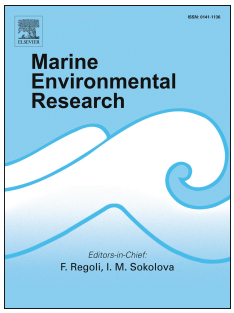
2019-01

Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming

Lemasson, A

10.1016/j.marenvres.2018.11.006
Marine Environmental Research
Elsevier

Accepted Manuscript



Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming

A.J. Lemasson, J.M. Hall-Spencer, V. Kuri, A.M. Knights

PII: S0141-1136(18)30416-1

DOI: https://doi.org/10.1016/j.marenvres.2018.11.006

Reference: MERE 4643

To appear in: *Marine Environmental Research*

Received Date: 1 June 2018

Revised Date: 5 October 2018

Accepted Date: 15 November 2018

Please cite this article as: Lemasson, A.J., Hall-Spencer, J.M., Kuri, V., Knights, A.M., Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming, *Marine Environmental Research* (2018), doi: https://doi.org/10.1016/j.marenvres.2018.11.006.

Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming

Lemasson A.J.[a]*, Hall-Spencer J.M.[a,b], Kuri V.[c], and A.M. Knights[a]

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

[b] Shimoda Marine Research Centre, University of Tsukuba, Shimoda City, Shizuoka, Japan

[c] Food, Health and Nutrition, School of Biological and Marine Sciences, University of Plymouth, Plymouth, Drake Circus, Plymouth, PL4 8AA. UK

*Corresponding Author: A.J. Lemasson (anaelle.lemasson@plymouth.ac.uk)

Abstract:

Ocean acidification and warming may threaten future seafood production, safety and quality by negatively impacting the fitness of marine species. Identifying changes in nutritional quality, as well as species most at risk, is crucial if societies are to secure food production. Here, changes in the biochemical composition and nutritional properties of the commercially valuable oysters, *Magallana gigas* and *Ostrea edulis*, were evaluated following a 12-week exposure to six ocean acidification and warming scenarios that were designed to reflect the temperature (+3°C above ambient) and atmospheric pCO_2 conditions (increase of 350 – 600ppm) predicted for the mid- to end-of-century. Results suggest that *O. edulis*, and especially *M. gigas*, are likely to become less nutritious (i.e. containing lower levels of protein, lipid, and carbohydrate), and have reduced caloric content under ocean acidification and warming. Important changes to essential mineral composition under ocean acidification and warming were evident in both species; enhanced accumulation of copper in *M. gigas* may

be of concern regarding consumption safety. In light of these findings, the aquaculture industry may wish to consider a shift in focus toward species that are most robust to climate change and less prone to deterioration in quality, in order to secure future food provision and socio-economic benefits of aquaculture.

Keywords: Oyster; living resources; biochemistry; food security; global change; environmental stress; multi-stressors; *Magallana gigas; Crassostrea gigas*

1 Introduction

Seafood is the source of >15% of animal protein consumed globally, yet climate change is of increasing threat to the security of this resource (FAO, 2014: Golam et al., 2017; Rice and Garcia, 2011). This, as well as the continued burgeoning human population (Gerland *et al.*, 2014; United Nations, 2015), especially in coastal areas (Firth *et al.*, 2016), are placing increasing and arguably unsustainable demands on sources of animal protein, which are unlikely to be met by land farming (Campbell *et al.*, 2017; Cooley *et al.*, 2012; Delgado, 2003). Some argue the marine environment can make up the shortfall via a 'Blue Revolution'. But as overfishing, habitat destruction, and climate change are already causing decline in fish stocks in many areas (Macura *et al.*, 2016; McCauley *et al.*, 2015; Pauly *et al.*, 1998), there is growing concern about the resilience of the marine environment to withstand increased anthropogenic pressure and provide sustainable food provision in the future (Knights *et al.*, 2015; Porter *et al.*, 2014; UNEP, 2010; Weatherdon *et al.*, 2016).

Aquaculture is increasingly promoted as an alternative to land-based meat production and a solution for securing food provision in the future (Gentry *et al.*, 2017; Naylor *et al.*, 2000; Tacon & Metian, 2013). The aquaculture industry is the fastest growing food sector, with production increasing nearly year-on-year since the 1950s (FAO, 2016), which has now surpassed that of capture fisheries. Molluscan aquaculture is increasingly important, with many molluscs strategically chosen due to their low production cost compared to that of other fish and shellfish due to no requirement for feed (Tacon & Metian, 2013; Troell *et al*. 2014). In 2015, ~15% of the total aquaculture production volume was attributed to molluscan aquaculture (over 16 million tonnes; worth over US$18billion) (FAO 2018). Additionally, mollusc aquaculture has been found as having the lowest environmental production impacts

of all animal source food, and therefor may constitute a more sustainable source of protein (Froehlich *et al*., 2018; Hilborn *et al*., 2018).

The increased prevalence of obesity in several regions of the world (Abarca-Gómez *et al.*, 2017) is leading to greater public awareness and desire to consume a healthy and balanced diet. A healthy diet should include sufficient proteins, amino acids, essentials fats such as long-chain omega-3 fatty acids, vitamins and minerals (FAO, 2016; Simopoulos, 2002). The proximate composition can be used as a measure of nutritional quality (see EFSA NDA Panel, 2014; Hart & Fisher, 1971; Nielsen, 2006; Tate *et al.*, 2017), dividing the food into fractions including moisture, ash, protein, lipids and minerals. Seafood contains high levels of these important components compared to other meats (reviewed in Tacon & Metian, 2013) and is therefore viewed as highly nutritious, and key to human health and well-being (FAO/WHO, 2011; Lloret *et al.*, 2016; Simopoulos, 2002). Oysters, in particular, are a popular and well-known natural source of these nutrients (Asha *et al.*, 2014; Cochet *et al.*, 2015; Orban *et al.*, 2004; Pogoda *et al.*, 2013; Sprague *et al.*, 2017).

In 2015, global oyster production exceeded 5.4 million tonnes, and was valued at >US$4 billion. In the UK, oysters are one of the major aquaculture species (Pinnegar *et al.*, 2017) with ~1600 tonnes produced in 2015, and worth more than US$6.4 million. Yet there is increasing concern over the long-term future of shellfish production due to the effects of environmental stressors associated with rising atmospheric CO_2 levels such as warming and marine heat waves, falling levels of seawater carbonate and oxygen plus rising sea levels and increased storminess (Branch *et al.*, 2013; Cooley *et al.*, 2015; Dupont *et al.*, 2014; Ekstrom *et al.*, 2015; Lemasson *et al.*, 2017b). Ocean acidification and sea-surface warming are changing animal physiology and affecting the quality of seafood (Dupont *et al.*, 2014; Tate *et*

al., 2017). In oysters, the effects of ocean acidification are already being detected (Lemasson *et al.*, 2017a), with several hatcheries experiencing declines in production, jeopardising economic revenues and necessitating adaptive actions (Barton *et al.*, 2015; Cooley *et al.*, 2017). However, physiological effects of ocean acidification and warming in oysters appear species-specific (Lemasson *et al.*, 2018).

To date, there has been limited consideration of potential changes in the quality of shellfish under warming and acidification. The few published studies have shown changes, such as reductions in protein and lipid content, and reductions in omega-3 fatty acids (Ab Lah *et al.*, 2018; Clements *et al.*, 2018; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015). A better understanding is needed if we are to shed light on the future of shellfish aquaculture. Here, using two economically and commercially important species of oysters, *Magallana gigas* – a non-native introduced species – and *Ostrea edulis* – a native species –, we tested if ocean acidification and warming conditions predicted under future climate scenarios has the potential to impact seafood nutritional quality. We also consider how condition indices – a widely used metric in aquaculture for evaluating health and value of bivalves because they are correlated with meat yield (Knights, 2012; Orban *et al*. 2002, 2006) – might change under ocean acidification and warming scenarios.

2 Methods

2.1 Organism collection and treatments

Collection of organisms, acclimation, treatments, and mesocosm set-up followed the protocol described in Lemasson *et al.* (2018). Following 14 days of acclimation to laboratory conditions (16°C, salinity 33, ~400 ppm pCO_2, 12:12 dark:light cycle, fed *ad libitum* with a

mixed algal diet (Shellfish Diet, 1800; Reed Mariculture)), each oyster was placed in its own 3 L experimental tank and exposed for 12 weeks to three pCO_2 concentrations (ambient ~400 ppm, intermediate ~750 ppm, elevated ~1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C) in an orthogonal experimental design (*M. gigas* n= 4; *O. edulis* n=8 individuals per treatment). This design aimed to simulate current and future ocean acidification and warming scenarios, using scenarios in line with conditions predicted by the IPCC (IPCC, 2013) and for the UK for mid- to end-century (see also Lemasson *et al.*, 2018). Throughout the study, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet, 1800; Reed Mariculture).

Temperature, salinity, and pH were measured daily in all replicate tanks. 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 (AT) 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 CO_2 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 KSO_4 dissociation constant from Dickson (1990).

Table 1: Seawater chemistry for *Magallana gigas* and *Ostrea edulis* in each treatment. Data shown are means (±SD) values. T=Temperature in °C. ppm= parts per million. Ω_{Ca}= saturation state of calcite. Ω_{Ar}= saturation state of aragonite. A_T= Total alkalinity in mmol/kg seawater.

