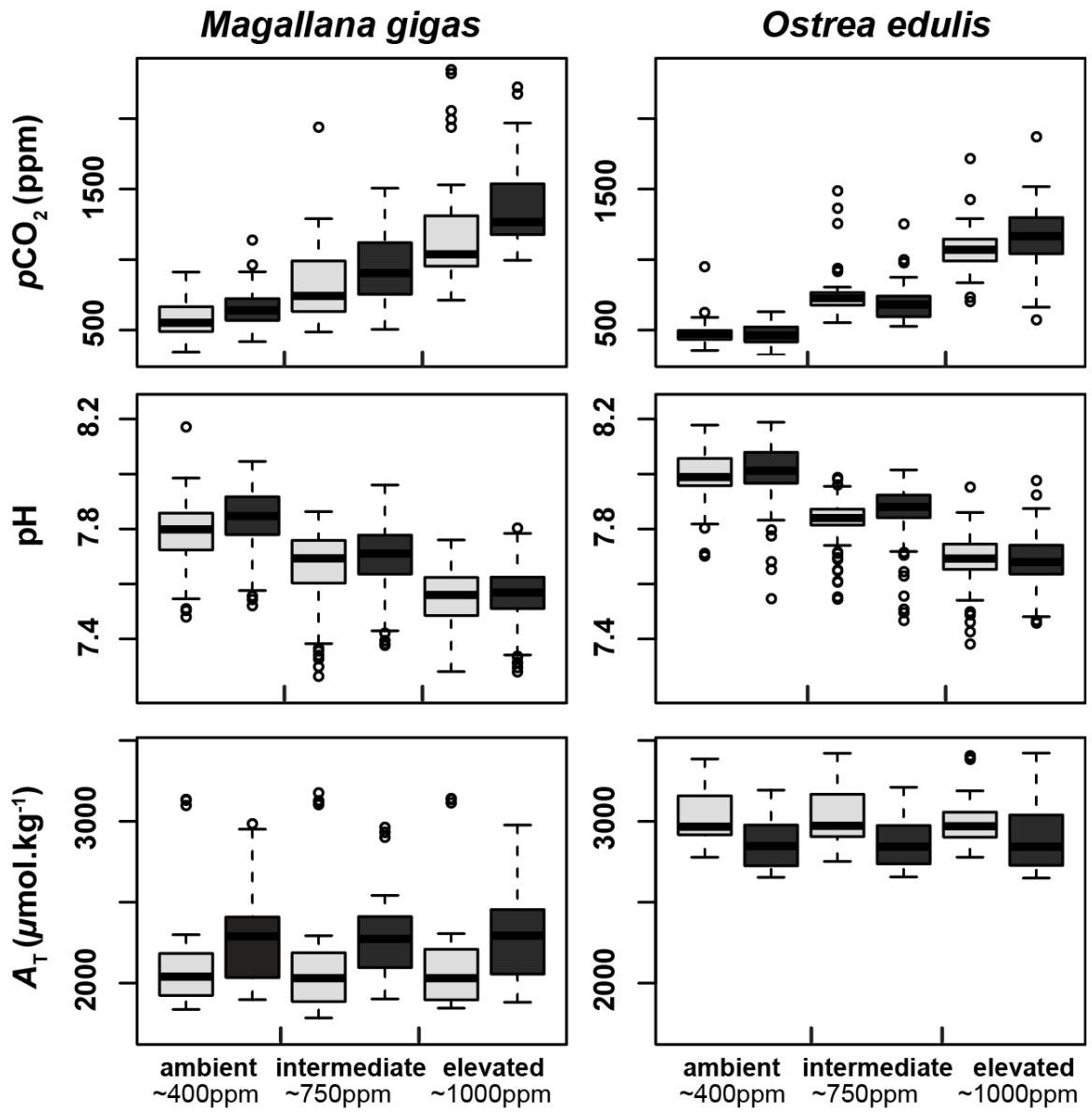


Fig 2. Variation in pCO_2, pH, and total alkalinity (A_T), of seawater in the experimental treatments. ppm=part per million. Grey= control temperature (~16.8°C, black= elevated temperature (~20.0°C). Data are pooled based on daily (pH) and weekly (A_T) measurements over the 12 week experimental duration. Weekly pCO_2 values were calculated using CO2 SYS (Pierrot D *et al.*, 2006).

Physiological measurements

309 Following 10 days, 5, 9, and 12 weeks of exposure to each OAW scenario, metabolic
310 activity and energy acquisition were measured for each oyster (N=24 per species; 4
311 per OAW scenario). To limit post-prandial metabolism of food and excretion of
312 faeces that could alter the results, oysters were not fed for 24h prior to
313 measurements.
314

315 ## Standard metabolic rate

316 Respiration rates were measured as proxy for Standard Metabolic Rates (SMR),
317 using microfiber optic oxygen sensors (Firebox 4, PreSens Germany,
318 www.presens.de). All oysters (N=24 per species; 4 per OAW scenario) was placed in
319 a 1.2 L air-tight container, filled with 1 L of seawater filtered to 2 μm and pre-
320 equilibrated to their respective experimental pCO_2 and temperature treatment. To
321 maintain stable temperature in the chambers, all measurements were conducted in
322 controlled-temperature rooms. The seawater in each chamber was stirred using a
323 magnetic rod for the duration of the assay (350 rpm). Respiration measurements
324 started when the oyster resumed filtration, and ended either when O_2 saturation
325 reached 80% to prevent the organisms from experiencing hypoxic conditions, or
326 when the oyster shut its valves. O_2 measurements were corrected for temperature,
327 salinity, and barometric pressure using Green and Carritt's (1967) oxygen solubility
328 coefficients and Weiss' (1970) vapour pressure values, as well as corrected for
329 background bacterial respiration (the reduction in dissolved oxygen in each tank
330 without shellfish was subtracted from total O_2 reductions in the same tank with
331 shellfish) and the individuals' volume and dry weight, to obtain absolute quantities of
332 oxygen consumed. Temperature and salinity was recorded at the start of each assay
333 as described above. Barometric pressure data were obtained from the Plymouth Live

Weather Station (http://www.bearsbythesea.co.uk). Dry weight was assessed at the end of the 12-wk exposure (see below "Condition Index" section for details). Volume was determined using the water displacement method. SMR was calculated as follows:

$$SMR = \frac{V_r(L) \times \Delta C_w O_2 (mgO_2.L^{-1})}{\Delta t(h) \times bw(g)} \quad [1]$$

where SMR is the oxygen consumption normalized to 1 g of dry tissue mass (DW) in $mgO_2.g^{-1}$ DW $.h^{-1}$; V_r is the volume of the respirometry chamber minus the volume of the oyster (L); $\Delta C_w O_2$ is the change in water oxygen concentration measured ($mgO_2.L^{-1}$); Δt is measuring time (h); and bw is the dry tissue mass (g) of the oyster.

Clearance rates

Directly following the respirometry assay, the Clearance Rate (CR) of all oysters from each treatment (n=4) was calculated using methods previously described in Coughlan (1969) and Sanders *et al.,* (2013). Individuals selected for clearance rate measurements were the same individuals used for the respirometry assay described above. Oysters were placed in a 1.2 L chamber, filled with 1 L of seawater filtered to 2 μm and pre-equilibrated at their respective experimental pCO_2 and temperature treatment. To maintain stable temperature in the chambers, all measurements were conducted in controlled-temperature rooms. ~20 mL of the same live algae culture (mix of *Tetraselmis* sp. and *Isochrysis galbana*) was added to each chamber when oysters started filtering. To allow homogeneous mixing of algae, the seawater in each chamber was stirred using a magnetic rod (350 rpm). Three replicate 5mL water samples were taken from haphazard locations throughout the chamber (1) prior to the addition of food (t_i); (2) immediately after addition of food (t_0) to check the

initial algal concentration; and (3) at 10 minute intervals following food addition for a duration of 40 minutes, providing 6 sampling times (i.e. t_i, t_o, t_1, t_2, t_3, and t_4). If the oyster shut its valves, the chronometer was stopped and restarted once the valves re-opened. Counts of algae in all water samples were performed in triplicate using a Coulter Counter (Beckman Coulter Z2). Clearance rates (CR) were calculated using the following equation after Coughlan (1969):

$$CR = \frac{V \times ln\left(\frac{C_{n-1}}{C_n}\right)}{t_n - t_{n-1}} \quad [2]$$

where CR is the clearance rate measured during the 10 minute interval between sampling times t_{n-1} and t_n, normalized to 1 g of dry tissue mass ($L^{-1}.g^{-1}DW.h^{-1}$), V is the volume of the chamber in L, C_{n-1} is the concentration ($cell.L^{-1}$) in the sample taken at time t_{n-1} (hour), and C_n is the concentration ($cell.L^{-1}$) in the sample taken at time t_n (hour). Results are presented as CRmax, the maximum clearance rate observed during the 40-minute incubation.

Condition index

The Condition Index (CI) of oysters was calculated at the end of each experiment based on dry weight following the method recommended by Lucas and Beninger (1985) and described in equation [3]. Condition indices are useful tools widely used in the aquaculture sector to evaluate the overall quality and health of bivalves (Knights, 2012; Marin *et al.*, 2003). They reflect their ability to withstand adverse conditions (Marin *et al.*, 2003) by describing the quantity of organic tissue present (Bodoy *et al.*, 1986).

$$CI = \frac{dry\ meat\ weight}{dry\ shell\ weight} \times 100 \quad [3]$$

Dry tissue weight was determined after each oyster was shucked using an oyster knife and oven-dried at 105°C until a constant mass was achieved.

