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OCEAN ACIDIFICATION AND WARMING IMPACTS ON NATIVE AND NON-NATIVE SHELLFISH: A MULTIDISCIPLINARY ASSESSMENT

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OCEAN ACIDIFICATION AND WARMING IMPACTS ON NATIVE AND NON-NATIVE SHELLFISH: A MULTIDISCIPLINARY ASSESSMENT

By

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"Mighty oaks from little acorns grow"

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Abstract

OCEAN ACIDIFICATION AND WARMING IMPACTS ON NATIVE AND NON-NATIVE SHELLFISH: A MULTIDISCIPLINARY ASSESSMENT

ANAËLLE JULIE LEMASSON

Ocean acidification and warming have been shown to affect a wide range of marine organisms and impact assemblages and ecosystems. Many of the species experiencing negative biological effects provide valuable ecosystem services, yet it is unclear how these biological effects will affect ecosystem services provision. This thesis aimed to appraise the consequences of ocean acidification and warming on important shellfish species, from physiology to provision of ecosystem services, using a multidisciplinary approach. The responses to ocean acidification and warming of two ecologically and commercially important species of oysters – the native European Flat oyster *Ostrea edulis*, and the non-native Pacific oyster *Magallana gigas* – were assessed in laboratory mesocosms following long-term exposures to a range of scenarios predicted for 2050 and 2100.

Oysters provide numerous ecosystem services, including improvement of water quality, reef formation, and food provision, but are at risks from ocean acidification and other stressors due to negative impacts occurring at multiple life-stages and threatening reef maintenance and functioning (Chapter 1). The physiology of adult oysters appeared susceptible to ocean acidification and warming, with evident sub-lethal effects (Chapter 2). *Magallana gigas* experienced a greater degree of stress than *O. edulis*, displaying increased Standard Metabolic Rate, reduced Clearance Rate, and poorer Condition Indices. Reductions in Clearance Rates of *M. gigas* are especially concerning and may have important ecological impacts by limiting their ability to improve water quality in the future. The physiological changes experienced by individual oysters held important implications for the functioning of the reefs through changes in predation resistance. Again, *M. gigas* appeared to undergo more pronounced changes than *O. edulis*, displaying increased muscle strength but weakened shell strength. These changes are expected to alter its susceptibility to predators and influence community level interactions. Both *O. edulis* and *M. gigas* also underwent important changes to their biochemical

composition with trends for impoverished nutritional quality, which holds direct implications on the provision of sea food. In particular, *M. gigas* contained lower lipid, carbohydrate, and protein levels, but higher contaminant concentration (copper); this change holds concerns for both future food security and future food safety. It was apparent that the physiological stress experienced (Chapter 2), led to significant energy reallocation from somatic growth to metabolism by depleting energetic reserves (Chapter 4), at the detriment of its nutritional quality. No negative effects on the eating quality of *M. gigas* (appearance, aroma, texture, taste, and overall acceptability) were recorded following a short-term exposure to ocean acidification and warming (Chapter 5), which was considered positive for the aquaculture sector. In order to secure future food provision and economic revenue, the UK aquaculture industry might need to reconsider its management strategy in the future, and encourage the production and consumption of *O. edulis*, in addition to the already popular *M. gigas*.

It is clear that the impacts of ocean acidification and warming on oysters are multifaceted and occurring at multiple scales and levels of organisation. The risks to oysters and oyster reefs appear species-specific; in the UK, introduced *M. gigas* may be more vulnerable than native *O. edulis*. To secure benefits and minimise costs related to the management of introduced species, these findings could be integrated into the current management and conservation measures in place for these species and the reefs they can form.

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"A little knowledge is a dangerous thing"

A version of this chapter has been published and is available online as:

Lemasson, A.J., Fletcher, S., Hall-Spencer, J.M., and A.M. Knights. 2017. Linking the biological impacts of ocean acidification on oysters to changes in ecosystem services: A review. *Journal of Experimental Marine Biology and Ecology*. 10.1016/j.jembe.2017.01.019

1 General Introduction – Linking the biological impacts of ocean acidification on oysters to changes in ecosystem services: A review

Abstract:

Continued anthropogenic carbon dioxide emissions are acidifying our oceans, and hydrogen ion concentrations in surface oceans are predicted to increase 150% by 2100. Ocean acidification (OA) is changing ocean carbonate chemistry, including causing rapid reductions in calcium carbonate availability with implications for many marine organisms, including biogenic reefs formed by oysters. The impacts of OA are marked. Adult oysters display both decreased growth and calcification rates, while larval oysters show stunted growth, developmental abnormalities, and increased mortality. These physiological impacts are affecting ecosystem functioning and the provision of ecosystem services by oyster reefs. Oysters are ecologically and economically important, providing a wide range of ecosystem services, such as improved water quality, coastlines protection, and food provision. OA has the potential to alter the delivery and the quality of the ecosystem services associated with oyster reefs, with significant ecological and economic losses. This general introduction provides a summary of current knowledge of OA on oyster biology, but then links these impacts to potential changes to the provision of ecosystem services associated with healthy oyster reefs. In a final section, it also outlines the thesis aims and goals, and provides an overview of the model species used.

1.1 Introduction

The risks arising from climate change are now widely acknowledged as a major cause for concern, yet awareness of ocean acidification is far less prevalent (Gattuso *et al.*, 2015). It is clear that delay in reducing emissions amplifies the consequences: the more CO_2 emitted the greater the scale of the ecological, social, and economic consequences that current and future generations are left to cope with (Henson *et al.*, 2017; Reum *et al.*, 2015). Consequently, our understanding of the scope and severity of ocean acidification (OA herein) and its impacts on the marine environment remain relatively limited, and especially, the implications of OA to the continued provision of valuable ecosystem services.

Since the industrial revolution (*circa* 1750), the oceans have absorbed approximately 30% of anthropogenic CO_2, altering oceanic carbonate chemistry by reducing carbonate ion concentrations (CO_3^{2-}), and reducing the saturation states of calcite and aragonite. The result – lower pH, or 'ocean acidification' (Gattuso *et al.*, 2014). Oceanic pH has since decreased by 0.1 units, and the latest IPCC assessment predicts an increase in atmospheric CO_2 to 490-1290 ppm by 2100 (Stocker et al., 2013), corresponding to a decrease in seawater pH of 0.3-0.5 units. Historic ocean acidification linked to the Permian-Triassic mass extinction led to the disappearance of ~90% of marine species (Clarkson *et al.*, 2015). Today, without significant cuts in CO_2 emissions, a 150% increase in the concentration of surface ocean H^+ is predicted by 2100 (Stocker *et al.*, 2013). Despite increased awareness of the potential risks from ocean acidification (Gattuso *et al.*, 2014; Gattuso *et al.*, 2015), an understanding of the scope and severity of its impacts on the contemporary marine environment remains limited.

Ocean acidification has been shown to affect a wide range of marine organisms, from fish, microalgae, seaweeds, crustaceans, echinoderms or molluscs (Barry *et al.*, 2011; Hendriks *et al.*, 2010; Kroeker *et al.*, 2010). However, a large number of publications are still restricted to a few taxa; therefore reviews have mostly considered the overall response of marine fauna (Aze *et al.*, 2014; Barry *et al.*, 2011; Dupont & Thorndyke, 2009; Fabry *et al.*, 2008; Harvey *et al.*, 2013; Hofmann *et al.*, 2010; Kroeker *et al.*, 2013; Kroeker *et al.*, 2010; Ross *et al.*, 2011), with the exception of reviews focusing on molluscs (Gazeau *et al.*, 2013; Parker *et al.*, 2013) or coral reefs (Albright, 2011; Andersson & Gledhill, 2013; Brander *et al.*, 2012; Hoegh-Guldberg *et al.*, 2007; Pandolfi *et al.*, 2011). In recent decades there has been a surge in research efforts aimed at deciphering the physiological and ecological impacts of ocean acidification. While OA may be of benefit to some organisms, such as jellyfish and toxic species of algae (Hall-Spencer & Allen, 2015; Somero, 2010; Uthicke *et al.*, 2015), for other species OA is expected and has been shown to cause considerable direct harm, with major concerns existing regarding the fate of calcifying organisms. As they rely on various forms of calcium carbonate, calcifiers such as corals and molluscs are thought to be particularly at risk from ocean acidification, even more so given that many regions are predicted to see their calcium carbonate saturation states decrease. Studies have focused on ecologically or economically important calcifying species, such as mussels and oysters (Goncalves *et al.*, 2017; Gray *et al.*, 2017; Scanes *et al.*, 2017; Thomsen *et al.*, 2017;

Zhao *et al.*, 2017), and charismatic ecosystems, such as coral reefs (Allen *et al.*, 2017; Brander *et al.*, 2014; Comeau *et al.*, 2015; Smith *et al.*, 2016b; Speers *et al.*, 2016).

However, the ecosystem effects and loss of ecosystem services associated with OA remain conspicuously absent, despite the increased prevalence of ecosystem-based approaches in environmental legislation and management. Here, we address that gap and introduce the current state of knowledge required to underpin a multidisciplinary evaluation (Knights *et al.*, 2014), that considers the ecological, social and economic consequences of OA.

In particular, we assess the biological, social and economic consequences of the ways in which ocean acidification affects individual oysters, their populations, and the habitats they form. Being ecosystem engineers (Jones et al., 1996), oysters are important species both ecologically and economically, therefore their fate potentially matters to a wide range of stakeholders. They provide a large number of goods and services to human society, whether it is from the extensive reefs they create or by directly providing food from wild harvest and aquaculture. For habitat-forming species such as oysters, valuing both the species and the associated habitat is necessary (Rodrigues *et al.*, 2013). Oyster reefs worldwide have dramatically declined in the past century and are now at the centre of many conservation measures and restoration strategies (Beck *et al.*, 2011; Grabowski & Peterson, 2007). However, these efforts could be unsuccessful if they do not take into account all potential factors affecting oyster reef formation and maintenance, including ocean acidification. A plethora of recent reviews and meta-analyses have highlighted the threat of OA to marine fauna (see references above), but are often restricted to the description of biological effects on a range of taxa, and do not focus on specific species or groups of organisms (but see Albright, 2011; Gazeau *et al.*, 2013; Hoegh-Guldberg *et al.*, 2007; Parker *et al.*, 2013, for reviews on corals and shelled molluscs). Despite many experts emphasizing the need for an approach that encompasses biological, social and economic sciences (Garrard *et al.*, 2012; Hilmi *et al.*, 2013; Knights *et al.*, 2014), research so far still largely lacks the 'multidisciplinary approach' required to fully appraise the scope and nature of the consequences of ocean acidification. Reviews and meta-analyses have looked into the different consequences of ocean acidification separately, by focusing on the biology and ecology (Doney, 2009; Kroeker *et al.*,

2010), the societal impacts (Cooley, 2012) or the economic impacts (Cooley & Doney, 2009), but rarely have they tried to draw a comprehensive picture interlinking those different fields. Nevertheless, in recent years, efforts have been made towards building such comprehensive picture (Fernandes *et al.*, 2017; Speers *et al.*, 2016).

This general introduction is in three parts: firstly, we undertake a brief review of the biological and ecological impacts of OA on oysters. This includes an assessment of the effects of OA on individual life history stages (planktic larvae, sessile juveniles, and adults), populations and ecosystem-level responses. We then review the range of ecosystem services that are provided by oysters, including an assessment of their economic value and associated metrics. We conclude by considering how impacts at the organismal-level can affect the provision of ecosystem services. Finally, we give an outline of the thesis and an overview of the model species within the context of this thesis.

1.2 The biological impacts of ocean acidification on oysters

1.2.1 Effects of OA on reproduction and planktic life-history stages

Like many marine species, bivalves have a biphasic life-cycle, comprising a benthic sessile and a pelagic dispersive phase (Figure 1.1). The planktic larval stage is a crucial life-history stage for many benthic organisms and changes in development, performance or survival of this stage can critically influence juvenile-adult population dynamics and ecosystem functioning (Bachelet, 1990; Green *et al.*, 2004; Rumrill, 1990). A review by Kurihara (2008) emphasized the vulnerability of the early developmental stages of marine calcifiers to ocean acidification, and concluded that "future changes in ocean acidity will potentially impact the population size and dynamics, as well as the community structure of calcifiers, and will therefore have negative impacts on marine ecosystems". The importance of the larval stage has been widely recognised in the past few years, and the subsequent increase in early-life stage studies has provided an increasing amount of evidence describing their response to ocean acidification (reviewed in Albright, 2011; Byrne, 2011; Przeslawski *et al.*, 2015; Ross *et al.*, 2011).

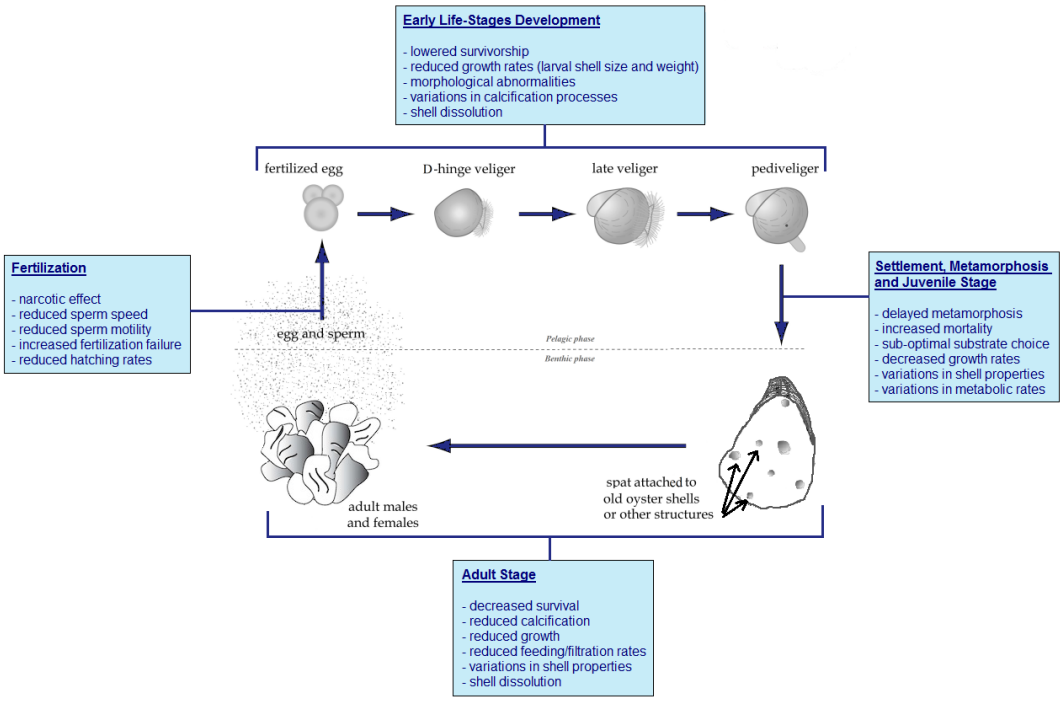

Figure 1.1: Summary of the negative effects of ocean acidification observed on the embryonic, larval, juvenile and adult life history stages of oysters.

OA has been shown to induce narcotic effects on motile life-history stages, reducing fertilisation success (Byrne, 2011). In a number of instances, OA effects include reduced sperm motility, reductions in fertilisation success and hatching rates of embryos (Barros *et al.*, 2013; Parker *et al.*, 2009; Parker *et al.*, 2010), although in the case of Parker *et al.,* (2009), changes could not be solely attributed to OA due to the effects being conflated with suboptimal culture temperatures. However, OA-induced narcosis has not been consistently shown, with disparity between studies of the same species (e.g. Kurihara *et al.*, 2007; Parker *et al.*, 2012). Parker et al. (2012) suggest this disparity may be the result of intraspecific phenotypic plasticity, whereas Byrne (2011) argues that the fertilisation process in marine invertebrates can be resilient to fluctuations in pH and may not be a reliable end-point. Neither Parker *et al.,* (2012) nor Byrne's (2011) theories have been tested, but the inconsistencies shown highlight the need for comparative studies using discrete populations to determine if OA has consistent and repeatable effects, irrespective of scale or location.

In contrast to the fertilisation process, embryos and larvae are considered less tolerant to the effects of OA (Cole *et al.*, 2016; Parker *et al.*, 2017), in part because molluscs and other calcareous shell-forming species commonly lack the specialised ion-regulatory epithelium used to maintain acid-base status (reviewed in Lannig *et al.*, 2010). The process of shell mineralisation begins at the trochophore

(prodissoconch I) stage (reviewed in Gazeau *et al.*, 2013). Larvae use two types of calcium carbonate, firstly mineralising highly soluble amorphous calcium carbonate (ACC) (Brečević & Nielsen, 1989) before switching to aragonite (Weiner & Addadi, 2002; Weiss *et al.*, 2002). In juvenile and adult stages, this again changes to the use of low solubility calcite instead (Lee *et al.*, 2006; Stenzel, 1964). Because the calcium carbonate structures formed in these early life-history stages play a crucial role in protection, feeding, buoyancy and pH regulation, disruption of calcification from OA could have significant consequences for survival (Figure 1.2) (Barros *et al.*, 2013; Simkiss & Wilbur, 2012). In other taxa, OA has been shown to greatly alter the structure of the important larval shell of calcifying organisms, including affecting dissolution rates and causing shell malformation, stunted growth, altered mineral content, and weaker skeletons (reviewed in Byrne, 2011; but see Fitzer *et al.*, 2016; and Kurihara, 2008).

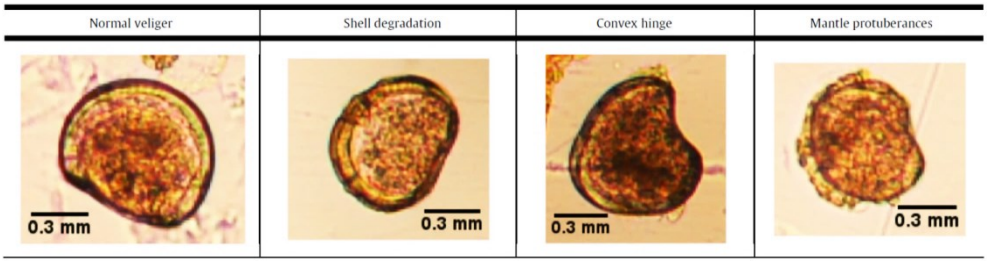

Figure 1.2 Changes in the morphology of *Magallana gigas*[1] larvae during exposure to ocean acidification (from Barros *et al.*, 2013).

OA can also affect development rates. Multi-stressor experiments manipulating pCO_2, pH, total alkalinity, and Ω_{arag} in order to simulate future acidification scenarios have shown that oyster larvae are highly sensitive to predicted future conditions. Responses include lower survivorship, abnormal development, smaller body size, and altered shell properties (Cole *et al.*, 2016; Guo *et al.*, 2015; Parker *et al.*, 2017; Timmins-Schiffman *et al.*, 2013). However, the response remains inconsistent, with differences between species and regions apparent (see Gazeau *et al.*, 2011; Kurihara *et al.*, 2007; Parker *et al.*, 2010, for a regional comparison of *Magallana gigas* performance), with the differences within species suggestive of pre-adaptation determined by exposure in their respective natural environment (sensu environmental filtering Kraft *et al.*, 2015).

[1] *Magallana gigas* is the currently accepted taxonomic name for the Pacific oyster, formerly known as *Crassostrea gigas* (Marshall, 2016 - http://www.marinespecies.org/aphia.php?p=taxdetails&id=836033)

OA places individuals under stress as they try to regulate or maintain physiological function. Processes including shell mineralisation, maintenance of internal acid-base balance, somatic growth, swimming and feeding are energetically expensive (Pörtner *et al.*, 2004), and require additional energy for maintenance under OA (Pörtner, 2008). As such, the planktic larval stage can be extended to allow individuals to compensate for inefficient feeding and delayed development, but doing so may subsequently affect the fitness, competitive ability and survivorship of the individual at later life-history stages (Anil *et al.*, 2001; Gazeau *et al.*, 2010; Rumrill, 1990; Talmage & Gobler, 2009). Trade-offs between calcification and other physiological aspects are expected to occur, but the extent to which these occur and their impact, will depend on an individuals' ability to obtain sufficient energy from their environment to counteract any negative effects of acidification (Hettinger *et al.*, 2013a).

1.2.2 Carry-over or latent effects: metamorphosis to juvenile-adult stages

Changes in larval fitness are expected to impact adult population success through a combination of latent/carry-over effects (see Pechenik, 2006) and bottleneck effects (Schneider *et al.*, 2003). These effects may only be transient, for instance, in cases where larval development is slower and the increased risks associated with extended larval duration (e.g. mortality from predation, starvation) enhance bottleneck effects. However, if larval development is unchanged and larval duration does not vary, the full suite of consequences will be transferred to the juvenile (carry-over effects). Consequences may include reduced environmental tolerance, decreased predation resistance, and increased mortality, which can introduce an additional bottleneck for the adult population (see Gaylord *et al.*, 2011).

The negative impacts of OA on both pre- and post-settlement processes in oysters are clear. These include: reduced metamorphosis success (Hettinger *et al.*, 2013a); greater mortality of juveniles (Beniash *et al.*, 2010; Dickinson *et al.*, 2012); shell weakening (Dickinson *et al.*, 2012) and greater prevalence of micro-fractures, a reduction in shell dry mass, soft-tissue mass (Beniash *et al.*, 2010; Dickinson *et al.*, 2012), and growth (Hettinger *et al.*, 2013b; Hettinger *et al.*, 2012; Parker *et al.*, 2011). Metamorphosis is a crucial step in population development and growth and mortality is often high due to the high energetic cost (Gosselin & Qian, 1997; Videla *et al.*, 1998). OA can lead to the

depletion of energy reserves (e.g. lipids), impair larval fitness and decrease the likelihood of successful metamorphosis by up to 30% (e.g. Talmage & Gobler, 2009). A delay in metamorphosis can reduce energy reserves and lead to settlement in suboptimal habitat, such that post-settlement competence is impaired and mortality rates increased (see the extensive work by Pechenik, including Pechenik, 2006; Pechenik *et al.*, 1998). Given that post-settlement mortality often exceeds 90% under natural environmental conditions (Thorson, 1950), any additional impact on juveniles fitness or survival associated with OA impacts is likely to lead to significant consequences in terms of adult population density.

It has been suggested that negative consequences of OA on juvenile and adult oysters are carried-over as a result of energetic deficits experienced during the early (planktonic) life stage (Hettinger *et al.*, 2013b); a less fit larva will likely become a less fit (e.g. smaller) juvenile/adult (Hettinger *et al.*, 2012). These consequences may, in general, be negative but individuals from specific regions or some species appear to have developed coping mechanisms. Ko *et al.,* (2013) provided evidence of compensatory mechanisms in *M. gigas* juveniles raised under acidified conditions, in which individuals displayed more rapid calcification (without a reduction in shell thickness or change in microcrystalline structure). Parker *et al.,* (2011) demonstrated that some bred populations of *Saccostrea glomerata* were more resistant to OA than wild populations. While both bred and wild-caught individuals displayed significant reduction in shell growth at pH 7.8, wild populations were more susceptible to OA conditions than the bred population. The authors argue that these differences could be due to phenotypic plasticity emanating from different parental history, and/or differences in enzymatic activity of carbonic anhydrase - an enzyme linked with acid-base regulation and shell formation. Irrespective of the mechanism(s) of resilience, some species may be better suited for future OA conditions, and identification of resilient lineages may provide important insights for future food biosecurity and decision-making (see part 1.3.3).

1.2.3 OA impacts on adult oysters

The early days of ocean acidification studies focused on adult physiological responses, and as they appeared relatively robust, focus then turned towards the effects on the pelagic phases (see review by

Gazeau *et al.*, 2013; Parker *et al.*, 2013). However, the consequent increase in early-life stages studies had led to the adult stage being somewhat overlooked, despite displaying negative responses.

Impacts include reduced calcification and shell growth (Beniash *et al.*, 2010; Gazeau *et al.*, 2007; Parker *et al.*, 2009; Ries *et al.*, 2009; Waldbusser *et al.*, 2011b; Wright *et al.*, 2014), and reduction in survival (Beniash *et al.*, 2010; Dove & Sammut, 2007; Speights *et al.*, 2017). A decrease in shell density and weight was recorded in *M. gigas* and *O. edulis* (Bamber, 1990), while an increase in shell dissolution was observed for *Crassostrea virginica* (Waldbusser *et al.*, 2011a) and a reduction in shell strength was seen in *Pinctada fucata* (Welladsen *et al.*, 2010) and in *C. virginica* (Speights *et al.*, 2017). Crucial metabolic activities such as feeding are also impacted by acidification. Bamber (1990) observed impairments of feeding activity at pH ≤7.0 in *M. gigas*, and pH ≤7.2 for *O. edulis*. Similarly, adults of *S. glomerata* decrease filtration rate at low pH (Dove & Sammut, 2007). Impaired filtration and feeding has the potential to effect energy supply and metabolic maintenance in adult oysters, and may affect resilience and persistence to OA in the long term. Li *et al.* (2009) observed that metabolic and immunological activities of *M. gigas* were impeded under food deprivation, which slowed down recovery after the additional stress of spawning. However, as a limited number of studies have looked into changes in metabolic activities and stress responses of adult oysters under OA conditions, additional studies focusing back on adults are needed to depict a more consistent and reliable picture.

The effect of ocean acidification on the immune system of organisms has received little attention, but may have an important role in the persistence of the species. Increased temperature had been shown to compromise the immune system of commercially important shellfish species, thereby increasing the threat to future food security (Rowley *et al.*, 2014). Equally, increased CO_2 can affect the immune response of several taxa (bivalves, echinoderms, Asplund *et al.*, 2014; Beesley *et al.*, 2008; Bibby *et al.*, 2008; Dupont & Thorndyke, 2012; Matozzo *et al.*, 2012), although some taxa appear unaffected (decapods, Small *et al.*, 2010). Li *et al.* (2009) noted impediment to metabolic activities of *M. gigas* under food deprivation and extended recovery time post-spawning, which they linked to an impaired immunological response. Wang *et al.,* (2016) recently demonstrated that reduced pH (≤7.8) negatively impacted the immune system of *M. gigas* by increasing haemocyte apoptosis and reactive

oxygen species production, inhibiting the activity of antioxidant enzymes, and influencing the mRNA expression pattern of immune related genes.

In *C. virginica, Crassostrea angulata*[2], and *M. gigas*, suppression of immune-related functions including haemocyte production and antioxidant defence was compromised leading to greater sensitivity to metal pollutants (Ivanina *et al.*, 2014; Moreira *et al.*, 2016). Li *et al.* (2015) found that short-term ocean acidification and warming (OAW) of 31°C significantly altered immune parameters of the Pearl oyster *P. fucata*, with ramifications for its acid-base regulation capacity, the functioning of its immune system, and its bio-mineralization process. Beesley *et al.,* (2008) found that CO_2-acidified water negatively affected the health of *M. edulis* by allowing a 'leaky lysosome'. The lysosome is a crucial organelle involved notably in the immune response, and any damage could alter its function, and therefore an organism's susceptibility to pathogens. *Vibrios* are known pathogens of marine molluscs (Ellis *et al.*, 2011), and a few studies on host-pathogen interactions of *Vibrio tubiashii* on the blue mussel *M. edulis* showed that ocean acidification can potentially favour pathogens and induce increased metabolic costs of immune response maintenance (Asplund *et al.*, 2014; Bibby *et al.*, 2008; Ellis, 2013). *V. tubiashii* is also a well-known pathogen of oysters that causes significant losses to the aquaculture sector (Elston *et al.*, 2008; Richards *et al.*, 2015a). Dorfmeier (2012) found that while elevated pCO_2 did not directly affect the susceptibility of *M. gigas* larvae to *V. tubiashii*, it increased the growth of the pathogen, hence having indirect consequences for *M. gigas* by increasing the likelihood of outbreaks. The impacts of ocean acidification on the health and immune response of marine organisms and particularly economically important species such as oysters is still a grey area, and the question remains as to how OA will affect their susceptibility to pathogens.