	Treatment	Measured				Calculated		
	(pCO_2 + Temperature)	pH	T	A_T	S	pCO_2	ΩAr	ΩC_a
Magallana gigas	Ambient + Control	7.79±0.10	16.9±0.2	2.13±0.32	33.9±1.1	597.2±146.1	1.70±0.32	2.64±0.50
	750 ppm + Control	7.67±0.12	16.9±0.2	2.13±0.33	33.9±1.2	816.9±296.4	1.4±0.36	2.10±0.55
	1000 ppm + Control	7.55±0.10	16.8±0.2	2.13±.032	33.9±1.2	1174.6±420.9	0.99±0.22	1.53±0.34
	Ambient + Elevated	7.84±0.10	20.4±0.3	2.32±0.29	34.3±1.2	669.7±155.9	2.02±0.31	3.11±0.47
	750 ppm + Elevated	7.70±0.11	20.6±0.4	2.33±0.29	34.2±1.1	945.1±275.7	1.60±0.34	2.46±0.52
	1000 ppm + Elevated	7.56±0.10	20.2±0.3	2.34±0.31	34.3±1.2	1376.8±280.8	1.14±0.17	1.76±0.26
Ostrea edulis	Ambient + Control	8.00±0.08	16.5±0.3	3.04±0.18	34.2±0.8	481.4±90.9	3.70±0.65	5.75±1.01
	750 ppm + Control	7.84±0.08	16.6±0.2	3.04±0.19	34.2±0.8	760.1±178.2	2.68±0.51	4.17±0.80
	1000 ppm + Control	7.72±0.16	16.6±0.2	3.00±0.16	34.3±0.7	1053.6±223.3	2.15±1.20	3.34±1.87
	Ambient + Elevated	8.00±0.08	19.8±0.3	2.86±0.15	34.4±0.9	467.9±78.4	3.80±0.65	5.85±1.01
	750 ppm + Elevated	7.90±0.07	20.2±0.5	2.87±0.15	34.4±09	694.7±135.4	2.94±0.47	4.52±0.72
	1000 ppm + Elevated	7.70±0.09	19.8±0.3	2.6±0.22	34.4±0.9	1165.0±226.8	2.01±0.47	3.10±0.73

2.2 Condition index, proximate composition, and energy content

After 12 weeks exposure, oysters were manually shucked and their wet tissue mass (g) was recorded on an electronic balance (Mettler AE240), before being oven-dried at 105°C for 24 hours until constant mass was achieved.

The Condition Index (CI) of each oyster was calculated following the method recommended by Lucas and Beninger (1985) as follows:

$$CI = (dry\ meat\ weight / dry\ shell\ weight) \times 100$$

Moisture percentage of the meat was calculated for each individual oyster according to the following formula:

$$Moisture\ (\%) = ((\text{Total weight} - \text{Dry weight})/\text{Total weight}) \times 100$$

For each species, following estimation of Condition Index and moisture content for all individuals, the dried meat samples were then pooled by treatment to provide sufficient tissue material for proximate composition and energy content analyses. Pooled samples were homogenised, then ground into a fine powder using a coffee grinder. Complete or partial pooling of specimens from the same treatment or sampling site for biochemical analysis has been reported in several studies (Fernandez *et al.*, 2015; Marin *et al.*, 2003; Soto-Jiménez *et al.*, 2001). While not allowing individual comparisons, this approach provides nutritional information at the population level. The following assays were performed in triplicate.

Ash content (a measure of the total amount of minerals present within a food) was determined using 500mg of dried tissue samples and an adaptation of the Association of Official Agricultural Chemists official method (AOAC, 1995). Lipid content was determined by continuous extraction of fat from 2 g material using petroleum ether as a solvent following the Soxhlet method (Luque de Castro & García-Ayuso, 1998; Manirakiza *et al.*, 2001) in a Soxtherm Rapid Extraction Unit (C. Gerhardt GmbH & Co. KG). Total protein content was determined using the Kjeldahl method (Kjeldahl, 1883) on ~150 mg samples with a Gerhardt Kjeldatherm digestion block, a Gerhardt Turbosog scrubber unit and a Gerhardt Vapodest 50s distillation unit (Gerhardt Laboratory Instruments, Bonn, Germany). Glycogen content was determined indirectly by calculating carbohydrate content using the above results for moisture, ash, lipid, and protein content following Maclean *et al*. (2003) as follows:

$$Carbohydrates\ (\%) = 100 - (\%\text{M} + \%\text{A} + \%\text{L} + \%\text{P})$$

Where:

C=carbohydrate, M=moisture, A=ash, L=lipid, and P=protein. All values used were as percentage of total weight.

Caloric (energy) content was measured as gross energy content ($kJ.g^{-1}$) by bomb calorimetry using an isoperibol oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA) on ~1 g of material per sample.

2.3 Macro and micro-minerals

Macro-mineral (calcium [Ca], potassium [K], magnesium [Mg], sodium [Na]) and micro-mineral (copper [Cu], iron [Fe], zinc [Zn]) content was determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; iCAP 7000series Thermo Scientific) following standard protocols as reported elsewhere (Rider *et al.*, 2010). The content of the micro-mineral selenium [Se] was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Xseries2, Thermo Scientific) following standard protocols. Mineral composition was determined on ~100-150 mg of material per sample. Before use, both ICP-OES and ICP-MS were calibrated using mixed, matrix-matched standards (0–100 $\mu g.L^{-1}$) prepared from certified Aristar plasma emission grade solutions. Quality control was assured by carrying out accuracy checks using a known standard or blank every 10 samples during the analysis. Also, 2% internal iridium and indium standards (P/N/4400-013 CPI, Quality control standard 26) were added to each sample. DORM-3 (dogfish certified reference material − CRM) from National Research Centre Canada (NRCC) was used to verify the digestion procedure as reported elsewhere (Rossbach *et al.*, 2017).

2.4 Statistical analyses

The logistical constraints of the experimental infrastructure meant oyster species had to be tested sequentially. There were natural variations in the chemistry of the seawater used between experiments due to natural seasonal fluctuations in seawater properties combined with differences in atmospheric partial pressure (barometric pressure). These fluctuations in pCO_2 also led to fluctuations in CO_2 adsorbed by the seawater. The resulting pCO_2, and pH, conditions were therefore different between experiments, but also within experiments were different from the expected levels of ~400ppm, ~750ppm and ~1000ppm. Nevertheless, the effect size (magnitude of difference in pCO_2 and pH between treatments within experiments) were comparable. These constraints prevented a formal comparison of the two species in a factorial design, so these were analysed separately. Analyses were performed using R Version 3.2.5 (R Core Development Team, 2018) using the *base*, *MASS*, *stat* and *vegan* packages. $p < 0.05$ was used as statistical threshold.

Condition Index and Moisture content

Condition index and moisture data (obtained before pooling of the samples) were tested for homogeneity of variances using Levene's test (*car* package). Tests for differences in condition index and moisture content between treatments were done using 2-factor ANOVA with 'temperature' and 'pCO_2' as fixed factors.

Proximate composition, energy content, and mineral composition

As oyster tissues were pooled in order to provide sufficient material to perform these analyses, there were no 'true replicates' (*sensu* Hurlbert, 1984). Triplicate measures of protein, lipid, ash, calorimetry, and mineral analysis were performed to determine within sample variability.Data were pooled, and averages used for statistical analysis to avoid Type I error.

The pooled samples used were therefore homogenised samples of multiple individuals (*M. gigas* n=4; *O. edulis* n=8), and in effect the single values associated with each assay are means without the variances. Analyses were thus performed on these single values.

Calorimetry data were analysed using a 2-way ANOVA with 'temperature' and 'pCO_2' as fixed factors (n=3 per Temperature level; n=2 per pCO_2 level). Due to this experimental design, interactions between the two factors could not be assessed.

To compare proximate and mineral compositions across species, temperature and pCO_2, nonmetric multidimensional scaling (nMDS) coupled with Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) was used to evaluate the significance of observed patterns (based on Euclidian distance and 1000 permutations of the data) and test hypotheses related to changes in composition due to experimental treatment.

For tests using ANOVA, when significant differences were present, *post-hoc* Tukey tests were performed to assess differences between treatment levels.

3 Results

3.1 Condition Index and Moisture

Temperature, but not pCO_2, had a significant effect on the condition index of *M. gigas* ($F_{1,12}$ = 12.298; p<0.01), with condition index negatively impacted by elevated temperature (Figure 1a). Mean condition index decreased 35% with increased temperature, from ~3.2 (±0.3) at ambient temperature to ~2.1 (±0.20) at elevated temperature. While marginally not significant ($F_{2,18}$=2.97; p=0.07), there were clear differences in condition indexbetween *M. gigas* cultured under different temperature and pCO_2 regimes, with a trend toward increasing

condition indexwith increasing pCO_2 at the control temperature, and decreasing condition indexwith increasing pCO_2 at elevated temperature (Figure 1a). The condition indexof *O. edulis* was unaffected by any of the treatments. Moisture – the principal contributor to oyster flesh – was also unaffected by temperature or pCO_2 treatment and ranged between 70-80% for both species (Table 2; Figure 1b).

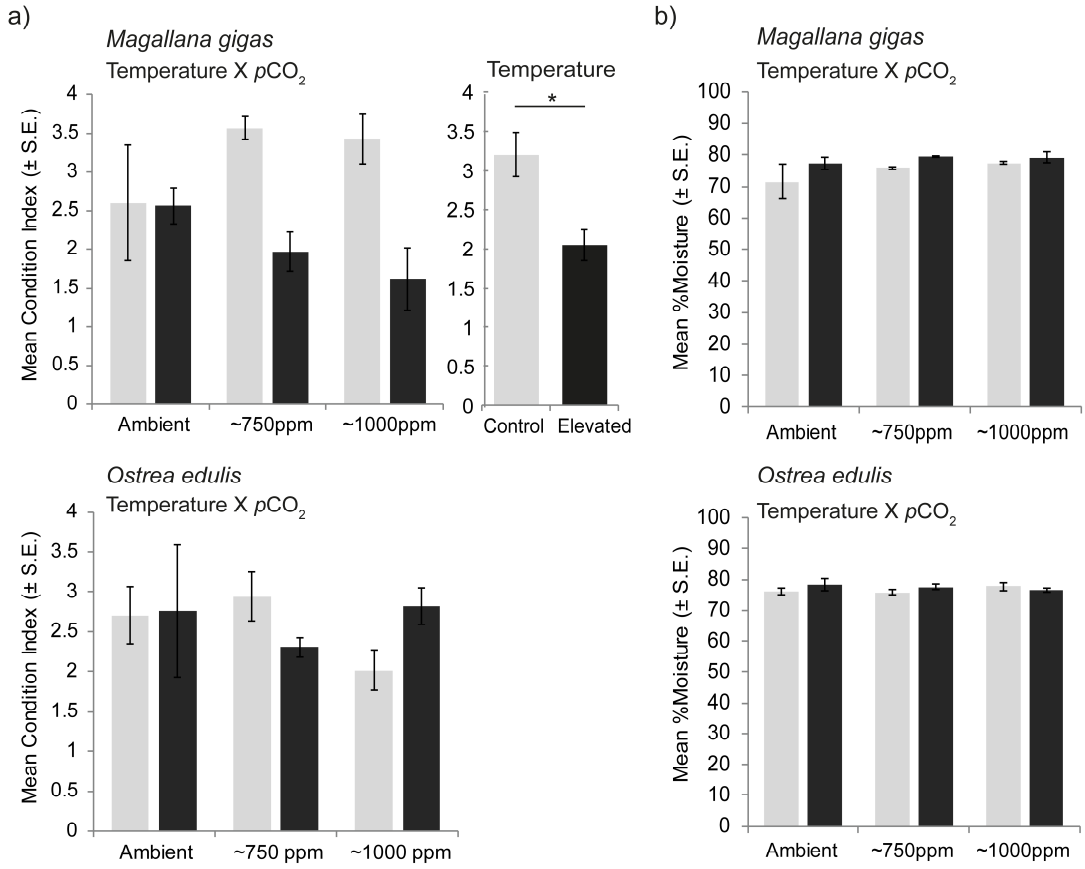


Figure 1 Difference in a) Condition Index and b) moisture content (as % of total weight), of *Magallana gigas* and *Ostrea edulis* across temperature and pCO_2 treatments. ppm= part per million; s.e. = standard error. Grey = control temperature. Black= elevated temperature. * indicates significant difference.