Statistical analyses

All data were tested for the assumption of homogeneity of variances, and where not met, data were transformed using logarithmic or square-root transformations. If after transformations assumptions were still not met, equivalent non-parametric tests were conducted. Differences were considered statistically significant if $p < 0.05$. All data were analysed using the public domain software *R* (version 3.2.5 R Core Team, 2016). 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, and therefore, species were not formally compared and data analysed separately.

SMR and CR

SMR and CR data were analysed using linear mixed effects (lme) models with an autocorrelation argument (nlme package; see Zuur *et al.,* (2009)). 'Temperature' and 'pCO_2' were considered as fixed factors to assess differences in species' response to the treatments, and 'Exposure' (levels: 10 days, 5 wk, 9 wk, 12 wk) nested within 'Replicate' to partition differences due to individual oysters. If significant differences were present, *post-hoc* test was performed to assess differences between treatment levels (TukeyC and Multicomp packages). For each species, data were interrogated for the presence of fundamental relationships between the two physiological traits using the Pearson's correlation test.

406 Condition Index

407 Differences in CI with treatment were analysed using 2-factor ANOVA with
408 'temperature' (levels: 'control'; 'elevated') and 'pCO_2' (levels: 'ambient ~400ppm',
409 'intermediate ~750ppm', 'elevated ~1000ppm') as fixed factors. If significant
410 differences were present, *post-hoc* pairwise comparisons (Tukey HSD) were
411 performed to determine differences between treatment levels.

412

413 Results

414 Standard metabolic rate

415 For both species, there was clear inter-individual variability in responses (Fig 3.). The
416 linear mixed-effects model revealed differences in metabolic response depending on
417 exposure and OAW scenario S2 Fig.). For *M. gigas*, higher temperature, but not
418 pCO_2, increased SMR by >43% (Fig 3. $F_{1,18} = 11.51$, $p < 0.01$). For *O. edulis*,
419 exposure time led to a statistically significant decrease in SMR after 5 weeks ($F_{1,71} =$
420 25.55, $p < 0.001$), and temperature led to a statistically significant increase in SMR
421 of >39% ($F_{1,18} = 9.52$, $p < 0.01$). However, it should be noted that for *O. edulis,*
422 while the interaction between temperature and pCO_2 was marginally not significant
423 ($F_{1,66} = 3.50$, $p = 0.052$), clear trends were apparent. SMR decreased by up to 36%
424 under elevated pCO_2 conditions (750 and 1000 ppm) when oysters were kept at the
425 control temperature, but when the temperature was elevated, there was no change
426 in SMR even when pCO_2 was increased. This was especially notable under 1000
427 ppm pCO_2, where SMR was ~46% lower in the control temperature than in the warm
428 temperature treatment.

429

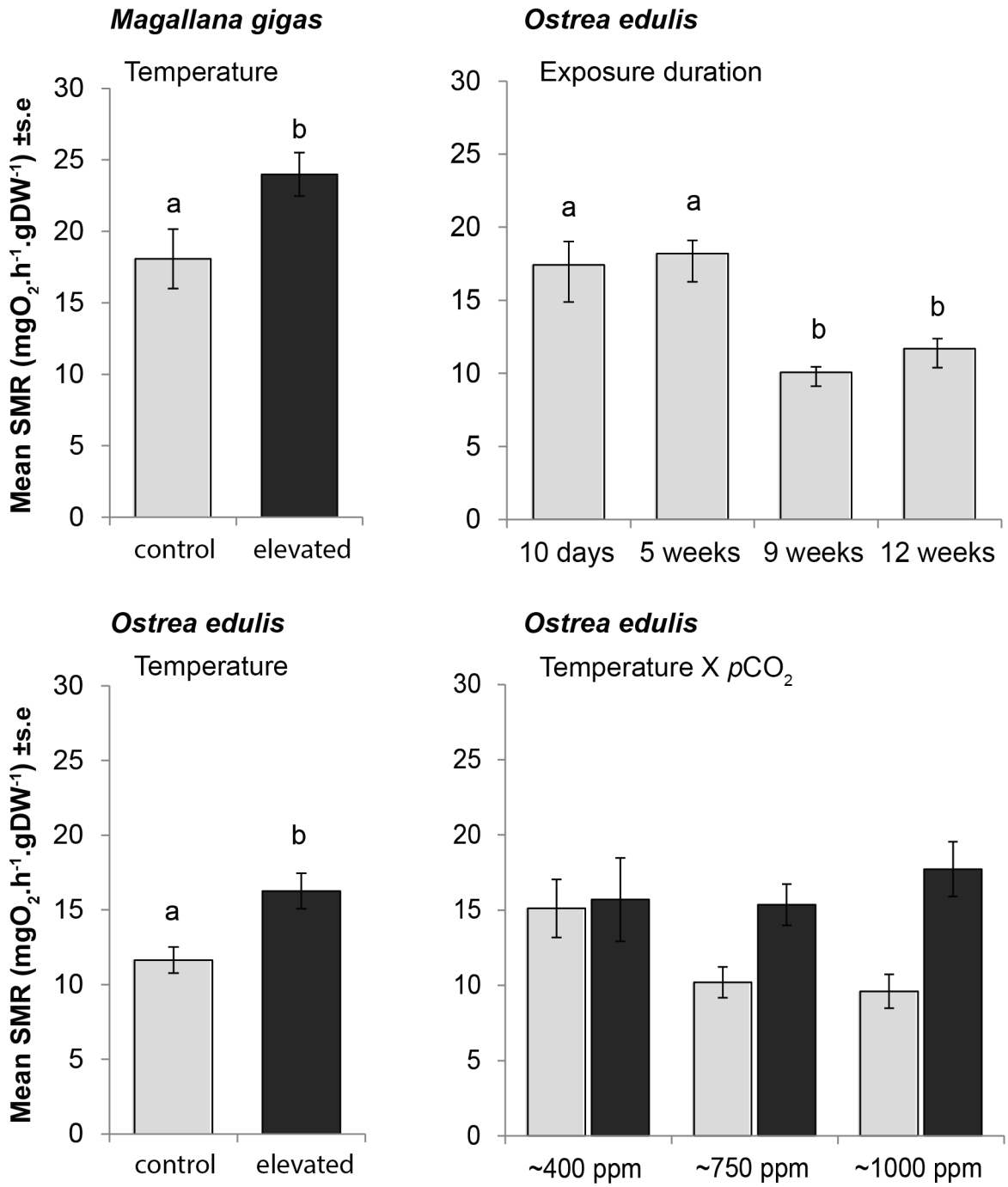


Fig 3. Changes in standard metabolic rates (SMR) of *M. gigas* (top left) and *O. edulis* (bottom left) with temperature treatment; and of *O. edulis* with exposure duration (top right) and the interaction of temperature and pCO_2 treatments (bottom right). Grey = control temperature. Black = elevated temperature. DW = dry weight. Treatment groups that do not share a letter are significantly different.