1.2.4 From individual to ecosystem-level effects: consequences for the oyster reef

It is apparent that oysters, like many other marine invertebrates, are vulnerable to OA, yet consequences at the population and ecosystem level remain largely unknown. Some critical questions are still to be answered: how will OA impact oyster population sustainability? How will it alter oysters'

[2] The status of *Crassostrea angulata* as being a distinct species from *Magallana gigas* (previously known under *Crassostrea gigas*) is still debated (Gofas, S. (2015). *Crassostrea angulata*. In: MolluscaBase (2015). See: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=146900). Hereafter, they will be considered as one species and referred to as *M. gigas*.

susceptibility to predation? How oysters' vulnerability might shift the competitive balance within the reef system? How might that affect ecosystem function and alter the provision of goods and services provided by oyster reefs (e.g. food provision, improved water quality, shoreline protection…)? Many of the effects of OA are non-lethal, but substantial ecosystem changes can be expected as systems become restructured, with 'winners' and 'losers', through environmental filtering and niche partitioning (Barry *et al.*, 2011; Fulton, 2011; Kraft *et al.*, 2015; Somero, 2010).

Oysters can potentially become more vulnerable to predation pressures by developing thinner or weaker shells that lose their integrity, hence modifying predator-prey interactions (Kroeker *et al.*, 2014), as observed in the blue mussel *M. edulis* (Mackenzie *et al.*, 2014). However, the observed oysters' susceptibility to predation pressure under OA treatments was not a direct result of a loss in shell integrity, but a reduction in prey shell size which allowed for a lower energy expenditure from the predator (Sanford *et al.*, 2014), and pressure for the predator to consume more prey as a result of its own compensatory energy requirements from OA (Wright *et al.*, 2014).

Future levels of OA are likely to shift competitive dominance for food and space in response to variations in the relative tolerance of species. It has been suggested that species with a brooding reproductive strategy, such as the widely invasive slipper limpet *Crepidula fornicata* - a competitor of oysters for space and food (Blanchard *et al.*, 2008; De Montaudouin *et al.*, 2002) - would have a higher competitive advantage due to the increased protection provided by the encapsulation of its embryo (Noisette *et al.*, 2014a). However, recent studies have proven this suggestion to be wrong in the case of *C. fornicata*, although the species does display a great tolerance to OA, potentially providing the species with a competitive dominance over other native species (Noisette *et al.*, 2014a; Noisette *et al.*, 2014b). *Crepidula fornicata* shares the same habitat niche as oysters and can form dense aggregations. In an OA scenario where *C. fornicata* out-competes oysters at specific sites, there could be significant consequences in terms of the goods and services provided by the local ecosystem. For instance, as oysters are ecosystem engineers (Jones *et al.*, 1996), their loss would not only affect seafood provision, but would also potentially destabilize ecosystem structure and function.

As previously described, some species appear more robust than others, with a trend for oysters to be more resilient than other molluscs (Gobler & Talmage, 2014; Guo *et al.*, 2015; Talmage & Gobler, 2009), with the possible exception of *C. fornicata*. Differences in tolerance also exist even between closely related species of oysters (*M. gigas* more resistant than *S. glomerata*, Parker *et al.*, 2010). Inter- and intra-specific variations in vulnerability are of potentially high significance to food security. By understanding these variations, societies can adapt to OA and secure future food provision (see part 1.2.2). Nevertheless, the overall vulnerability of marine calcifiers, such as oysters, to OA could shift competitive advantage towards non-calcifiers or to more resistant calcifying species in the natural environment (see also Brown *et al.*, 2017; Diaz-Pulido *et al.*, 2011; Sampaio *et al.*, 2017).

Sites with naturally acidified conditions, such as volcanic seeps, lagoons and upwelling areas, can provide insights into competitive dominance and long-term community responses to OA, particularly in areas subjected to anthropogenic stress (see studies by Basso *et al.*, 2015; Range *et al.*, 2012; Smith *et al.*, 2016a; Thomsen *et al.*, 2010). A study conducted by Cigliano *et al.* (2010) in the waters of the volcanic island of Ischia (Italy) showed a change in community structure along the natural pH gradient due to impacts on recruitment, competition for space, and a significant biodiversity loss from the disappearance of calcifiers. In another study of Ischia island communities, Kroeker *et al.* (2012) observed a shift in ecosystem composition along the pH gradient from a system dominated by calcifiers to one dominated by fleshy seaweeds. They concluded that the observed shift was due to altered competitive dynamics resulting from the naturally occurring OA. Diaz-Pulido *et al.* (2011) also observed a shift in the competitive balance of the coral-seaweed *Lobophora papenfussii* and the coral *Acropora intermedia,* with OA conditions benefiting the seaweed. However, issues have been raised regarding the applicability of in situ studies at naturally acidified sites to the future anthropogenic OA scenarios. Indeed, at natural sites, no barriers exist that separate them from surrounding locations unaffected by acidification, leading to communities at sites possibly receiving external influences, such as larval recruitment, from those locations at ambient conditions. Therefore, natural sites are not fully representative of future OA conditions that will occur globally in the marine environment. Nevertheless, they give critical insights into large-scale effects of OA on marine ecosystems. The use of mesocosms studies can help obviate this effect. Christen *et al.* (2013) used a

mesocosm experiment to investigate change in macro-faunal communities under OA and found a significant variation in community structure and a loss of biodiversity with decreasing pH, shifting from a system dominated by calcareous species to one dominated by non-calcareous species, highlighting the differential vulnerability amongst taxa.

1.2.5 Potential for acclimatization and adaptation

Responses of individuals to OA will dictate the future state of adult populations. Changes in recruitment and survival rates will alter adult densities, potentially impacting on ecological relationships and ecosystem functions.

Ocean acidification is occurring alongside other environmental and anthropogenic stressors that are likely to affect the responses of organisms. Multi-stressors can lead to additive effects (the sum of the effect of each stressor acting separately), synergistic (exacerbating the response, the combined effect is stronger than the sum of each effect separately), or antagonistic effects (having opposite effects compensating each other). The outcomes of multi-stressor interactions are difficult to predict and seem highly context-specific (see the reviews on the impacts of ocean warming and acidification by Byrne, 2011; Byrne & Przeslawski, 2013; and Harvey *et al.*, 2013). Few studies have looked at multi-stressor effects on oysters, compared to mussels (Gazeau *et al.*, 2014; Kroeker *et al.*, 2014; Rodolfo-Metalpa *et al.*, 2011; Wang *et al.*, 2015). Ko *et al.* (2014) observed complex interactive effects of OA, elevated temperatures and reduced salinity (both expected under future climate change scenarios) on the early life stages of *M. gigas* from the Yellow Sea, with the interaction of the three stressors delaying larval growth rate and negating the positive effect of warmer temperature alone. This study appears in contradiction with an earlier study by Thiyagarajan & Ko (2012) on *M. gigas* from the East China Sea whose larvae appeared tolerant to elevated temperature, reduced salinity, and reduced pH. This discrepancy again highlights the need for more studies to determine the complex pattern of inter- and intra-specific responses.

If species cannot tolerate future climate conditions, the question of whether species can adapt over time between generations is a crucial one (Somero, 2010; Sunday *et al.*, 2014). Perhaps of biggest

concern is if species can adapt quickly enough given documented rates of change in ocean conditions (Visser, 2008). To date there is little evidence to answer this question. Collins and Bell (2004) found no evidence of adaptation in the fast-generating pond algae, *Chlamydomonas,* to elevated pCO_2 after 1000 generations. For species such as oysters, generation times are considerably longer and it seems unlikely that they will be capable of evolving rapidly enough (Barry *et al.*, 2011; Byrne, 2011). This notion has been supported by the lack of evidence of adaptation in tropical sea urchins (Uthicke *et al.*, 2013). However, studies by Parker *et al.* (2015; 2012) demonstrated positive carry-over effects of adults' exposure onto offspring, which highlights the significance of maternal effects in adjustments to external conditions, but also brings hope that sensitive marine organisms may be capable of efficient adjustments. The authors suggest that carry-over effects can hold positive consequences through time by improving the resilience of the following generations. Nonetheless, variations in the scope for adaptation are likely to occur amongst species (*M. gigas* > *S. glomerata*) and populations (bred line populations > wild populations of *S. glomerata*), as demonstrated by Parker *et al.* (2010; 2011, respectively) and Thompson *et al.* (2015). Further trans-generational studies are necessary to better comprehend the capacity of species to adjust to environmental changes and to be able to integrate the knowledge into future management plans, whether applied to natural oyster reefs or aquaculture practices.

A possible mechanism through which resilience to environmental stress can be achieved may be linked to the 'quality' of the environment. In the Baltic Sea, seasonal upwelling of eutrophic water leads to very high CO_2 levels and phytoplankton blooms, nevertheless, blue mussel reefs are able maintain their structure and function (Thomsen *et al.*, 2017). Thomsen *et al.*, (2012) argued that the high food supply allows the mussels to offset the metabolic costs of hypercapnia; a hypothesis that would support why aquaculture-reared individuals may be more tolerant to OA than wild individuals. However, what is not yet clear is the extent to which resilience and survival is achieved at the expense of ecosystem service (ES) provision?

1.3 The impacts of ocean acidification on oyster-reef ecosystem services

1.3.1 Using ESs to assess the impacts of OA

1.3.1.1 Assessing and valuing ESs

ESs allow society to evaluate and estimate the social and economic impacts of changes in resource availability (Beaumont *et al.*, 2007; Cooley, 2012). They provide a tangible link between ecosystem health and human use (Figure 1.3) that helps to inform decision-making of how to sustainably use and protect ecosystems (e.g. deciding whether to provide financial incentives, (Knights *et al.*, 2014). This is especially important as human well-being is linked to the sustained provision of resources including food, fuel, shelter, and water (Díaz *et al.*, 2006). However, clear context and definition of ESs is critical to their perceived 'value' (Friedrich *et al.*, 2015).

There are a number of ways to value ESs. The most obvious - '*monetary value'* - can be useful in conjunction with frameworks, such as the *Total Economic Valuation* (TEV) framework, which provides a classification of the different types of economic values associated with ESs (DEFRA, 2013; Herbert *et al.*, 2012). In TEV, ESs are classified as either 'use values' – those derived from human interactions with a particular resource – or 'non-use values' – derived solely from the knowledge that the resource exists currently and will continue to exist in the future (Figure 1.4).

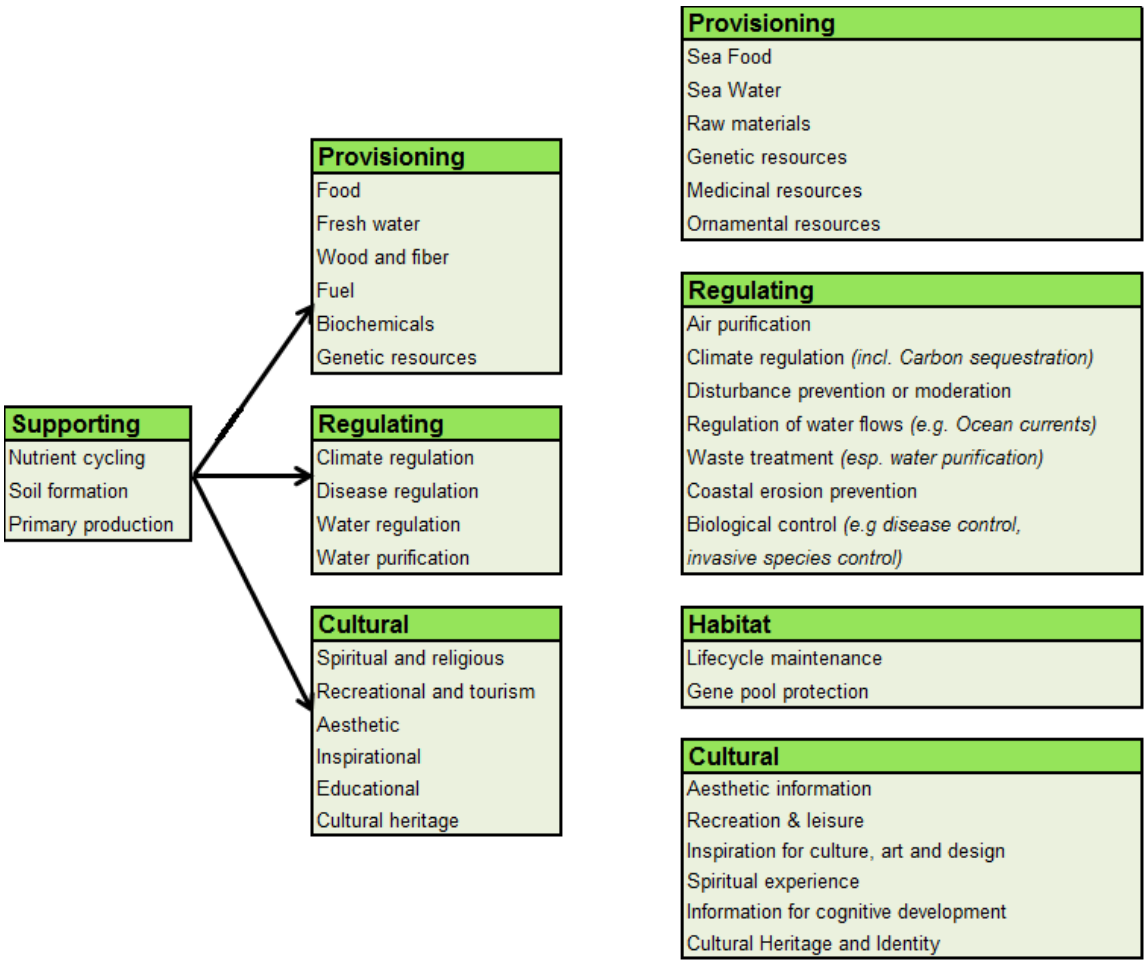

Figure 1.3: Categories of ecosystem goods and services, as described (left) in the Millennium Ecosystem Assessment (MEA, 2005), and additional examples (right) from the ODEMM Linkage Framework (White et al., 2013).

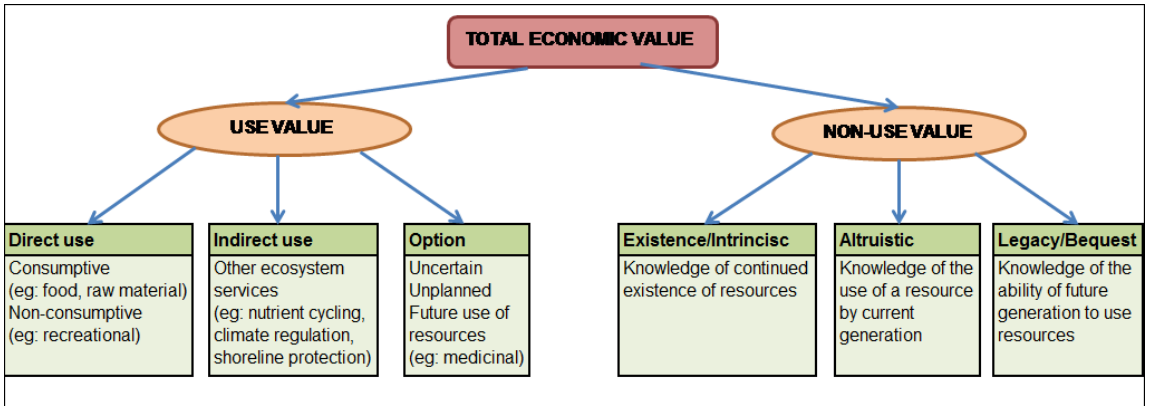

Figure 1.4: Total Economic Value framework (modified from Herbert et al., 2012).

1.3.1.2 Valuing oyster-derived ESs

The value of a service can be relatively easily estimated when a particular ES has a market price. For example, in 2009, Pacific oysters bought for the food industry were valued at £1,815/tonne (Herbert *et al.*, 2012; Humphreys *et al.*, 2014). Other services, such as carbon sequestration (carbon credit values), or raw materials (shell cultch) can be valued in a similar way. However, when no market price exists or the ES value is subjective, estimating value can be a difficult task. In these instances, approaches such as 'Willingness-to-Pay' (WTP. e.g.De Groot *et al.*, 2002; Fletcher *et al.*, 2014) can provide a 'value' based on what people are willing to pay to avoid or counteract the adverse effects of the loss of the ES. The WTP approach is less precise than using market values, but can still be used to infer ecosystem or ES value.

1.3.1.3 ESs and OA

Future OA conditions are predicted to negatively affect the availability and quality of ESs through direct or indirect effects on species and the ecosystem (Frommel *et al.*, 2012) with major social and economic consequences (Figure 1.5) (Cooley *et al.*, 2009). Studies have typically focused on particular habitats, such as coral reefs (Brander *et al.*, 2012), locations of significant value (Bosello *et al.*, 2015; Hilmi *et al.*, 2014; Lacoue-Labarthe *et al.*, 2016; Rodrigues *et al.*, 2013), or an economic sector (Cooley & Doney, 2009; Cooley *et al.*, 2015; Moore, 2011; Narita & Rehdanz, 2016; Narita *et al.*, 2012). Although an increasing number of studies have tried to quantify the economic implications of OA on ES provision (Brander *et al.*, 2012; Cooley & Doney, 2009; Moore, 2011; Narita *et al.*, 2012), they are often qualitative in nature due to a lack of quantitative data (Cooley, 2012; Hilmi *et al.*, 2014; Hilmi *et al.*, 2013). While qualitative assessments are valuable for indicating directionality of change in ES provision, it is challenging to include the findings in management decisions and investment prioritization without clear quantitative estimates of change. Therefore, future studies providing more quantitative estimates are critically needed in order to bridge those gaps (Runting *et al.*, 2017).

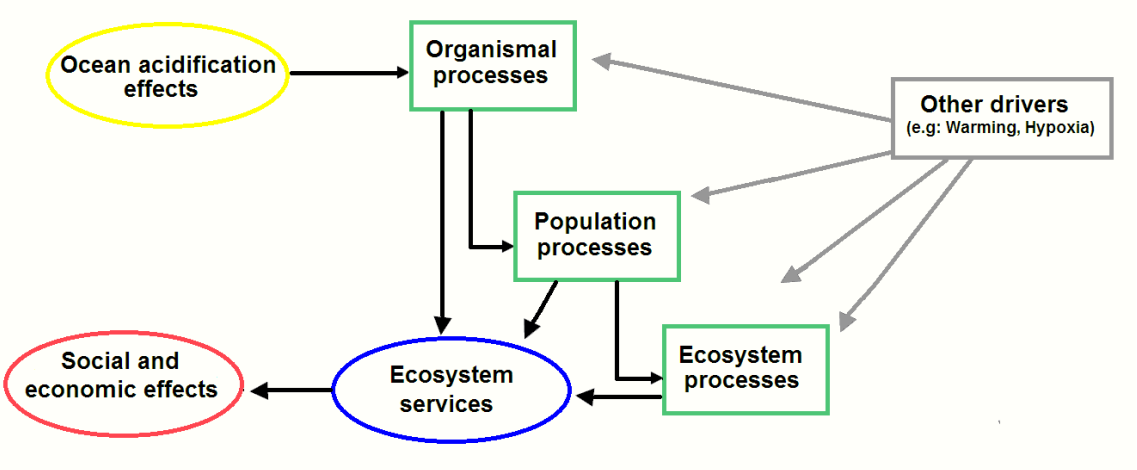

Figure 1.5: Relationships between ocean acidification effects, various levels of biological complexity, the provision of ecosystem services, and the associated social and economic effects (adapted from Le Quesne & Pinnegar, 2012).

1.3.2 Oyster-reefs ESs, their value, and impacts of OA

1.3.2.1 Oyster reef ecosystem services

Biogenic reefs provide a wide range of ecosystem services including supporting, provisioning, regulating, and cultural services (MEA, 2005) (Figure 1.3). Oysters are ecosystem engineers (Jones *et al.*, 1996; Jones *et al.*, 1997), in that they form biogenic reefs providing habitat for a range of other species and contribute a number of ecosystem functions and services (Fletcher *et al.*, 2012; Herbert *et al.*, 2016; Herbert *et al.*, 2012) (Figure 1.6, Table 1.1). Importantly, oysters are both allogenic and autogenic engineers, and ESs originate from both individual oysters and the wider reef structure (Walles *et al.*, 2015). Allogenic ESs include water filtration, benthic-pelagic coupling, nutrient cycling, carbon sequestration, and food provision (from oyster harvesting), while autogenic ESs include habitat formation, food provision, erosion protection and shoreline stabilization (Figure 1.6, Table 1.1). Additionally, cultural services associated with oyster reefs include recreational harvesting, educational use (research) and cultural heritage (Paolisso & Dery, 2010; Scyphers *et al.*, 2014).

The perceived and relative importance of oyster-derived ESs is context-specific (Scyphers *et al.*, 2014). Local or regional characteristics, such as environmental conditions, but also local economy and communities, can influence the significance of each service, but also their susceptibility to OA. For instance, biofiltration is a particularly valued ES in areas that are: polluted or susceptible to eutrophication; intensely used for recreation; or located near to seagrass beds (Cerco & Noel, 2007).

Alternatively, shoreline stabilisation and protection from erosion by oysters is of importance in coastal areas under threat of climate change and extreme weather events (Brumbaugh *et al.*, 2010; La Peyre *et al.*, 2014), and in other areas, seafood is considered most important (Cooley *et al.*, 2012).

Determining the importance of ESs to society and the economy can be achieved using one of the valuation methods described in brief above. Several studies have estimated the value of ESs associated with oyster reefs (Beseres-Pollack *et al.*, 2013; Grabowski *et al.*, 2012; Grabowski & Peterson, 2007; Henderson & O'Neil, 2003; Kroeger, 2012; Volety *et al.*, 2014) but estimates are wide-ranging (e.g. Grabowski *et al.,* (2012) valued oyster reefs at between \$5500-\$99000 ha^{-1} yr^{-1}, excluding the economic value from harvesting). Given local/regional priorities may vary, such varied estimates are unsurprising, but makes predicting the economic impact of OA on oyster reefs challenging and perhaps explains why no study has attempted to do so to date. In lieu of such an analysis, studies that estimate the economic losses emanating from damaged oyster reefs may be a useful proxy. Here, we use these studies to provide a first assessment of OA impacts on ES provision from oyster reefs. While it can be argued that drawing conclusions on the effects of OA from damaged reefs is unrealistic or inaccurate, it has the merit to reinforce the high value of oyster reef ESs, allow an initial estimate of losses to be undertaken, and provides direction for future studies.

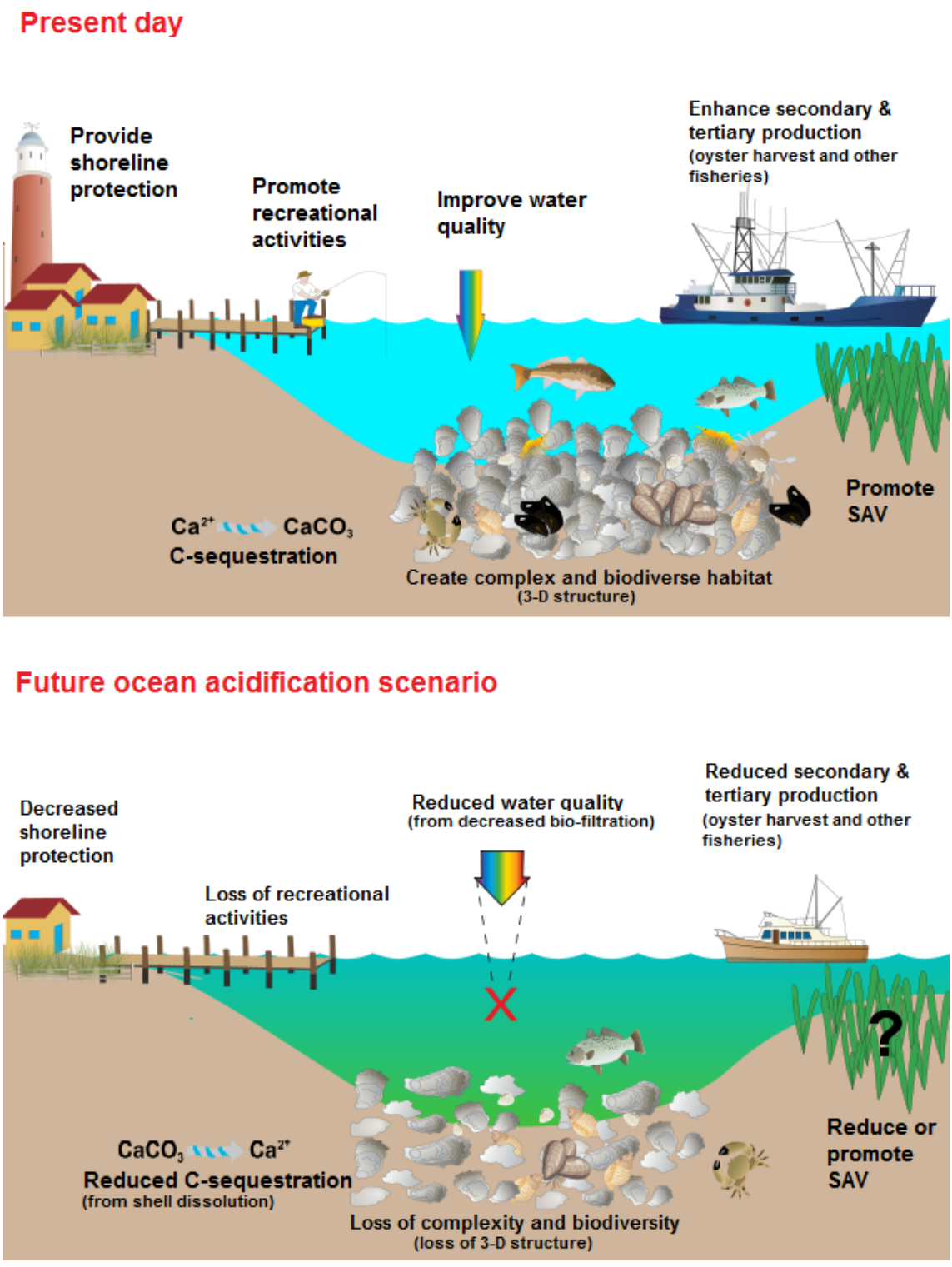

Figure 1.6: Conceptual diagram depicting some of the ecosystem services provided by oyster reefs (above) and the potential effects of ocean acidification on their provision (below). SAV=Submerged Aquatic Vegetation (Figure created by the authors, symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/)).