3.2 Proximate composition analysis

There were significant differences in proximate composition with temperature and pCO_2 for both species (*Magallana gigas:* perm $F_{2,24}$=75.41; $p < 0.001$; *Ostrea edulis:* perm $F_{2,24}$=14.37; $p < 0.001$) (Table 2, Figures 2 & 3a-d). For all treatments, after moisture, protein was the second largest component in both *M. gigas* and *O. edulis* under ambient pCO_2 and control temperature, representing 16.6% and 9.9% of the total composition, respectively (Table 2; Figure 2). *Ostrea edulis* was characterised by higher carbohydrates (10.5g per 100g) than *M. gigas* (4.5g per 100g), but *M. gigas* had higher percentages of proteins and lipids (Table 2,). Temperature (Figure 3a-b) and pCO_2 (Figure 3c-d) both led to clear dissimilarity in proximate composition between species, with greater changes apparent in *M. gigas*, largely driven by reductions in proteins and lipids (Table 2, Figure 2) from 16.6g to 11.8g and from 4.8g to 1.4g, respectively. The treatments also appeared to a lesser degree to negatively impact the carbohydrate proportion in *M. gigas*, which dropped from 4.9g to 3.3g (Table 2; Figure 2). There were significant differences in proximate composition between all pCO_2 treatments in *M. gigas,* but in *O. edulis*, there was no difference between oysters cultured in 400 and 1000 ppm, whereas those cultured in intermediate pCO_2 (750ppm) were significantly different (Figure 3c). This was particularly evident in *O. edulis* for the lipid proportions, which were reduced from 1.3g to 0.9g at elevated temperature and intermediate pCO_2 (~750 ppm) (Table 2; Figure 2).

Table 2: Proximate composition of *Magallana gigas* and *Ostrea edulis* under six ocean acidification and warming scenarios. Moisture represents the remaining component, adding up to 100g. In g per 100g sample (wet weight).

	Treatment (pCO_2 x T)	Protein	Carbo-hydrate	Lipid	Ash	Moisture
Magallana gigas	Ambient x Control	16.6	4.5	4.8	2.7	71.4
	~750ppm x Control	14.6	4.6	2.9	1.9	76.0
	~1000ppm x Control	12.6	3.9	3.9	2.1	77.5
	Ambient x Elevated	12.8	4.9	2.7	2.2	77.4
	~750ppm x Elevated	13.7	3.3	1.4	2.4	79.6
	~1000ppm x Elevated	11.8	4.3	2.6	2.1	79.2
Ostrea edulis	Ambient x Control	9.9	10.5	1.3	2.1	76.2
	~750ppm x Control	10.3	10.3	1.3	2.1	75.9
	~1000ppm x Control	9.7	8.7	1.5	2.4	77.7
	Ambient x Elevated	8.8	9.3	1.6	2.0	78.3
	~750ppm x Elevated	10.4	8.7	0.9	2.5	77.6
	~1000ppm x Elevated	9.2	11.1	1.3	1.9	76.4

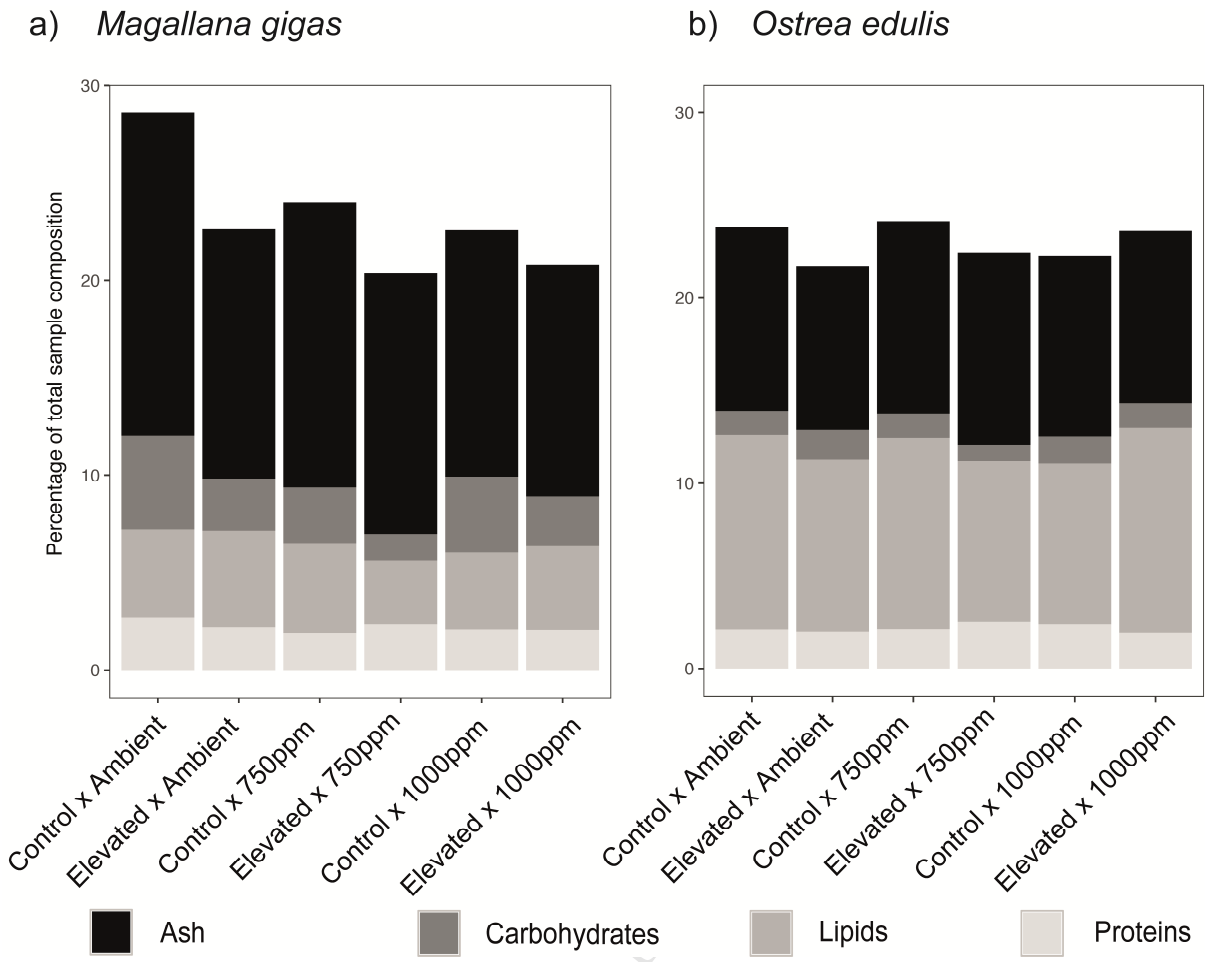
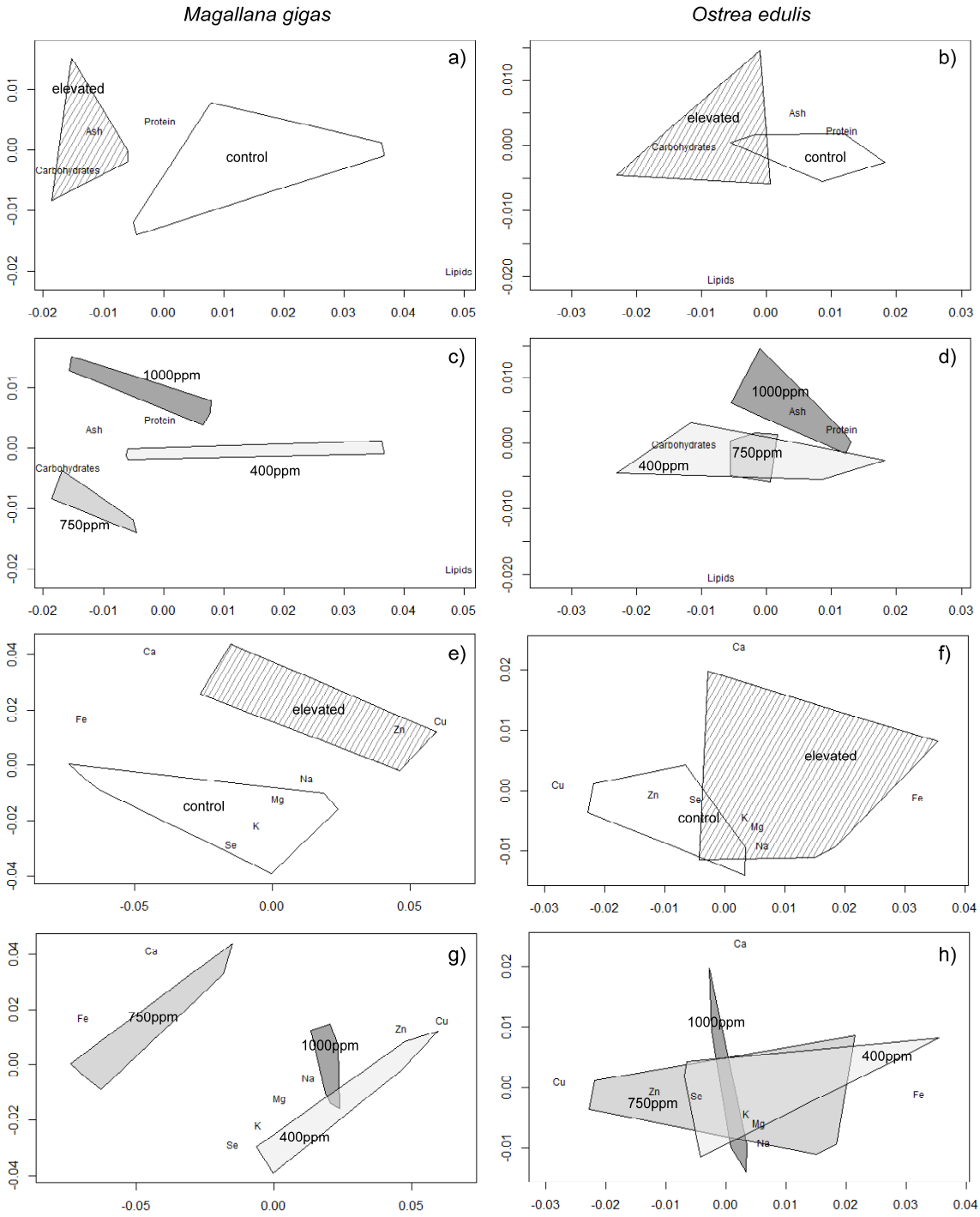


Figure 2: Relative composition of proximate components present in a) *Magallana gigas* and b) *Ostrea edulis* across temperature and pCO_2 treatments. The values for each treatment represent the means of the three procedural replicates of the pooled samples. ppm= part per million.