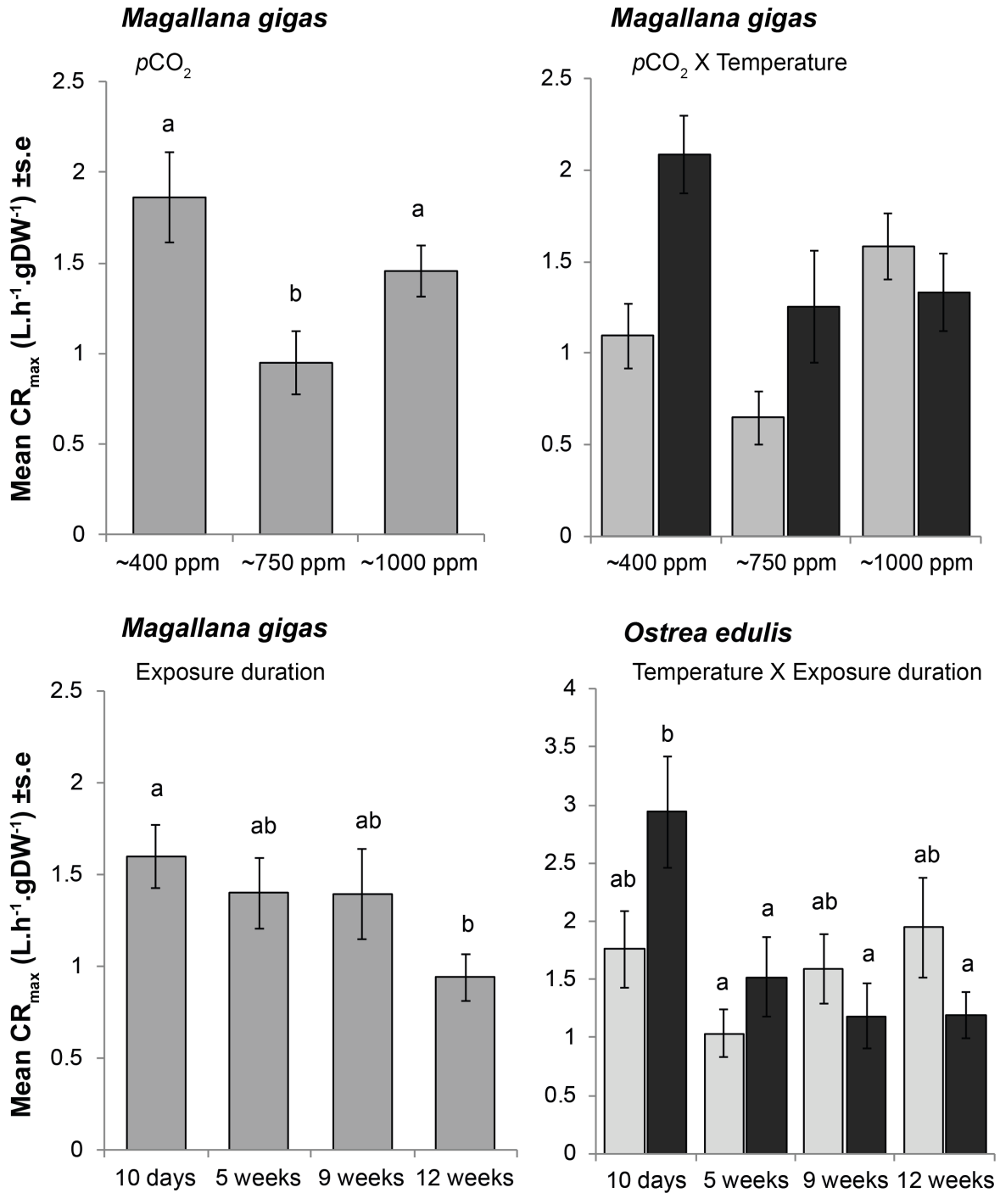
Clearance rate

Again, for both species, there was clear inter-individual variability in responses (S3 Fig.). The linear mixed-effects model revealed differences in feeding response depending on exposure and OAW scenario (S3 Fig). For *M. gigas*, pCO_2 ($F_{2,18} = 5.8$, $p < 0.05$) and exposure time ($F_{1,66} = 11.3$, $p < 0.001$) had significant effects on CRmax (Fig 4.). Intermediate pCO_2 (~750 ppm) led to ~40% decrease in CRmax in comparison to ambient pCO_2 conditions (Fig 4 top left). While not statistically significant, there was evidence that suggests an interaction between temperature and pCO_2 on CRmax (Fig 4 top right). Under control temperature, CRmax was 1.1 ± 0.2 $L.h^{-1}.gDW^{-1}$ at ambient pCO_2 but when oysters were exposed to elevated pCO_2, CRmax either decreased by ~41% (750ppm) or increased by ~45% (1000ppm). Elevating the temperature led to an increase in CRmax (~91%) under ambient pCO_2; an effect that was then lost under the 750ppm and 1000ppm OA treatments, with CRmax returning to a level comparable with this species held under control temperature and ambient pCO_2 conditions (Fig 4 top right). After 12 wk, CRmax had decreased by ~41% of the starting clearance rate (Fig 4 bottom left).

For *O. edulis*, CRmax was affected by a combination of temperature and exposure time, but not pCO_2 ($F_{1,70} = 11.2$, $p < 0.001$)(Fig 4 bottom right). Under control temperature, CRmax was not different at 10d, 9 and 12 wk, although there was a reduction in CRmax of ~41% at wk-5. Under elevated temperature, CRmax of *O. edulis* was 2.9 ± 0.5 $L.h^{-1}.gDW^{-1}$ after 10d exposure (an increase of ~67% over control temperature oysters), but which subsequently dropped back to a rate

461 comparable to oysters reared under control temperature for the remainder of the

462 study.



463

464 **Fig 4. Changes in maximum clearance rate (CRmax) of: *M. gigas* with pCO_2**

465 **treatment (top left), pCO_2 and temperature (top right),exposure duration**

466 **(bottom left); and *O. edulis* with exposure duration (bottom right)**. Grey =

control temperature. Black = elevated temperature. Treatments that do not share a letter are significantly different. DW = dry weight.

Relationship between the physiological traits

There was no correlation between SMR and CRmax for either *M. gigas* or *O. edulis*.

Condition index

At the end of the exposure duration, none of the oysters were reproductive. For *M. gigas*, the effects of temperature and pCO_2 were only marginally not significant most likely due to statistical power ($F_{2,18}$ = 3.46, p = 0.053), but clear trends were apparent. Under ambient temperature, there was no change in mean CI irrespective of pCO_2, but when temperature was elevated, there was a sustained reduction in mean CI with increasing pCO_2 (Fig. 5b). Considering temperature or pH alone, temperature led to a 40% reduction in CI from 3.5 ± 0.2 to 2.1 ± 0.2 ($F_{1,18}$ = 12.5, p < 0.01, Fig. 5a) but pCO2 had no effect ($F_{2,18}$ = 0.56, p = 0.58). In *O. edulis*, neither temperature ($F_{1,17}$ = 0.85, p = 0.37) or pCO_2 ($F_{1,17}$ = 0.10, p = 0.902) had any effect on CI, which averaged at 2.6 ± 0.1 (Fig 5c).

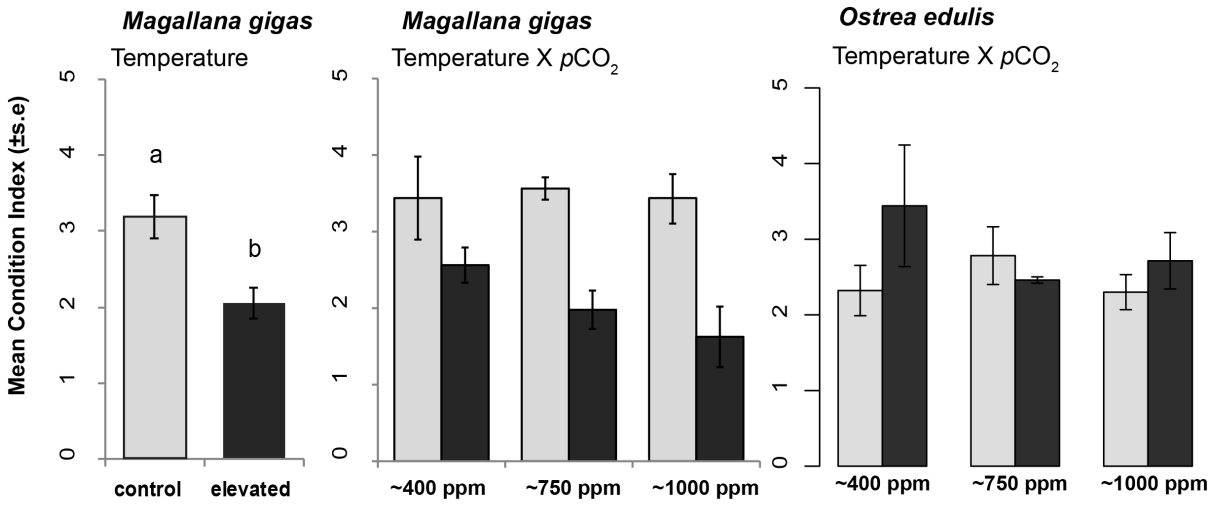


Fig 5. Variations in the condition index of *M. gigas* and *O. edulis* across temperature and pCO_2 treatments after 12 weeks exposure. Grey = control temperature. Black = elevated temperature. *M. gigas*: n=4; *O. edulis*: n=4.

Discussion

Climate change represents an important selection pressure dictating the distribution of species and the functioning of marine ecosystems. Today, there is pressure to understand the effects of multiple stressors on species that provide important ecosystem goods and services (Osborn *et al.*, 2017), and to mitigate any negative impacts in order to ensure the sustainable delivery of these goods and services. Here, following exposure to temperature and pCO_2 scenarios predicted for the near future, we show species-specific changes in metabolic rate, feeding rate, and condition of two ecologically and economically important species of oysters. Contrary to expectations, non-native *M. gigas* experienced more pronounced negative effects of warming and acidification than the native *O. edulis*, displaying increased metabolic rate under elevated temperature to 20°C, but decreased feeding rate under ~750

ppm pCO_2, which led to reduced overall condition after 12 weeks. *O. edulis* appeared relatively unimpacted by future OAW scenarios.

Metabolism

In marine organisms, the performance of routine activities such as growth, reproduction, and feeding is supported by the metabolism of oxygen, which is modulated by environmental conditions such as temperature (Pörtner & Farrell, 2008). Throughout our experiment, the metabolic rate of *M. gigas* was affected by elevated temperature only. Overall, a ~3°C temperature increase led to a >43% increase in the SMR of *M. gigas*. Similarly, the metabolism of *O. edulis* also increased with elevated temperature by ~39%, although unlike *M. gigas*, this increase coincided with highest pCO_2 (~1000 ppm) concentrations. This suggests that both *Magallana gigas* and *Ostrea edulis* display some capacity to withstand ocean acidification and warming scenarios in the short term, but elevated temperatures may pose a threat to functioning should increases in metabolism approach maxima.