Table 1.1: Marine ecosystem goods and services (MEGS) provided by oysters and oyster reefs, following the ODEMM Linkage Framework description (White et al., 2013), and the potential impacts of ocean acidification (OA). '√' indicates that the service is provided by oysters, 'χ' indicates the service is not provided (according to the available literature). Direction of arrows indicates expected change in the ecosystem service (i.e. ↑ indicates increase in ES provision, ↓ a decrease in ES provision). '~' indicates that no consensus is reached or the change is context-specific.

	MEGS	Provided by Oysters or Oyster Reefs?	Estimated Value?	Affected by OA?
Habitat	Lifecycle maintenance	√ *(assumed from 3-D structure)*	*Unknown*	*Yes* ↓~
	Gene pool protection	√ *(assumed from 3-D structure)*	*Unknown*	*Yes* ↓~
Provisioning	Sea Food	√ *(harvest, aquaculture, extended fisheries)*	$20 850-$52 224/hectare of reef *(oyster harvest value- Grabowski et al. (2012))* $4123/year/hectare *(extended fisheries- Grabowski et al. (2012))* 809.7$/tonne *(aquaculture production (FAO data †))*	*Yes* ↓ or ↑ ~
	Sea Water	χ	χ	χ
	Raw materials	√ *(clutch material, construction material)*	$7-10 lb^{-1} *(Baywater Oyster Seeds LLC)* $17 yd^{-3} *(Pontchartrain Materials Corp., New Orleans)* $17 yd^{-3} *(LDWF, 2004)* ~$126/ton *(Kwon et al., 2004)*	*Yes* ↓~
	Genetic resources	√ *(aquaculture bred lines/triploid)*	*Unknown*	*Unknown*
	Medicinal resources	χ	χ	χ
	Ornamental resources	√ *(shell collection)*	*~$13m^{-3}$* (Lemasson, unpublished)	*Yes* ↓~
Regulating	Air purification	χ	χ	χ
	Climate regulation (incl. carbon sequestration)	√ *(carbon sequestration)*	*Unknown*	*Yes* ↓~
	Disturbance prevention or moderation	√	Upward of $6 million *(for coastal defence structures- Firth et al., 2014)*	*Yes* ↓~
	Regulation of water flows	χ	χ	χ
	Waste treatment (esp. water purification)	√ *(water purification, Chl-a removal, nutrient cycling)*	$1385-$6716/yr/ha. (Grabowski et al., 2012) $314 836/yr *(Newell et al., 2005)* $18 135/million oysters *(Kasperski and Wieland, 2009)* $3000/ac./yr *(Piehler and Smyth, 2011)* $293 993/yr *(Beseres-Pollack et al., 2013)*	*Yes* ↓~
	Coastal erosion prevention	√ *(shoreline stabilization, erosion prevention)*	*Upward of $6 million for coastal defence structures (Firth et al. 2014)*	*Yes* ↓~
	Biological control	√ *(facilitate submerged aquatic vegetation)*	*Unknown*	*Yes* ↓~
Cultural	Aesthetic information	χ	χ	χ
	Recreation & leisure	√	$222 million *(U.S. National Research Council, 2004)*	*Yes* ↓~
	Inspiration for culture, art and design	χ	χ	χ
	Spiritual experience	√ *(assumed)*	*Unknown*	*Unknown*
	Information for cognitive development	√ *(assumed)*	*Unknown*	*Unknown*
	Cultural Heritage and Identity	√ *(sense of tradition, oyster festivals)*	*Unknown*	*Unknown*

1.3.2.2 Current state of oyster reefs

Despite the numerous ESs provided by healthy oyster reefs, reef conservation and health is rarely considered unless populations are in danger of collapsing or are threatened (Kirby, 2004). It is only in these instances that the value of oyster reefs is assessed; the outcomes used to direct restoration efforts (Beck *et al.*, 2011; Coen *et al.*, 2007; Grabowski *et al.*, 2012; Grabowski & Peterson, 2007; La Peyre *et al.*, 2014; Volety *et al.*, 2014). A recent study estimated that 85% of native oyster reefs had been lost globally and that in many bays and ecoregions, reefs were less than 10% of their historical abundance and 'functionally extinct' (Beck *et al.*, 2011). As such, there is an urgent need for an assessment of ESs provided by oyster reefs.

There is, however, on-going debate over what is considered an acceptable level of 'ecological health' for oyster reefs (Alleway & Connell, 2015). While it is recognised that reef degradation can lead to a decrease in, or even loss of, provision of many ESs (Table 1.1 Coen *et al.*, 2007; Jackson *et al.*, 2001; Paerl *et al.*, 1998; zu Ermgassen *et al.*, 2013), the impact of OA on ESs derived from oyster reefs is less clear. In fact, the potential consequences of OA are largely speculative, and based either on the physiological and ecological impacts observed during laboratory experiments, or using *in situ* field experiments in naturally acidified sites (see Section 1.2.4). These studies suggest that population sizes may decrease below the minimum threshold required for the desired level of ESs (Figure 1.4) and indicate an overall negative effect of OA on oysters' early life stages, with direct consequences on juvenile and adult physiology as well as recruitment and population dynamics (see discussion in Section 1.2.4). However, while there have been studies examining the effects of OA on filtration rates, growth, and survival of oysters, there has been no attempt to date to link those effects to ES provision.

1.3.2.3 Ecosystem services associated with oyster reefs

Oyster reefs provide a number of important provisioning, regulating, habitat and cultural ESs (Table 1.1). Below we describe each of the ESs in turn, grouped using the MEA (MEA, 2005) and more recent ODEMM assessment (White *et al.*, 2013), and consider how OA is likely to affect the provision of those services in the future.

1.3.2.3.1 Habitat/Structural Services

Oysters can create large complex 3-dimensional structures (Knights & Walters, 2010), providing a unique habitat for many organisms, assuring life-cycle maintenance, and securing a diverse gene pool (Table 1.1). Reef condition is a function of oyster abundance (Beck *et al.,* 2011). When abundances are low, the reef structure can be lost, and the remaining oyster populations are referred to as "beds" of free-laying individuals. The maintenance of the reef structure relies notably on the successful recruitment of oyster larvae and juveniles into the adult population. By creating a mismatch between environmental conditions and larval/juvenile performance (see Section 1.2), OA can have direct negative impacts on the formation and replenishment of the reef structure (Table 1.1). Moreover, it can hold further negative consequences for the reef, by lowering gene pool diversity and creating additional bottlenecks. OA-induced reef deterioration is likely to alter the available niches for other species, and restructure the overall habitat, which can hold critical consequences for the provision of other ESs.

1.3.2.3.2 Provisioning Services

Biodiversity and Seafood (other than oysters)

Biogenic reefs are important to food webs and fisheries (Peterson & Lipcius, 2003) and many organisms use reefs as refugia from predators, as nests, or feeding grounds. Oyster reefs create critical habitat for a number of species, including other economically important molluscs, crustaceans, and various species of fish (reviewed in Coen *et al.*, 2007; Coen *et al.*, 1999; La Peyre *et al.*, 2014; Scyphers *et al.*, 2011; Tolley & Volety, 2005; Volety *et al.*, 2014). Grabowski *et al.,* (2012) estimated that one ha. of healthy oyster reef increases biodiversity and enhances commercial fish value by up to ~$4123 yr^{-1} (Table 1.1; see also Grabowski & Peterson, 2007), although that value-added benefit may only be apparent when the oyster reef is not located near to other biogenic habitats that provides a similar function (Geraldi *et al.*, 2009). The value of oyster reefs can be used to justify a management action (Knights *et al.*, 2014), for example, the decision to restore an oyster reef following degradation (an effective method for increasing fish and large crustaceans production, (Peterson *et al.*, 2003)). At the time of writing, there is no indication or means of disentangling the 'value' of oyster reefs to

biodiversity and seafood in differing states of health, nor a clear understanding of the likely state of oyster reefs under OA beyond an expectation that some reefs will be more susceptible to damage and lead to a reduction in ES provision. Current research is on-going to determine if other/non-native species, appearing more robust to OA, can provide redundancy for the loss of biogenic reef species threatened by OA.

Ornamental and raw materials

Oyster shell is valuable to a number of sectors (Yao *et al.*, 2014), including construction, agriculture, and wastewater treatment. The cost of oyster cultch is relatively low (~$126/tonne, (Kwon *et al.*, 2004); Table 1.1) and the calcium carbonate from the shells is used as grit for the rearing of poultry (Çath *et al.*, 2012; Scott *et al.*, 1971); as a construction material and substitute for aggregate in concrete or mortar (Yang *et al.*, 2010; Yang *et al.*, 2005; Yoon *et al.*, 2003; Yoon, 2004); as a liming material and soil stabilizer (Lee *et al.*, 2008; Ok *et al.*, 2010); and to treat discharged wastewaters to remove phosphates and traces of toxic heavy metals such as cadmium, copper and nickel (Hsu, 2009; Kwon *et al.*, 2004). As shell dissolution rates increase with OA, the availability of oyster shell for use as a raw material may decline in the future (in terms of abundance and/or 'quality'), increasing the cost of the material to industry and raising the cost to the consumer.

The use of oysters for ornamental purposes has decreased in modern times, but there are historical references of the use of oyster shells as raw material for the creation of glass (Wedepohl & Baumann, 2000). Shells are still available commercially, with values ranging from £5-15 a shell (Lemasson et al. *unpublished*). OA is likely to impact on the provision of raw materials, by negatively impacting on the calcification process of oysters, promoting adult shell dissolution, and may well reduce the value of shells as a raw material. The economic costs that would be incurred because of the loss of this service are unclear, and no estimates are found in the literature to date.

1.3.2.3.3 Regulating Services

Climate regulation and carbon sequestration

A number of studies have suggested that oysters provide climate change mitigation as a result of carbon sequestration during the calcification process (Dehon, 2010; Grabowski & Peterson, 2007; Lee *et al.*, 2010; Peterson & Lipcius, 2003; Wingard & Lorenz, 2014). Although it can be argued that oysters are net CO_2 producers on their own, this service is particularly valid when oysters are present in association with algae (Hall *et al.*, 2011). While the calcification process in oysters has been extensively researched, the extent to which climate can be mitigated is not easily quantifiable, and the impact on the carbon cycle not easily determined (Hickey, 2008). Under OA, shell calcification is negatively affected as shell dissolution increases (Welladsen *et al.*, 2010) as a result of corrosive waters. The carbon sequestration process and efficiency of the climate regulation service is therefore at risk. At the time of writing, there was no data assessing the value of such service, although estimates could be derived from the price of carbon credits, or be related to bequest value of the bio-sequestration of CO_2 (Herbert *et al.*, 2012).

Disturbance prevention and coastal erosion protection

The 3-dimensional structure of oyster reefs provides protection from erosion and stabilising shorelines. Adjacent critical habitats, such as seagrass beds or saltmarshes, positively benefit from the attenuation of wave action and the modification of the local water flow (Coen *et al.*, 2007; Scyphers *et al.*, 2011). It is argued that shore stabilisation and erosion protection are the most valuable ESs provided by oyster reefs (Grabowski *et al.*, 2012). How OA will affect the maintenance of the 3-dimensional reef structure is unclear (see also provisioning services above), but the impacts on early life history, juvenile and adult forms (discussed in Section 1.2) are predicted to lead to a reduction in reef size, and therefore, a reduction or loss of coastal protection and shoreline stabilisation properties. The 'value' of this ES is likely to continue to erode under climate change as the number and intensity of extreme weather events increases, such that the ability of reefs to attenuate wave action may be reduced (Michener *et al.*, 1997).

The cost of using engineered shoreline stabilization solutions as an alternative to reefs can provide some insights into the value of this ES (termed 'avoidance costs'), but as Grabowski *et al.,* (2012) points out, this value is context-specific and highly dependent on factors including the location,

infrastructure types and prices, local economy, and the level of exposure. Nevertheless, costs of coastal defence structures can be in the a few dollars to millions of dollars depending on the setting and requirements (Table 1.1; see Firth *et al.*, 2014, for a review)

Water treatment and quality

Oysters are important bio-filters that improve water quality (Grizzle *et al.*, 2008; Hoellein *et al.*, 2015; La Peyre *et al.*, 2014; Nelson *et al.*, 2004; Newell, 2004) and affect nutrient cycling (Beseres-Pollack *et al.*, 2013; Coen *et al.*, 2007; Hoellein *et al.*, 2015; Kellogg *et al.*, 2013; Newell *et al.*, 2005; Piehler & Smyth, 2011). A number of approaches have been used to attribute value to this ES. Grabowski *et al.,* (2012) used nitrogen permits as a proxy for market price, estimating that one ha. of oyster reef removed a quantity of nitrogen valued to \$1385-\$6716 yr^{-1}. Beseres-Pollack *et al.,* (2013) used the costs of adding a biological nutrient removal system to a wastewater treatment plant, valuing the nitrogen removal service at \$293,993 yr^{-1} in the Mission-Aransas Estuary (NB this is equivalent to between 44 and 212 hectares of oyster reef based on Grabowski *et al.*, 2012, estimates). Oyster-mediated nitrogen removal was valued at \$314,836 yr^{-1} in the Choptank estuary, using an average monetary value of ~\$24 kg^{-1} of nitrogen removed specifically applied to their study site (Newell *et al.*, 2005), \$18,136 per million oysters in Chesapeake Bay (Kasperski & Wieland, 2009), and \$3000 ac^{-1} yr^{-1} in Bogue Sound, using values derived from the North Carolina nutrient offset trading program of \$13 per kg of nitrogen removed (Piehler & Smyth, 2011). These values are, however, difficult to compare as they are based on multiple assumptions of what constitutes 'value' and the bodies of water are of different sizes and properties, but nevertheless highlight the important economic value of this ecosystem service provided by oyster reefs.

The continued provision of this ES has important indirect benefits to other ES provision that are often not considered. For example, improved water quality affects the provision of ESs by other healthy and functioning species and habitats, such as economically important seagrass beds (Cerco & Noel, 2007; Coen *et al.*, 2007; Dennison *et al.*, 1993; Grabowski *et al.*, 2012; Kahn & Kemp, 1985; Meyer *et al.*, 1997). Increased seagrass bed coverage that comes with improved water quality can also be incorporated into the economic valuation of oyster reefs. Grabowski *et al.,* (2012) estimated that

one ha. of oyster reef promoted 0.005 hectares of additional seagrass bed, valued at \$2584 ha^{-1}. Therefore, under OA, further economic losses can originate from adversely impacted adjacent habitats.

1.3.2.3.4 Cultural Services

Recreation and leisure

Improved water quality associated with healthy oyster reefs increases human well-being by reducing the likelihood of eutrophication (Lipton, 2004), and increasing recreational use of the environment. The value of recreation and tourism can therefore be used as an indirect estimate of the value of oyster reefs. Oyster reefs are socially recognised as a valuable resource; the U.S. National Research Council (2004) valued reefs using willingness-to-pay estimates at ~\$222 million (Grabowski & Peterson, 2007; see also Volety *et al.*, 2014). Reduced bio-filtration rates under OA will likely lead to increased eutrophication and reduced water quality, diminishing public appeal and generating financial losses from lower recreational use.

Spiritual experience and cultural heritage

Oysters hold a significant place in local culture, traditions and history. Many countries have a long history of oyster harvest and consumption (Kirby, 2004), including the USA (Dyer & Leard, 1994), France (Heral, 1989), and the UK (Humphreys *et al.*, 2014; Mac Con Iomaire, 2006), which is celebrated during oyster festivals, such as the 'Bluff Oyster' in New Zealand (Panelli *et al.*, 2008; Rusher, 2003), 'Oysterfest' in Australia (Lee & Arcodia, 2011), and the 'Whitstable Oyster Festival' and the 'Falmouth Oyster Festival' in the UK. Traditional oyster harvesting can be an important part of the local economy and creates a sense of community and heritage, with the desire for this activity to be sustainable and prosper (Dyer & Leard, 1994; Paolisso & Dery, 2010; Scyphers *et al.*, 2014). The impact of OA on the provision of cultural services is difficult to assess, but a reduction in the persistence of oyster reefs (and the number of harvestable oysters) will likely impact on the sense of heritage and affect local economies and communities that rely on a long tradition of oyster harvesting. In the worst case, OA could lead to the disappearance of oyster festivals, leading to the loss of sense

of tradition and community well-being, as well as negative economic impacts locally through a reduction in tourism.

1.3.3 Economic Impacts of OA on oyster harvest and aquaculture

The vulnerability of shellfisheries to OA and the likely economic consequences is of growing concern (Cooley *et al.*, 2015; Ekstrom *et al.*, 2015; Haigh *et al.*, 2015; Seijo *et al.*, 2016). Although the 'value' of reefs is highly variable and context-dependent in terms of harvest yield, OA is likely to negatively affect the resilience, persistence, and sustainable use of wild oyster reefs into the future. In response, wild harvests now represent only a small proportion of oyster production worldwide, and have been increasingly replaced by aquaculture (146,828 tonnes of wild harvest compared to 5,321,737 tonnes of aquaculture production; valued at $ 4,094,411,000 in 2015 - FAO data[3]).

Aquaculture is the fastest growing food sector, and production reached an all-time high of 106 million tonnes in 2015 (FAO data[2]). In the UK, the decline of the native oyster, *O. edulis*, led to the introduction of the Pacific oyster, *M. gigas*, which now represents over 90% of the country's oyster production (Humphreys *et al.*, 2014). Approximately 1200 tonnes of Pacific oysters are estimated to be produced each year in the UK (Herbert *et al.*, 2012) worth an estimated £10.14 million (Humphreys *et al.*, 2014). Given the demand and value of shellfish aquaculture, determining how OA will affect food security in the future is a crucial question that remains unanswered, although in 2010, the United Nations Environment Programme (UNEP) cited OA as a major threat to food security (UNEP, 2010). At locations where the effects of OA are already being felt, damages to the oyster aquaculture industry have been disastrous. In the US, the Pacific North-west region hosts an oyster industry worth over $72 million, but since 2007, several oyster hatcheries have suffered from mass mortalities of oyster larvae of up to 70-80% due to the upwelling of waters that were acidified, highly saline and rich in *V. tubiashii* (Barton *et al.*, 2015; Elston *et al.*, 2008; Feely *et al.*, 2008). Similar impacts are expected in UK shellfisheries and elsewhere under combined scenarios of acidification and warming (Callaway *et al.*, 2012).

[3] http://www.fao.org/fishery/statistics

Concerns have been expressed regarding the likely imbalance in the social and economic consequences of OA experienced by different communities (Ciuriak, 2012; Cooley *et al.*, 2012). Islands and coastal communities that rely heavily on seafood as a source of protein and for their livelihood (e.g. tourism), are expected to suffer most, particularly as their potential for adaptation and mitigation is restricted due to lower financial means and limited access to technologies (Cooley *et al.*, 2009; Hilmi *et al.*, 2014; Hilmi *et al.*, 2013; UNEP, 2010). Seafood aquaculture holds the potential to provide food security in a future where growing population and growing income are expected to increase the demand for food. However, for aquaculture to be sustainable, there is a need to recognise that the sector is nested in a sensitive system interconnecting economic, social and ecological spheres, whereby impacts on one sphere will likely disrupt the others (Bailey, 2008; Schmitt & Brugere, 2013; Soto *et al.*, 2008).

Some argue that shellfish aquaculture will be less impacted by OA than wild harvests (Rodrigues *et al.*, 2013), for instance, the rearing of larval stages in tanks may mitigate the impacts of OA. Further, the use of informed 'climate-proof' management measures, designed to buffer the effect(s) of OA, such as quality control and close monitoring of water quality may minimise any potential effects (Hilmi *et al.*, 2014; see also the case study on the mitigation of the effects of ocean acidification on prawn and scallop fisheries in Australia by Richards *et al.*, 2015b). Despite potentially being 'climate-proofed' by hosting the vulnerable early life stages in isolated systems, oyster aquaculture systems hold a phase spent in the marine environment, where young spat are reared to adult sizes, during which they are exposed to the deleterious effects of ocean acidification. Climate-proofing' of natural oyster reefs, however, remains a greater challenge, for which a better understanding of the effect of ocean acidification on ecosystem services provision is necessary. In the Pacific North-west, oyster hatcheries have benefitted from close monitoring of seawater quality, which has greatly improved yield and reduced mortality (Barton *et al.*, 2015). Relocation of hatcheries and farms to areas of higher water quality and environmental conditions more suited to the rearing of larvae is an option already considered by shellfish producers (Barton *et al.*, 2015), although this may represent a costly alternative. Aquaculture farms could focus on more resistant species, such as *C. virginica* and *M. gigas* (Gobler & Talmage, 2014; Guo *et al.*, 2015; Parker *et al.*, 2010), and hatcheries could select for lines that produce

the highest survival rates, such as selectively bred lines (Goncalves *et al.*, 2017; Parker *et al.*, 2011; Thompson *et al.*, 2015). Indeed, aquaculture is already using bred-line and triploid specimen, as they display increased growth rates and diseases resistance (Nell, 2002). Using resistant-bred lines, or switching to more robust species, can in theory reduce the mortality in aquaculture practices induced by ocean acidification, thereby increasing the yield and help securing food demand. However, such scenarios must rely on robust scientific knowledge of the response of the different life stages of different species of oysters to OA. Moore (2011) claims that damage to individuals, and especially their shell, which appear to be susceptible to low pH (Welladsen *et al.*, 2010), are unlikely to hold significant economic impacts as long as the organism survives the culture process. However, OA has the potential to impact on the final quality and value of the product by affecting the wet tissue mass, the shell appearance (due to corrosive waters), tissue texture and taste (Dupont *et al.*, 2014), and potentially their nutritional value, although this aspect has been little studied yet.

OA is likely to increase production costs due to the necessary buffering measures taken during husbandry procedures, such as pH and Ω_{ar} manipulations and increased feeding, as well as the costs associated with the longer developmental time of the early life stages, unless the production is focused on OA-resistant species or lines. New models should be investigated in order to link future levels of OA with variations in the production of oysters, and to try and predict to what extent those variations in production are likely to indirectly impact the economy due to welfare losses, and welfare effects of price increase due to the reduced supply (Narita *et al.*, 2012), changes in job opportunities, and general reverberation into the wider economy. As Richards *et al.,* (2015b) stated "OA (*sic*) itself cannot be mitigated through fisheries management, however management can be used to reduce the negative effects and take advantage of positive effects associated with this phenomenon".

1.4 Conclusion

OA is likely to have negative effects throughout the life cycle of oysters, although the effects may be difficult to decipher due to other stressors acting on multiple life-history stages (Byrne & Przeslawski, 2013; Knights & Walters, 2010). Acclimation, parental (hereditary) traits, or adaptation may well

reduce the risk of negative consequences from OA, although the extent to which individuals can respond to the threat remains to be seen.

Oysters are a key ecological species that provide a plethora of ES to humans, but the provision, quantity, and quality of ESs in the future under climate change and OA remains uncertain. OA is occurring alongside other environmental and anthropogenic stressors, such as warming, hypoxia, variations in salinity, eutrophication, or metal contamination, that are likely to affect the organisms' responses. The outcomes of multi-stressor interactions are difficult to predict and seem highly context-specific (see the reviews on the impacts of ocean warming and acidification by Byrne, 2011; Byrne & Przeslawski, 2013; and Harvey *et al.*, 2013). Future studies should consider the combination of multiple stressors, but should also focus on adult oysters with the aim to link individual and population responses with the provision of associated ESs. The consequences of OA on oysters are already being felt in parts of the world (Cooley *et al.*, 2016), where natural populations and hatcheries have been negatively impacted upon; therefore ocean acidification will likely put increased pressure on food security in the future, by reducing harvest and aquaculture productions. Predicted levels of OA are likely to hold a number of other significant social and economic consequences that goes beyond oyster production from harvest and aquaculture, such as impoverished water quality, reduced shoreline protection, or altered well-being.

The few studies to date that estimate the value of oyster-derived ESs give an insight into the importance and value of oysters to the environment and society. Whilst there is some variation in the economic value of oyster-derived ESs due to local and/or regional differences in societal value placed on those services, the role of oysters in supporting a healthy and functioning ecosystem is clear, but which is under threat from OA. Further assessments of the social and economic impacts of OA on oysters and oyster reefs are needed to emphasise the 'value' of oysters to society, such that necessary steps are taken to ensure their long-term future.

1.5 Thesis aim, context and outline

1.5.1 Thesis aim

The general aim of this thesis is to investigate the possible impacts of ocean acidification and warming, based on the latest local predictions, on two valuable species of oysters found in the UK: the native European Flat oyster *Ostrea edulis*, and the non-native Pacific oyster *Magallana gigas*, originally introduced for aquaculture purposes. Findings will be linked to changes in ecosystem functioning, and in the provision and delivery of associated ecosystem services, in order to assess the potential consequences for society.

1.5.2 Oyster fishery and historical introduction

In the UK, *O. edulis*, and *M. gigas* provide numerous ecosystem services (Herbert *et al.*, 2012). As detailed previously, these services include, but are not limited to, improvement of water quality (through cycling and purification), reef formation, and food provision (through aquaculture and fisheries) (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 (Laing *et al.*, 2006; Lallias *et al.*, 2010; Woolmer *et al.*, 2011). Despite being protected, harvest of wild beds of *O. edulis* still takes place, using both light hand-hauled dredges and heavy dredges (Woolmer *et al.,* 2011). In contrast, *M. gigas* was introduced as broodstock and spat to the UK within regulated aquaculture settings in the mid-20th Century in response to the decline of *O. edulis* (Mills, 2016). This species is cultivated using various bottom (i.e: oysters lay directly onto the seabed) and off-bottom (e.g: cages, racks, trays, stakes, pontoons) culture methods depending on the environmental conditions (see Mills, 2016), with spat originating from UK hatcheries. Today, represents over 90% of UK oyster production (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 populations, but 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; Troost, 2010). Despite the occurrence of wild populations, the harvest of *M. gigas* is currently mostly limited to regulated aquaculture sites (Herbert *et al.,* 2012) A number of management measures aimed at preventing the further proliferation of *M. gigas* or its eradication are being considered, including mechanical removal and the use of sterile triploids in aquaculture (Herbert *et al.*, 2016), at the same time, promoting the

recovery of *O. edulis* through seeding (Harding *et al.*, 2016), development of disease-resistant stocks (Laing *et al.*, 2006), pest control and improvement of fishery management (Woolmer *et al.*, 2011). Whether to implement efforts to eradicate *M. gigas* is the subject of much debate, due to the conflicting aims of conserving protected habitats/species, while maintaining ecosystem services and securing sustainable socio-economics benefits from aquaculture.