286



287

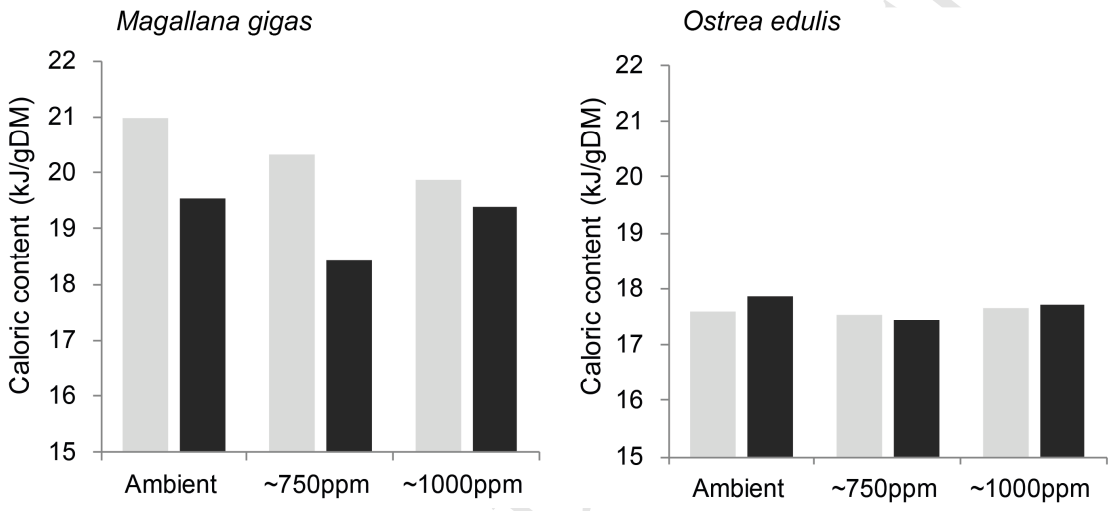
288 **Figure 3:** nMDS plots of proximate (a-d) and mineral compositions (e-h) of *Magallana*
289 *gigas* (left column) and *Ostrea edulis* (right column). Dispersion of points within temperature
290 (plots a, b, e, f) and pCO_2 (plots c, d, g, h) treatments are illustrated using cluster hulls plotted
291 using the R package '*ggplot2*'. Centroids of each proximate composition and mineral
292 components are shown. 2D stress for all plots < 0.08.

293

294 3.3 Energy content

295 There was a 13% reduction in the caloric content of *M. gigas* with temperature and pCO_2
296 from 20.97 kJ/gDW at control temperature and ambient pCO_2 to 18.41 kJ/gDW at elevated
297 temperature and intermediate pCO_2 (~750 ppm) (Figure 4). The energetic value of *O. edulis*
298 did not change (17.63 kJ/gDW) with treatment (Figure 4).

299



300

301 **Figure 4:** Variations in the caloric content of oysters across temperature and pCO_2 treatments
302 for *Magallana gigas* (left) and *Ostrea edulis* (right). ppm= part per million; Light grey =
303 control temperature. Dark grey= elevated temperature. The value for each treatment
304 represents the mean of the three procedural replicates of the pooled samples, therefore no
305 error bars were obtained. Values are per kg of oyster dry matter (DM).

306

307 3.4 Trace elements

308 There were significant differences in trace elements composition with, temperature and pCO_2
309 levels for both species (*Magallana gigas:* perm $F_{2,24}$=166.75; $p < 0.001$; *Ostrea edulis:* perm
310 $F_{2,24}$=16.27; $p < 0.001$) (Table 3, Figure 3e-h). *Magallana gigas* was characterised by higher
311 Se, Fe, K, Mg, and Na content than *O. edulis* (Table 3, Figure 3e-h), but *O. edulis* displayed
312 higher Zn levels. Temperature led to clear dissimilarity in the mineral composition of each

oyster species, with greater change apparent in *M. gigas* (Figure 3e-fa). pCO_2 also had notable effects, but only on *M. gigas* (Table 3, Figure 3g-h). Overall, the mineral composition of *M. gigas* was clearly affected by the treatments (Table 3), with notable increases in Cu and Zn content, and decreases in Fe and Se contents.

Table 3: Mineral composition of *Magallana gigas* and *Ostrea edulis* under six ocean acidification and warming scenarios. T= temperature. ppm= part per million. Ca=calcium; Cu=copper; Fe=Iron; K=potassium; Mg=magnesium; Na=sodium; Zn=zinc; Se=selenium. All values are in $mg.kg^{-1}$, except Se which is in $\mu g.kg^{-1}$.

	Treatment (pCO_2 x T)	Ca	Cu	Fe	K	Mg	Na	Zn	Se
Magallana gigas	Ambient x Control	1250.0	64.7	205.5	322.1	847.1	4626.3	450.3	537.1
	~750ppm x Control	492.8	60.0	35.7	268.1	553.5	3101.7	404.9	313.1
	~1000ppm x Control	618.1	149.0	54.0	270.2	676.7	3811.5	749.2	459.7
	Ambient x Elevated	1409.6	92.5	74.7	219.2	509.6	3111.3	566.8	248.8
	~750ppm x Elevated	544.5	202.0	57.7	260.4	793.6	5476.6	1063.2	309.4
	~1000ppm x Elevated	719.1	143.5	69.4	214.2	609.5	3969.1	798.3	314.0
Ostrea edulis	Ambient x Control	1567.2	196.2	48.4	277.4	546.0	3747.0	1077.3	263.6
	~750ppm x Control	1535.5	127.1	39.3	253.3	496.8	3390.0	862.9	232.8
	~1000ppm x Control	1353.5	103.0	42.0	270.7	636.7	4571.7	940.0	252.1
	Ambient x Elevated	1267.3	87.6	55.9	257.4	501.3	3503.5	623.8	192.0
	~750ppm x Elevated	1560.6	111.1	64.8	258.8	539.5	3842.1	879.7	256.1
	~1000ppm x Elevated	2142.2	113.4	37.8	258.0	545.5	3672.5	898.1	248.8

4 Discussion

The ability of human society to feed the ever-growing population is a major ongoing concern, particularly as climate change is already negatively impacting food production from both terrestrial and marine environments (Brander *et al.*, 2017; Campbell *et al.*, 2017; UNEP, 2010). Mollusc aquaculture is increasingly recognised as a solution to this issue. Here, following exposure to temperature and pCO_2 levels predicted for 2050 to 2100, we show species-specific variations in the nutritional quality of two commercially important oyster species. Both *O. edulis* and *M. gigas* displayed changes in biochemical (proximate and mineral) composition; in particular *M. gigas* had lower lipid, carbohydrate, and protein levels, but higher copper concentration, which could pose concerns for both future food safety and security.

4.1 Condition Index and Moisture content

Condition indices are widely used in aquaculture to evaluate the overall health and value of bivalves, and select specimens of the highest quality (Knights, 2012; Orban *et al.*, 2006; Orban *et al.*, 2002). These indices are correlated with the meat yield, which declines in bivalves under stressful environmental conditions the require significant energetic expenditure (Orban *et al.*, 2002). The condition indexof *M. gigas* was negatively impacted by elevated temperature but not elevated pCO_2, whereas the condition indexof *O. edulis* was unaffected by any of the treatment conditions, suggesting that the two species did not experience or respond to environmental change in the same way. Changes in condition index reflect the respective changes in feeding and respiration rates to ocean acidification and warming of the two species, as observed by Lemasson *et al.* (2018). In the Lemasson *et al.* (2018) study, *M. gigas* increased its metabolic rate at elevated temperatures and reduced its

feeding rate at elevated levels of pCO_2 (~750 ppm), leading to reduced condition index, whereas the metabolic rate and feeding rate of *O. edulis* was unaffected by ocean acidification and warming. These results are in contrast to those of Lannig *et al*., (2010) on *M. gigas* who recorded a decrease of ~20% in condition index between individuals exposed to ambient and elevated pCO_2 (see further discussion on the effects of ocean acidification and warming on bivalves in Lemasson *et al.,* 2018). In bivalves, declines in condition index usually suggest depletion of reserves following energetic reallocation, which can lead to changes in individuals biochemical composition (proximate and mineral) and consequently in their nutritional value (see EFSA NDA Panel, 2014; Tate *et al.*, 2017).

Water constitutes the major part of oysters (Asha *et al.,* 2014). This component is linked to juiciness, which is an important sensory trait of oysters and influences their marketability (Cruz-Romero *et al.*, 2004). Sensory traits, such as juiciness, texture, appearance, odour or taste, are linked to biochemical composition, and have recently been shown to be unchanged in oysters under ocean acidification and warming (Lemasson *et al*., 2017b). As was also observed in *Turbo militaris* (Ab Lah, 2018), here the moisture content of either *M. gigas* or *O. edulis* did not change when exposed to ocean acidification and warming conditions.

4.2 Energetic reserves

Protein, lipids, and carbohydrates constitute the main energy storage compounds in bivalves, which all have important functions in physiological processes, for instance gametogenesis and reproduction (Dridi *et al.,* 2007). By influencing oysters physiology and metabolic responses, environmental conditions, such as ocean acidification and warming, can dictate the accumulation and depletion of energetic reserves in bivalves (Clements *et al.,* 2018)..