Temperature increasing the metabolism of organisms is common in ectotherms; an effect previously shown in oysters (Bougrier *et al.*, 1998; Saucedo *et al.*, 2004; Shpigel *et al.*, 1992) and other bivalves (Artigaud *et al.*, 2014; Matoo *et al.*, 2013). This is not necessarily problematic if temperature elevations are within the thermal window of the organism, but ocean warming is expected to push species closer to or beyond their upper thermal limit with physiological and ecological consequences. This is especially true for individuals already living close to their upper thermal limit (Pörtner & Farrell, 2008). In the UK, *M. gigas* is considered to be living in the middle

526 of its thermal range; its capacity to increase metabolic rate under elevated
527 temperature supports this assertion. The thermal limits of *O. edulis* are less well
528 known, but here, individuals were able to increase metabolic rate under elevated
529 temperatures, suggesting some biological scope to withstand the climate scenarios
530 predicted for the future.
531
532 In our study, adult *M. gigas* and *O. edulis* displayed complex responses to variations
533 in pCO_2 conditions, although none significantly changed their SMR, indicating that
534 acidification levels tested here (~750 ppm; ~1000 ppm pCO_2) might not constitute
535 stressful conditions for them. It is likely that these levels are not unusual in coastal
536 and estuarine waters, and organisms may well have been subjected to these pCO_2
537 levels before (Hales *et al.*, 2016). However, the metabolic response of bivalves to
538 elevated pCO_2 appears species and population-specific. Several other studies
539 examining the effect of pCO_2 on respiration rate in bivalves at concentrations
540 equivalent to those tested here also revealed no change in SMR (e.g. *Crassostrea*
541 *virginica* (at 800 ppm - Matoo *et al.*, 2013), Mediterranean mussels *Mytillus*
542 *galloprovinciallis* (at ~1090 ppm - Gazeau *et al.*, 2014), and scallops, *Pecten*
543 *maximus* (at either 750 ppm and 1140 ppm - Sanders *et al.*, 2013)). Pronounced
544 changes in respiration rates can be shown when pCO_2 levels greatly exceed those
545 tested here (e.g. increasing in *C. virginica* (at 3500 ppm - Beniash *et al.*, 2010) and
546 *Mytilus edulis* (at 1120 ppm and 2400 ppm - Thomsen & Melzner, 2010), but
547 reducing in *Ruditapes decussatus* (between 1698 ppm and 4345 ppm - Fernández-
548 Reiriz *et al.*, 2011)). It is argued that increases in metabolic rates allow individuals to
549 maintain their internal acid-base balance and mainain routine physiological activities,
550 such as biomineralization (Melzner *et al.*, 2009; Pörtner & Farrell, 2008) although the

conditions used to stimulate these changes greatly exceed pCO_2 concentrations predicted for the next 80 years.

Previously, interactive effects of pCO_2 and temperature on metabolism have been shown (e.g. Lannig *et al.*, 2010, at ~1480 ppm and 20°C or 25°C); an effect reinforced in *O. edulis* in this study which showed that elevated temperature could compensate for the decreasing trend in SMR under elevated pCO_2 (~1000 ppm) and lead to an overall increase in SMR. Increasing metabolic rate is energetically expensive. This may be a physiological response developed to cope with stressful conditions in the short-term but could also be an involuntary change caused by a speed-up of biochemical reactions. Irrespective of the mechanism, this suggests a higher energy demand necessary for the maintenance, active metabolism, and overall survival of oysters. However, long-term elevation in SMR may not be sustainable for organisms due to the added energetic costs, particularly if left uncompensated, unless they become adapted over multiple generations.

Clearance rate

In the literature, the terms feeding rate, ingestion rate, clearance rate, and filtering rate are often used in concomitance or interchangeably (e.g. Coughlan, 1969; Fernández-Reiriz *et al.*, 2011; Sanders *et al.*, 2013). All are related to the amount of particles or the volume of water being processed over time. Previous studies have shown that respiration and feeding in oysters are related (Giomi *et al.*, 2016; Haure *et al.*, 2003; Haure *et al.*, 1995). Higher metabolism leads to higher energetic demands, commonly met through enhanced food consumption. Here however, contrary to predictions, no relationships between respiration and feeding rates were

found for either species. Nevertheless, in our study, the clearance rate of *M. gigas* followed an increasing trend under elevated temperature, particularly at ambient and intermediate pCO_2 (Fig 5.), suggesting a mechanism working towards enhanced food acquisition and energy supply. While increased feeding activity with temperature has been previously shown in several species of mollusc (e.g: *O. edulis* (non-linear increase from 10°C to 30°C - Haure *et al.*, 1998, and references therein), *M. galloprovinciallis* (from 12°C to 18°C - Kroeker *et al.*, 2014), and *Mytilus chilensis* (between 12°C and 16°C - Navarro *et al.*, 2016)), it was observed in *O. edulis* in this study only after 10 days of exposure, following which clearance rates returned to control levels. This suggests an initial acclimation response to experimental conditions rather than a longer-term response to the treatment.

Elevated pCO_2 reduced the clearance rate of *M. gigas* by up to 40%; an effect not observed in *O. edulis.* There is a burgeoning literature on the effects of elevated pCO_2 on the feeding behaviour and clearance rate of juvenile and adult bivalves, both of which are increasingly recognised as potential key physiological traits that govern an organisms' responses to ocean acidification (Vargas *et al.*, 2015). Although feeding is an energetically expensive process (Pörtner *et al.*, 2004), it has the potential to alleviate the negative effects of ocean acidification by providing the required additional energy to overcome the increased cost of metabolism. Indeed, several studies have shown that high food availability can counteract the effects of acidification on molluscan larvae and juveniles (Hettinger *et al.*, 2013a; Sanders *et al.*, 2013; Thomsen *et al.*, 2012). However, elevated pCO_2 has also been shown to negatively impact on the clearance and ingestion rates of several species of molluscs as found here for *M. gigas*. Juveniles of the mussel *Perumytilus purpuratus*

601 decreased their clearance rates by up to 70% under similar pCO_2 levels (at 700 ppm
602 and 1000 ppm - Vargas *et al.*, 2015). Elevated pCO_2 to 1000 ppm also led to reduced
603 clearance rate and absorption efficiency in *M. chilensis* (Navarro *et al.*, 2016), and to
604 a weak decrease in feeding rates in *M. galloprovinciallis* at 1200 ppm (Kroeker *et al.*,
605 2014). Additionally, more extreme pCO_2 levels have also been linked to reduced
606 clearance and ingestion rates in juveniles of the clam *R. decussatus* (between 1698
607 ppm and 4345 ppm - Fernández-Reiriz *et al.*, 2011). Impairment of filtration and
608 feeding can prevent organisms from resisting ocean acidification or compensating for
609 its effects, with subsequent starvation leading to increased mortality within the
610 population. In accordance with our results for *O. edulis,* no marked effects of
611 elevated pCO_2 on clearance rate were recorded in *P. maximus* (at levels up to 1140
612 ppm - Sanders *et al.*, 2013). These results reinforce the idea that responses to
613 acidification conditions are not only species-specific, but also dependent on the
614 range and number of pCO_2 levels considered.
615

616 Condition index

617 The higher metabolic costs associated with increased respiration rates under future
618 OAW conditions, particularly in *M. gigas*, were not compensated for by added energy
619 through enhanced feeding. However, added energetic demands can also be met by
620 other trade-offs with calcification, reproduction, and growth of somatic tissues.
621

622 Condition Indices (CI) are recognised as useful tools to evaluate the overall status
623 and health of bivalves (Knights, 2012), and reflect their ability to withstand adverse
624 environmental conditions (Marin *et al.*, 2003). Stressful environmental conditions
625 requiring significant energetic expenditure result in low CI in bivalves over time

626 (Orban *et al.*, 2002). Here, the CI of *M. gigas* was negatively impacted by elevated
627 temperature but not elevated pCO_2, an effect also seen for the mussel *M. edulis*
628 (Mackenzie *et al.*, 2014). Our results for pCO_2-exposed individuals are in contrast to
629 those of Lannig *et al*., (2010) on *M. gigas* who recorded a decrease of ~20% in CI
630 between control individuals and those exposed to elevated pCO_2. However, similar
631 decreases in CI with elevated temperature were recorded in several other bivalves
632 (Gabbott & Walker, 1971; Hiebenthal *et al.*, 2012; Shpigel *et al.*, 1992). Bivalves
633 have the capacity to reallocate energy reserves by reabsorbing somatic tissues and
634 gonads to sustain routine maintenance when needed. Declines in CI usually suggest
635 depletion of these reserves and are often associated with long-term stressful
636 conditions (Lannig *et al.*, 2010) or alterations in energy budget (Melzner *et al.*, 2009).
637
638 As reduced condition index is associated with depletion of energetic reserves, it
639 suggests that the long-term costs associated with increased metabolism in *M. gigas*
640 were met by a reallocation of reserves from somatic and gonadal tissues to sustain
641 maintenance and insure survival. While no mortality of *M. gigas* occurred during the
642 experiment, the lack of acclimation in respiration and clearance rates responses after
643 12 weeks exposure suggests that, if left uncompensated, the added metabolic costs
644 could compromise survival once all somatic and gonadal reserves are depleted.
645
646 The CI of *O. edulis* was unaffected by any of the treatment conditions, suggesting
647 that the experimental environmental conditions were not equally experienced by both
648 species, and *M. gigas* only may be stressed. A potential explanation for the
649 maintenance of *O. edulis* CI when exposed to the elevated temperature and ~1000
650 ppm pCO_2 treatment despite increased metabolic rates is that its sustained clearance

651 rates provided sufficient energy supply to compensate for the additional metabolic
652 costs over the 12 weeks. Nevertheless, exposure beyond the 12 week period of this
653 study might produce *O. edulis* displaying lowered CI from longer-term accumulated
654 and uncompensated energetic costs.
655

656 # Conclusion:

657 This study has shown that two important physiological traits of oysters are affected
658 by warming and/or acidification, however the responses appear species-specific.
659 Due to logistic limitations inherent to the OAW system used during the experiment,
660 the sample size for each species was limited to n=4 per treatment and as such, there
661 was high variability in the responses recorded, which led to lack of statistical power
662 for the analysis. Yet despite this, clear biological effects were apparent. If
663 anthropogenic CO_2 emissions continue to rise and temperatures continue to increase,
664 increased metabolic cost to oysters are predicted. *Magallana gigas* in particular may
665 find it difficult to meet these costs due to decreased feeding activity at ~750 ppm
666 pCO_2 levels. Non-native and invasive species are often more resilient to
667 environmental fluctuations and other biotic or abiotic stressors, yet in oysters
668 sampled from wild Plymouth populations, *M. gigas* was more negatively impacted by
669 the OAW scenarios tested than its native counterpart, *O. edulis* – which contradicted
670 our initial predictions. The non-native oysters had elevated metabolism, reduced
671 feeding, and decreased condition, signs that it could not cope well with the warming
672 and acidification conditions. Krassoi *et al.* (2008) demonstrated that differences exist
673 with respect to abiotic environmental tolerances of extreme physical conditions
674 between exotic and native oyster species, with the native species able to withstand
675 harsher environmental conditions. This was also recently observed in Brazil, where

676 the native *Crassostrea brasiliana* was more tolerant to high temperatures than the
677 non-native *M. gigas* (Moreira *et al.*, 2017). However, it should be noted that, although
678 here only two factors were tested, the interaction of multiple environmental drivers
679 has been shown to influence the sensitivity of organisms to a single specific factor
680 (Parker *et al.*, 2017a; Parker *et al.*, 2017b).
681
682 Due to poorer performance and condition of individual *M. gigas*, as found here,
683 warming and acidification may threaten populations maintenance and functioning,
684 degrading the provision of ecosystem services such as erosion control, improved
685 water quality, and fisheries from unharvested wild beds, while reducing aquaculture
686 productivity at designated aquaculture sites. The latter is especially important in the
687 UK where harvest of cultured *M. gigas* populations constitutes 90% of the oyster
688 aquaculture production, worth an estimated £10.14 million annually (Humphreys *et*
689 *al.*, 2014). Additionally, reduced clearance rates of *M. gigas* under OAW may have
690 important ecological impacts by limiting their ability to reduce turbidity and improve
691 water quality. Similar concerns have been expressed regarding the fate of waste
692 bioremediation service by mussels under future ocean acidification, as their filtration
693 rates might be negatively impacted (Broszeit *et al.*, 2015). Wild unharvested oyster
694 beds consisting in majority of *M. gigas* might see their surrounding water quality
695 diminish, with negative consequences for further associated ecosystem services
696 such as allowing for recreational use and promoting the maintenance of submerged
697 vegetation. In contrast, it appears that under future OAW corresponding to the levels
698 tested in this study, *O. edulis* will be able to continue delivering its important bio-
699 filtration service, and consequently the provision of improved water quality will
700 remain secure, if abundances recover and beds become functional again.

Such findings are of importance in terms of species ecological status, population conservation, and management measures. Oyster-related ecosystem services are mostly associated with 'reef' formations, which would require high recruitment and abundant populations (Herbert *et al.*, 2012). As such, further efforts to promote the restoration of native *O. edulis* beds should be pursued, and efforts to eradicate *M. gigas* populations may be reconsidered, in order to secure not only food provision, but also good water quality and associated beneficial ecosystem services in the future from functional populations of both species. However, ecological and economic trade-offs will need to be considered carefully, as the delivery of some of these ecosystem services from wild populations (food provision *vs* water quality) may be at odds given their opposing effects on oyster abundances.

Acknowledgements

We wish to thank the following staff, student volunteers, and friends from the Marine Biology and Ecology Research Centre, Plymouth University, for their help with organism collection and their laboratory assistance during this experiment: Marie Palmer, Martin Canty, Ann Torr, Richard Ticehurst, Roger Haslam, Lucy Jupe, Chloé Tisserand. We also wish to acknowledge Nils Piechaud for his patience and help towards producing Fig 1. We are grateful to the National Trust, the Carew Pole Garden Charitable Trust, and Mr Brian Langley for granting us access to the collection site.

Funding source

This research is supported by a grant awarded to AMK by the School of Biological and Marine Science, Plymouth University, as part of the PhD research of AJL.

References

Airoldi L, Beck MW. Loss, status and trends for coastal marine habitats of Europe. Oceanography and Marine Biology - An Annual Review. 2007;45:345-405.

Angles d'Auriac MB, Rinde E, Norling P, Lapegue S, Staalstrom A, Hjermann DO, et al. Rapid expansion of the invasive oyster *Crassostrea gigas* at its northern distribution limit in Europe: Naturally dispersed or introduced? PLoS One. 2017;12(5):e0177481. doi: 10.1371/journal.pone.0177481

Artigaud S, Lacroix C, Pichereau V, Flye-Sainte-Marie J. Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus* spp. Comp Biochem Physiol A Mol Integr Physiol. 2014;175:135-40. doi: 10.1016/j.cbpa.2014.06.005.

Barry JP, Widdicombe S, Hall-Spencer JM. Chapter 10: Effects of ocean acidification on marine biodiversity and ecosystem function. In: Ocean Acidification 2011. p. 192-209.

Barton A, Waldbusser G, Feely R, Weisberg S, Newton J, Hales B, et al. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography. 2015;25(2):146-59. doi: 10.5670/oceanog.2015.38.

Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, et al. Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience. 2011;61(2):107-16. doi: 10.1525/bio.2011.61.2.5.

749 Beiras R, Camacho AP, Albentosa M. Short-term and long-term alterations in the
750 energy budget of young oyster *Ostrea edulis* L. in response to temperature
751 change. J Exp Mar Biol Ecol. 1995;186(2):221-36. doi:
752 http://dx.doi.org/10.1016/0022-0981(94)00159-B.
753 Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM. Elevated level of carbon
754 dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*
755 (Gmelin). Mar Ecol Prog Ser. 2010;419:95-108. doi: 10.3354/meps08841.
756 Bertness MD, Gaines SD. Larval dispersal and local adaptation in acorn barnacles.
757 Evolution. 1993;47(1):316-20.
758 Bodoy A, Prou J, Berthome J-P. Etude comparative de différents indices de
759 condition chez l'huître creuse (*Crassostrea gigas*). Haliotis. 1986;15:173-82.
760 Bougrier S, Collet B, Geairon P, Geffard O, He´ral M. Respiratory time activity of the
761 Japanese oyster *Crassostrea gigas* (Thunberg). J Exp Mar Biol Ecol.
762 1998;219:205- 16.
763 Broszeit S, Hattam C, Beaumont N. Bioremediation of waste under ocean
764 acidification: Reviewing the role *of Mytilus edulis*. Mar Pollut Bull. 2015. doi:
765 http://dx.doi.org/10.1016/j.marpolbul.2015.12.040.
766 Buxton C, Newell R, Field J. Response-surface analysis of the combined effects of
767 exposure and acclimation temperatures on filtration, oxygen consumption and
768 scope for growth in the oyster *Ostrea edulis*. Mar Ecol Prog Ser. 1981;6(1):73-82.
769 Byrne M, Przeslawski R. Multistressor impacts of warming and acidification of the
770 ocean on marine invertebrates' life histories. Integr Comp Biol. 2013;53(4):582-96.
771 doi: 10.1093/icb/ict049.

772 Calosi P, Turner LM, Hawkins M, Bertolini C, Nightingale G, Truebano M, et al.
773 Multiple physiological responses to multiple environmental challenges: an
774 individual approach. Integr Comp Biol. 2013;53(4):660-70. doi: 10.1093/icb/ict041.
775 Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, et al.
776 Ecosystem services related to oyster restoration. Mar Ecol Prog Ser.
777 2007;341:303-7.
778 Cole VJ, Parker LM, O'Connor SJ, O'Connor WA, Scanes E, Byrne M, et al. Effects
779 of multiple climate change stressors: ocean acidification interacts with warming,
780 hyposalinity, and low food supply on the larvae of the brooding flat oyster *Ostrea*
781 *angasi*. Mar Biol. 2016;163(5). doi: 10.1007/s00227-016-2880-4.
782 Comeau LA, Pernet F, Tremblay R, Bates SS, LeBlanc A. Comparison of eastern
783 oyster (*Crassostrea virginica*) and blue mussel (*Mytilus edulis*) filtration rates at
784 low temperatures: Fisheries and Oceans Canada; 2008.
785 Coolen JWP. North Sea Reefs. Benthic biodiversity of artificial and rocky reefs in the
786 southern North Sea: Wageningen University; 2017.
787 Cooley SR, Cheney JE, Kelly RP, Allison EH. 2 Ocean acidification and Pacific
788 oyster larval failures in the Pacific Northwest United States. Global Change in
789 Marine Systems: Societal and Governing Responses2017.
790 Coughlan J. The estimation of filtering rate from the clearance of suspensions. Mar
791 Biol. 1969;2(4):356-8. doi: 10.1007/bf00355716.
792 Davis CH, Calabrese A. Survival and growth of larvae of the European oyster
793 (*Ostrea edulis* L.) at different temperatures. Biol Bull. 1969;136(2):193-9. doi:
794 doi:10.2307/1539813.