1.5.3 Species biology and ecology

Notable differences between *O. edulis* and *M. gigas* exist regarding their biology and ecology, questioning the assumption that they do provide similar ecological function and ecosystem services. Both species are successive protandrous hermaphrodites (Guo *et al.*, 1998; Shelmerdine & Leslie, 2009), but while most *O. edulis* reach sexual maturity during their second summer (Korringa, 1952), *M. gigas* can reach sexual maturity as soon as their first winter (Guo *et al.*, 1998). *Ostrea edulis* is also a brooding species – an embryo is brooded within the mantle cavity following internal fertilization and then released into the external environment, where its dispersal is limited (O Foighil & Taylor, 2000) – whereas *M. gigas* is a broadcast spawner, releasing gametes into the water column, where fertilization leads to the production of larvae capable of dispersing relatively much further. Additionally, their vertical distribution on UK shores also vary. *Ostrea edulis* settle on hard substrates (e.g: hard silt, muddy gravel, sand and rocks) but can also be found on soft sediment where shells are present. This species mostly occurs from the low intertidal to the subtidal, where its abundance is greatest (see Shelmerdine & Leslie, 2009). In contrast, *M. gigas* colonise both hard substrates – including littoral rock, boulders, piers and seawalls – but also soft habitats such as mud and sand flats, where dense aggregations can drastically change the local hydro-dynamism and biodiversity (Herbert *et al.,* 2012). In rocky areas, *M. gigas* occur solely in the intertidal, but this species can also occur subtidally on soft sediment areas (Herbert *et al.*, 2012). While their respective vertical distributions on the shore are largely different, beds comprised of both *M. gigas* and *O. edulis* have been recorded (Zwerscke *et al.*, 2017), such as sites along the South-West coast of the UK (pers. observations; Figure 1.7a,b). Although it is likely that they compete for space and resources, the impact of *M. gigas* on *O. edulis* remains unclear.

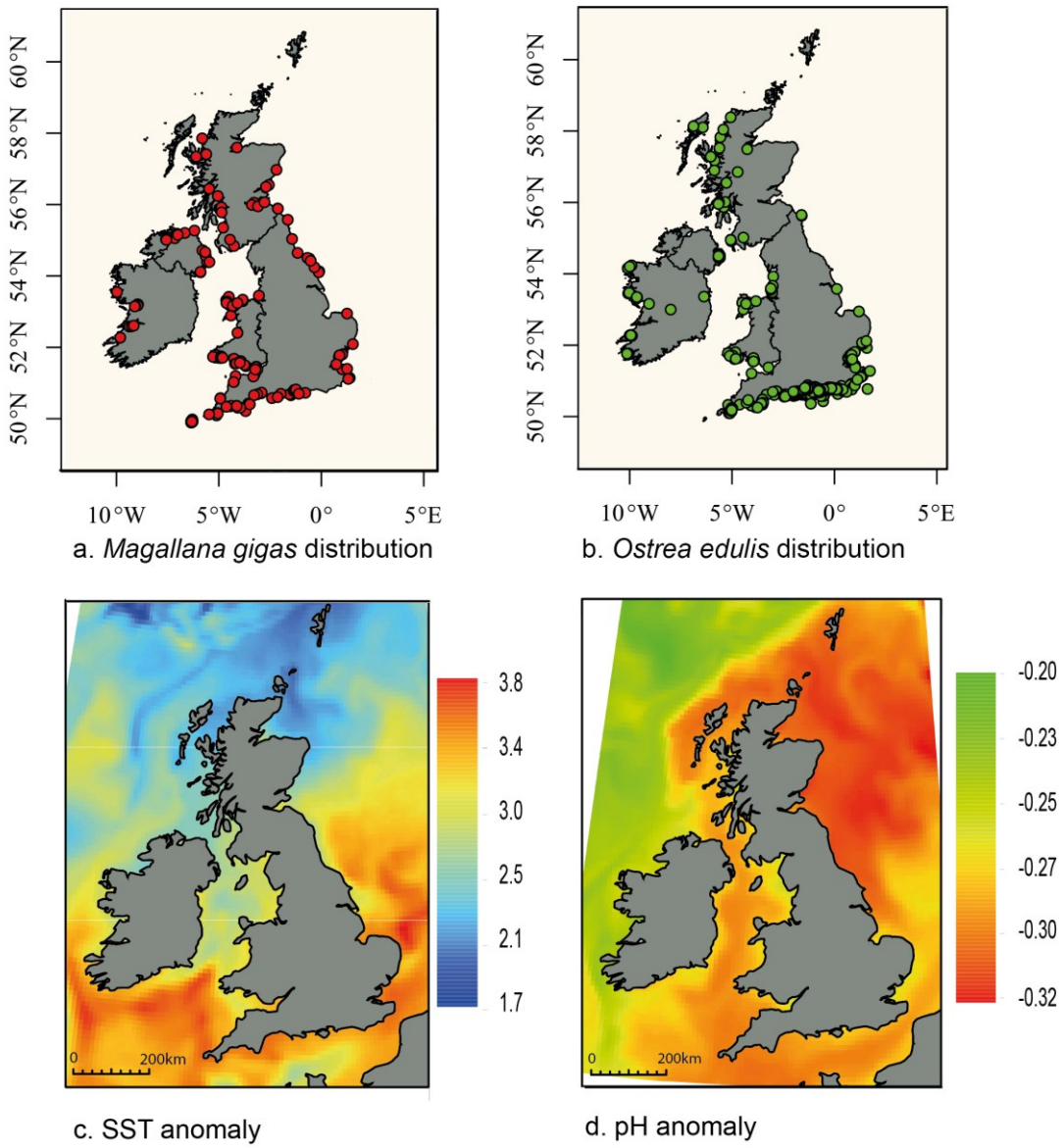

Figure 1.7: Current UK wild distribution of (a) *Magallana gigas* (red) and (b) *Ostrea edulis* (green) (data obtained from the Global Biodiversity Information Facility (GBIF) database), (c) maximum mean annual sea surface temperature anomaly (SST, in °C; medium emission scenario IPCC SRES: A1B for 2070-2099, 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).

1.5.4 Climate change context

In the UK, following medium CO_2 emission scenarios, local models predict an increase in sea surface temperatures by 1.7-3.8°C, along with a decrease in pH by 0.20-0.32 units by mid- to end century (Figure 1.7c,d). *Magallana gigas* is currently spreading northward on European shores, facilitated by increasing sea surface temperatures (Rinde *et al.*, 2016). In contrast, the extent of *O. edulis* is continuing

to decline, and further increase in SST is expected to skew its sex-ratio (Eagle *et al.*, 2017), although some argue that ocean warming may also enhance *O. edulis* stocks by favouring larval production, recruitment, and post-settlement growth (Beiras *et al.*, 1995; Davis & Calabrese, 1969). Given the negative impacts on oysters and other calcifiers described previously, both species are expected to be negatively impacted by ocean acidification. Non-native and invasive species are often highly tolerant of fluctuations in environmental conditions, and are able to withstand greater stress than their native counterparts (Hall-Spencer & Allen, 2015; Lodge, 1993; Stachowicz *et al.*, 2002). It is therefore unclear how OAW, from continued CO_2 emissions, will affect *M. gigas* and *O. edulis* and what the consequences for ecological functioning and provisioning of ecosystem services will be. Substitution of one species for another, partially or completely, can produce significant ecological impacts (Krassoi *et al.*, 2008), but since *M. gigas* is able to provide similar ecological functions and ecosystem services as *O. edulis* (Herbert *et al.*, 2012; Zwerschke *et al.*, 2016) efforts to eradicate it may be unwise.

1.5.5 Thesis outline

Changes in physiological responses under stress can have important consequences in terms of energy allocation and trade-offs, with long-term implications for individual survival and population maintenance, and therefore implications for ecosystem functioning and ES provisions. Chapter 2 investigates changes in physiological responses of adult specimens of *M. gigas* and *O. edulis*, looking at condition, metabolism, and feeding activity, in order to determine the potential ecosystem service contribution of both species today and in the future.

Sustainable populations of marine organisms are key for continued provision of marine ecosystem services. However, it is well established that OAW can alter a multitude of aspects related to the fitness of molluscs, affecting their physiology and shell properties, and altering their energy budget (the focus of Chapter 2). Important implications for the stability and resilience of impacted populations, as a result of changes in the strength of population-regulating mechanisms and community interactions, can arise from changes in the fitness of individuals. For instance, any negative effects of OAW on traits of oysters that reduce predation is likely to alter predator-prey interactions and reshape reef structure and functioning. Thus, Chapter 3 tested the effects of OAW

on aspects of oysters' physiology and morphology linked with predation resistance, in order to assess future stability and sustainability of oyster populations.

Seafood contributes greatly to the provision of protein and to global food security, with over 4.3 billion people relying on fish and shellfish as their main source of animal protein. However, with the ever-growing population, the need for highly nutritious seafood will intensify in the future. Future OAW has been shown to negatively impact the quantity of aquaculture production, but there is uncertainty regarding how the nutritional quality will be affected. To date, only one study has looked at changes in the quality of seafood with future environmental conditions, showing negative effects on important nutritional properties. Given the necessity to secure future food provision, it is crucial to investigate potential changes in the nutritional quality of seafood species. Chapter 4 therefore investigates changes in the delivery of the food provision service from a nutritional point of view, focusing on adults of both *M. gigas* and *O. edulis*. The findings of this chapter will linked back to the physiological responses described in Chapters 2 and 3, and will hold a direct link with Chapter 5.

Another crucial aspect of the food provision service is related to the eating quality – *sensu* sensorial quality – of food products. Changes to the eating quality can greatly impact on the market, particularly when dealing with high value species such as oysters. To date, only one study had looked at changes in taste of shellfish with future environmental conditions, showing negative effects. Therefore, there is a clear need to understand potential impacts on the eating quality of oysters, to predict variations in consumer preferences and market fluctuations. Chapter 5 looks into changes in the sensorial quality of oysters under climate change, focusing on *M. gigas,* as it is a major aquaculture species.

Some of these studies may appear repetitive of what is found in the literature, as a large amount of recently published work investigates the responses of molluscs to acidification. However, there is a need for studies investigating the interaction of acidification and warming, as those stressors will co-occur in the future. Moreover, very few studies have focused on UK oyster populations, particularly, the responses of *O. edulis* to environmental change, which remains largely unknown. This thesis appears particularly novel in the sense that very few studies try to decipher the links between

biological responses and ecosystem function, and the consequences in terms of ecosystem services. Ultimately, this thesis aims to provide an integrated picture of the effects of future climate scenarios on UK oysters, with the hope that findings will inform society and measures will be taken in order to secure some of those significant ecosystem services.

Chapter 2: Acidification and warming impact the physiology of invasive oysters *Magallana gigas* more than native *Ostrea edulis* in UK waters.

"To be fit as a fiddle"

A version of this chapter is currently under review as:

Lemasson, A.J.,Hall-Spencer, J.M., Fletcher, S., Provstgaard-Morys, S., and A.M. Knights. Acidification and warming impact the physiology of introduced oysters *Magallana gigas* more than native *Ostrea edulis* in UK waters, for publication in *PlosOne*.

2 Acidification and warming impact the physiology of invasive oysters *Magallana gigas* more than native *Ostrea edulis* in UK waters.

Abstract:

The effects of ocean acidification and warming on metabolic rate, feeding rate, and somatic growth was assessed using two co-occuring species of oysters collected off Plymouth (UK) – the invasive Pacific oyster *Magallana gigas*, and native flat oyster *Ostrea edulis*. Biological responses were measured for all combinations of temperature (16.8°C, 20.0°C) and pCO_2 (~400 ppm, ~750 ppm, ~1000 ppm). Effects differed 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 ~750ppm pCO_2. Similarly, the condition index of *O. edulis* was unimpacted, but *M. gigas* was negatively impacted by warming, likely due to increased energetic demands that were not compensated for by increased feeding. These findings suggest that the consequences of anthropogenic CO_2 emissions will not be equally stressful for both species. Contrary to expectations, invasive *M. gigas* experienced a higher level of stress due to warming and acidification than the native *O. edulis*. If these laboratory findings hold true for wild populations, then continued CO_2 emissions can be expected to adversely affect the functioning of *M. gigas* populations and have significant economic repercussions on aquaculture. Our findings strengthen arguments in favour of *O. edulis* restoration in UK waters.

2.1 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), raising concerns for population persistence and the continued provision of important ecosystem services (Lacoue-Labarthe *et al.*, 2016; Lemasson *et al.*, 2017a; Sunday *et al.*, 2016; Weatherdon *et al.*, 2016). Calcifying ectotherms are especially at risk as they are susceptible to alterations in ocean chemistry (Chapter 1), manifested by increased metabolism, respiration and energy expenditure (Pörtner & Farrell, 2008). A common way for marine organisms to balance their energy intake and expenditure is to increase their feeding rate, and the species most resilient to OAW may well be those best able to enhance their energy

assimilation (Lardies *et al.*, 2017; Ramajo *et al.*, 2015; Sanders *et al.*, 2013; Thomsen *et al.*, 2012; Towle *et al.*, 2015) or reallocate energy through partitioning and trade-offs (Leung *et al.*, 2017a). Species less able to manipulate their feeding activity to offset OAW stress (Houlbrèque *et al.*, 2015; Mackenzie *et al.*, 2014; Speights *et al.*, 2017; Vargas *et al.*, 2015) may show reduced energetic levels and capacity for metabolic maintenance (Harvey & Moore, 2017; Ong *et al.*, 2017). OAW may therefore be an important selection pressure that dictates the distribution of species and functioning of marine ecosystems (Poloczanska *et al.*, 2016). 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.

As previously described in Chapter 1, the native European flat oyster, *Ostrea edulis*, and the non-native invasive Pacific oyster, *Magallana gigas* are two ecologically and economically valuable species in the UK, that provide relatively similar and numerous ecosystem services (see Table 1.1). These services include, but are not limited to, reef formation (if abundances are high enough), erosion control, improvement of water quality (through cycling and purification), raw material supply, and food provision (through aquaculture and fisheries). Despite historically being highly abundant in the UK and Europe (Coolen, 2017; Orton, 1937), today *O. edulis* is a protected species with active restoration efforts underway to counteract the declining populations (Laing *et al.*, 2006; Lallias *et al.*, 2010; Woolmer *et al.*, 2011). In contrast, *Magallana gigas* – introduced to the UK for aquaculture in the mid-20th Century in response to the decline of *O. edulis* – has now formed wild populations on UK and Irish shores where it is considered invasive (Dolmer *et al.*, 2014; Herbert *et al.*, 2016; Troost, 2010). Beds comprised of both free-laying *M. gigas* and *O. edulis* occur, such as sites along the South-West coast of the UK (pers. observations; Figure 1.7a,b), and although it is likely that they compete for space and resources, the impact of *M. gigas* on *O. edulis* and the surrounding habitat remains unclear. A number of management measures aimed at preventing the further proliferation of *M. gigas* or its eradication are being considered (see Chapter 1, section 1.5.2), at the same time, promoting the recovery of *O. edulis* (Harding *et al.*, 2016; Woolmer *et al.*, 2011). Whether to implement efforts to eradicate *M. gigas* is the subject of much debate, due to the conflicting aims of conserving protected

habitats/species, while maintaining ecosystem services and securing sustainable socio-economics benefits from aquaculture.

The increase in sea surface temperatures is currently facilitating the northward spread of *Magallana gigas* on European shores (Rinde *et al.*, 2016; Thomas *et al.*, 2016). In contrast, the extent of *Ostrea edulis* is continuing to decline, and native oyster reefs and beds are considered some of the most endangered coastal habitats in Europe (Airoldi *et al.,* 2007; Beck *et al.,* 2011). Nevertheless, both species are 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 maintenance) 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 have rapidly increased in number, but show highly variable responses (detailed in Chapter 1). Some show altered immune response (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 (Speights *et al.*, 2017; Welladsen *et al.*, 2010). Crucial metabolic activities, such as respiration and feeding, may also be impacted (Comeau *et al.*, 2008; Scanes *et al.*, 2017; Speights *et al.*, 2017), the resulting stress leading to mortality and reduced population resilience, which would undermine the provision of ecosystem services (Lemasson *et al.*, 2017a).

Non-native and invasive species are often highly tolerant of fluctuations in environmental conditions, and are able to withstand greater stress than their native counterparts (Hall-Spencer & Allen, 2015; Lodge, 1993; Stachowicz *et al.*, 2002). In the UK, *M. gigas* is living well within its thermal range (1.8-35°C, FAO factsheet; Figure 1.7a) but, surprisingly, the thermal limits of *O. edulis* are less clearly defined (Shelmerdine & Leslie, 2009). Temperatures higher than 20°C are suboptimal, negatively affecting growth, metabolism and filtration activity in juvenile *O. edulis* (Buxton *et al.*, 1981), but earlier studies have also suggested cold-limitation (Orton, 1940; Walne, 1958). It is therefore unclear how continued CO_2 emissions will affect the metabolism of *M. gigas* and *O. edulis,* and what the

consequences for ecological functioning and provisioning of ecosystem services will be. Substitution of one species for another, partially or completely, can produce significant ecological impacts (Krassoi *et al.*, 2008), but since *M. gigas* is able to provide similar ecological functions and ecosystem services as *O. edulis* (Herbert *et al.*, 2012; Zwerschke *et al.*, 2016) efforts to irradicate it may be unwise.

In this study, we test the effects of OAW on the physiological responses of a native and a non-native species of UK oyster in order to determine the potential respective ecosystem service contribution of both species today and in the future. Individual measures of fitness were assessed using Standard Metabolic Rates (SMR), Clearance Rates (CR), and Condition Indices (CI) under simulated warming and acidification scenarios over a 12 week period. SMR was used as a proxy for metabolic costs and energetic requirements, while CR informed us of energy uptake. CI was used to assess overall fitness and the availability of energy reserves within somatic tissues. Our hypotheses were that future OAW conditions would induce metabolic stress for both species of oysters, along with compensatory increases in energy acquisition through enhanced feeding. Additionally, we hypothesised that *M. gigas* would show evidence of higher tolerance to warming and acidification than *O. edulis*.

2.2 Methods

2.2.1 Organism collection and acclimation

Adult Pacific oysters (*M. gigas;* 112.4 ± 6.9 mm in length and weighing 285.9 ± 13.4 g) and European flat oysters (*O. edulis*; 78.8 ± 4.6 mm in length and weighing 97.7 ± 17.0 g) were wild-collected from a low-intertidal site located at the meeting point of the Lynher River and the River Tamar, in Plymouth Sound, UK (50°23'29.95"N, 004°13'16.77"W), in July 2015 and January 2016, respectively. This site is primarily composed of muddy sand, however hard substrata is also present in the form of rocks from Rat Island (50°23'36.1"N 4°13'08.2"W) and from important accumulation of shells from dead organisms. No long-term data on the environmental conditions at this site are currently available (but see records at the coastal L4 station for long-term environmental data for Plymouth Sound[1]).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. Over an

[1] http://www.westernchannelobservatory.org.uk

acclimation period of 14 days, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

2.2.2 Experimental design, mesocosm set-up, and measurements of seawater parameters

OAW experimental treatments

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 scenarios are in line with warming and acidification conditions predicted for the UK (see Figure 1.7c,d). Twenty-four 3 L experimental tanks (four per OAW scenario) were set-up for the experiment (Figure 2.1). One individual of *M. gigas* was placed in each tank and exposed to the treatment conditions for 12 weeks. 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, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater. This experiment was repeated with *O. edulis* following the same procedures.

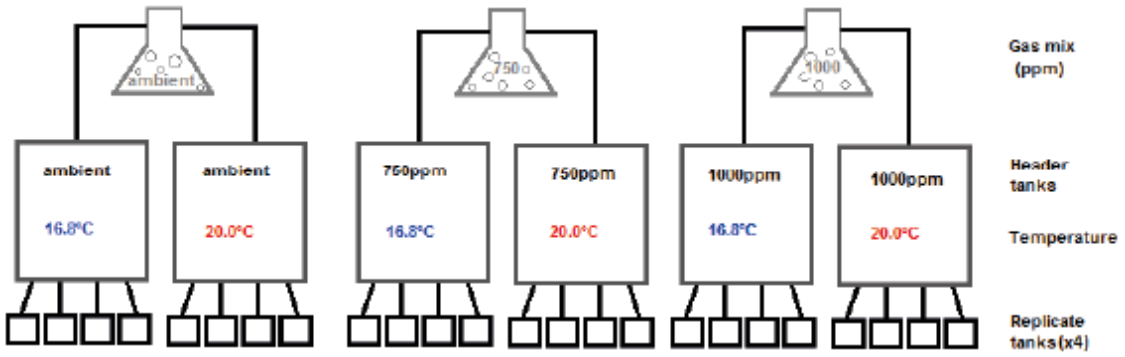

Figure 2.1: Experimental design used to maintain adult oysters under OAW scenarios. Each combination of pCO_2 and temperature had four replicates containing the oysters. ppm= part per million.

Experimental design and mesocosm set-up

The ocean acidification and warming system used during the experiment is a modified version of the one described by Calosi *et al.,* (2013). Briefly, each treatment consisted of a header tank (volume=80 L) of seawater, supplied from one of two sumps (16.5 °C and 20 °C), and aerated with either the ambient air pipe (pCO_2 ~400 ppm) or one of the two CO_2- enriched air pipes (pCO_2 ~750 ppm,

pCO_2~1000 ppm). Mixing in all header tanks was achieved using a submersible pump (Hydor Koralia Nano 900, Italy). CO_2 gas mix were obtained by slowly releasing CO_2 into two Buchner flasks where it mixed with ambient air, achieving two different levels of pCO_2, using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK) (Figure 2.1). As such, throughout the experiment the three CO_2 levels varied in a similar manner following natural variations in CO_2 in the ambient air. As such, the treatments took account of natural daily variability, which has been suggested as a critical consideration point for climate change experimental studies (Humphreys, 2016; Reum *et al.*, 2015). CO_2 levels in the two CO_2-enriched pipes were recorded using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA) and adjusted manually to the desired level twice daily. CO_2 levels in the ambient air pipe were also recorded to monitor for the control treatments. Seawater was gravity-fed from the header tanks to each of the four corresponding replicate tanks (3 L transparent sealed containers) at a constant rate of ~60 mL/min. The replicate tanks were held within four larger 300 L holding trays, each sump supplying seawater to two of the holding trays, effectively creating water baths maintaining the replicate tanks at the desired temperature. Each tray held two replicates of each CO_2 levels. 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 was added and replaced on a daily basis, and 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.

Measurement of seawater parameters

Temperature, salinity, and pH were measured daily in all replicate tanks (Figure 2.2, Table 2.1, see Appendix 1). 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 bottles 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 D *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) (Figure 2.2, Table 2.1).

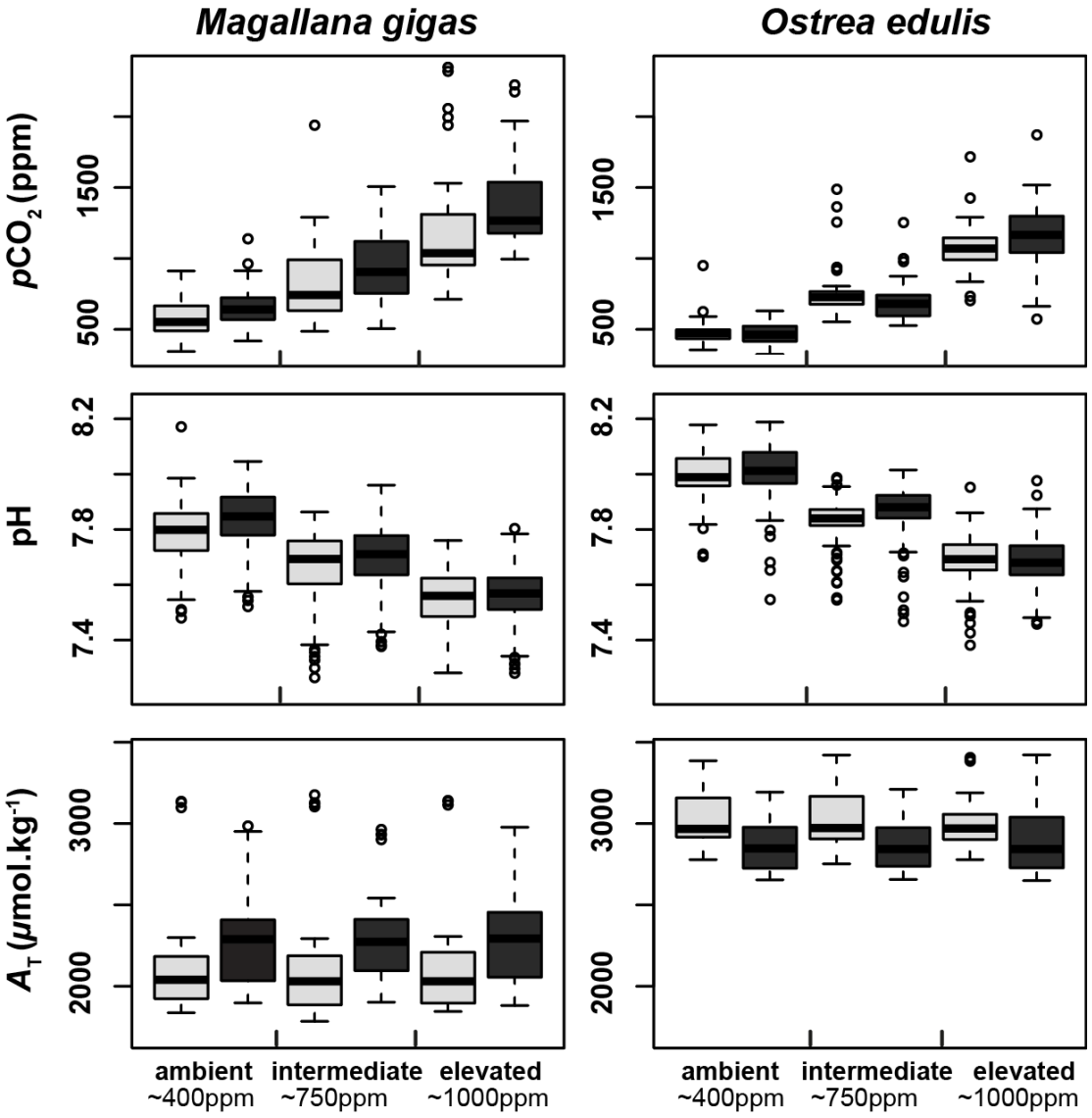

Figure 2.2: Variation in pCO_2, pH, and total alkalinity (A_T), of seawater over 12 weeks. ppm=part per million. Light grey= control temperature (~16.8°C), dark grey= warm temperature (~20.0°C).

Table 2.1: 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.

	Treatment (pCO_2 X Temperature)	T(°C)	S	Ω_a	Ω_c
Magallana gigas	Ambient X Control	16.9±0.2	33.9±1.1	1.70±0.32	2.64±0.50
	750ppm X Control	16.9±0.2	33.9±1.2	1.4±0.36	2.10±0.55
	1000ppm X Control	16.8±0.2	33.9±1.2	0.99±0.22	1.53±0.34
	Ambient X Elevated	20.4±0.3	34.3±1.2	2.02±0.31	3.11±0.47
	750ppm X Elevated	20.6±0.4	34.2±1.1	1.60±0.34	2.46±0.52
	1000ppm X Elevated	20.2±0.3	34.3±1.2	1.14±0.17	1.76±0.26
Ostrea edulis	Ambient X Control	16.5±0.3	34.2±0.8	3.70±0.65	5.75±1.01
	750ppm X Control	16.6±0.2	34.2±0.8	2.68±0.51	4.17±0.80
	1000ppm X Control	16.6±0.2	34.3±0.7	1.98±0.37	3.07±0.57
	Ambient X Elevated	19.8±0.3	34.4±0.9	3.80±0.65	5.85±1.01
	750ppm X Elevated	20.2±0.5	34.4±09	2.94±0.47	4.52±0.72
	1000ppm X Elevated	19.8±0.3	34.4±0.9	2.01±0.47	3.10±0.73

2.2.3 Condition index

The Condition Index (CI) of oysters was calculated at the end of the experiment using two methods to compare accuracy: dry weight, as used by Bodoy *et al.,* (1986) and Brown and Hartwick (1988), and wet weight, as used by (Knights, 2012). According to Bodoy *et al.,* (1986) CI based on dry weights are generally more accurate, however assessments based on wet weight would represent a quicker and cheaper method (Knights, 2012). Condition indices are useful tools widely used in the aquaculture sector to evaluate the overall physiological status and health of bivalves (Knights, 2012), and reflect their ability to withstand stress (Marin *et al.*, 2003).

$$CI1 = \frac{dry\ meat\ weight}{dry\ shell\ weight} \times 100 \quad [1]$$

$$CI2 = \frac{wet\ meat\ weight}{wet\ meat\ weight + dry\ shell\ weight} \times 100 \quad [2]$$

Wet and dried weight were determined after each oyster was shucked using an oyster knife. The wet tissue weight of each oyster was weighed using an electronic balance (Mettler AE240), after being

drained on a sieve and pat-dried to remove excess moisture. The dry weight of oysters was determined after being oven-dried at 105°C until a constant mass was achieved.