Carbohydrates

Carbohydrate, in the form of glycogen, is thought to be the energy reserve present in the highest quantity in bivalves and is used to sustain routine metabolic processes (Anacleto *et al.*, 2014, and references therein). A decline in glycogen content under environmental stress (e.g. hypercapnia, hyposalinity, increased temperature) is common in oysters and can indicate physiological stress (Dickinson *et al.*, 2012). Here, the carbohydrate content of *M. gigas* was reduced under ocean acidification and warming, particularly at ~750 ppm pCO_2 and elevated temperature, but was unaffected in *O. edulis*. Carbohydrate content remained high in *O. edulis* (>8.5% wet weight) compared to *M. gigas* (<5% wet weight). Native bivalve species have previously been shown to have higher glycogen content than non-native and invasive species, this may be a metabolic adaptive strategy to cope with environmental change (Anacleto *et al.*, 2014), and may account for the lack of response of *O. edulis* to ocean acidification and warming here. Given the importance of carbohydrates for oyster maintenance, condition, and their ability to sustain physiological processes, depletion of carbohydrate reserves in *M. gigas* might jeopardize organisms survival in the long term, which aligns with results showing reduced condition (Lemasson et al., 2018).

Proteins

Proteins supply structural elements and have a crucial role in metabolic reactions. Under sustained stress, bivalves can catabolise proteins to mobilise energy once carbohydrates and lipids have been depleted (Barber & Blake, 1985). *Magallana gigas* and *O. edulis* were both high in protein, but under ocean acidification and warming *M. gigas* displayed important reductions. A similar response was shown in the whelk *Dicathais orbita* , large declines (>50%) in protein content under ocean acidification and warming (Tate *et al.*, 2017), but in *Mytilus edulis* (Clements *et al*., 2018) and *T. militaris* (Ab Lah *et al.*, 2018*)*, no reductions in protein were observed suggesting taxon-specific responses.

400

401 *Lipids*

402 The array of lipids in molluscs, with a low proportion of saturated fatty acids and high
403 proportion of polyunsaturated fatty acids (including Ω-3), offer numerous health benefits to
404 people (Sprague *et al.*, 2017). Here, *M. gigas* contained higher levels of lipids (~1.4-4.8%
405 wet weight) compared to *O. edulis*, in the range reported elsewhere (Cochet *et al.*, 2015;
406 Pogoda *et al.*, 2013; Shpigel *et al.*, 1992). In contrast, *O. edulis* used in this study were
407 relatively poor in crude lipids (~0.8-1.6% wet weight), with values below those reported
408 elsewhere (Pogoda *et al.*, 2013). Ocean acidification and warming scenarios led to a decrease
409 in lipid content in both species, particularly notable at ~750 ppm pCO_2. Larger absolute
410 reductions occurred in *M. gigas* (30%; wet weight percentage) which possessed a higher
411 baseline lipid content under control conditions, but larger percentage reductions were
412 apparent in *O. edulis* (~50%; wet weight percentage). Reductions in total lipid content and
413 differences in fatty acid composition (including decreases in polyunsaturated fatty acids)
414 under ocean acidification and warming have been shown in other molluscs (Ab Lah *et al.*,
415 2018; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015 but see Clements *et al*. 2018), with
416 variation attributed to differential deposition and energy use rates between species (Child &
417 Laing, 1998; Pogoda *et al.*, 2013).

418

419 Although carbohydrates (and especially glycogen) are often the preferred source of energy
420 for oysters, species-specific differences may exist (see discussion in Pogoda *et al.*, 2013). It
421 has previously been suggested that *O. edulis* preferentially use lipids whereas *M. gigas* use
422 proteins as their principal energy source for metabolic activity when subjected to food
423 limitation (Child & Laing, 1998). Here, both oyster species when exposed to ocean
424 acidification and warming appeared to use lipids and carbohydrates as their primary source of

425 energetic reserves, but to a lesser extent by *O. edulis*, possibly because they did not have
426 important lipid reserves in the first place. Depletions of energetic reserves were indeed
427 particularly apparent for *M. gigas*, with additional reductions in proteins reflected in the
428 reduced condition index and caloric content, especially at intermediate pCO_2 level. The
429 differential use of energetic reserves by oysters is therefore likely a consequence of the
430 differential physiological stress endured when exposed to ocean acidification and warming
431 conditions (Lemasson et al., under review).
432
433 4.3 Mineral content
434 Seafood quality also varies based on the proportion (total ash) and composition of inorganic
435 minerals. In particular, minerals are an essential component of a healthy diet in humans
436 (EFSA NDA Panel, 2014). Here, the two oyster species displayed similar ash content (~1.9-
437 2.6%), which was unimpacted by ocean acidification and warming. In fact, a modest increase
438 in ash content in *O. edulis* under elevated pCO_2 might indicate mineral accumulation within
439 the tissue. Ab Lah *et al*., (2018) have also found no changes in ash content of *T. militaris*
440 under ocean warming and acidification.
441
442 Although nutritionists often focus on macronutrients, such as calcium (Ca) and magnesium
443 (Mg), which are beneficial for teeth and bones (Lambert *et al.*, 2017), there is an increasing
444 understanding of the dietary benefits of trace minerals (FAO, 2016). For instance, potassium
445 (K)-rich foods are considered particularly healthy; selenium (Se) strengthens the immune
446 system and reduces oxidative stress in tissue (Rayman, 2000); and zinc (Zn) and iron (Fe) are
447 critical for stamina and disease resistance (Knez *et al.*, 2017; Solomons & Schümann, 2017).
448 Moreover, micronutrient deficiencies afflict an enormous proportion of the population. For

449 instance, over 2 billion people are diagnosed as iron-deficient, and an estimated 800,000
450 children die every year from zinc deficiency (FAO, 2016).
451
452 In this study, large differences in the levels of macro and micro-nutrients between *M. gigas*
453 and *O. edulis* were evident, which is unsurprising as species-specific differences in mineral
454 composition is common in bivalves (Bray *et al.*, 2015). Notably, *M. gigas* exposed to the
455 current climate conditions were relatively high in K, sodium (Na), Mg, Fe, and Se when
456 compared to *O. edulis*, which was high in Zn. While the values presented here for macro- and
457 micro-minerals are within the ranges described in other studies on oysters and may not be
458 locally dependent (Se: Cantillo, 1998; Fe: Diaz Rizo *et al.*, 2010; Na, K, Mg, Fe, Se: Orban
459 *et al.*, 2004), concentrations of copper (Cu) and Zn in this study were significantly higher
460 than those commonly found in literature (Cantillo, 1998). High Cu and Zn contents have been
461 described for oysters growing in contaminated locations associated with mining and harbour
462 activities (Diaz Rizo *et al.*, 2010; Frias-Espericueta *et al.*, 2009). Plymouth Sound – the
463 location of oyster collection for this study – has a long history of mining that has led to
464 significant contamination of its waters and substrates (see Knights *et al.*, 2016 and references
465 therein), which could explain the elevated Cu and Zn levels obtained here.
466
467 Here, exposure to ocean acidification and warming conditions led to species-specific changes
468 in the concentration of those minerals, with the mineral composition of *M. gigas* being
469 especially affected. A recent study found increased levels of Zn in *T. militaris* exposed to
470 ocean acidification and warming conditions, but without changes in other micro- and macro-
471 elements concentrations (Ab Lah *et al.*, 2018). Here, the reductions in Ca, Fe, and Se content
472 in *M. gigas* exposed to ocean acidification and warming to levels similar or lower than
473 *O. edulis*, coupled to the accumulation of Cu, represent a measurable change to its nutritional

474 value, which could have nutritional and safety implications. Copper, along with other trace
475 metals such as arsenic, copper and lead, can become toxic to marine organisms in high
476 concentrations (Götze *et al*., 2014; Moreira *et al.,* 2016), and threaten human health through
477 seafood ingestion (Bhupander *et al*., 2011; Han *et al.,* 1998). Since bivalves are filter feeders,
478 they readily accumulate metals present in the surrounding waters into their edible tissue (Lu
479 *et al.,* 2017; Raposo *et al.,* 2009). This process can be modulated by ocean acidification, for
480 instance enhancing the bioaccumulation of Cu in oysters (Belivermiş *et al*., 2015; Götze *et al.*,
481 2014; Hawkins & Sokolova, 2017; Ivanina *et al.*, 2015; Ivanina *et al*., 2016). While Cu
482 accumulation under ocean acidification and warming can come at metabolic costs to
483 organisms (Hawkins & Sokolova, 2017), the implications for human consumption are still
484 unclear. For instance, in Plymouth Sound where background levels are already high, further
485 bioaccumulation of potentially harmful minerals, such as Cu or Zn, in *M. gigas* could exceed
486 safe levels for consumption.
487
488
489 4.4 Implications for food security and aquaculture management
490 Our results suggest that the nutritional quality of *M. gigas*, but not *O. edulis*, is likely to be
491 affected by short-termwarming and acidification of coastal seawater caused by CO_2
492 emissions. These changes include reduced proteins, lipids, energetic value, as well as changes
493 to their essential mineral contents. Oysters are seldom a major contributor to human diet,
494 however islands and countries with little agricultural land rely on wild-caught seafood and
495 aquaculture for protein (Cooley *et al*., 2012). Should the changes observed in oysters be
496 widespread in seafood species, then the nutritional benefits of seafood to human health and
497 its role in food security may be further compromised (Cooley *et al.*, 2012; Ding *et al.*, 2017).
498 Given the need for additional and sustainable sources of proteins, the current exponential

expansion of the aquaculture industry is inevitable; nevertheless a careful evaluation of this industry as well as the development of appropriate mitigations plans (Clements and Chopin, 2017) are needed to ensure that aquaculture is a wise investment in the face of ocean acidification and warming. Diversifying the target species and promoting those currently under-utilized may supplement the industry with 'novel' sources of protein. However, this in practicality might face new challenges, such as selecting species that also thrive under aquaculture conditions and avoiding selecting non-native species (Arismendi *et al.,* 2009), and might require strategic management plants. In order to optimise protein supply and secure socio-economic benefits of mollusc aquaculture, research needs to focus on identifying and selecting native aquaculture species that are resilient to future climate conditions, and able to retain their beneficial nutritional properties (Cooley *et al.*, 2012; Sato *et al*., 2018), without introducing new challenges.