795 Dickinson GH, Ivanina AV, Matoo OB, Portner HO, Lannig G, Bock C, et al.
796 Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters,
797 *Crassostrea virginica*. J Exp Biol. 2012;215(Pt 1):29-43. doi: 10.1242/jeb.061481.
798 Dickson AG, Millero FJ. A comparison of the equilibrium constants for the
799 dissociation of carbonic acid in seawater media. Deep-Sea Research Part a-
800 Oceanographic Research Papers. 1987;34:1733:43.
801 Dickson AG. Thermodynamics of the dissociation of boric acid in synthetic seawater
802 from 273.15 to 318.15 K. Deep-Sea Research Part a-Oceanographic Research
803 Papers. 1990;37:755:66.
804 Dolmer P, Holm MW, Strand Å, Lindegarth S, Bodvin T, Norling P, et al. The invasive
805 Pacific oyster*, Crassostrea gigas*, in Scandinavian coastal waters: A risk
806 assessment on the impact in different habitats and climate conditions. Institute of
807 Marine Research: 2014 0071-5638.
808 Dove MC, Sammut J. Impacts of estuarine acidification on survival and growth of
809 Sydney rock oysters *Saccostrea glomerata* (Gould 1850). J Shellfish Res.
810 2007;26(2):519-27.
811 Fernández-Reiriz MJ, Range P, Álvarez-Salgado XA, Labarta U. Physiological
812 energetics of juvenile clams *Ruditapes decussatus* in a high CO_2 coastal ocean.
813 Mar Ecol Prog Ser. 2011;433:97-105. doi: 10.3354/meps09062.
814 Gabbott PA, Walker AJM. Changes in the condition index and biochemical content of
815 adult oysters (*Ostrea edulis* L.) maintained under hatchery conditions. Journal du
816 Conseil. 1971;34(1):98-105. doi: 10.1093/icesjms/34.1.98.
817 Gazeau F, Alliouane S, Correa ML, Gentile M, Bock C, Hirse T, et al. Impact of
818 ocean acidification and warming on the Mediterranean mussel (*Mytilus*
819 *galloprovincialis*). Front Mar Sci. 2014;1:62. doi: 10.3389/fmars.2014.00062.

820 Gestoso I, Arenas F, Olabarria C. Ecological interactions modulate responses of two
821 intertidal mussel species to changes in temperature and pH. J Exp Mar Biol Ecol.
822 2016;474:116-25. doi: 10.1016/j.jembe.2015.10.006.
823 Giomi F, Mandaglio C, Ganmanee M, Han GD, Dong YW, Williams GA, et al. The
824 importance of thermal history: costs and benefits of heat exposure in a tropical,
825 rocky shore oyster. J Exp Biol. 2016;219(Pt 5):686-94. doi: 10.1242/jeb.128892.
826 Gray MW, Langdon CJ, Waldbusser GG, Hales B, Kramer S. Mechanistic
827 understanding of ocean acidification impacts on larval feeding physiology and
828 energy budgets of the mussel *Mytilus californianus*. Mar Ecol Prog Ser.
829 2017;563:81-94. doi: 10.3354/meps11977.
830 Green EJ, Carritt DE. New tables for oxygen saturation of seawater. J Mar Res.
831 1967;25:140-7.
832 Hales B, Suhrbier A, Waldbusser GG, Feely RA, Newton JA. The carbonate
833 chemistry of the “Fattening Line,” Willapa Bay, 2011–2014. Estuar Coasts.
834 2016;40(1):173-86. doi: 10.1007/s12237-016-0136-7.
835 Hall-Spencer J, Allen R. The impact of CO_2 emissions on 'nuisance' marine species.
836 Res Rep biodivers Stud. 2015;33:33-46. doi: 10.2147/rrbs.s70357.
837 Harding S, Nelson L, Glover T. Solent Oyster Restoration Project Management Plan.
838 Blue Marine Foundation, 2016.
839 Haure J, Huvet A, Palvadeau H, Nourry M, Penisson C, Martin JLY, et al. Feeding
840 and respiratory time activities in the cupped oysters *Crassostrea gigas*,
841 *Crassostrea angulata* and their hybrids. Aquaculture. 2003;218(1-4):539-51. doi:
842 10.1016/s0044-8486(02)00493-3.

843 Haure J, Penisson C, Bougrier S, Baud J. Influence of temperature on clearance and
844 oxygen consumption rates of the flat oyster *Ostrea edulis*: determination of
845 allometric coefficients. Aquaculture. 1998;169(3):211-24.
846 Haure J, Penisson C, Bougrier S, Baud JP. Allometric relationships and effects of
847 temperature on clearance and oxygen consumption rates of *Crassostrea gigas*.
848 Aquaculture. 1995;169:211-24.
849 Herbert RJH, Humphreys J, Davies CJ, Roberts C, Fletcher S, Crowe TP. Ecological
850 impacts of non-native Pacific oysters (*Crassostrea gigas*) and management
851 measures for protected areas in Europe. Biodivers Conserv. 2016;25(14):2835-
852 65. doi: 10.1007/s10531-016-1209-4.
853 Herbert RJH, Robert C, Humphreys J, Fletcher S. The Pacific Oyster (*Crassostrea*
854 *gigas*) in the UK: economic, legal and environmental issues associated with its
855 cultivation, wild establishment and exploitation. Report for the Shellfish
856 Association of Great Britain. 2012.
857 Hettinger A, Sanford E, Hill T, Hosfelt J, Russell A, Gaylord B. The influence of food
858 supply on the response of Olympia oyster larvae to ocean acidification.
859 Biogeosciences. 2013;10(10):6629-38.
860 Hettinger A, Sanford E, Hill TM, Lenz EA, Russell AD, Gaylord B. Larval carry-over
861 effects from ocean acidification persist in the natural environment. Glob Chang
862 Biol. 2013;19(11):3317-26. doi: 10.1111/gcb.12307.
863 Hettinger A, Sanford E, Hill TM, Russell AD, Sato KNS, Hoey J, et al. Persistent
864 carry-over effects of planktonic exposure to ocean acidification in the Olympia
865 oyster. Ecology. 2012;93(12):2758-68. doi: 10.1890/12-0567.1.

866 Hiebenthal C, Philipp E, Eisenhauer A, Wahl M. Interactive effects of temperature
867 and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis*
868 and *Arctica islandica*. Aquat Biol. 2012;(14):289-98.
869 Hofmann GE, Barry JP, Edmunds PJ, Gates RD, Hutchins DA, Klinger T, et al. The
870 effect of ocean acidification on calcifying organisms in marine ecosystems: An
871 organism-to-ecosystem perspective. Annu Rev Ecol Evol Syst. 2010;41(1):127-
872 47. doi: 10.1146/annurev.ecolsys.110308.120227.
873 Houlbrèque F, Reynaud S, Godinot C, Oberhänsli F, Rodolfo-Metalpa R, Ferrier-
874 Pagès C. Ocean acidification reduces feeding rates in the scleractinian coral
875 *Stylophora pistillata*. Limnol Oceanogr. 2015;60(1):89-99. doi: 10.1002/lno.10003.
876 Humphreys J, Herbert RJH, Roberts C, Fletcher S. A reappraisal of the history and
877 economics of the Pacific oyster in Britain. Aquaculture. 2014;428-429:117-24. doi:
878 10.1016/j.aquaculture.2014.02.034.
879 Humphreys MP. Climate sensitivity and the rate of ocean acidification: future
880 impacts, and implications for experimental design. ICES J Mar Sci.
881 2016;74(4):934-40. doi: 10.1093/icesjms/fsw189.
882 Knights AM. Spatial variation in body size and reproductive condition of subtidal
883 mussels: Considerations for sustainable management. Fish Res. 2012;113(1):45-
884 54. doi: 10.1016/j.fishres.2011.09.002.
885 Kochmann J, O'Beirn F, Yearsley J, Crowe TP. Environmental factors associated
886 with invasion: modelling occurrence data from a coordinated sampling programme
887 for Pacific oysters. Biol Invasions. 2013;15(10):2265-79.
888 Krassoi FR, Brown KR, Bishop MJ, Kelaher BP, Summerhayes S. Condition-specific
889 competition allows coexistence of competitively superior exotic oysters with native
890 oysters. J Anim Ecol. 2008;77(1):5-15. doi: 10.1111/j.1365-2656.2007.01316.x.

Kroeker KJ, Gaylord B, Hill TM, Hosfelt JD, Miller SH, Sanford E. The role of temperature in determining species' vulnerability to ocean acidification: a case study using *Mytilus galloprovincialis*. PLoS One. 2014;9(7):e100353. doi: 10.1371/journal.pone.0100353.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, et al. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob Chang Biol. 2013;19(6):1884-96. doi: 10.1111/gcb.12179.

Kurihara H. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser. 2008;373:275-84. doi: 10.3354/meps07802.

Lacoue-Labarthe T, Nunes PALD, Ziveri P, Cinar M, Gazeau F, Hall-Spencer JM, et al. Impacts of ocean acidification in a warming Mediterranean Sea: An overview. Regional Studies in Marine Science. 2016;5:1-11. doi: http://dx.doi.org/10.1016/j.rsma.2015.12.005.

Laing I, Walker P, Areal F. Return of the native – is European oyster (*Ostrea edulis*) stock restoration in the UK feasible? Aquat Living Resour. 2006;19(3):283-7. doi: 10.1051/alr:2006029.