2.2.4 Physiological measurements

Following 10 days, 5, 9, and 12 weeks of exposure to each OAW scenario, metabolic activity and energy budget were measured. To limit post-prandial metabolism of food and excretion of faeces that could alter the results, oysters were not fed for 24h prior to measurements.

Standard metabolic rate

Respiration rates were measured as proxy for Standard Metabolic Rates (SMR), using microfiber optic oxygen sensors (PreSens Germany, www.presens.de). Oysters were placed in sealed 1.2 L containers, 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. Salinity was also recorded using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK). The seawater in each chamber was stirred using a magnetic rod (350 rpm). Respiration measurements started when the oyster resumed filtration, and ended either when O_2 saturation reached 80% to prevent the organisms from experiencing hypoxic conditions, or when the oyster shut its valves. O_2 measurements were corrected for temperature, salinity, and barometric pressure using Green and Carritt's (1967) oxygen solubility coefficients and Weiss' (1970) vapour pressure values, as well as corrected for the individuals' volume and dry weight, to obtain absolute quantities of oxygen consumed. Barometric pressure data were obtained from the Plymouth Live Weather Station (http://www.bearsbythesea.co.uk). SMR was calculated as follows:

$$SMR = \frac{V_r(L) \times \Delta C_w O_2 (mgO_2.L^{-1})}{\Delta t(h) \times bw(g)} \quad [2]$$

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

Clearance Rates (CR) were 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 random 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):

$$CR = \frac{V \times ln\left(\frac{C_{n-1}}{C_n}\right)}{t_n - t_{n-1}} \quad [3]$$

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.

2.2.5 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. The differences were considered significant if $p < 0.05$, unless stated otherwise. All data were analysed using the public domain package

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.

Condition index

The two condition indices were compared using Pearson's correlation to assess any apparent differences in condition estimation. CI1 and CI2 were plotted as independent variables, and a linear model was used to determine the correlation. ANOVA (single factor) was used to test the significance of the relationship. Differences in CI with treatment were analysed using 2-factor ANOVA with 'temperature' (levels: 'control'; 'elevated') and 'pCO_2' (levels: 'ambient ~400ppm', 'intermediate ~750ppm', 'elevated ~1000ppm') as fixed factors. If significant differences were present, *post-hoc* pairwise comparisons (Tukey HSD) were performed to determine differences between treatment levels.

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, 9, 12 weeks) 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, as well as between each of them and the CI using the Pearson's correlation test.

2.3 Results

2.3.1 Condition index

For both oyster species, the two condition indices were highly correlated (Figure 2.3), and CI1 only was used for further analysis and referred to as CI. Temperature, but not OA, had a significant effect

on the condition index of *M. gigas* (CI: $p<0.01$), with CI negatively impacted by elevated temperature (Figure 2.4). Mean CI decreased with increased temperature, from ~3.2 (±0.3) at ambient temperature to ~2.1 (±0.20) at elevated temperature. While the interaction between temperature and pCO_2 was not significant ($F_{2,18}=2.967$, $p=0.07$), there were marked reductions in CI under elevated temperature and elevated pCO_2 treatments (Figure 2.4). There was no effect of temperature or OA on the CI of *O. edulis* ($F_{2,17}=0.327$, $p>0.05$), which averaged at ~2.7 (±0.2) (Figure 2.4).

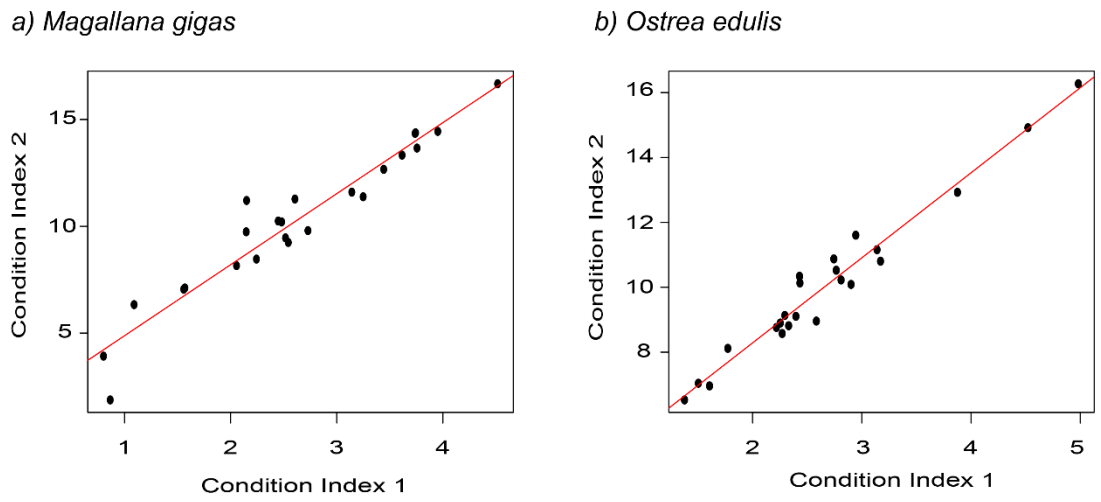

Figure 2.3: Correlation between the two condition indices for a) *M. gigas* and b) *O. edulis* fitted with their respective regression lines (linear model; *M. gigas*: $y=3.3282x+1.5442$; $R^2=0.92$ $p<0.001$ – *O. edulis*: $y=2.6204x+3.0448$; $R^2=0.96$; $p<0.001$).

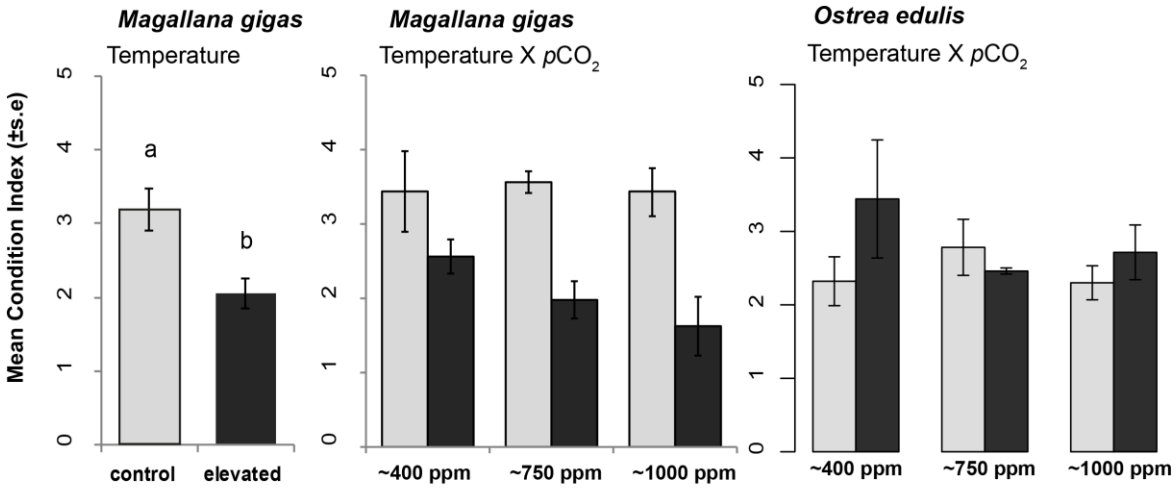

Figure 2.4: Variations in the condition index of *M. gigas* (left and middle) and *O. edulis* (right) across temperature and pCO_2 treatments after 12 weeks exposure. Light grey = control temperature. Dark grey= elevated temperature. *M. gigas*: n=4; *O. edulis*: n=4. Treatments that do not share a letter are significantly different.

2.3.2 Standard metabolic rate

The linear mixed-effects model revealed differences in metabolic response depending on species, exposure, and OAW scenario. For *M. gigas*, only temperature had a significant effect on SMR (Figure 2.5), whereas for *O. edulis*, only temperature and exposure has significant effects on SMR. However, it should be noted that the interaction between temperature and OA was only marginally not significant (p=0.052), and clear trends were apparent. Overall, elevated temperature led to a >43% increase in *M. gigas'* SMR, and a >39% increase in *O. edulis'* SMR. For both species, there was clear inter-individual variability in responses (Figure 2.6)

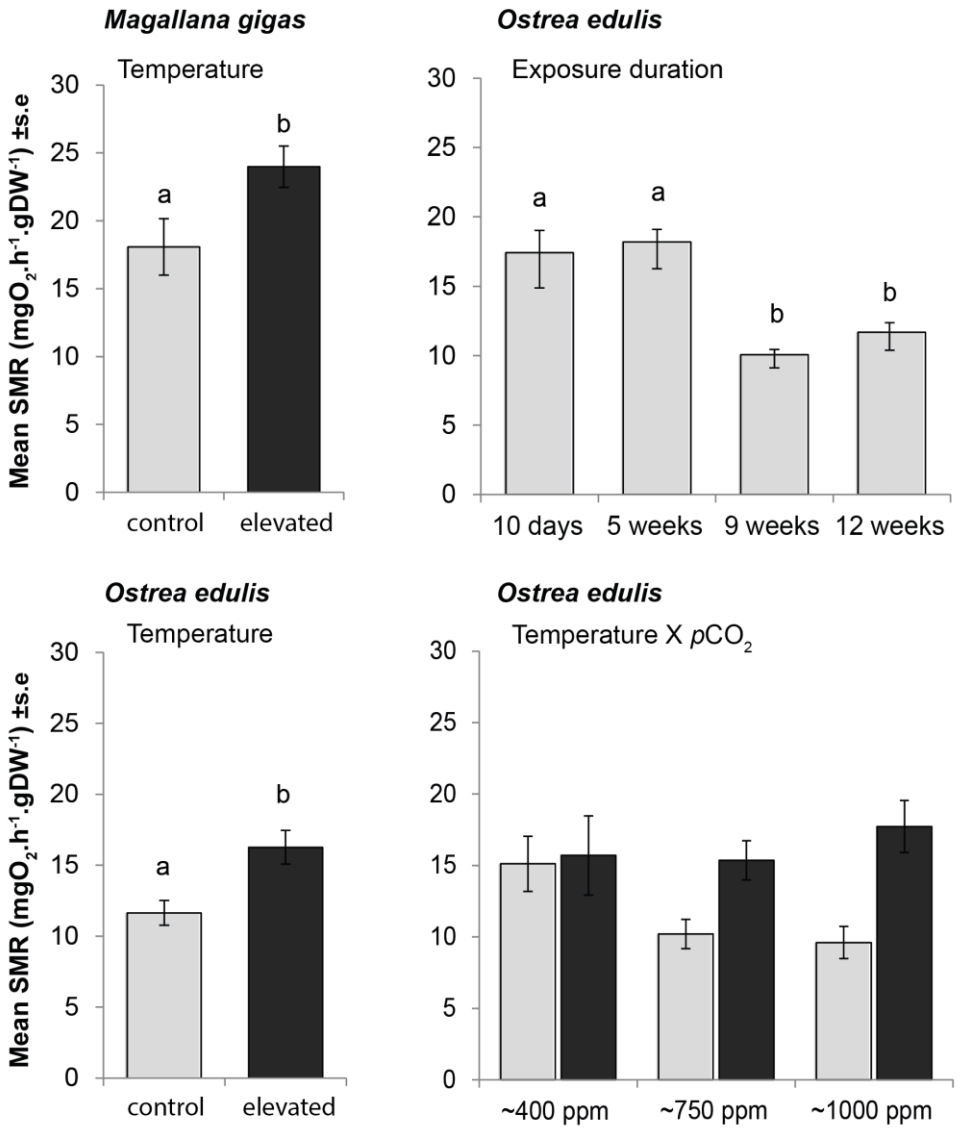

Figure 2.5: Changes in standard metabolic rates (SMR) of *M. gigas* (top left) with temperature; and of *O. edulis* with exposure duration (top right), temperature (bottom left) and the interaction of temperature and pCO_2 (bottom right). Light grey = control temperature. Dark grey = elevated temperature. DW = dry weight. Treatments that do not share a letter are significantly different.

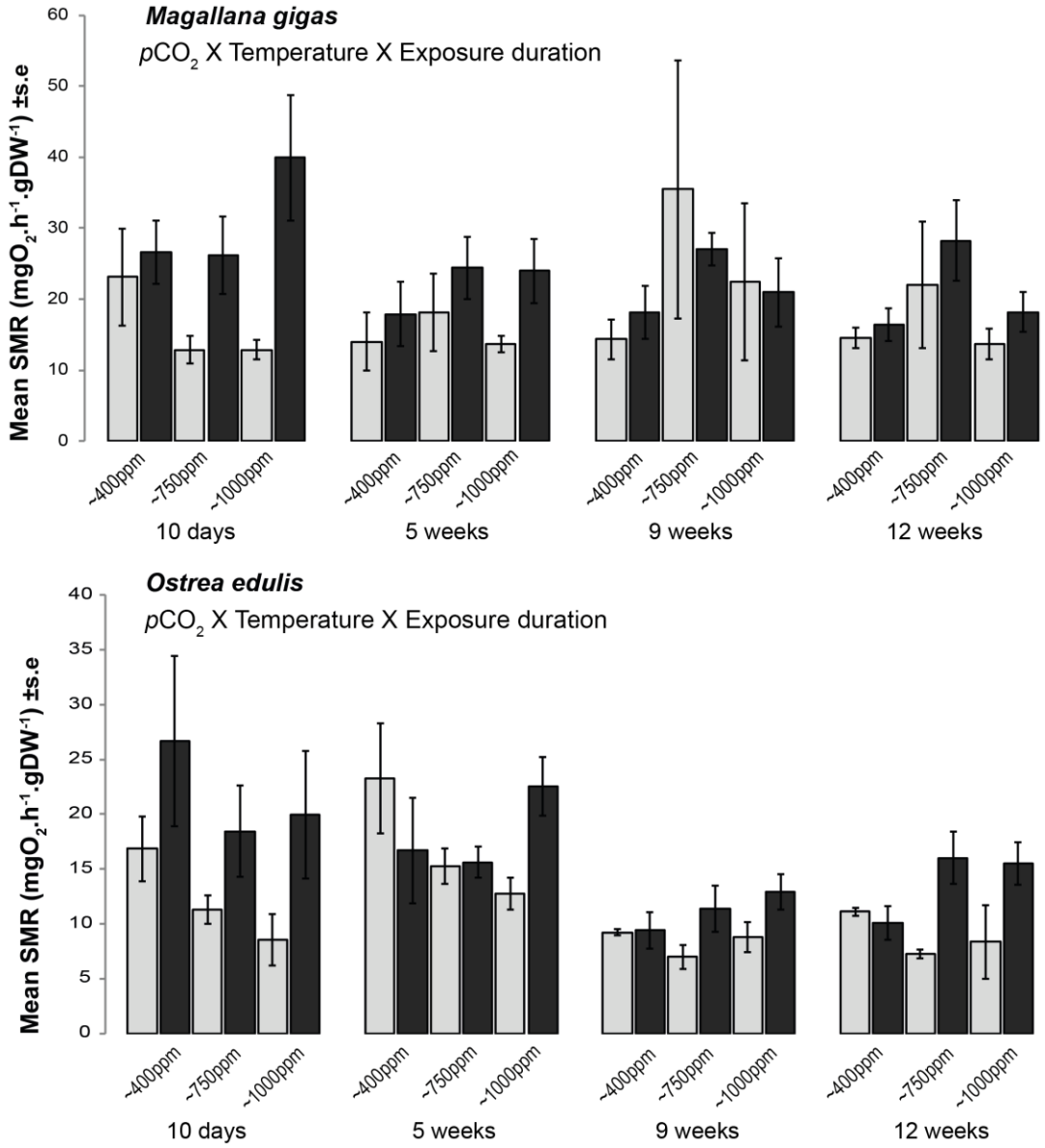

Figure 2.6: Standard metabolic rate (SMR) of *M. gigas* (top) and *O. edulis* (bottom) over 12 weeks exposure to temperature and pCO_2 combinations. Light grey = control temperature. Dark grey= elevated temperature. DW = dry weight.

2.3.3 Clearance rate

The linear mixed-effects model revealed differences in feeding response depending on species, exposure, and OAW scenario. For *M. gigas*, pCO_2 ($p<0.05$) and exposure time ($p<0.01$) had significant effects on CRmax (Figure 2.7), whereas for *O. edulis*, only the interaction of temperature and exposure time had a significant effect on CRmax ($p<0.01$). Overall, intermediate pCO_2 (~750ppm) led to a ~40% decrease in *M. gigas'* CRmax. Although not significantly different, there was clear evidence of changes in CRmax with temperature and pCO_2 for *M. gigas* (Figure 2.8). Responses were variable, and temperature led to increases in CRmax at ambient and intermediate

pCO_2 (~750ppm) by 90.9% and 88.7%, respectively, and decreases in CRmax at elevated pCO_2 (~1000ppm) by 16.0% (Table 2.2). For both species, there was clear inter-individual variability in responses (Figure 2.8.).

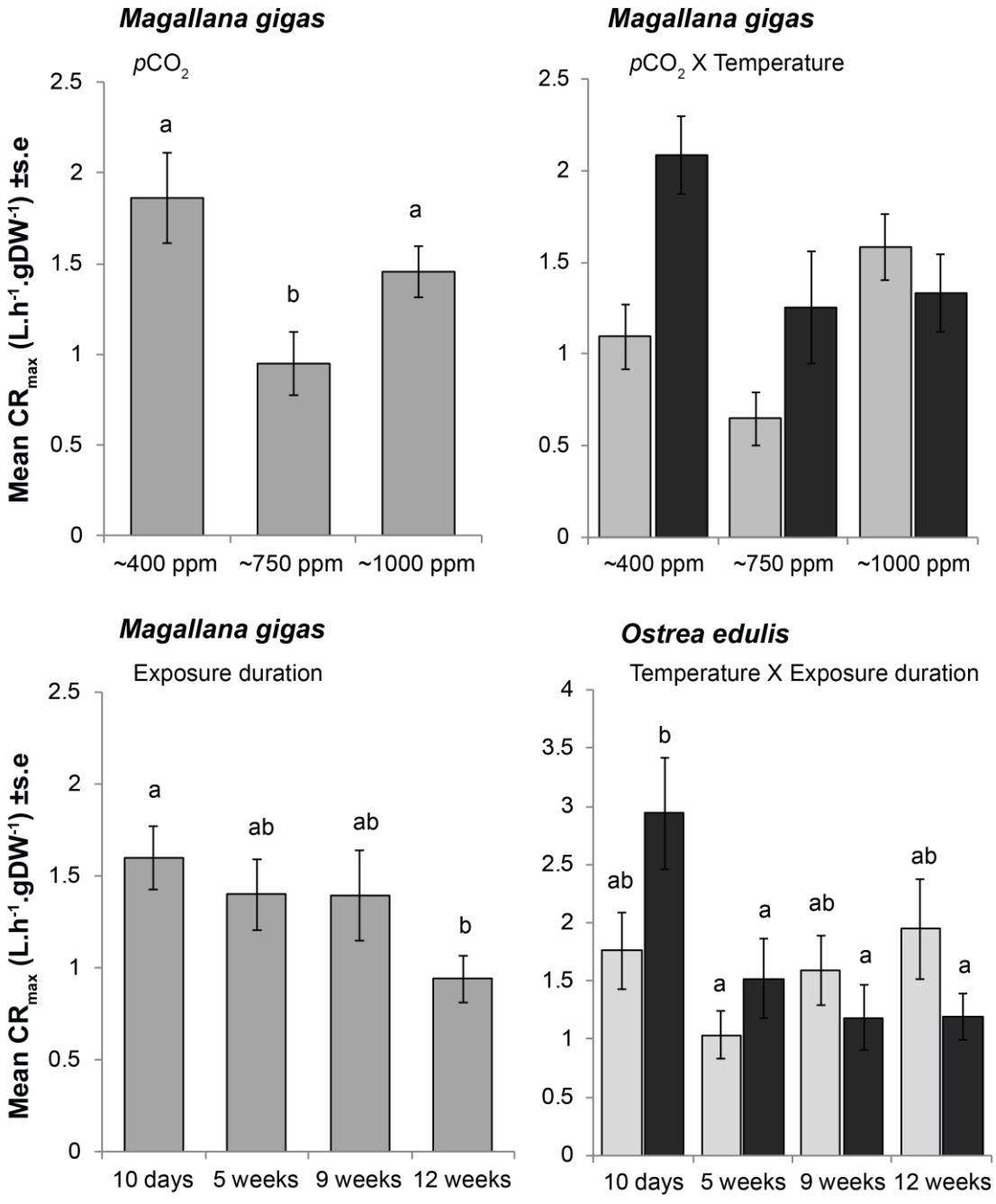

Figure 2.7: Changes in maximum clearance rate (CRmax) of: *M. gigas* with pCO_2 treatment (top left), the interaction of pCO_2 and temperature (top right), and exposure duration (bottom right); and *O. edulis* with exposure duration (bottom right). Light grey = control temperature. Dark grey = elevated temperature. Treatments that do not share a letter are significantly different. DW = dry weight.

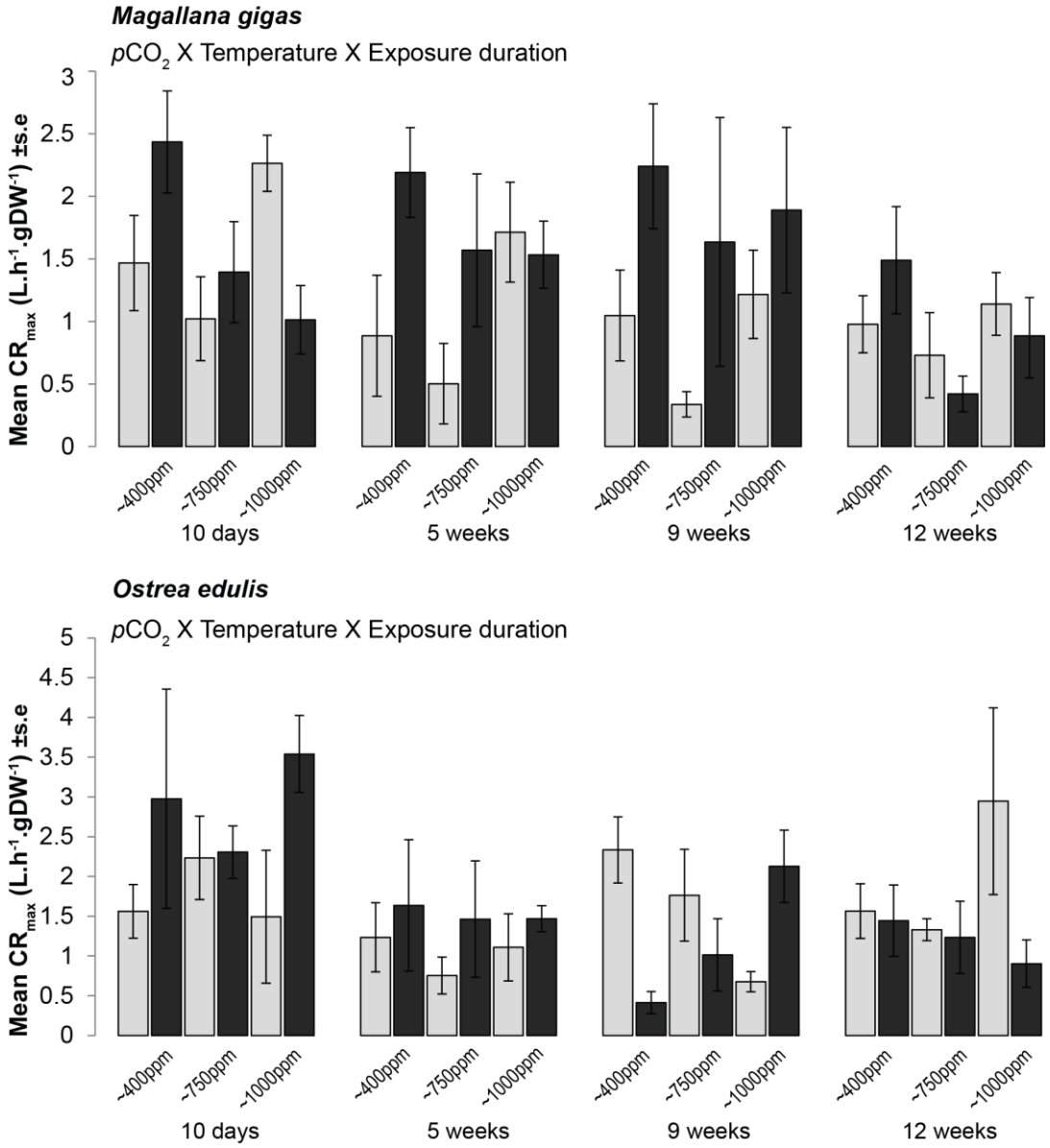

Figure 2.8: Maximum clearance rate (CRmax) of *M. gigas* (top) and *O. edulis* (bottom) over 12 weeks exposure to temperature and pCO_2 combinations. Light grey = control temperature. Dark grey= elevated temperature. DW = dry weight.

Table 2.2: Effects of pCO_2 and temperature treatments on the maximum clearance rate (CRmax) of *M. gigas* (as means ± s.d), and details of the biological response and effect size.

Treatment (pCO_2 X temperature)	Mean CRmax	Biological response	Effect size
Ambient X Control	1.09 ± 0.71	90.9 % increase	1.4
Ambient X Elevated	2.09 ± 0.85		
750ppm X Control	0.65 ± 0.59	88.7 % increase	0.98
750ppm X Elevated	1.26 ± 1.22		
1000ppm X Control	1.58 ± 0.73	16.0 % decrease	-0.35
1000ppm X Elevated	1.33 ± 0.85		

2.3.4 Relationship between the physiological traits

SMR and CI were significantly negatively correlated ($p<0.05$) albeit relatively weakly ($R^2 = 0.17$). For *M. gigas*, there was no correlation between CRmax and SMR, or between CRmax and CI (Figure 2.9). There was no correlation between any of the physiological traits for *O. edulis*.