Our results suggest *M. gigas* is at higher risk of reduction in nutritional quality than its native counterpart *O. edulis* under future ocean acidification and warming scenarios. In the UK, a reduction in the nutritional quality of oysters may not quickly be recognised by consumers, but lower energetic reserves and condition of *M. gigas* may hold an economic relevance to the aquaculture industry, since this species currently represents >90% of the production (Humphreys *et al.*, 2014). Additionally, the biochemical composition can dictate meat appearance, aroma, taste and texture (Cochet *et al.*, 2015; Fratini *et al.*, 2013), and any changes in biochemical composition occurring because of ocean acidification and warming can impact on the sensory quality (Lemasson *et al.*, 2017b). Therefore changes in biochemical composition under ocean acidification and warming can influence the consumer appeal for the product, reducing the demand for it and depressing its economic value (Cooley *et al.*, 2012). As such, the UK aquaculture industry might need to reconsider the management

strategy for the future (Fernandes *et al.*, 2017; Jennings *et al.*, 2016) and consider a shift in focus toward species more robust to climate change, such as *O. edulis*, in order to secure future food provision and economic revenue.

5 Acknowledgements

We wish to thank Liz Preston, Victoria Cammack, and Natalie Sweet, technical staff in the Food and Nutrition Unit at Plymouth University, for their help and assistance towards the proximate composition analysis and calorimetry procedures. We also are grateful to Dr Robert Clough and Dr Andy Fisher for their guidance in using the ICP-OES and ICP-MS equipment. Additionally, we are indebted to the following undergraduate students for their continued assistance throughout this study: Samuel Provstgaard-Morys and Lucy Jupe. Finally, we are grateful to Mr Brian Langley, the National Trust, and the Carew Pole Garden Charitable Trust for granting us access to the collection site.

6 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.

7 References

Ab Lah, R., Kelaher, B. P., Bucher, D., & Benkendorff, K. (2018). Ocean warming and acidification affect the nutritional quality of the commercially-harvested turbinid snail *Turbo militaris*. *Marine environmental research*. https://doi.org/10.1016/j.marenvres.2018.08.009

Abarca-Gómez, L., Abdeen, Z. A., Hamid, Z. A., Abu-Rmeileh, N. M., Acosta-Cazares, B., Acuin, C., Adams, R. J., Aekplakorn, W., Afsana, K. & Aguilar-Salinas, C. A. (2017) 'Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults'. *The Lancet*, 390 (10113). pp 2627-2642.

Anacleto, P., Maulvault, A. L., Bandarra, N. M., Repolho, T., Nunes, M. L., Rosa, R. & Marques, A. (2014) 'Effect of warming on protein, glycogen and fatty acid content of native and invasive clams'. *Food Research International*, 64 pp 439-445.

AOAC (1995) *Official Methods of Analysis of the Association of Official Analytical Chemistry.* 16th Edn., AOAC International, Washington, USA., Pages: 1141

Arismendi, I., Soto, D., Penaluna, B., Jara, C., Leal, C., & León‐Muñoz, J. O. R. G. E. (2009). Aquaculture, non‐native salmonid invasions and associated declines of native fishes in Northern Patagonian lakes. *Freshwater Biology*, 54(5), 1135-1147

Asha, K. K., Anandan, R., Mathew, S. & Lakshmanan, P. T. (2014) 'Biochemical profile of oyster *Crassostrea madrasensis* and its nutritional attributes'. *The Egyptian Journal of Aquatic Research*, 40 (1). pp 35-41.

Barber, B. J. & Blake, N. J. (1985) 'Intra-organ biochemical transformations associated with oogenesis in the bay scallop, *Argopecten irradians concentricus* (Say), as indicated by 14C incorporation'. *The Biological Bulletin*, 168 (1). pp 39-49.

Barton, A., Waldbusser, G., Feely, R., Weisberg, S., Newton, J., Hales, B., Cudd, S., Eudeline, B., Langdon, C., Jefferds, I., King, T., Suhrbier, A. & McLauglin, K. (2015) 'Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response'. *Oceanography*, 25 (2). pp 146-159.

Belivermiş, M., Warnau, M., Metian, M., Oberhänsli, F., Teyssié, J.-L. & Lacoue-Labarthe, T. (2015) 'Limited effects of increased CO_2 and temperature on metal and radionuclide bioaccumulation in a sessile invertebrate, the oyster *Crassostrea gigas*'. *ICES Journal of Marine Science*

Branch, T. A., DeJoseph, B. M., Ray, L. J. & Wagner, C. A. (2013) 'Impacts of ocean acidification on marine seafood'. *Trends in Ecology and Evolution*, 28 (3). pp 178-186.

Brander, K., Cochrane, K., Barange, M. & Soto, D. (2017) 'Chapter 3. Climate Change Implications for Fisheries and Aquaculture'. *Climate Change Impacts on Fisheries and Aquaculture: A Global Analysis.* pp 45.

Bray, D. J., Green, I., Golicher, D. & Herbert, R. J. H. (2015) 'Spatial variation of trace metals within intertidal beds of native mussels (*Mytilus edulis*) and non-native Pacific oysters *(Crassostrea gigas*): implications for the food web?'. *Hydrobiologia*, 757 (1). pp 235-249.

Bhupander, K., & Mukherjee, D. P. (2011). Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. *Advances in Life Science and Technology*, 2, 13-24.

584 Campbell, B. M., Beare, D. J., Bennett, E. M., Hall-Spencer, J. M., Ingram, J. S. I., Jaramillo,
585 F., Ortiz, R., Ramankutty, N., Sayer, J. A. & Shindell, D. (2017) 'Agriculture production as a
586 major driver of the Earth system exceeding planetary boundaries'. *Ecology and Society*, 22
587 (4).

588 Cantillo, A. Y. (1998) 'Comparison of results of Mussel Watch Programs of the United States
589 and France with Worldwide Mussel Watch Studies'. *Marine Pollution Bulletin*, 36 (9). pp 712-
590 717.

591 Child, A. R. & Laing, I. (1998) 'Comparative low temperature tolerance of small juvenile
592 European, *Ostrea edulis* L., and Pacific oysters, *Crassostrea gigas* Thunberg'. *Aquaculture*
593 *Research*, 29 (2). pp 103-113.

594 Clements, J. C., & Chopin, T. (2017). Ocean acidification and marine aquaculture in North
595 America: potential impacts and mitigation strategies. *Reviews in Aquaculture,* 9(4), 326-341.

596 Clements, J. C., Hicks, C., Tremblay, R., & Comeau, L. A. (2018). Elevated seawater
597 temperature, not p CO2, negatively affects post-spawning adult mussels (*Mytilus edulis*)
598 under food limitation. *Conservation physiology*, 6(1), cox078.

599 Cochet, M., Brown, M., Kube, P., Elliott, N. & Delahunty, C. (2015) 'Understanding the
600 impact of growing conditions on oysters: a study of their sensory and biochemical
601 characteristics'. *Aquaculture Research*, 46 (3). pp 637-646.

602 Cooley, S. R., Cheney, J. E., Kelly, R. P., & Allison, E. H. (2017). 2 Ocean acidification and
603 Pacific oyster larval failures in the Pacific Northwest United States'. *Global Change in Marine*
604 *Systems: Societal and Governing Responses*.

605 Cooley, S. R., Lucey, N., Kite-Powell, H. & Doney, S. C. (2012) 'Nutrition and income from
606 molluscs today imply vulnerability to ocean acidification tomorrow'. *Fish and Fisheries*, 13 (2).
607 pp 182-215.

608 Cooley, S. R., Rheuban, J. E., Hart, D. R., Luu, V., Glover, D. M., Hare, J. A. & Doney, S. C.
609 (2015) 'An integrated assessment model for helping the United States sea scallop
610 (*Placopecten magellanicus*) fishery plan ahead for ocean acidification and warming'. *PLoS*
611 *One*, 10 (5). pp e0124145.

612 Cruz-Romero, M., Smiddy, M., Hill, C., Kerry, J. & Kelly, A. (2004) 'Effects of high pressure
613 treatment on physicochemical characteristics of fresh oysters (*Crassostrea gigas*)'.
614 *Innovative Food Science & Emerging Technologies.*, 5 (2). pp 161-169.

615 Delgado, C. L. (2003) *Fish to 2020: Supply and demand in changing global markets.* vol. 62.
616 Penang, Malaysia: WorldFish, 2003.

617 Diaz Rizo, O., Olivares Reumont, S., Viguri Fuente, J., Diaz Arado, O., Lopez Pino, N.,
618 D'Alessandro Rodriguez, K., de la Rosa Medero, D., Gelen Rudnikas, A. & Arencibia
619 Carballo, G. (2010) 'Copper, zinc and lead enrichments in sediments from Guacanayabo
620 Gulf, Cuba, and its bioaccumulation in oysters, *Crassostrea rhizophorae*'. *Bulletin of*
621 *Environmental Contamination and Toxicology*, 84 (1). pp 136-140.

622 Dickinson, G. H., Ivanina, A. V., Matoo, O. B., Portner, H. O., Lannig, G., Bock, C., Beniash,
623 E. & Sokolova, I. M. (2012) 'Interactive effects of salinity and elevated CO_2 levels on juvenile
624 eastern oysters, *Crassostrea virginica*'. *J Exp Biol*, 215 (Pt 1). pp 29-43.

625 Ding, Q., Chen, X., Hilborn, R. & Chen, Y. (2017) 'Vulnerability to impacts of climate change
626 on marine fisheries and food security'. *Marine Policy*, 83 pp 55-61.

627 Dridi, S., Romdhane, M. S. & Elcafsi, M. h. (2007) 'Seasonal variation in weight and
628 biochemical composition of the Pacific oyster, *Crassostrea gigas* in relation to the
629 gametogenic cycle and environmental conditions of the Bizert lagoon, Tunisia'. *Aquaculture*,
630 263 (1-4). pp 238-248.

631 Dupont, S., Hall, E., Calosi, P. & Lundve, B. (2014) 'First evidence of altered sensory quality
632 in a shellfish exposed to decreased pH relevant to ocean acidification'. *Journal of Shellfish*
633 *Research*, 33 (3). pp 857-861.

634 EFSA NDA Panel (2014) 'Scientific Opinion on health benefits of seafood (fish and shellfish)
635 consumption in relation to health risks associated with exposure to methylmercury'. *EFSA*
636 *Journal*, 12 (7). pp 3761.