Lallias D, Boudry P, Lapègue S, King J, Beaumont A. Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes. Conserv Genet. 2010;11(5):1899-910. doi: 10.1007/s10592-010-0081-0.

Lannig G, Eilers S, Portner HO, Sokolova IM, Bock C. Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*-changes in metabolic pathways and thermal response. Mar Drugs. 2010;8(8):2318-39. doi: 10.3390/md8082318.

916 Lemasson AJ, Fletcher S, Hall-Spencer JM, Knights AM. Linking the biological
917 impacts of ocean acidification on oysters to changes in ecosystem services: A
918 review. J Exp Mar Biol Ecol. 2017;492:49-62. doi: 10.1016/j.jembe.2017.01.019.
919 Leung JY, Russell BD, Connell SD. Mineralogical plasticity acts as a compensatory
920 mechanism to the impacts of ocean acidification. Environ Sci Technol. 2017. doi:
921 10.1021/acs.est.6b04709.
922 Liu S, Shi W, Guo C, Zhao X, Han Y, Peng C, et al. Ocean acidification weakens the
923 immune response of blood clam through hampering the NF-kappa beta and toll-
924 like receptor pathways. Fish Shellfish Immunol. 2016;54:322-7. doi:
925 10.1016/j.fsi.2016.04.030.
926 Lodge DM. Biological invasions: lessons for ecology. Trends Ecol Evol.
927 1993;8(4):133-7.
928 Lucas A, Beninger PG. The use of physiological condition indices in marine bivalve
929 aquaculture. Aquaculture. 1985;44(3):187-200. doi:
930 http://dx.doi.org/10.1016/0044-8486(85)90243-1.
931 Mackenzie CL, Ormondroyd GA, Curling SF, Ball RJ, Whiteley NM, Malham SK.
932 Ocean warming, more than acidification, reduces shell strength in a commercial
933 shellfish species during food limitation. PLoS One. 2014;9(1):e86764. doi:
934 10.1371/journal.pone.0086764.
935 Marin MG, Moschino V, Deppieri M, Lucchetta L. Variations in gross biochemical
936 composition, energy value and condition index of *T. philippinarum* from the
937 Lagoon of Venice. Aquaculture. 2003;219(1-4):859-71. doi: 10.1016/s0044-
938 8486(03)00035-8.
939 Matoo OB, Ivanina AV, Ullstad C, Beniash E, Sokolova IM. Interactive effects of
940 elevated temperature and CO_2 levels on metabolism and oxidative stress in two

941 common marine bivalves (*Crassostrea virginica* and Mercenaria mercenaria).
942 Comparative Biochemistry and Physiology Part A: Molecular & Integrative
943 Physiology. 2013;164(4):545-53. doi: 10.1016/j.cbpa.2012.12.025.
944 Mehrbach C, Culberson CH, Hawley JE, R.M. P. Measurement of the apparent
945 dissociation constants of carbonic acid in seawater at atmospheric pressure.
946 Limnol Oceanogr. 1973;18:897:907.
947 Melzner F, Gutowska M, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, et
948 al. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-
949 adaptation through lifestyle and ontogeny? Biogeosciences. 2009;6(10):2313-31.
950 Moreira A, Figueira E, Pecora IL, Soares A, Freitas R. Native and exotic oysters in
951 Brazil: Comparative tolerance to hypercapnia. Environ Res. 2018;161:202-11. doi:
952 10.1016/j.envres.2017.10.035.
953 Moreira A, Figueira E, Pecora IL, Soares AM, Freitas R. Biochemical alterations in
954 native and exotic oyster species in Brazil in response to increasing temperature.
955 Comp Biochem Physiol C Toxicol Pharmacol. 2017;191:183-93. doi:
956 10.1016/j.cbpc.2016.10.008. PubMed PMID: 27816652.
957 Navarro JM, Duarte C, Manríquez PH, Lardies MA, Torres R, Acuña K, et al. Ocean
958 warming and elevated carbon dioxide: multiple stressor impacts on juvenile
959 mussels from southern Chile. ICES J Mar Sci. 2016;73(3):764-71. doi:
960 10.1093/icesjms/fsv249.
961 Orban E, Di Lena G, Nevigato T, Casini I, Marzetti A, Caproni R. Seasonal changes
962 in meat content, condition index and chemical composition of mussels (*Mytilus*
963 *galloprovincialis*) cultured in two different Italian sites. Food Chem. 2002;77:57-65.
964 Orton JH. Effect of the severe frost of the winter of 1939-40 on the Fauna of the
965 Essex oyster beds. Nature. 1940;145:708-9.

966 Orton JH. Oyster Biology and Oyster Culture. Butler and Tanner Ltd, London.
967 UK.1937.
968 Osborn D, Dupont S, Hansson L, Metian M. Chapter 19. Ocean acidification: impacts
969 and governance. Handbook on the Economics and Management of Sustainable
970 Oceans: Edward Elgar Publishing; 2017. p. 396.
971 Parker LM, O'Connor WA, Byrne M, Coleman RA, Virtue P, Dove M, et al. Adult
972 exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster
973 *Saccostrea glomerata* in the presence of multiple stressors. Biol Lett. 2017;13(2).
974 doi: 10.1098/rsbl.2016.0798.
975 Parker LM, Ross PM, O'Connor WA. Comparing the effect of elevated pCO_2 and
976 temperature on the fertilization and early development of two species of oysters.
977 Mar Biol. 2010;157(11):2435-52. doi: 10.1007/s00227-010-1508-3.
978 Parker LM, Ross PM, O'Connor WA, Portner HO, Scanes E, Wright JM. Predicting
979 the response of molluscs to the impact of ocean acidification. Biology.
980 2013;2(2):651-92. doi: 10.3390/biology2020651.
981 Parker LM, Scanes E, O'Connor WA, Coleman RA, Byrne M, Portner HO, et al.
982 Ocean acidification narrows the acute thermal and salinity tolerance of the Sydney
983 rock oyster *Saccostrea glomerata*. Mar Pollut Bull. 2017;122(1-2):263-71. doi:
984 10.1016/j.marpolbul.2017.06.052.
985 Pierrot D, Lewis E, DWR W. MS Excel program developed for CO_2 system
986 calculations, ORNL/CDIAC-105. Oak Ridge National Laboratory, U.S. Department
987 of Energy, Oak Ridge (TN): Carbon Dioxide Information Analysis Center. 2006.
988 Pörtner H, Langenbuch M, Reipschläger A. Biological impact of elevated ocean CO_2
989 concentrations: lessons from animal physiology and Earth history. J Oceanogr.
990 2004;60(4):705-18. doi: 10.1007/s10872-004-5763-0.

991 Pörtner HO, Farrell AP. Physiology and Climate Change. Sci. 2008;322(5902):690-2.
992 doi: 10.1126/science.1163156.
993 Pörtner H-O, Karl DM, Boyd PW, Cheung WWL, Lluch-Cota SE, Nojiri Y, et al.
994 Ocean Systems. Climate Change 2014: Impacts, Adaptation, and Vulnerability
995 Part A: Global and Sectoral Aspects Contribution of Working Group II to the Fifth
996 Assessment Report of the Intergovernmental Panel on Climate Change.
997 Cambridge University Press, Cambridge, United Kingdom and New York, NY,
998 USA2014. p. pp. 411-84.
999 Przeslawski R, Byrne M, Mellin C. A review and meta-analysis of the effects of
1000 multiple abiotic stressors on marine embryos and larvae. Glob Chang Biol.
1001 2015;21(6):2122-40. doi: 10.1111/gcb.12833.
1002 R Core Team. R: A language and environment for statistical computing. R
1003 Foundation for Statistical Computing, Vienna, Austria. 2016. Available from:
1004 https://www.R-project.org/.
1005 Ramajo L, Marba N, Prado L, Peron S, Lardies MA, Rodriguez-Navarro A, et al.
1006 Biomineralization changes with food supply confer juvenile scallops (*Argopecten*
1007 *purpuratus*) resistance to ocean acidification. Glob Chang Biol. 2015. doi:
1008 10.1111/gcb.13179.
1009 Reum JCP, Alin SR, Harvey CJ, Bednaršek N, Evans W, Feely RA, et al.
1010 Interpretation and design of ocean acidification experiments in upwelling systems
1011 in the context of carbonate chemistry co-variation with temperature and oxygen.
1012 ICES J Mar Sci. 2015;73(3):582-95. doi: 10.1093/icesjms/fsu231.
1013 Rinde E, Tjomsland T, Hjermann D, Kempa M, Norling P, Kolluru VS. Increased
1014 spreading potential of the invasive Pacific oyster (*Crassostrea gigas*) at its

1015 northern distribution limit in Europe due to warmer climate. Mar Freshw Res.
1016 2016. doi: 10.1071/mf15071.
1017 Robert R, Vignier J, Petton B. Influence of feeding regime and temperature on
1018 development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae.
1019 Aquacult Res. 2017;48(9):4756-73. doi: 10.1111/are.13297.
1020 Sanders MB, Bean TP, Hutchinson TH, Le Quesne WJ. Juvenile king scallop,
1021 Pecten maximus, is potentially tolerant to low levels of ocean acidification when
1022 food is unrestricted. PLoS One. 2013;8(9):e74118. doi:
1023 10.1371/journal.pone.0074118..
1024 Sanford E, Kelly MW. Local adaptation in marine invertebrates. Ann Rev Mar Sci.
1025 2011;3:509-35. doi: 10.1146/annurev-marine-120709-142756.
1026 Saucedo PE, Ocampo La, Monteforte M, Bervera H. Effect of temperature on oxygen
1027 consumption and ammonia excretion in the Calafia mother-of-pearl oyster,
1028 *Pinctada mazatlanica* (Hanley, 1856). Aquaculture. 2004;229(1-4):377-87. doi:
1029 10.1016/s0044-8486(03)00327-2.
1030 Sawusdee A. PhD Thesis. Restoration of the European flat oyster *Ostrea edulis*
1031 using elevated broodstock reefs: University of Southampton; 2015.
1032 Scanes E, Parker LM, O'Connor WA, Stapp LS, Ross PM. Intertidal oysters reach
1033 their physiological limit in a future high-CO_2 world. J Exp Biol. 2017;220(Pt 5):765-
1034 74. doi: 10.1242/jeb.151365.
1035 Shelmerdine R, Mouat B, Shucksmith R. The most northerly record of feral Pacific
1036 oyster *Crassostrea gigas* (Thunberg, 1793) in the British Isles. BioInvasions
1037 Records. 2017;6(1):57-60. doi: 10.3391/bir.2017.6.1.09.