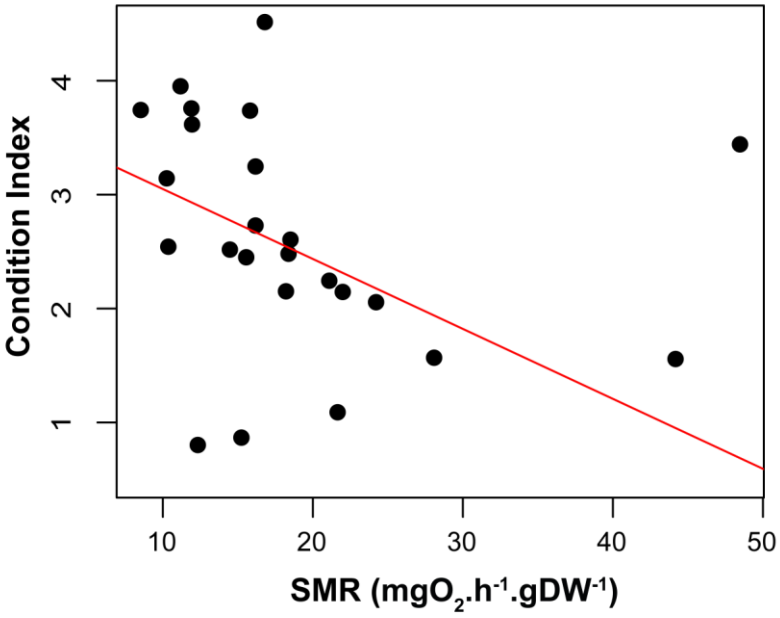

Figure 2.9: Relationship between the standard metabolic rate (SMR) and condition index of *M. gigas*. The two physiological traits are significantly correlated ($y=-0.06141x+3.66357$; $R^2=0.17$; $p<0.05$). DW = dry weight.

2.4 Discussion

2.4.1 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 the 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*. The change in the metabolic response of *O. edulis* between 5 and 9 weeks of exposure suggests an acclimation period to the experimental condition of over 5 weeks. Similarly to *M. gigas*, the metabolism of *O. edulis* was significantly increased by elevated temperature, with an increase of over 39%.

A positive effect of temperature on the metabolism of organisms is common amongst ectotherms; an effect previously shown in various species of oysters (Bougrier *et al.*, 1998; Saucedo *et al.*, 2004;

Shpigel *et al.*, 1992) and other bivalves (Artigaud *et al.*, 2014; Lardies *et al.*, 2017; Matoo *et al.*, 2013) as long as the temperatures lie within the thermal window of those organisms. Future warming of the ocean is expected to push species closer to or beyond their upper thermal limit with physiological and ecological consequences, particularly if a species already currently lives close to its upper thermal limit (Pörtner & Farrell, 2008). For instance, reduced respiration rates in *Mytilus coruscus* were found at the upper thermal limit of this species (Wang *et al.*, 2015). In the UK, *M. gigas* is living in the middle of its thermal range and this can explain its ability to increase its metabolic rates under elevated temperature. *O. edulis* could also increase its metabolic rate without signs of metabolic depression; this response appears to agree with the claims that the species can withstand warmer temperatures (Orton, 1940; Walne, 1958), and may not yet be close to its upper thermal limit in the UK. As such, future warming does not appear detrimental to *M. gigas* and *O. edulis* in the future as both species appear capable of coping with the added stress.

In this study, adult *M. gigas* and *O. edulis* displayed complex responses to variations in pCO_2 conditions, although none significantly changed their SMR, indicating that future acidification might not constitute stressful conditions for them. However, the metabolic response of bivalves to elevated pCO_2 appears species and population-specific. Several studies, in accordance with our results, reported no effect of reduced pH on the respiration rate of the eastern oysters *Crassostrea virginica* (Matoo *et al.*, 2013), Mediterranean mussels (Gazeau *et al.*, 2014; Range *et al.*, 2014), scallops *Pecten maximus* (Sanders *et al.*, 2013), and clams *Chamelea gallina* (Range *et al.*, 2014). However, increases in respiration rates were found for the mussel, *Mytilus edulis* (Beniash *et al.*, 2010; Thomsen & Melzner, 2010), and the common cockle *Cerastoderma edule* (Ong *et al.*, 2017), while reduced respiration rates were observed in *Mytlius galloprovincialis* (Michaelidis *et al.*, 2005), *Ruditapes decussatus* (Fernández-Reiriz *et al.*, 2011; Range *et al.*, 2014), and *Chlamys nobilis* (Liu & He, 2012).

Several studies have demonstrated clear interactive effects of pCO_2 and temperature on metabolism (e.g. Lannig *et al.*, 2010) and is reinforced in this study. Although the overall interaction between temperature and pCO_2 was not significant, there was a significant difference in the metabolic rate of *O. edulis* between the two temperature treatments at ~1000ppm, showing an increase in metabolic

rates at elevated pCO_2 (~1000ppm) and elevated temperature conditions. The absence of statistical significance may be explained by a lack of power from the low number of replicates (n=4 might be insufficient). Increases in metabolic rates are an energetically expensive response aimed at coping with stressful conditions, and suggest a higher energy demand necessary for the maintenance, active metabolism, and overall survival of oysters.

2.4.2 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. In our study, the clearance rate of *M. gigas* followed an increasing trend under elevated temperature, particularly at ambient and intermediate pCO_2 (Figure 2.8, Table 2.2), suggesting a mechanism working towards enhanced food acquisition and energy supply. Increased clearance rate with temperature was also observed in *O. edulis*, but only after 10 days of exposure, following which clearance rates returned to levels similar to the control, which suggests a shock response after being introduced to the experimental conditions rather than a longer-term response to the treatment. Elevated temperature has previously been shown to increase the clearance rate of several species of mollusc, such as *Ostrea edulis* (Haure *et al.*, 1995, and references therein), *Crassostrea virginica*, *Mytilus edulis* (Comeau *et al.*, 2008), *Mytilus galloprovinciallis* (Kroeker *et al.*, 2014), *Mytilus chilensis* (Navarro *et al.*, 2016), *Argopecten purpuratus* (Lardies *et al.*, 2017), and *Cerastoderma edule* (Ong *et al.*, 2017). In contrast, decreased clearance rates have been shown in one species of mussel (Wang *et al.*, 2015), indicating variation in the response of species to OAW scenarios.

This study also showed a significant negative effect of pCO_2 on the clearance rate of *M. gigas*, particularly at intermediate pCO_2 (~750ppm) with a decrease by ~40%, not observed in *O. edulis*. These results reinforce the idea that responses to acidification conditions are species-specific. The effect of elevated pCO_2 on the feeding behaviour and clearance rate of filter-feeders has not been widely researched, despite being recognised as a potential key physiological trait governing 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 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* decreased their clearance rates by up to 70% under elevated pCO_2 (Vargas *et al.*, 2015). Similarly, juveniles of the clam *Ruditapes decussatus* displayed reduced clearance and ingestion rates under decreased pH (Fernández-Reiriz *et al.*, 2011; Range *et al.*, 2014). Elevated pCO_2 also led to reduced clearance rate and absorption efficiency in *Mytilus chilensis* (Navarro *et al.*, 2016) and in *Cerastoderma edule* (Ong *et al.*, 2017), and to a weak decrease in feeding rates in *Mytilus galloprovinciallis* (Kroeker *et al.*, 2014). In accordance with our results for *O. edulis,* no marked effects of elevated pCO_2 on clearance rate were recorded in *Mytilus coruscus* (Wang *et al.*, 2015), *Pecten maximus* (Sanders *et al.*, 2013), and *Austrocochlea constricta* (Leung *et al.*, 2017a).

Impairment of filtration and feeding can negatively affect the energy supply and metabolic maintenance of adult oysters, and can prevent organisms from resisting ocean acidification or compensating for its effects. Subsequent starvation would lead to an increase in mortalities within the populations.

2.4.3 Condition index

Condition indices (CI) are recognised as useful tools to evaluate the overall physiological status and health of bivalves (Knights, 2012), and reflect their ability to withstand stress (Marin *et al.*, 2003). Stressful environmental conditions requiring significant energetic expenditure result in low CI in bivalves (Orban *et al.*, 2002). The CI of *M. gigas* was negatively impacted by elevated temperature but not elevated pCO_2, an effect also seen for the mussel *M. edulis* (Mackenzie *et al.*, 2014). Our results are in contrast to those of Lannig *et al.*, (2010) on *M. gigas* who recorded a decrease of ~20% in CI between control and treatment specimens. Similar decreases in CI with elevated temperature were recorded in several other bivalves (Gabbott & Walker, 1971; Hiebenthal *et al.*, 2012; Shpigel *et al.*,

1992). Declines in CI usually suggest depletion of energy reserves, leading to reduced growth efficiency, and are often associated with stressful conditions (Lannig *et al.*, 2010). Bivalves have the capacity to reallocate energy reserves to sustain routine maintenance by reabsorbing tissues and gonads, resulting in a reduced CI. In contrast with *M. gigas*, the CI of *O. edulis* was unaffected by any of the treatment conditions, suggesting that they did not experience environmental stress unlike *M. gigas*.

2.4.4 Relationships between the physiological traits

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. Contrary to predictions, no relationships between respiration and feeding rates were found in this study. Therefore, the higher metabolic costs from increased respiration rates under future OAW conditions were not compensated for by added energy through enhanced feeding. On the contrary, decreased feeding was recorded ~750ppm pCO_2 for *M. gigas*.

While no relationship was found between the condition index and the feeding rate in either species, the condition index of *M. gigas* was negatively correlated with metabolic rate. As reduced condition index is associated with depletion of energetic reserves, this suggests that the costs associated with increased metabolism were met by a reallocation of reserves from somatic and gonadal tissues to sustain maintenance and survival. While no mortality of *M. gigas* occurred during the experiment, the lack of acclimation in respiration and clearance rates responses after 12 weeks exposure suggests that, if left uncompensated, the added metabolic costs could compromise survival once soft tissue reserves are depleted.

No relationships between these physiological traits were found for *O. edulis*. A potential explanation for the maintenance of its CI despite increased metabolic rates with elevated temperature is that its sustained clearance rates allowed sufficient energy supply to compensate for the additional metabolic

costs. Nevertheless, exposure beyond the 12 week period of this study might produce *O. edulis* displaying lowered CI from accumulated uncompensated energetic costs.

2.5 Conclusion

This study has shown that two important physiological traits of oysters are affected by warming and acidification, but that the responses are species-specific. Due to logistic limitations inherent to the OAW system used during the experiment, the sample size for each species was limited to n=4 per treatment and as such, there was high variability in the responses recorded, which led to lack of statistical power for the analysis. Yet despite this, there were clear biological effects apparent (see discussion in section 6.5.2). If anthropogenic CO_2 emissions continue to rise, oysters may experience a higher cost of living due to the effect of elevated temperature on their metabolism. *Magallana gigas* in particular may find it difficult to meet these costs as it decreased its feeding activity at ~750 ppm pCO_2 levels. Non-native and invasive species are often more resilient to environmental fluctuations and stress, yet in oysters sampled from Plymouth populations, *M. gigas* was more negatively impacted by future OAW scenarios than its native counterpart, *O. edulis*. The invasive oysters had elevated metabolism, reduced feeding, and decreased condition and could not cope as well as *O. edulis* with the warming and acidification conditions. Krassoi *et al.* (2008) demonstrated that differences exist with respect to abiotic environmental tolerances of extreme physical conditions between exotic and native oyster species, with the native species able to withstand harsher environmental conditions. Due to poorer performance and condition of oysters, warming and acidification may threaten the maintenance and functioning of *M. gigas* populations, degrading the provision of ecosystem services such as erosion control, raw material supply, and fisheries and aquaculture. This is especially important in the UK where *M. gigas* constitutes 90% of the oyster aquaculture production, worth an estimated £10.14 million annually (Humphreys *et al.*, 2014). Reduced clearance rates of *M. gigas* under OAW may also have important ecological impacts by limiting their ability to improve water quality. Similar concerns have been expressed regarding the fate of waste bioremediation service by mussels under future ocean acidification, as their filtration rates might be negatively impacted (Broszeit *et al.*, 2015). Oyster beds consisting in majority of *M. gigas* might see their surrounding water quality diminish, with negative consequences for further associated ecosystem services. It appears that under

future OAW, *O. edulis* will be able to continue to deliver its important bio-filtration service, and consequently the provision of improved water quality will remain secure.

Admittedly, submerging intertidal organisms for the duration of the study and exposing them to static conditions may not have accurately reflected what they could experience in situ. However, recent work by Mangan *et al.*, (2017) suggests that responses to static conditions may actually be underestimated, when compared to responses following exposure to fluctuating conditions (see discussion in section 6.5.1). Therefore, these findings do hold biological and ecological significance, in terms of species ecological status, population conservation, and management measures. Further efforts to promote the restoration of native oyster 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.

Chapter 3: Differential responses in anti-predation traits of the native oyster *Ostrea edulis* and invasive *Magallana gigas* to ocean acidification and warming.

"To kill two birds with one stone"

A version of this chapter is currently in preparation as:

Lemasson, A.J. and A.M. Knights. Differential responses in anti-predation traits of two oyster species to ocean acidification and warming, for publication in a marine ecology and ecosystem research journal, such as *Marine Ecology Progress Series*.

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

Abstract:

Ocean acidification and warming (OAW) poses a threat to marine organisms, with particular negative effects on molluscs, and can jeopardize the provision of marine ecosystem services (MES). As predation is an important factor shaping populations in the marine environment, the ability of organisms to retain traits valuable in predation resistance under OAW may be decisive for population maintenance and MES provision in the future. Here, we examine how exposure to temperature (16.8°C, 20.0°C) and pCO_2 (ambient, ~750 ppm, ~1000 ppm) conditions affects traits linked to predation resistance (i.e. adductor muscle strength and shell strength) in two ecologically and economically important species of oysters – *Magallana gigas* and *Ostrea edulis* – and relate them to changes in physiology (Condition Index, muscle and shell metrics). We show that *O. edulis* remained unimpacted following exposure to OAW scenarios. In contrast, the adductor muscle of *M. gigas* was 52% stronger under elevated temperature and ~750 ppm pCO_2, and its shell 44% weaker under elevated temperature and ~1000 ppm pCO_2, suggesting changes to its susceptibility to predators. For both *Magallana gigas* and *Ostrea edulis*, individuals with more somatic tissue held an ecological advantage when it came to predator resistance, and consequently smaller oysters may be favoured by predators under OAW. Overall, by affecting fitness and predation resistance, OAW is expected to induce shifts in predator-prey interactions and reshape assemblage structure due to species- and size- selections, which may consequently modify reef functioning. Changes to oyster reef functioning could have implications for the provision of associated ecosystem services.

3.1 Introduction

Sustainable populations of marine organisms are key for the continued provision of marine ecosystem services, and importantly food biosecurity. However, food biosecurity appears threatened by environmental stress and future climate change (Ekstrom *et al.*, 2015; Kibria *et al.*, 2017; Lloret *et al.*, 2016), with studies of important commercial fisheries such as cod (Koenigstein *et al.*, 2017), scallops (Cooley *et al.*, 2015; Richards *et al.*, 2015b), and prawns (Richards *et al.*, 2015b), displaying reductions

in recruitment success, altered growth, and declines in harvests under ocean acidification and warming scenarios (OAW thereafter). Molluscs, which constituted in excess of 15% of aquaculture production in 2015 (FAO data[1]), are of particular concern under future OAW scenarios (Cooley *et al.*, 2012; Gazeau *et al.*, 2013; Parker *et al.*, 2013).

It is well established that OAW can alter a multitude of aspects related to the fitness of molluscs, from changes to their physiology and shell properties, and altered energy budgets (Spalding *et al.*, 2017). Increased respiration rates (Ong *et al.*, 2017, Lemasson et al., in review/see Chapter 2), decreased feeding rates (Vargas *et al.*, 2015), and reduced condition (Ong *et al.*, 2017, Lemasson et al., in review/see Chapter 2) are also evident and indicative of stress. Important calcification and mineralogical processes may also undergo crucial changes in functioning (Duquette *et al.*, 2017; Fitzer *et al.*, 2016; Leung *et al.*, 2017a), and as a consequence, shells can be less dense (Chatzinikolaou *et al.*, 2016), lighter (Lagos *et al.*, 2016), thinner (Lagos *et al.*, 2016), and weaker (Coleman *et al.*, 2014; Duquette *et al.*, 2017; Speights *et al.*, 2017). It is predicted that these fitness consequences for individuals will have important implications for the stability and resilience of impacted populations as a result of changes in the strength of population-regulating mechanisms and community interactions, particularly in the case of ecosystem engineers (Gribben *et al.*, 2009; Gribben *et al.*, 2013; McCann, 2000).

Predation is a well-recognised top-down driver of population dynamics in the marine environment (Myers *et al.*, 2007; Peckarsky *et al.*, 2008), the strength of which can change when exposed to multiple environmental stressors such as OAW (Dixson *et al.*, 2010; Jellison *et al.*, 2016). The extent to which changes occur and their effect on population and community dynamics are not well understood, but a number of negative consequences of OAW have been shown. These include changes from avoidance to attraction to predator olfactory cues by prey (reviewed in Ashur *et al.*, 2017; Dixson *et al.*, 2010), altered predator consumption rates (Harvey & Moore, 2017; Sampaio *et al.*, 2017; Sanford *et al.*, 2014; Wright *et al.*, 2014), the induction of important non-consumptive effects on prey (Lord *et al.*, 2017) or a reduction in prey shell strength (Landes & Zimmer, 2012). Individually or collectively,

[1] http://www.fao.org/figis/

these changes point toward significant alterations to community dynamics and highlight a particular concern that assessing the physiological effects of OAW on a target species (e.g. a commercially-valuable prey species), may alone be insufficient to accurately predict sustainability outcomes for the future.

There are a number of predators of commercially important bivalves with wide range of predation strategies. Mechanical approaches are common: for instance, gastropods drill holes into the shell, starfish pry open the two valves and tear the muscles responsible for valve closure (Lavoie, 1956; Reimer & Tedengren, 1996), crabs and other durophagous predators crush the shell (Elner, 1978; Menzel & Nichy, 1958 - although some species of crabs such as *Carcinus maenas* can also pry the valves open (Sanchez-Salazar *et al.,* 1987)), and some birds are known to also target the hinge. The ability of individuals to resist predation is crucial for survival and long-term population maintenance (Knights *et al.*, 2012). Bivalve resistance to predation is closely linked to their ability to build robust protective shells (therefore linked to shell metrics such as size, thickness, strength) as well as strong adductor muscle to control their gaping behaviour, affecting predator handling time (Beadman *et al.*, 2003; Bishop & Peterson, 2006; Boulding, 1984; Reimer & Tedengren, 1996; Soledad López *et al.*, 2010).

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

Here, I tested the effects of OAW on aspects of oysters' physiology and morphology linked with predation resistance, comparing a native (*Ostrea edulis*) and a non-native (*Magallana gigas*) species of oyster. Both species are of high economic value in the UK (Herbert *et al.*, 2012), and the basis of a major shellfish fishery value at ~US$6.5 million in 2015 (FAO data[1)]. Changes in adductor muscle strength and shell fracture strength were evaluated under simulated warming and acidification scenarios over a 12-week period, and linked to changes in physiological (Condition Indices, tissue weight) and morphological (muscle diameter and area, shell density, shell thickness, shell weight) traits. I had three hypotheses: 1) OAW scenarios would negatively impact on the physiology and morphological properties of oysters valuable to predation resistance (Condition Index, muscle diameter and area, tissue weight, shell density and thickness), and would in turn affect the 2) adductor muscle strength, and 3) shell fracture strength of oysters, linked to two distinct mechanical predation strategies (prying and crushing, respectively).

3.2 Methods

3.2.1 Adult collection

Adult Pacific oysters (*Magallana gigas*: 79.5 ± 5.9 mm in length and weighing 45.1.9 ± 8.5 g) and European flat oysters (*Ostrea edulis:*70.3 ± 5.5 mm in length and weighing 41.4 ± 19.5 g) were wild-collected from the same low-intertidal site in Plymouth Sound as described in Chapter 2 in August and November 2016, respectively. Organisms were brought back to the *Marine Biology and Ecology Research Centre* at Plymouth University within one hour of collection and kept in large stock tanks (200 L) at ambient laboratory conditions of ~16.5°C and atmospheric pressure of ~400 ppm pCO_2 for a holding period of two-weeks, during which oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

3.2.2 Experimental design and set-up, treatments, and measurements of seawater parameters

OAW experimental treatments

Effects of OAW scenarios were tested on oyster species separately. Three levels of pCO_2 (ambient, ~750 ppm, ~1000 ppm), and two temperatures (control 16.8 °C, warm 20 °C), were tested in an orthogonal experimental design to simulate current and future OAW scenarios predicted for the UK.

Ninety-six 3 L experimental tanks (eight per OAW scenario) were set-up for the experiment (Figure 3.1). Individual oysters were placed in each tank and exposed to the treatment conditions for 12 weeks. Throughout the duration of the experiment, oysters were fed daily with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture) to obtain a concentration of approximately 10^8 cell.L^{-1} within the experimental tank. Three times a week, tanks were gently brushed and siphoned, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater.

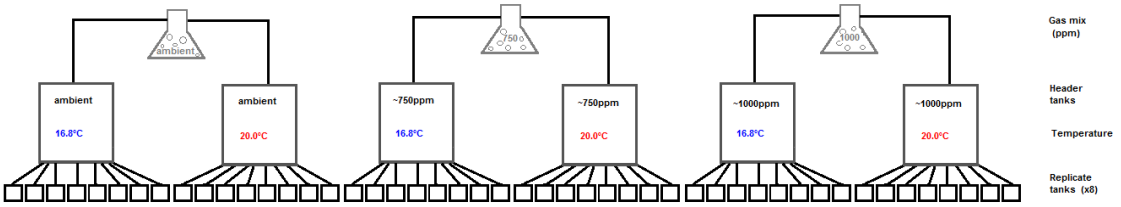

Figure 3.1: Experimental design used to maintain adult oysters under OAW scenarios. Eight replicate tanks per OAW scenarios, each containing an individual oyster. ppm= part per million.

Experimental design and mesocosm set-up

The experimental design and mesocosm set-up is as described in Chapter 2.

Measurements of seawater parameters

Measurements of seawater parameters followed the same procedures as described in Chapter 2, with the exception of Total Alkalinity (A_T). Total alkalinity (A_T) measurements were conducted once weekly on 125mL water samples in triplicate for each treatment directly from the header tanks. Prior validation was performed to ensure that the replicate tanks had consistently the same A_T as each other and as the header tank. Water samples were directly analysed for A_T within 15min of being sampled by automatic Gran titration (Titralab AT1000 © Hach Company). Partial pressure of carbon dioxide (pCO_2) and saturation states of calcite and aragonite (Ωca and Ωar) were calculated as in Chapter 2 (see Table 3.1 and Appendix 2).

Table 3.1: Seawater chemistry for a) *Magallana gigas* and b) *Ostrea edulis*. Data shown are means (±sd) values. Ppm= parts per milliom. ΩCa= saturation state of calcite. ΩAr= saturation state of aragonite. SW= seawater. A_T= Total Alkalinity.

a)

Treatment (pCO_2 X Temperature)	Salinity	Temperature (°C)	A_T (μmol/kg-SW)	pH	pCO_2	ΩCa	ΩAr
Ambient X Control	33.8±1.8	16.9±0.3	2680.4±446.0	7.89±0.09	587.1±116.0	4.0±1.0	2.6±0.6
750 ppm X Control	33.8±1.8	16.9±0.3	2659.7±448.2	7.78±0.07	797.6±157.5	3.1±0.6	2.0±0.4
1000 ppm X Control	33.8±1.7	16.8±0.3	2647.5±467.0	7.65±0.07	1127.1±189.4	2.3±0.6	1.5±0.4
Ambient X Warm	34.7±1.7	20.2±0.4	2848.0±508.9	7.94±0.06	628.9±93.8	4.8±1.3	3.1±0.8
750 ppm X Warm	34.7±1.7	20.6±0.6	2860.0±515.7	7.84±0.06	834.3±137.9	4.1±1.0	2.7±0.6
1000 ppm X Warm	34.6±1.8	20.2±0.4	2850.4±528.1	7.73±0.08	1176.0±129.3	3.0±1.0	2.0±0.7

b)

Treatment (pCO_2 X Temperature)	Salinity	Temperature (°C)	A_T (μmol/kg-SW)	pH	pCO_2	ΩCa	ΩAr
Ambient X Control	33.0±1.8	16.7±0.6	1683.1±144.7	7.96±0.05	492.4±68.7	2.0±0.3	1.3±0.2
750 ppm X Control	33.1±1.6	16.7±0.6	1670.3±150.7	7.86±0.07	626.7±79.3	1.6±0.3	1.0±0.2
1000 ppm X Control	33.3±1.7	16.7±0.6	1665.3±146.7	7.77±0.10	818.5±140.4	1.3±0.4	0.8±0.2
Ambient X Warm	33.6±2.2	19.8±0.8	1978.3±154.7	8.04±0.08	464.8±59.3	3.1±0.6	2.0±0.4
750 ppm X Warm	33.4±2.1	20.3±1.2	1981.4±140.3	7.92±0.06	731.2±84.4	2.3±0.4	1.5±0.3
1000 ppm X Warm	33.5±2.1	19.8±0.9	1979.6±143.3	7.84±0.06	864.3±104.6	1.9±0.3	1.2±0.2

3.2.3 Adductor muscle and shell strengths

At the end of the exposure duration, each oyster were removed from the experimental system, dried, scrubbed of epibionts, and a stainless steel hook glued a few centimetres from the edge of the right valve with EPAFD low viscosity epoxy resin mixed with rapid hardener (Reactive Resins, EP Resins Limited). Their left valves were glued with the same epoxy resin/hardener combination onto a 150 X 150mm metal plate. Adductor muscle strength was determined by applying a vertical pulling (extensive) force to the right valve, using a force transducer (Instron Testing System, Instron, USA) connected to the hook of the oyster (Figure 3.2a). The metal plate glued to the lower valve of the oyster was securely held in place onto the base of the force transducer using clamps, to obtain an immobile base and allow accurate measurement of the vertical force exerted. The force profile for each oyster was recorded (Figure 3.2b), along with corresponding visual observations. Three data-points were extracted from the force profile: 1) the initial resisting force of the oyster against the pulling pressure, defined as the initial slope of the force curve from onset of the pulling force to the first opening of the valves; 2) the force necessary for the valves to start opening (Point 2); 3) the maximum force applied before the onset of muscle rupture (Point 3) (Figure 3.2b).