637 Ekstrom, J. A., Suatoni, L., Cooley, S. R., Pendleton, L. H., Waldbusser, G. G., Cinner, J. E.,
638 Ritter, J., Langdon, C., van Hooidonk, R., Gledhill, D., Wellman, K., Beck, M. W., Brander, L.
639 M., Rittschof, D., Doherty, C., Edwards, P. E. T. & Portela, R. (2015) 'Vulnerability and
640 adaptation of US shellfisheries to ocean acidification'. *Nature Climate Change*, 5 (3). pp 207-
641 214.

642 FAO (2014) 'The State of World Fisheries and Aquaculture 2014. Rome. 223 pp.'.

643 FAO (2016) *The State of World Fisheries and Aquaculture 2016.* Contributing to food
644 security and nutrition for all. Rome. 200 pp. Available.

645 FAO/WHO (2011) *Report of the Joint FAO/WHO Expert Consultation on the Risks and*
646 *Benefits of Fish Consumption. Rome, Food and Agriculture Organization of the United*
647 *Nations; Geneva, World Health Organization, 50pp.* Available.

648 Fernandes, J. A., Papathanasopoulou, E., Hattam, C., Queirós, A. M., Cheung, W. W. W. L.,
649 Yool, A., Artioli, Y., Pope, E. C., Flynn, K. J., Merino, G., Calosi, P., Beaumont, N., Austen,
650 M. C., Widdicombe, S. & Barange, M. (2017) 'Estimating the ecological, economic and social
651 impacts of ocean acidification and warming on UK fisheries'. *Fish and Fisheries*, 18 (3). pp
652 389-411.

653 Fernandez, A., Grienke, U., Soler-Vila, A., Guiheneuf, F., Stengel, D. B. & Tasdemir, D.
654 (2015) 'Seasonal and geographical variations in the biochemical composition of the blue
655 mussel (*Mytilus edulis* L.) from Ireland'. *Food Chemistry*, 177 pp 43-52.

656 Firth, L. B., Knights, A. M., Thompson, R., Mieszkowska, N., Bridger, D., Evans, A., Moore,
657 P., O'Connor, N., Sheehan, E. & Hawkins, S. J. (2016) 'Ocean sprawl: challenges and
658 opportunities for biodiversity management in a changing world'. *Oceanography and Marine*
659 *Biology: An Annual Review*
660 Fratini, G., Medina, I., Lupi, P., Messini, A., Pazos, M. & Parisi, G. (2013) 'Effect of a
661 finishing period in sea on the shelf life of Pacific oysters (*C. gigas*) farmed in lagoon'. *Food*
662 *Res Int*, 51 (1). pp 217-227.

663 Frias-Espericueta, M. G., Osuna-Lopez, I., Banuelos-Vargas, I., Lopez-Lopez, G., Muy-
664 Rangel, M. D., Izaguirre-Fierro, G., Rubio-Carrasco, W., Meza-Guerrero, P. C. & Voltolina, D.
665 (2009) 'Cadmium, copper, lead and zinc contents of the mangrove oyster, *Crassostrea*
666 *corteziensis*, of seven coastal lagoons of NW Mexico'. *Bulletin of Environmental*
667 *Contamination and Toxicology.*, 83 (4). pp 595-599.

668 Froehlich, H. E., Runge, C. A., Gentry, R. R., Gaines, S. D., & Halpern, B. S. (2018).
669 Comparative terrestrial feed and land use of an aquaculture-dominant world. *Proceedings of*
670 *the National Academy of Sciences*, 201801692.

671 Gentry, R. R., Froehlich, H. E., Grimm, D., Kareiva, P., Parke, M., Rust, M., Gaines, S.D. &
672 Halpern, B. S. (2017). Mapping the global potential for marine aquaculture. *Nature ecology &*
673 *evolution*, 1(9), 1317.

674 Gerland, P., Raftery, A. E., Ševčíková, H., Li, N., Gu, D., Spoorenberg, T., Alkema, L.,
675 Fosdick, B. K., Chunn, J. & Lalic, N. (2014) 'World population stabilization unlikely this
676 century'. *Science*, 346 (6206). pp 234-237.

677 Golam, K., Haroon Yousuf, A. K., & Dayanthi, N. (2017). Climate change impacts on tropical
678 and temperate fisheries, aquaculture, and seafood security and implications-A review.
679 *Livestock Research for Rural Development,* 29.

680 Götze, S., Matoo, O. B., Beniash, E., Saborowski, R. & Sokolova, I. M. (2014) 'Interactive
681 effects of CO2 and trace metals on the proteasome activity and cellular stress response of
682 marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*'. *Aquatic Toxicology*, 149
683 pp 65-82.

684 Han, B. C., Jeng, W. L., Chen, R. Y., Fang, G. T., Hung, T. C., & Tseng, R. J. (1998).
685 Estimation of target hazard quotients and potential health risks for metals by consumption of
686 seafood in Taiwan. A*rchives of environmental contamination and toxicology*, 35(4), 711-720.

687 Hart, F. L. & Fisher, H. J. (1971) 'Introduction—General methods for proximate and mineral
688 analysis'. *Modern Food Analysis.* Berlin, Heidelberg: Springer Berlin Heidelberg, pp 1-27.

689 Hawkins, C. A. & Sokolova, I. M. (2017) 'Effects of elevated CO_2 levels on subcellular
690 distribution of trace metals (Cd and Cu) in marine bivalves'. *Aquatic Toxicology*, 192
691 (Supplement C). pp 251-264.

692 Hilborn, R., J. Banobi, S. J. Hall, T. Pucylowski, and T. E. Walsworth. (2018). The
693 environmental costs of animal source foods. *Frontiers in Ecology and the Environment* doi:
694 10.1002/fee.1822.

695 Humphreys, J., Herbert, R. J. H., Roberts, C. & Fletcher, S. (2014) 'A reappraisal of the
696 history and economics of the Pacific oyster in Britain'. *Aquaculture*, 428-429 pp 117-124.

697 Hurlbert, S. H. (1984) 'Pseudoreplication and the Design of Ecological Field Experiments'.
698 *Ecological Monograph*, 54 (2). pp 187-211.

699 IPCC (2013): Summary for Policymakers. In: *Climate Change 2013: The Physical Science*
700 *Basis. Contribution of Working Group I to the Fifth Assessment Report of the*
701 *Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor,
702 S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge
703 University Press, Cambridge, United Kingdom and New York, NY, USA.

704 Ivanina, A. V., Hawkins, C., Beniash, E. & Sokolova, I. M. (2015) 'Effects of environmental
705 hypercapnia and metal (Cd and Cu) exposure on acid-base and metal homeostasis of
706 marine bivalves'. *Comparative Biochemistry and Physiology Part C: Toxicology &*
707 *Pharmacology*,

708 Ivanina, A. V., Hawkins, C. & Sokolova, I. M. (2016) 'Interactive effects of copper exposure
709 and environmental hypercapnia on immune functions of marine bivalves *Crassostrea*
710 *virginica* and *Mercenaria mercenaria*'. *Fish and Shellfish Immunology*, 49 pp 54-65.

711 Jennings, S., Stentiford, G. D., Leocadio, A. M., Jeffery, K. R., Metcalfe, J. D., Katsiadaki, I.,
712 Auchterlonie, N. A., Mangi, S. C., Pinnegar, J. K., Ellis, T., Peeler, E. J., Luisetti, T., Baker-
713 Austin, C., Brown, M., Catchpole, T. L., Clyne, F. J., Dye, S. R., Edmonds, N. J., Hyder, K.,
714 Lee, J., Lees, D. N., Morgan, O. C., O'Brien, C. M., Oidtmann, B., Posen, P. E., Santos, A.

715 R., Taylor, N. G. H., Turner, A. D., Townhill, B. L. & Verner-Jeffreys, D. W. (2016) 'Aquatic
716 food security: insights into challenges and solutions from an analysis of interactions between
717 fisheries, aquaculture, food safety, human health, fish and human welfare, economy and
718 environment'. *Fish and Fisheries*, 17 (4). pp 893-938.

719 Kjeldahl, J. (1883) 'Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern'.
720 *Zeitschrift für analytische Chemie,* 22 (1). pp 366-383.

721 Knez, M., Nikolic, M., Zekovic, M., Stangoulis, J. C. R., Gurinovic, M. & Glibetic, M. (2017)
722 'The influence of food consumption and socio-economic factors on the relationship between
723 zinc and iron intake and status in a healthy population'. *Public Health Nutrition*, 20 (14). pp
724 2486-2498.

725 Knights, A. M. (2012) 'Spatial variation in body size and reproductive condition of subtidal
726 mussels: Considerations for sustainable management'. *Fisheries Research*, 113 (1). pp 45-
727 54.

728 Knights, A. M., Firth, L. B., Thompson, R. C., Yunnie, A. L. E., Hiscock, K. & Hawkins, S. J.
729 (2016) 'Plymouth — A World Harbour through the ages'. *Regional Studies in Marine Science*,
730 8 (Part 2). pp 297-307.

731 Knights, A. M., Piet, G. J., Jongbloed, R. H., Tamis, J. E., White, L., Akoglu, E., Boicenco, L.,
732 Churilova, T., Kryvenko, O. & Fleming-Lehtinen, V. (2015) 'An exposure-effect approach for
733 evaluating ecosystem-wide risks from human activities'. *ICES Journal of Marine Science:*
734 *Journal du Conseil*, 72 (3). pp 1105-1115.

735 Lambert, H., Hakim, O. & Lanham-New, S. A. (2017) 'Chapter 8: Major minerals: calcium
736 and magnesium'. *Essentials of Human Nutrition.* pp 131.

737 Lannig, G., Eilers, S., Pörtner, H.O., Sokolova, I.M. and Bock, C. (2010). Impact of ocean
738 acidification on energy metabolism of oyster, *Crassostrea gigas*—changes in metabolic
739 pathways and thermal response. *Marine drugs*, 8(8), pp.2318-2339.

740

741 Lemasson, A. J., Fletcher, S., Hall-Spencer, J. M. & Knights, A. M. (2017a) 'Linking the
742 biological impacts of ocean acidification on oysters to changes in ecosystem services: A
743 review'. *Joiurnal of Experimental Marine Biology and Ecology*, 492 pp 49-62.