1038 Shelmerdine RL, Leslie B. Restocking of the native oyster, *Ostrea edulis*, in
1039 Shetland: habitat identification study Scottish Natural Heritage Commissioned
1040 Report No. 396, 2009.
1041 Shpigel M, Barber BJ, Mann R. Effects of elevated temperature on growth,
1042 gametogenesis, physiology, and biochemical composition in diploid and triploid
1043 Pacific oysters, *Crassostrea gigas* Thunberg. J Exp Mar Biol Ecol.
1044 1992;161(1):15-25. doi: http://dx.doi.org/10.1016/0022-0981(92)90186-E.
1045 Stachowicz JJ, Fried H, Osman RW, Whitlatch RB. Biodiversity, invasion resistance,
1046 and marine ecosystem function: reconciling pattern and process. Ecology.
1047 2002;83(9):2575-90.
1048 Sunday JM, Fabricius KE, Kroeker KJ, Anderson KM, Brown NE, Barry JP, et al.
1049 Ocean acidification can mediate biodiversity shifts by changing biogenic habitat.
1050 Nat Clim Change. 2016;7(1):81-5. doi: 10.1038/nclimate3161.
1051 Thomas Y, Pouvreau S, Alunno-Bruscia M, Barillé L, Gohin F, Bryère P, et al. Global
1052 change and climate-driven invasion of the Pacific oyster (*Crassostrea gigas*) along
1053 European coasts: a bioenergetics modelling approach. J Biogeogr.
1054 2016;43(3):568-79. doi: 10.1111/jbi.12665.
1055 Thomsen J, Casties I, Pansch C, Kortzinger A, Melzner F. Food availability
1056 outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field
1057 experiments. Glob Chang Biol. 2012;19(4):1017-27. doi: 10.1111/gcb.12109.
1058 Thomsen J, Melzner F. Moderate seawater acidification does not elicit long-term
1059 metabolic depression in the blue mussel *Mytilus edulis*. Mar Biol.
1060 2010;157(12):2667-76.

1061 Towle EK, Enochs IC, Langdon C. Threatened Caribbean coral is able to mitigate
1062 the adverse effects of ocean acidification on calcification by increasing feeding
1063 rate. PLoS One. 2015;10(4):e0123394. doi: 10.1371/journal.pone.0123394.
1064 Townhill B, Pinnegar J, Tinker J, Jones M, Simpson S, Stebbing P, et al. Non-native
1065 marine species in north-west Europe: Developing an approach to assess future
1066 spread using regional downscaled climate projections. Aquat Conserv: Mar
1067 Freshwat Ecosyst. 2017;27(5):1035-50. doi: 10.1002/aqc.2764.
1068 Troost K. Causes and effects of a highly successful marine invasion: Case-study of
1069 the introduced Pacific oyster *Crassostrea gigas* in continental NW European
1070 estuaries. J Sea Res. 2010;64(3):145-65. doi: 10.1016/j.seares.2010.02.004.
1071 Vargas CA, Aguilera VM, Martín VS, Manríquez PH, Navarro JM, Duarte C, et al.
1072 CO_2-driven ocean acidification disrupts the filter feeding behavior in Chilean
1073 gastropod and bivalve species from different geographic localities. Estuar Coasts.
1074 2015;38(4):1163-77. doi: 10.1007/s12237-014-9873-7.
1075 Waldbusser GG, Gray MW, Hales B, Langdon CJ, Haley BA, Gimenez I, et al. Slow
1076 shell building, a possible trait for resistance to the effects of acute ocean
1077 acidification. Limnol Oceanogr. 2016. doi: 10.1002/lno.10348.
1078 Waldbusser GG, Hales B, Langdon CJ, Haley BA, Schrader P, Brunner EL, et al.
1079 Ocean acidification has multiple modes of action on bivalve larvae. PLoS One.
1080 2015;10(6):e0128376. doi: 10.1371/journal.pone.0128376.
1081 Waldbusser GG, Steenson RA, Green MA. Oyster shell dissolution rates in estuarine
1082 waters: effects of pH and shell legacy. J Shellfish Res. 2011;30(3):659-69. doi:
1083 10.2983/035.030.0308.
1084 Waldbusser GG, Voigt EP, Bergschneider H, Green MA, Newell RIE. Biocalcification
1085 in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in

1086 Chesapeake Bay pH. Estuar Coasts. 2011;34(2):221-31. doi: 10.1007/s12237-
1087 010-9307-0.
1088 Walne P. Growth of oysters (*Ostrea edulis* L.). J Mar Biol Assoc UK.
1089 1958;37(03):591-602.
1090 Wang Q, Cao R, Ning X, You L, Mu C, Wang C, et al. Effects of ocean acidification
1091 on immune responses of the Pacific oyster *Crassostrea gigas*. Fish Shellfish
1092 Immunol. 2016;49:24-33. doi: http://dx.doi.org/10.1016/j.fsi.2015.12.025.
1093 Weatherdon LV, Magnan AK, Rogers AD, Sumaila UR, Cheung WWL. Observed
1094 and projected impacts of climate change on marine fisheries, aquaculture, coastal
1095 tourism, and human health: An Update. Front Mar Sci. 2016;3. doi:
1096 10.3389/fmars.2016.00048.
1097 Weiss RF. The solubility of nitrogen, oxygen and argon in water and seawater.
1098 Deep-Sea Research. 1970;17:721-35.
1099 Welladsen HM, Southgate PC, Heimann K. The effects of exposure to near-future
1100 levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia:
1101 Pteriidae). Molluscan Res. 2010;30(3):125.
1102 Woolmer AP, Syvret M, A. F, No: 960 p. Restoration of Native Oyster, *Ostrea edulis*,
1103 in South Wales: Options and Approaches. CCW Contract Science Report. 2011
1104 960.
1105 Wright JM, Parker LM, O'Connor WA, Williams M, Kube P, Ross PM. Populations of
1106 Pacific oysters *Crassostrea gigas* respond variably to elevated CO_2 and predation
1107 by *Morula marginalba*. Biol Bull. 2014;226(3):269-81.
1108 Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects models and
1109 extensions in ecology with R: Springer New York; 2009.

Zwerschke N, Emmerson MC, Roberts D, O'Connor NE. Benthic assemblages associated with native and non-native oysters are similar. Mar Pollut Bull. 2016;111(1-2):305-10. doi: 10.1016/j.marpolbul.2016.06.094

Zwerschke N, Kochmann J, Ashton EC, Crowe TP, Roberts D, O'Connor NE. Co-occurrence of native *Ostrea edulis* and non-native *Crassostrea gigas* revealed by monitoring of intertidal oyster populations. J Mar Biol Assoc UK. 2017:1-10. Epub 08/23. doi: 10.1017/S0025315417001448.

Supporting information

S1 Table. Physical and chemical characteristics of seawater in the six experimental treatments for *Magallana gigas* and *Ostrea edulis*. Presented as mean values over the duration of the experiment ± standard deviation (s.d.). T= temperature, S= salinity, Ω_a= saturation state of aragonite, Ω_c= saturation state of calcite.

S1 Fig. Temperature (left) and pH (right) data within the mesocosm set-up throughout 3-month exposure of a) *Magallana gigas* and b) *Ostrea edulis* exposed to two temperature levels: control (~16.5°C - blue); elevated (~20°C - red) three pCO_2 levels: Ambient (~400 ppm - white), ~750 ppm (yellow), ~1000 ppm (black).

S2 Fig. Changes in standard metabolic rate (SMR) of *M. gigas* (top) and *O. edulis* (bottom) over 12 weeks exposure to temperature and pCO_2 combinations. Grey = control temperature. Black = elevated temperature. DW = dry weight.

S3 Fig. Changes in maximum clearance rate (CRmax) of *M. gigas* (top) and *O. edulis* (bottom) over 12 weeks exposure to temperature and pCO_2 combinations. Grey = control temperature. Black = elevated temperature. DW = dry weight.

Highlights:

- Acidification and warming negatively impacted the physiology of *Magallana gigas*
- *Ostrea edulis* appeared unaffected by the treatment conditions
- Efforts to promote the restoration of native *O. edulis* beds should be pursued
- Efforts to eradicate *M. gigas* populations may need to be reconsidered