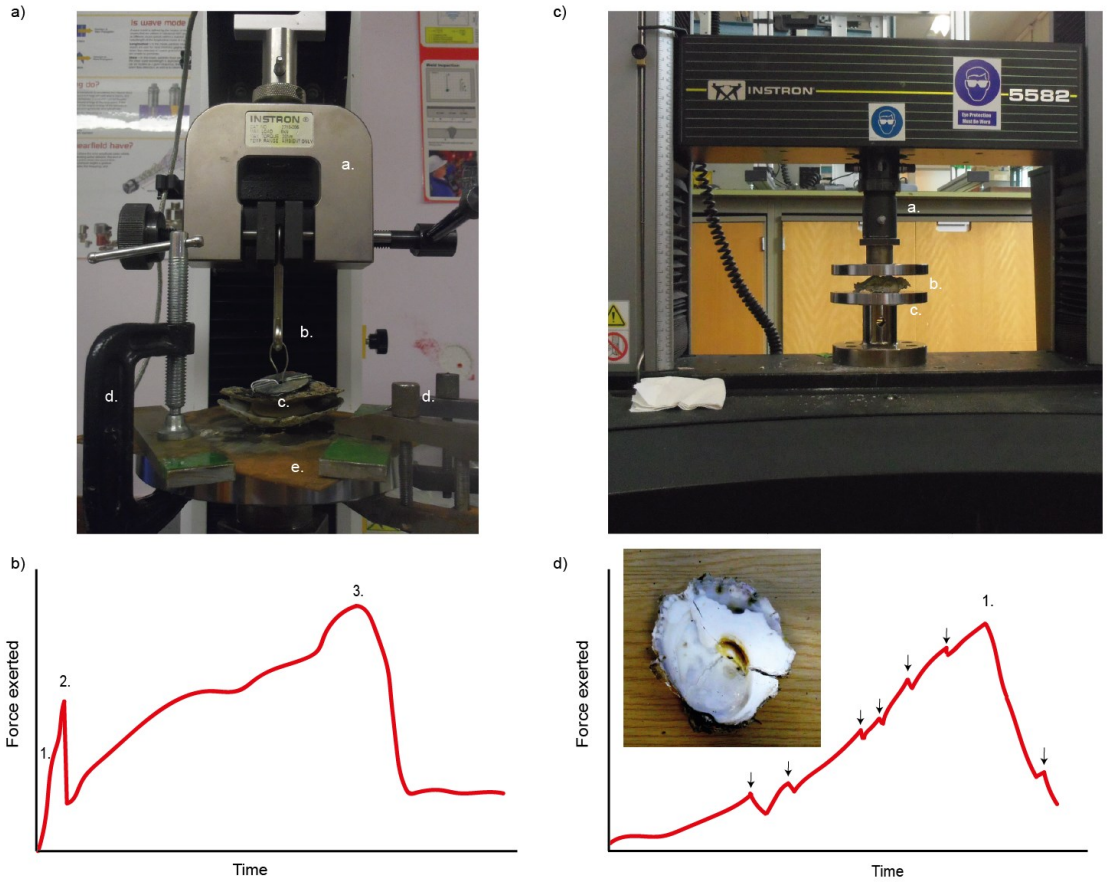

Figure 3.2: a) Experimental set-up of oysters' adductor muscle strength assessment. a. Instron cell load. b. hook glued to the c. oyster. d. clamps. e. metal plate. b) Typical curve for the extensive force exerted on the adductor muscle of oysters. 1. Initial resisting force against the pulling pressure (corresponding to the slope of the curve from onset of pulling force to the first opening of the valve (data point 2). 2. Force necessary for the valves to start opening. 3. Maximum force applied before the onset of muscle rupture. c) Experimental set-up of oysters' shell strength assessment. a. Instron cell load. b. oyster. c. fixed metal base. d) Typical curve for the compressive force exerted on the shell of oysters. Arrows: Minor cracks which did not lead to shell fracture. 1. Major crack which led to shell fracture (as per small picture top left)).

Following the adductor muscle strength assessment, the shell strength of the left valve of each individual oyster was determined using a vertical compressive force applied to the shell using a force transducer (Instron Testing System, Instron, USA). The left valve of each oyster shell was placed directly underneath the cell load (Figure 3.2c) and the force profile for each oyster recorded, along with corresponding visual observations. For each oyster, the force required to break the valve in half was recorded (Figures 3.2d, data point 1).

3.2.4 Morphometrics

Following the test for adductor muscle strength, the fresh tissue weight and the shell length of each oyster were measured using a digital scale (Fisher Scientific Precision series PP5413, precision 0.001g) and digital callipers (Mitutoya, Japan, precision 0.01mm), respectively. The inside of each valves was then photographed using a digital camera (Pentax OptioLS465) and the diameter and area of the adductor muscle was calculated using image analysis software (ImageJ) (Figure 3.3). Shell and soft tissue were then dried at 105°C for 24h or until a constant mass was achieved. Condition Indices were calculated using the following equation after Knights (2012):

$$CI = \frac{dry\ tissue\ weight}{dry\ shell\ weight} \times 100 \text{ [1]}$$

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

$$d = w \times \frac{d_{liq}}{w - y} \text{ [2]}$$

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

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

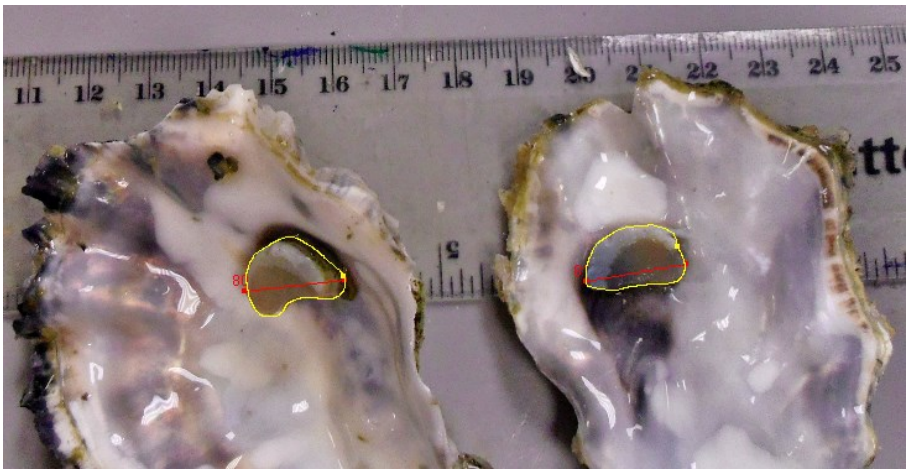

Figure 3.3: Diameter (red) and area (yellow) of the adductor muscle of *Magallana gigas* measured using ImageJ software.

3.2.5 Statistical analyses

The differences were considered significant if $p < 0.05$. All data were analysed using the public domain package *R* [version 3.3.1, (R Core Team, 2016)].

Morphometrics

Differences in muscle diameter and area, condition index, and shell thickness between treatments were assessed using two-way Analysis of Variance (ANOVA), with "pCO_2" and "Temperature" as fixed factors, after checking for homogeneity of variances. Differences in shell density were assessed using a three-factor ANOVA, with "pCO_2", "Temperature", and "Valve" (left of right) as fixed factors, after checking for homogeneity of variances. If variances were not homogeneous, log-transformation were applied. Where significant differences were identified, *post-hoc* Tukey test was performed to determine which treatment levels differed. Muscle diameter and area were plotted as independent variables, and a linear regression was used to determine the relationship. ANOVA (single factor) was used to test the significance of the relationship.

Adductor muscle and shell strengths

Data were analysed using three factor Analysis of Covariance (ANCOVA), with "pCO_2" and "Temperature" as fixed factors. The covariate was selected by comparing multiple models, with a different biometric for each model, using residual sum of squares. After determination of which biometric was the best suite predictor for each of the response variable, tissue dry mass (g) was selected as the continuous covariate in the model analysing the adductor muscle strength, and shell weight (g) as the continuous covariate in the model analysing the shell strength.

Diagnostics plots were used to visually assess model assumptions that the residuals were unbiased and homoscedastic. Where significant differences were present, *post-hoc* multiple comparisons ('multcomp' package in *R*) were performed to determine which treatment levels differed.

3.3 Results

3.3.1 Morphometrics

Muscle diameter and area

For both *M. gigas* and *O. edulis*, muscle diameter and area were positively correlated (Figure 3.4c), but neither were affected by Temperature, pCO_2, or their interaction (Table 3.2, Figure 3.4a,b).

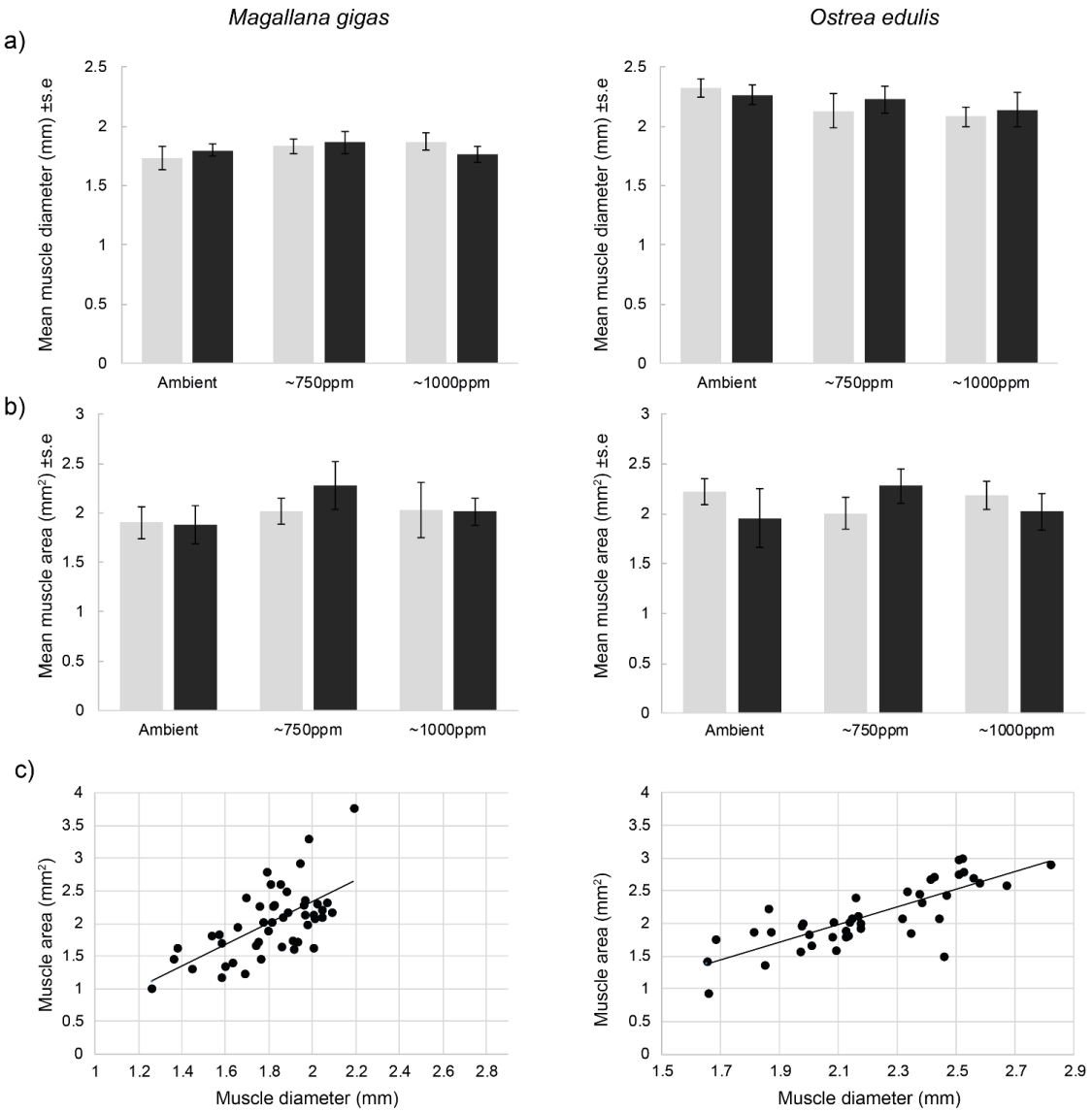

Figure 3.4: Variations in the a) diameter and b) area of the adductor muscle of oysters under temperature and pCO_2 scenarios. Ppm= part per million. Light grey: control temperature (16.8°C); Dark grey: warm temperature (20°C. c) For both oyster species, muscle diameter and area were correlated. *M. gigas*: y= 1.6374x -0.9451, R^2=0.38, p<0.001; *O. edulis*: y=1.3375x -0.8231, R^2=0.62, p<0.001.

Condition Index

There was an interactive effect of Temperature and pCO_2 on the Condition Index (CI) of *M. gigas* (Table 3.2, pCO_2:Temperature, p<0.05; Figure 3.5a). While the post-hoc tests were not able to differentiate between treatments, a clear increase in CI was apparent with increasing pCO_2 under the control temperature, with CI in the 1000 ppm being 1 unit greater than in the ambient pCO_2. Temperature increased the CI at ambient pCO_2 only by 0.7 unit from ~3.7 (±0.3) to ~4.4 (±0.3), but this positive effect disappeared at elevated pCO_2 with CI values similar to that of the control

temperature and ambient pCO_2 (±0.1 unit). In contrast, for *O. edulis*, all individuals had a CI of ~2.3 (±0.1) on average, irrespective Temperature and pCO_2 (Table 3.2).

Shell density

Only Valve had a significant effect on shell density of both species (Table 3.2, Valve, *M. gigas* $p<0.001$ *O. edulis* $p<0.001$). Left valves were approximately 8.5% and 9.7% denser than the right valves in both *M. gigas* and *O. edulis*, respectively (Figure 3.5b).

Shell thickness

There was a positive effect of Temperature on the shell thickness of *O. edulis*, with shells in the warm treatment being ~20.0% thicker (~0.63 mm thicker) than in the control temperature treatment (Table 3.2, pCO_2, $p<0.01$; Figure 3.5c), but no effects of the treatment on the shells of *M. gigas*. On average, the shells of *O. edulis* were ~52.6% thicker (~1.2 mm thicker) than that of *M. gigas* (Figure 3.5c).

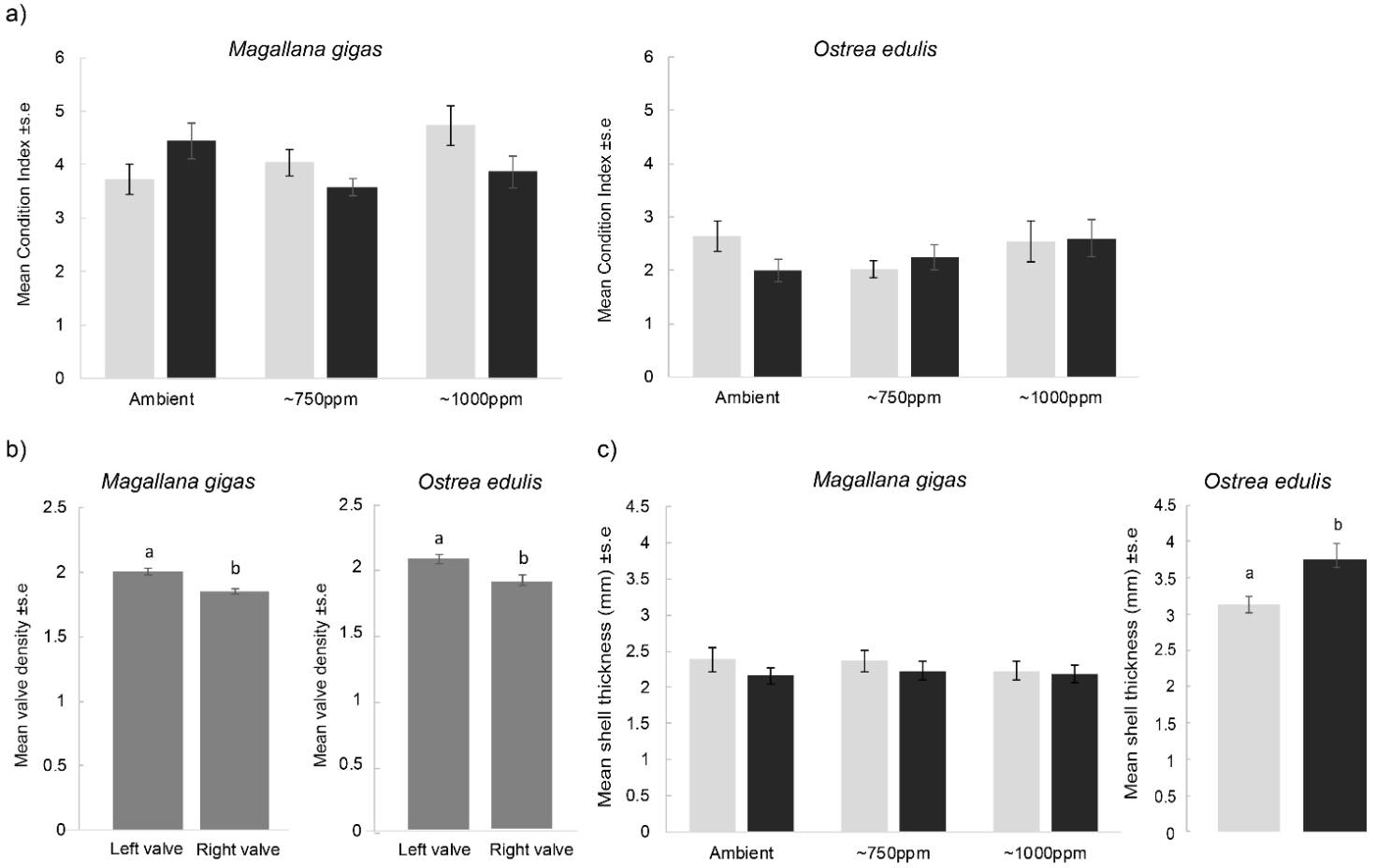

Figure 3.5: a) Oysters' Condition Index under Temperature and pCO_2 scenarios. b) Density of lower and upper shell valves of oysters. c) Thickness of the lower valve of *Magallana gigas* and *Ostrea edulis* under two temperature treatments. Ppm= part per million. Light grey: control temperature; Dark grey: warm temperature. Treatments that do not share a letter are significantly different.

Table 3.2: Summary of two-way ANOVA results for the effects of pCO_2, Temperature, and their interaction on *Magallana gigas* and *Ostrea edulis* morphometrics (Condition Index (CI), muscle diameter and area, shell thickness), and of the three-way ANOVA results for the effects of pCO_2, Temperature, Valve, and their interactions on their shell density. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded *p*-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas						***Ostrea edulis***					
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***	
Muscle diameter												
pCO_2	2	0.0565	0.0282	0.644	0.53		2	0.235	0.11752	1.395	0.261	
Temperature	1	0.0002	0.0002	0.004	0.947		1	0.0102	0.01023	0.121	0.73	
pCO_2:Temperature	2	0.0663	0.0332	0.756	0.476		2	0.0484	0.0242	0.287	0.752	
Residuals	42	1.8417	0.0439				36	0.0338	0.08427			
Muscle area												
pCO_2	2	0.518	0.259	0.866	0.428		2	0.403	0.20168	0.823	0.447	
Temperature	1	0.069	0.0694	0.232	0.632		1	0.125	0.12524	0.511	0.479	
pCO_2:Temperature	2	0.198	0.039	0.331	0.72		2	0.092	0.04577	0.187	0.83	
Residuals	42	12.557	0.299				36	8.823	0.24507			
CI												
pCO_2	2	1.927	0.9637	1.415	0.2543		2	1.241	0.6204	1.187	0.317	
Temperature	1	0.479	0.4792	0.703	0.4064		1	0.223	0.2233	0.427	0.518	
pCO_2:Temperature	2	5.443	2.7216	3.995	**0.0258**	*	2	1.563	0.7817	1.495	0.238	
Residuals	42	28.612	0.6812				36	18.82	0.522			
Shell density												
pCO_2	1	0.0088	0.0088	0.292	0.59		1	0.121	0.1214	1.842	0.17863	
Temperature	2	0.007	0.0035	0.117	0.89		2	0.333	0.1663	2.523	0.08678	.
Valve	1	0.5971	0.5971	19.886	**2.53E-05**	***	1	0.744	0.744	11.288	**0.00121**	**
pCO_2:Temperature	2	0.0225	0.0112	0.374	0.689		2	0.301	0.1505	2.283	0.10871	
Temperature:Valve	1	0.007	0.007	0.232	0.631		1	0.002	0.0023	0.034	0.85385	
pCO_2:Valve	2	0.078	0.039	1.299	0.278		2	0.024	0.0118	0.179	0.83635	
pCO_2:Temperature:Valve	2	0.0586	0.0293	0.976	0.381		2	0.021	0.0107	0.163	0.85021	
Residuals	84	2.522	0.03				78	5.141	0.0659			
Shell thickness												
pCO_2	1	0.64	0.6373	1.433	0.233		1	0.558	0.5581	8.489	**0.00421**	**
Temperature	2	0.22	0.1079	0.243	0.785		2	0.056	0.0279	0.425	0.65481	
pCO_2:Temperature	2	0.21	0.1055	0.237	0.789		2	0.162	0.081	1.231	0.29533	
Residuals	138	61.4	0.4449				129	8.481	0.0657			

3.3.2 Muscle strength

Initial resisting force (1)

There was considerable variation between individuals within treatments in initial resisting force. This variation led to no significant differences between treatments, however, differences in mean resistance force are clear (Table 3.3, Figure 3.6a). In particular marked decreases for both species at ~750 ppm pCO_2 in the control temperature treatment, with warm temperature counteracting the negative effect.

Force necessary to open the valves (2)

For *M. gigas*, there was a was an interactive effect of pCO_2 and tissue dry weight on the force required for the valves to start opening (Table 3.3, pCO_2:MeatDW, $p<0.01$; Figure 3.6b), but no effect of Temperature. Stronger force was necessary to open oysters exposed to elevated pCO_2 treatments, increasing from ~37.7N (±4.2) at ambient pCO_2, to ~42.0N (±6.0) and ~50.8N (±4.5) at ~750 ppm and ~1000 ppm, corresponding to 21.2% and 35.0% increases, respectively. In the ~750 ppm treatment, the increased force required to open the valves could be linked to a higher dry weight, although this relationship was not apparent in the other two pCO_2 treatments.

For *O. edulis*, all individuals opened under a ~36.4N (±3.9) extensive force on average irrespective of tissue dry weight, Temperature, or pCO_2 (Table 3.3, Figure 3.6b). However, differences in mean opening force between treatments are apparent. Increasing pCO_2 appeared to increase the force necessary to open the valves at the control temperature, from 24.8N (±7.5) at ambient pCO_2, to ~38.7N (±14.8) and ~43.3N (±13.1) at ~750 ppm and ~1000 ppm, corresponding to 56.4% and 74.7% increases, respectively. Warm temperatures led to a 63.4% increase in the force required at ambient pCO_2 to 40.5N (±13.5), but the additional effect of elevated pCO_2 did not lead to further increase.

Force required to induce muscle tear (3)

Temperature and pCO_2 affected the force required to induce muscle tear in *M. gigas* but not *O. edulis* (Table 3.3, *M gigas* pCO_2:Temperature, $p<0.01$). *Post-hoc* test revealed that this was due to significant

differences in temperature effects at ~750 ppm pCO_2 level ($p<0.01$). While ~750 ppm pCO_2 led to reduction in the required force at the control temperature, the opposite was true at the warm temperature, with an increase in the force necessary to tear the muscle fibres. Consequently, at ~750 ppm pCO_2 ~52.1% more force was required to tear the muscle fibres of oysters in the warm treatment compared to oysters in the control temperature treatment, corresponding to an increase in pulling force from 72.0N (±3.4) to 109.4N (±7.9). There also was an interactive effect of Temperature and tissue dry weight in *M. gigas* (Table 3.3, Temperature:MeatDW, $p<0.01$; Figure 3.6c). Increased dry weight had opposite effects on the force required to induce muscle tear in both cold and warm treatments. In the control temperature treatment, increased dry weight was linked with decreased force, while in the warm treatment it was linked with increased force, but the relationships were not significant ($p>0.05$).

For *O. edulis*, there was an effect of tissue dry weight (Table 3.3, MeatDW, $p<0.01$), with higher dry weight linked with increased force necessary to tear the muscle fibres (Table 3.3, Figure 3.6c). The maximum force withstood ranged from 40.9N in the smallest oyster to 144.8N in the second largest oyster.

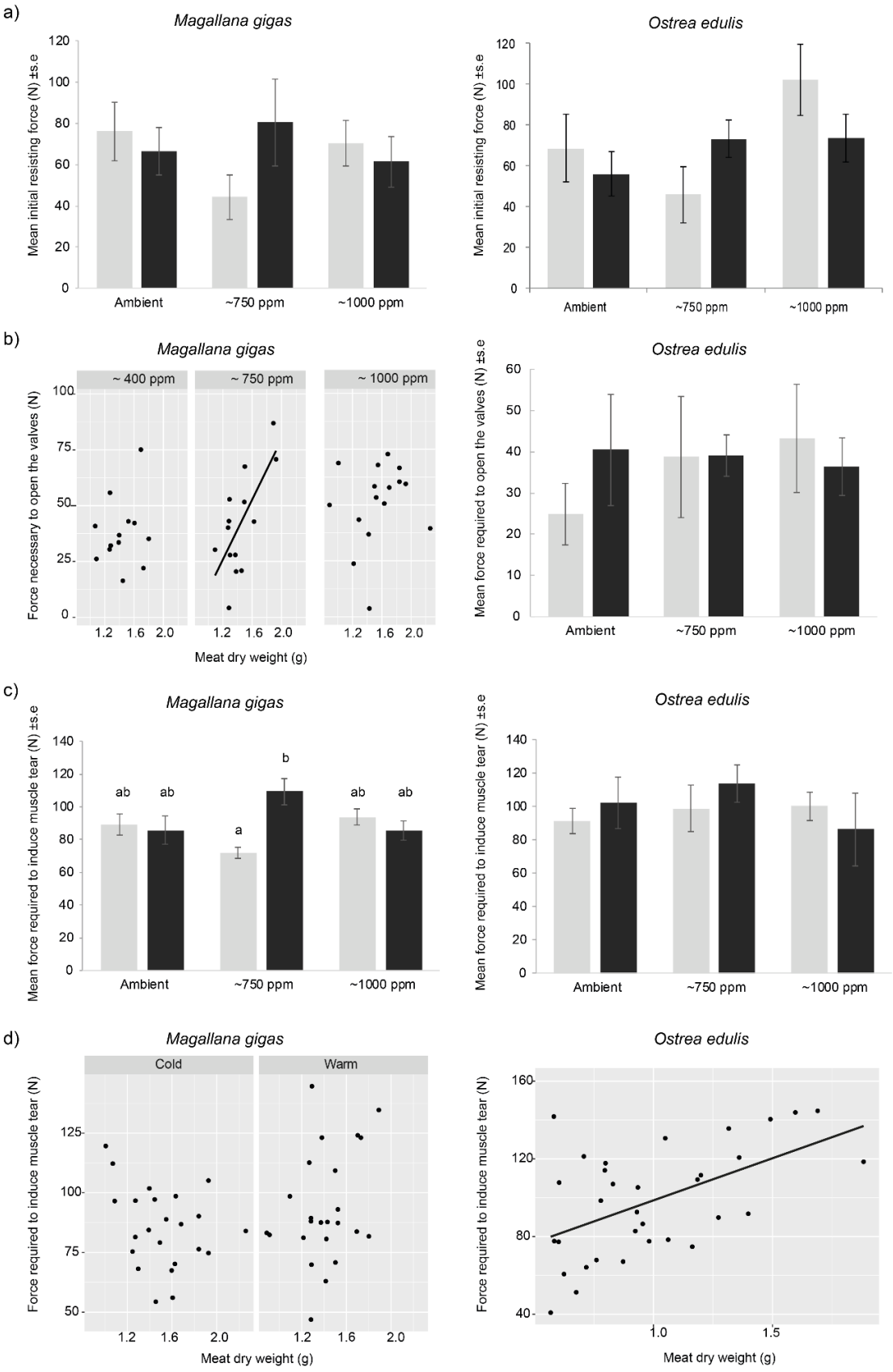

Figure 3.6: Changes in three adductor muscle traits of oysters exposed to six temperature and pCO_2 scenarios. a) Initial resistance to the extensive force. b) Extensive force required to open the valves. c) Extensive force required to induce muscle tear. Significant regressions are shown: *M. gigas* (b) at 750 ppm: y= 67.097x -54.669, R^2= 0.46, p<0.01; *O. edulis* (d) y= 43.40x +55.27; R^2=0.27; p<0.01. N= Newton. Ppm= part per million. Light grey: control temperature. Dark grey: warm temperature. Treatments that do not share a letter are significantly different.