744 Lemasson, A. J., Hall-Spencer, J. M., Fletcher, S., Provstgaard-Morys, S., & Knights, A. M.
745 (2018). Indications of future performance of native and non-native adult oysters under
746 acidification and warming. *Marine Environmental Research*.
747 https://doi.org/10.1016/j.marenvres.2018.10.003

748 Lemasson, A. J., Kuri, V., Hall-Spencer, J. M., S., F., Moate, R. & Knights, A. M. (2017b)
749 'Sensory qualities of oysters unaltered by a short exposure to combined elevated pCO_2 and
750 temperature'. *Frontiers in Marine Science*, 4 (352).

751 Lloret, J., Rätz, H.-J., Lleonart, J. & Demestre, M. (2016) 'Challenging the links between
752 seafood and human health in the context of global change'. *Journal of the Marine Biological*
753 *Association of the UK*, 96 (01). pp 29-42.

754 Lu, G. Y., Ke, C. H., Zhu, A. & Wang, W. X. (2017) 'Oyster-based national mapping of trace
755 metals pollution in the Chinese coastal waters'. *Environmental Pollution*, 224 pp 658-669.

756 Lucas, A. & Beninger, P. G. (1985) 'The use of physiological condition indices in marine
757 bivalve aquaculture'. *Aquaculture*, 44 (3). pp 187-200.

758 Luque de Castro, M. D. & García-Ayuso, L. E. (1998) 'Soxhlet extraction of solid materials:
759 an outdated technique with a promising innovative future'. *Analytica Chimica Acta*, 369 (1–2).
760 pp 1-10.

761 Maclean, W., Harnly, J., Chen, J., Chevassus-Agnes, S., Gilani, G., Livesey, G. & Warwick,
762 P. (2003) 'Food energy–Methods of analysis and conversion factors', *Food and Agriculture*
763 *Organization of the United Nations Technical Workshop Report*.

764 Macura, B., Lönnstedt, O. M., Byström, P., Airoldi, L., Eriksson, B. K., Rudstam, L. &
765 Støttrup, J. (2016) 'What is the impact on fish recruitment of anthropogenic physical and
766 structural habitat change in shallow nearshore areas in temperate systems? A systematic
767 review protocol'. *Environmental Evidence*, 5 (1). pp 1-8.

768 Manirakiza, P., Covaci, A. & Schepens, P. (2001) 'Comparative Study on Total Lipid
769 Determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and Modified Bligh & Dyer
770 Extraction Methods'. *Journal of Food Composition and Analysis*, 14 (1). pp 93-100.

771 Marin, M. G., Moschino, V., Deppieri, M. & Lucchetta, L. (2003) 'Variations in gross
772 biochemical composition, energy value and condition index of *T. philippinarum* from the
773 Lagoon of Venice'. *Aquaculture*, 219 (1-4). pp 859-871.

774 McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H. & Warner, R. R.
775 (2015) 'Marine defaunation: animal loss in the global ocean'. *Science*, 347 (6219). pp
776 1255641.

777 Moreira, A., Figueira, E., Soares, A. M., & Freitas, R. (2016). The effects of arsenic and
778 seawater acidification on antioxidant and biomineralization responses in two closely related
779 Crassostrea species. *Science of the Total Environment,* 545, 569-581.

780 Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C., Clay, J.,
781 Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000) 'Effect of aquaculture on world fish
782 supplies'. *Nature*, 405 (6790). pp 1017-1024.

783 Nielsen, S. S. (2006) 'Proximate Assays in Food Analysis'. *Encyclopedia of Analytical*
784 *Chemistry.* John Wiley & Sons, Ltd.

785 Orban, E., Di Lena, G., Masci, M., Nevigato, T., Casini, I., Caproni, R., Gambelli, L. &
786 Pellizzato, M. (2004) 'Growth, nutritional quality and safety of oysters (*Crassostrea gigas*)
787 cultured in the lagoon of Venice (Italy)'. *Journal of the Science of Food and Agriculture*, 84
788 (14). pp 1929-1938.

789 Orban, E., Di Lena, G., Nevigato, T., Casini, I., Caproni, R., Santaroni, G. & Giulini, G. (2006)
790 'Nutritional and commercial quality of the striped venus clam, *Chamelea gallina*, from the
791 Adriatic sea'. *Food Chemistry*, 101 (3). pp 1063-1070.

792 Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A. & Caproni, R. (2002) 'Seasonal
793 changes in meat content, condition index and chemical composition of mussels (*Mytilus*
794 *galloprovincialis*) cultured in two different Italian sites'. *Food Chemistry*, 77 pp 57-65.

795 Pauly, D., Christensen, V., Dalsgaard, J., Froese, R. & Torres, F. (1998) 'Fishing down
796 marine food webs'. *Science*, 279 (5352). pp 860-863.

797 Pinnegar, J. K., Buckley, P. & Engelhard, G. H. (2017) 'Chapter 12. Impacts of Climate
798 Change in the United Kingdom and Ireland'. *Climate Change Impacts on Fisheries and*
799 *Aquaculture: A Global Analysis.* pp 381.

Pogoda, B., Buck, B. H., Saborowski, R. & Hagen, W. (2013) 'Biochemical and elemental composition of the offshore-cultivated oysters *Ostrea edulis* and *Crassostrea gigas*'. *Aquaculture*, 400-401 pp 53-60.

Porter, J. R., Xie, L., Challinor, A. J., Cochrane, K., Howden, S. M., Iqbal, M. M., Lobell, D. B. & Travasso, M. I. (2014) 'Food security and food production systems'. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp pp. 485-533.

Raposo, J. C., Bartolome, L., Cortazar, E., Arana, G., Zabaljauregui, M., de Diego, A., Zuloaga, O., Madariaga, J. M. & Etxebarria, N. (2009) 'Trace metals in oysters, Crassotrea sps., from UNESCO protected natural reserve of Urdaibai: space-time observations and source identification'. *Bulletin of Environmental Contamination and Toxicology,* 83 (2). pp 223-229.

Rayman, M. P. (2000) 'The importance of selenium to human health'. *The Lancet*, 356 (9225). pp 233-241.

Rider, S. A., Davies, S. J., Jha, A. N., Clough, R. & Sweetman, J. W. (2010). Bioavailability of co-supplemented organic and inorganic zinc and selenium sources in a white fishmeal-based rainbow trout (*Oncorhynchus mykiss*) diet. *Journal of Animal Physiology and Animal Nutrition*, 94 (1), pp. 99-110.

Rice, J. C., and Garcia, S. M. (2011). Fisheries, food security, climate change, and biodiversity: characteristics of the sector and perspectives on emerging issues. – *ICES Journal of Marine Science*, 68: 1343–1353.

Rossbach, L. M., Shaw, B. J., Piegza, D., Vevers, W. F., Atfield, A. J. & Handy, R. D. (2017) Sub-lethal effects of waterborne exposure to copper nanoparticles compared to copper sulphate on the shore crab (*Carcinus maenas*). *Aquatic Toxicology*, 191 pp. 245-255.

Sato, K.N., Powell, J., Rudie, D., Levin, L.A. and Handling editor: Mary Hunsicker (2018). Evaluating the promise and pitfalls of a potential climate change–tolerant sea urchin fishery in southern California. *ICES Journal of Marine Science*, 75(3), pp.1029-1041.

Shpigel, M., Barber, B. J. & Mann, R. (1992) 'Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg'. *J Exp Mar Biol Ecol*, 161 (1). pp 15-25.

Simopoulos, A. P. (2002) 'The importance of the ratio of omega-6/omega-3 essential fatty acids'. *Biomedicine & pharmacotherapy*, 56 (8). pp 365-379.

Solomons, N. W. & Schümann, K. (2017) 'Iron and Zinc: Two Principal Trace Element Nutrients in the Context of Food Security Transitions'. in Biesalski, H.K., Drewnowski, A., Dwyer, J.T., Strain, J.J., Weber, P. and Eggersdorfer, M. (eds.) *Sustainable Nutrition in a Changing World.* Cham: Springer International Publishing, pp 205-222.

Soto-Jiménez, M., Páez-Osuna, F. & Morales-Hernández, F. (2001) 'Selected trace metals in oysters (*Crassostrea iridescens)* and sediments from the discharge zone of the submarine sewage outfall in Mazatlán Bay (southeast Gulf of California): chemical fractions and bioaccumulation factors'. *Environmental Pollution*, 114 (3). pp 357-370.

Sprague, M., Betancor, M. B., Dick, J. R. & Tocher, D. R. (2017) 'Nutritional evaluation of seafood, with respect to long-chain omega-3 fatty acids, available to UK consumers'. *Proceedings of the Nutrition Society*, 76 (OCE2).

845 Tacon, A. G. J. & Metian, M. (2013) 'Fish matters: importance of aquatic foods in human
846 nutrition and global food supply'. *Reviews in Fisheries Science*, 21 (1). pp 22-38.

847 Tate, R. D., Benkendorff, K., Ab Lah, R. & Kelaher, B. P. (2017) 'Ocean acidification and
848 warming impacts the nutritional properties of the predatory whelk, *Dicathais orbita*'. *Journal*
849 *of Experimental Marine Biology and Ecology*, 493 pp 7-13.

850 Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J.,
851 Barrett, S., Crépin, A.S., Ehrlich, P.R. and Gren, Å., (2014). Does aquaculture add resilience
852 to the global food system?. *Proceedings of the National Academy of Sciences*, 111(37),
853 pp.13257-13263.

854 UNEP (2010) *UNEP Emerging Issues: Environmental Consequences of Ocean Acidification:*
855 *A Threat to Food Security.* UNEP. Available.

856 United Nations, D. o. E. a. S. A., Population Division (2015) 'World Population Prospects:
857 The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241.'.

858 Valles-Regino, R., Tate, R., Kelaher, B., Savins, D., Dowell, A. & Benkendorff, K. (2015)
859 'Ocean warming and CO_2-induced acidification impact the lipid content of a marine predatory
860 gastropod'. *Marine Drugs*, 13 (10). pp 6019-6037.

861 Weatherdon, L. V., Magnan, A. K., Rogers, A. D., Sumaila, U. R. & Cheung, W. W. L. (2016)
862 'Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal
863 tourism, and human health: an update'. *Frontiers in Marine Science*, 3

864

865 **Highlights: (3-5)**

866 • Ocean acidification and warming can reduce oysters nutritional quality

867 • Changes to nutritional composition were more pronounced in the introduced species

868 • Oysters displayed decreased protein, lipid, and carbohydrate contents

869 • Multifaceted implications for the aquaculture sector and future food security