Table 3.3: Summary of ANCOVA results for the effects of pCO_2, Temperature, tissue dry weight (MeatDW), and their interactions on three traits related to the adductor muscle strength of *Magallana gigas* and *Ostrea edulis*. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded *p*-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas					***Ostrea edulis***				
	df	**SS**	**MS**	**F**	***p***	**df**	**SS**	**MS**	**F**	***p***
Initial resisting force										
pCO_2	2	355	177.7	0.1224	0.8853	2	4951	2475.26	1.8062	0.1836
Temperature	1	301	301	0.2073	0.6521	1	93	93.31	0.0671	0.7961
MeatDW	1	832	832.3	0.5731	0.4548	1	7	7.2	0.0053	0.9428
pCO_2:Temperature	2	4852	2425.9	1.6704	0.2047	2	4175	2097.69	1.5234	0.2361
pCO_2:MeatDW	2	6729	3364.4	2.3167	0.1154	2	1521	760.25	0.5548	0.5806
Temperature:MeatDW	1	2286	2285.6	1.5738	0.219	1	1754	1754.21	1.2801	0.2678
pCO_2:Temperature:MeatDW	2	872	435.9	0.3002	0.7428	2	136	67.83	0.0495	0.9518
Residuals	31	45021	1452.3			27	37001	1370.41		
Force necessary to open the valves										
pCO_2	2	1334	667.02	2.4322	0.104428	2	628.9	314.46	0.5731	0.5705
Temperature	1	459.9	459.87	1.6769	0.204903	1	195.9	195.93	0.3571	0.5551

MeatDW	1	1340.6	1340.63	4.8884	**0.034545**	*	1	1444.2	1444.21	2.6319	0.1164	
pCO_2:Temperature	2	493.4	246.71	0.8996	0.417085		2	785.4	392.72	0.7157	0.4979	
pCO_2:MeatDW	2	2977.9	1488.94	5.4292	**0.009516**	**	2	2708.6	1354.31	2.4681	0.1037	
Temperature:MeatDW	1	476.7	476.7	1.7382	0.197023		1	19.9	19.9	0.0363	0.8504	
pCO_2:Temperature:MeatDW	2	8.1	4.05	0.0148	0.985357		2	2497.7	1248.84	2.2759	0.1221	
Residuals	31	8501.6	274.25				27	14815.7	548.73			
Force required to induce muscle tear												
pCO_2	2	155.5	77.73	0.2646	0.7690719		2	403.6	201.8	0.3394	0.71602	
Temperature	1	762	762.12	2.5947	0.116207		1	288.4	288.4	0.4851	0.49376	
MeatDW	1	17.7	17.61	0.0602	0.807675		1	7731.3	7731.1	13.0035	**0.00166**	**
pCO_2:Temperature	2	4764	2382	8.1096	**0.001277**	**	2	1256	628	1.0562	0.36553	
pCO_2:MeatDW	2	508.3	254.15	0.8653	0.429748		2	209.5	104.7	0.1761	0.83972	
Temperature:MeatDW	1	2940.3	2940.3	10.0104	**0.003214**	**	1	163.8	163.8	0.2755	0.60516	
pCO_2:Temperature:MeatDW	2	257.1	128.56	0.4377	0.649016		2	3130.5	1565.2	2.6327	0.09545	.
Residuals	35	10280.4	293.72				21	12485.6	594.6			

5.3.3 Shell strength

Temperature and pCO_2 had an interactive effect on the shell strength of *M. gigas* (Table 3.4, pCO_2:Temperature, $p<0.01$). There is a clear trend for decreased shell strength with elevated pCO_2 at the control temperature, with shells able to withstand compressive force of ~619N after exposure to ambient pCO_2, but only ~390N and ~342N after exposure to ~750 ppm and ~1000 ppm pCO_2, respectively. These corresponded to shells being over 36% and 44% weaker (Figure 3.7a). While warm temperature reduced the mean strength of *M. gigas* shells in ambient pCO_2 conditions by over 40% (from ~619N down to ~367N), the effect of temperature and pCO_2 was not synergistic (i.e. temperature did not cause further reductions in strength at higher pCO_2 levels). *Post-hoc* tests revealed significant differences in strength occurred between shells at ambient and 1000 ppm pCO_2 treatments under control temperature, and between shells in the control and warm temperature treatments at ambient pCO_2 (Figure 3.7a). pCO_2 and shell weight also had an interactive effects on the shell strength of *M. gigas* (Table 3.4, pCO_2:ShellDW, $p<0.05$; Figure 3.7a). There was a positive relationship between shell weight and shell strength, but the strength of the relationship weakened with elevated pCO_2 and was only significant at ambient ~400 ppm, with little effect of shell weight on strength at ~1000 ppm (Figure 3.7a).

Shell weight influenced the shell strength of *O. edulis* (Table 3.4, ShellDW, $p<0.001$) with heavier shells being stronger, but without additional effect of Temperature or pCO_2 (Table 3.4, Figure 3.7b). *O. edulis* shells on average withstood a compressive force of 735.8N (±50.6) before fracturing, ranging from 148.5N in the lightest shell to 1627.3N in the heaviest shell.

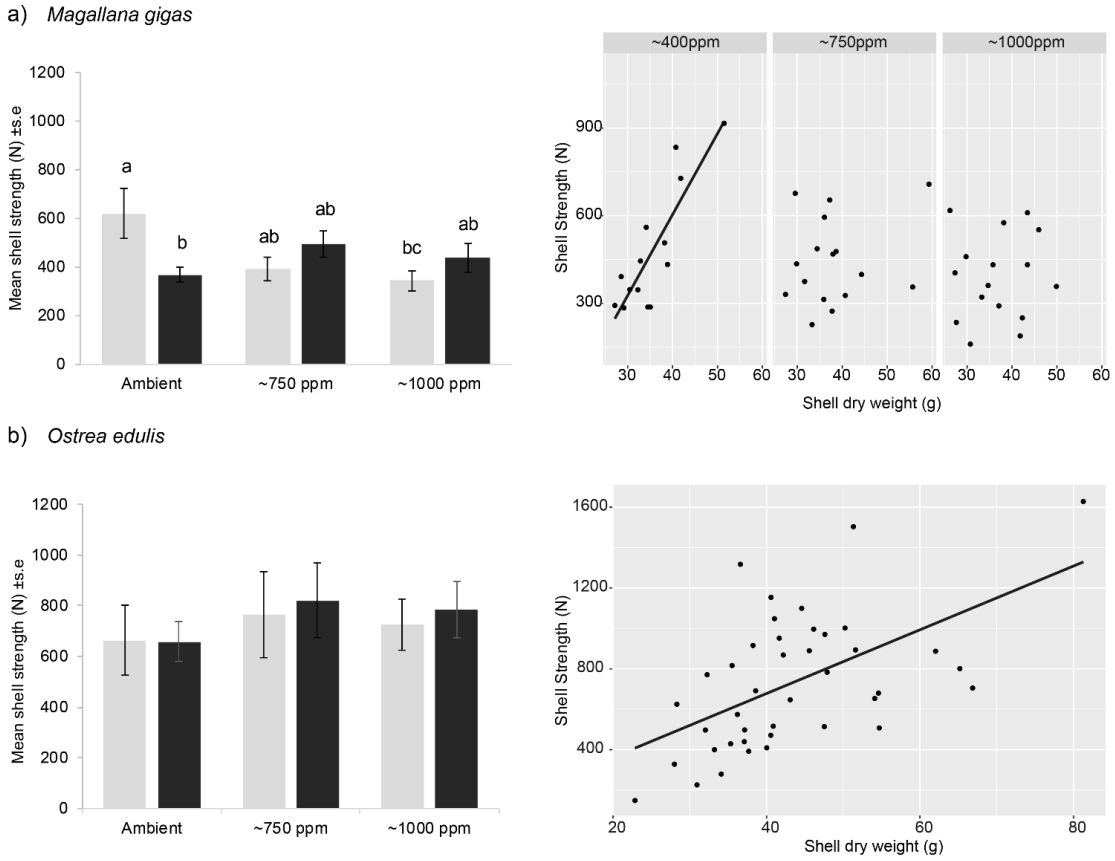

Figure 3.7: Strength of the left valve of a) *Magallana gigas* and b) *Ostrea edulis* shells under Temperature and pCO_2 treatments. Significant regressions are shown: *M. gigas* y=27.766x -507.291; R^2= 0.70; p<0.001; *O. edulis* y= 15.756x +47.274; R^2=0.28; p<0.001. N= Newton. ppm= part per million. Light grey: control temperature. Dark grey: warm temperature. Treatments that do not share a letter are significantly different.

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

Table 3.4: Summary of ANCOVA results for the effects of pCO_2, Temperature, shell dry weight (ShellDW), and their interactions on the shell strength of *Magallana gigas* and *Ostrea edulis*. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded *p*-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Maallana gigas						***Ostrea edulis***					
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***	
Shell strength												
pCO_2	2	56087	28044	1.4455	0.249754		2	138167	69084	0.7404	0.4857	
Temperature	1	331	331	0.017	0.896887		1	480	480	0.0051	0.943311	
ShellDW	1	166421	166421	8.5778	**0.006036**	**	1	1339909	1339909	14.3614	**0.000706**	***
pCO_2:Temperature	2	222324	111162	5.7296	**0.007171**	**	2	28371	14186	0.152	0.859631	
pCO_2:ShellDW	2	158710	79355	4.0902	**0.025601**	*	2	112678	56339	0.6038	0.553433	
Temperature:ShellDW	1	414	414	0.0213	0.884733		1	46453	46453	0.4979	0.486056	
pCO_2:Temperature:ShellDW	2	23800	11900	0.6134	0.547416		2	15719	7860	0.0842	0.919433	
Residuals	34	659641	19401				29	2705686	93300			

3.4 Discussion

Ongoing climate change causing ocean warming and acidification, poses a threat to oyster reefs, with a probable decline in the provision of ecosystem services predicted for the future (Lemasson *et al.*, 2017a). As predation is known to be an important factor shaping oyster populations (Knights *et al.*, 2012; O'Connor *et al.*, 2008), the ability of oysters to retain traits valuable in predation resistance in the future may be decisive for population maintenance. Despite the high variability in responses recorded across individuals of the same species, we show here two clearly contrasting responses of *Magallana gigas* and *Ostrea edulis* to exposure to warm temperature and pCO_2 reflecting predicted climate change for 2050 and 2100. In particular, whereas *O. edulis* remained unimpacted, the traits linked to predation resistance in *M. gigas* were altered following exposure to OAW scenarios.

3.4.1 Physiological traits and shell morphometrics

Surprisingly, there were no changes in the Condition Index (CI) – a trait related to organism's physiological status and overall health (see Chapter 2) – of either *M. gigas* or *O. edulis* under OAW

scenarios. This findings are in contrast with previous studies which observed marked decreases in the CI of molluscs from OAW due to energy reallocation towards other metabolic processes (Mackenzie *et al.*, 2014; Ong *et al.*, 2017). For *M. gigas*, this is in contrast with findings from Chapter 2, where its CI was negatively impacted by similar experimental conditions. This change could be due to the slightly different feeding regime (mixed diet of live algae *Isochrysis galbana* and *Tetraselmis* sp. in Chapter 2 vs Shellfish Diet here). Although live algal diet has been linked to higher growth rates in bivalves compared to artificial diets (Laing, 1987) with *I. galbana* being a crucial species, mono- and bi-specific diets have been associated with poorer performance, particularly when *C. calcitrans* was not present (Rico-Villa *et al.*, 2006). It is likely that, although artificial, Shellfish Diet 1800 led to better oyster condition due to its varied composition, which includes *I. galbana*, *C. calcitrans*, and four additional species. Food can be a crucial factor in organisms' response to OAW (Mackenzie *et al.*, 2014; Thomsen *et al.*, 2012), and differences in energetic value between the two feeding regimes are possibly the cause of the discrepancies in physiological responses recorded.

In oysters, the adductor muscle can weigh between 20% and 40% of the total soft tissue weight (Galtsoff, 1964). Here, muscle diameter also remained unaffected, suggesting that even under OAW scenarios, oysters were capable of allocating sufficient energy towards the maintenance of their adductor muscle.

In molluscs, reallocation of energetic resources can rapidly occur in response to OAW, and negatively impact on their shell characteristics, such as density and thickness (3 months exposure: Chatzinikolaou *et al.*, 2016; 18 days exposure: Lagos *et al.*, 2016), due to limited energy available for calcification. The absence of declines in shell density and thickness of *M. gigas* and *O. edulis* here suggests that calcification may not be compromised under OAW, at least over the short time scale used in this study. On the contrary, the shell of *O. edulis* grew thicker under warm temperature, suggesting enhanced calcification and protection. Growing thicker shells – a common plastic phenomenon in bivalves – has been shown to provide additional protection from predators (Beadman *et al.*, 2003; Leonard *et al.*, 1999; Reimer & Tedengren, 1996).

3.4.2 Muscle strength

The natural state of oysters is open and gaping, due to the mechanical spring-like effect of the ligament connecting the two valves (Kurita *et al.*, 2016). Oysters can control the opening and closing of the valves by contracting their adductor muscle, which also connects the two shell valves (Galtsoff, 1964; Kurita *et al.*, 2016). Being able to control valve closing has an important survival value for bivalves, as a means of protection against harmful environmental stressors and against predators (Reimer & Tedengren, 1996), but is also essential to undertake vital functions such as feeding, respiring, and waste elimination (Robson *et al.*, 2007). If the valves are forced apart by predators, the muscle fibres will eventually tear from the centre (Galtsoff, 1964).

To our knowledge, no studies to date have looked into the effects of OAW on the strength of bivalve's adductor muscle. Against our initial hypothesis, *O. edulis* retained the ability to resist pulling forces under OAW conditions, and it took similar amount of extensive force to open the valve and tear the adductor muscle across treatments. It is thus likely that the muscle strength and muscle fibre integrity of *O. edulis* were not affected by OAW. This is consistent with the lack of changes to the CI and muscle diameter of *O. edulis* individuals found in our study. Tearing the adductor muscle of *O. edulis* individuals with higher dry weight required more force, but remarkably the added weight did not confer additional strength to keep the valves shut. Bigger (*sensu* higher tissue weight) organisms may have developed more robust muscle fibre capable of resisting tearing forces, giving them an advantage compared to smaller organisms when it comes to predation resistance.

In contrast, OAW appeared to have positive effects of the muscle strength of *M. gigas*. Oysters displayed stronger muscles better able to resist forced-opening under elevated pCO_2, but also more resistant muscle fibres, particularly under intermediate OAW (~750 ppm X 20°C). Similarly to *O. edulis*, individuals with higher dry weight were able to sustain higher level of extensive force on their adductor muscle. As the adductor muscle constitutes 20-40% of the total soft tissue weight of oysters (Galtsoff, 1964), it is possible that individuals with higher dry weight had heavier muscle. Additionally, because the action of the adductor muscle is predominantly fuelled by soft-tissue

glycogen in oysters (Galtsoff, 1964), individuals with higher soft tissue weight would store more glycogen to allocate to muscle maintenance. As mentioned previously, possessing a strong muscle constitutes an important fitness advantage for oysters, and thus resisting more important pulling forces may confer *M. gigas* an ecological advantage over *O. edulis* in the future.

3.4.3 Shell strength

Shells constitute the first line of defence that oysters have to external threats, and therefore to possess a strong shell is to hold a fitness advantage (Currey & Hughes, 1982). The production of shell material is biologically controlled and energetically expensive, but modulated by changes in environmental conditions (Gazeau *et al.*, 2013). The burgeoning evidence from ocean acidification studies have shown that molluscan shell calcification is particularly vulnerable to increases in pCO_2 and decreases in seawater pH (Gazeau *et al.*, 2013). Previously observed changes to the mineralogy and structural integrity of shells (Duquette *et al.*, 2017; Fitzer *et al.*, 2016), and reductions in shell metrics (Chatzinikolaou *et al.*, 2016; Lagos *et al.*, 2016), are likely to negatively affect its protective function.

Contrary to our initial hypothesis, the strength of *O. edulis* shells was not altered following exposure to OAW. Similar findings were reported for four Mediterranean gastropods exposed to naturally acidified waters (Duquette *et al.*, 2017), whose shells mechanical strength were not compromised. It is possible that growing thicker shells under OAW allowed for greater resistance in *O. edulis*. In contrast, the shells of *M. gigas* experienced important weakening under OAW, with reductions in strength by over 44%. Shell weakening under acidification has previously been reported for the pearl oyster *Pinctada fucata* (Welladsen *et al.*, 2010), with reduction in strength by 25.9% at pH 7.8 and 26.8% at pH 7.6, for the zebra top snail *Austrocochlea porcata* under ~560 ppm and ~840 ppm pCO_2 (Coleman *et al.*, 2014), and for juveniles of the eastern oyster *Crassostrea virginica* exposed to seawater pH of 7.8 (Speights *et al.*, 2017). The crystals that form the shell of bivalves have been shown to hold modified orientation under OAW (Fitzer *et al.*, 2016), meaning that they had less control over the crystallization process, which could have implications for the mechanical integrity of the shell and its protective function. While crystallisation was not assessed here, and although no changes in shell thickness and density were recorded, it is likely that *M. gigas* allocated energy towards tissue production and

maintenance of its adductor muscle, at the detriment of calcification. Similarly to *M. gigas* in this study, *Mytilus edulis* was shown to reallocate energy from shell strength to maintenance under OAW (Mackenzie *et al.*, 2014). For both *O. edulis* and *M. gigas,* individuals with heavier shells were more resistant to crushing forces, but this advantage disappeared for *M. gigas* under elevated pCO_2, suggesting that building a heavier shell may not confer an ecological advantage to *M. gigas* in the future. It should be noted that here, shell strength was assessed as the force necessary to crush the main surface of the shell, but no attempt was made to assess the integrity and strength of the shell hinge, known to also be a strategic access point by predators.

Weakening of the shells represents a reduction in fitness that may put oysters at greater risks of predation, by shortening the prey handling time from current predators or allowing new species to crush shells that were previously stronger than they could manage (Beadman *et al.*, 2003). For instance, a potential predator with crushing force limited to 400N, such as the stone crab *Myomenippe hardwicki* which has a maximum crushing force of ~410N (Han *et al.*, 2008), would not have the capacity to crush the shells of *M. gigas* nor *O. edulis* under control temperature and ambient pCO_2 conditions, but under future OAW conditions such predator would then in theory have sufficient crushing force to predate on *M. gigas*. Such alteration in predator-prey interaction could then induce important community and ecosystem-level destabilization.

3.5 Conclusion

The two oysters had contrasting responses in terms of muscle strength and shell strength. There were no physiological and morphological effects of OAW on *O. edulis*, with the exception of thicker shells, and consequently its adductor muscle and shell strength remained unimpacted. Despite a lack of physiological and morphological effects, the adductor muscle of *M. gigas* appeared stronger and more difficult to tear, particularly for bigger organisms, but the shell weaker. As a consequence, it is likely that in the future *M. gigas* may see its susceptibility to predators change, becoming more resistant to valve-pulling predators, such as starfish, but more vulnerable to durophagous (shell-crushing) predators, such as crabs. Unlike *M. gigas*, *O. edulis* might retain the same level of predation resistance as it currently has. Reduction in predation pressure on *O. edulis* could potentially occur indirectly

through *M. gigas* selection by predators. However, considering that *M. gigas* is an exotic species, whether natural enemies would target it is uncertain (enemy release hypothesis: Colautti *et al.*, 2004; Keane & Crawley, 2002). For both species, oysters with more somatic tissue hold an ecological advantage when it comes to predator resistance. Under OAW, smaller oysters may be favoured by predators due to weaker adductor muscle and therefore shorter handling time.

These changes in fitness are likely to induce shifts in predator-prey interactions (Harvey & Moore, 2017), reshape assemblage structure due to species- and size- selections (Babarro *et al.*, 2017; Gooding & Harley, 2015; Leung *et al.*, 2017b; Lord *et al.*, 2017), and consequently induce important cascading modifications in the functioning of oyster reefs and the delivery of associated ecosystem services. In particular, the oyster aquaculture sector might need to adapt its culture and harvest practises, as well as species selection, according to predicted susceptibility in order to lower predation risks and optimize oyster survival, if it is to secure viable future production. It is apparent that the responses to OAW are highly species-specific, and further studies investigating alterations to predator-prey and community-level interactions are necessary to better understand the ecosystem-level implications, and inform aquaculture practices.

Chapter 4: Changes in the proximate composition of oysters exposed to ocean acidification and warming conditions – implications for future food security

"Don't bite the hand that feeds you"

A version of this chapter has been submitted as:

Lemasson, A.J., Hall-Spencer, J.M., Kuri, V., and A.M. Knights. Changes in the biochemical composition of seafood due to ocean acidification and climate change, for publication in *Marine Environmental Research*.

Chapter 4: Biochemical composition of oysters exposed to acidification and warming conditions – implications for future food security

Abstract:

Ocean acidification and warming (OAW) may threaten future seafood production and quality by negatively impacting the fitness of marine species. Deciphering the mechanisms behind changes in seafood nutritional quality, as well as identifying species most at risk, is crucial if societies are to secure food production in the future. Here, changes in the biochemical composition and nutritional properties of two economically important aquaculture species, the Pacific oyster *Magallana gigas* and the European Flat oyster *Ostrea edulis*, were evaluated following a 12-week exposure to six OAW scenarios. Results suggest that oysters are likely to become less nutritious in the near future, with reduced levels of protein, lipid, and carbohydrate, as well as reduced energetic value, particularly in *M. gigas*. Species-specific changes to the nutritional qualities resulted from differential use of energetic reserves under physiological stress, reflected in their Condition Index. Important changes to the essential mineral composition were also evident in both species. In particular, the accumulation of copper in *M. gigas* under OAW is cause for concern regarding its consumption safety. These findings hold important implications for the aquaculture industry, which may need to consider a shift in focus toward species more robust to climate change, in order to secure future food provision and secure socio-economic benefits of aquaculture.

4.1 Introduction

Seafood represents 15% of the main source of animal protein for more than 4.3 billion people around the globe, yet its security is at risk from climate change (FAO, 2014). Food security is commonly defined as "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life" (FAO, 1996; Porter *et al.*, 2014). Seafood contributes a significant part to the provision of protein and global food security, and is particularly important to coastal and island communities (Cooley *et al.*, 2012; FAO, 2016).

The world human population is rapidly rising and predicted to reach between 9.6 and 12.3 billion by the year 2100 (Gerland *et al.*, 2014; United Nations, 2015), with disproportionate growth in coastal areas (Firth *et al.*, 2016). This rise will undoubtedly increase demand for animal protein but this is unlikely to be met by land farming, placing increasing pressure on the marine environment to provide the shortfall (Cooley *et al.*, 2012; Delgado, 2003). 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 a growing concern regarding the sustainability of increased pressure being exerted on the marine environment in order to secure future food provision (Knights *et al.*, 2015; Porter *et al.*, 2014; UNEP, 2010; Weatherdon *et al.*, 2016).

The 'Blue Revolution' – the emergence of aquaculture as an important alternative to meat production from land agricultural activity – is thought by many to be a solution to securing food provision in the future (Naylor *et al.*, 2000; Tacon & Metian, 2013). The aquaculture industry is the fastest growing food sector, with a positive rise in production year-on-year (FAO, 2016). In 2015, total aquaculture production volume was estimated at over 106 million tonnes and valued in excess of US$162.9 billion; ~15% of this total is attributed to molluscan aquaculture (over 16 million tonnes; worth over US$18 billion) (FAO data[1]; Table 4.1.). While the production value of many molluscs is often not high compared to that of other fish and shellfish, several species (e.g. mussels, scallops, and oysters) are often seen as luxury items that attain high market values.

A healthy diet should include sufficient protein, amino acids, essentials fats such as long-chain omega-3 fatty acids, vitamins and minerals (FAO, 2016; Simopoulos, 2002),. In food science, the combination of important biochemical components present in food (i.e. moisture, ash, protein, fat [crude lipid], fibre, and nitrogen free extracts [digestible carbohydrates]) is referred to as the 'proximate composition' and is a measure of nutritional quality (Hart & Fisher, 1971; Nielsen, 2006). Seafood contains high levels of these important components and is therefore viewed as highly nutritious, and key to human health and well-being (Kris-Etherton *et al.*, 2003; Simopoulos, 2002). Remarkably, seafood composition includes a high protein content (higher than most terrestrial

[1]http://www.fao.org/figis/

meats); a high concentration of essential amino acids; a rich source of essential minerals, trace elements, and vitamins (more than terrestrial meats); a lower saturated fat content (compared to terrestrial meats); and the highest concentration of long-chain omega-3 polyunsaturated fatty acids in any other food (reviewed in Tacon & Metian, 2013). The nutritional attributes of seafood have been linked to several health promoting effects, such as reduced risk of cardiovascular diseases, strokes, and diabetes, but also protective and/or symptom relieving effects for inflammatory diseases, various forms of cancer, cognitive decline, and depression and anxiety (FAO/WHO, 2011; Larsen *et al.*, 2011; Lloret *et al.*, 2016).

Oysters are natural sources of proteins, lipids, fatty acids, vitamins, and minerals (Asha *et al.*, 2014; Cochet *et al.*, 2015; Orban *et al.*, 2004; Pogoda *et al.*, 2013; Sprague *et al.*, 2017), and are considered a delicacy in many developed countries. Global oyster production has been estimated to exceed 5.4 million tonnes in 2015, and worth in excess of US$4 billion (Table 4.1). Oysters are one of the major UK aquaculture species (Pinnegar *et al.*, 2017); the estimated 1600 tonnes produced in 2015 worth in excess of US$6.4 million (Table 4.1). This is particularly true of the native oyster *Ostrea edulis*, whose low natural availability boosts its status as luxury seafood item and therefore its market value, reaching US$13/kg (>£9/kg) in French markets[2].

In recent years, concerns have been expressed about the fate of shellfish production under future climate scenarios (Branch *et al.*, 2013; Cooley & Doney, 2009; Cooley *et al.*, 2015; Dupont *et al.*, 2014; Ekstrom *et al.*, 2015; Lemasson *et al.*, 2017a; Lemasson *et al.*, 2017b). Environmental stressors, such as acidification and warming, can also influence the physiology of marine organisms affecting meat quality (Borderias & Sanchez-Alonso, 2011; Dupont *et al.*, 2014), although a recent study simulating short-term perturbations, characteristics of environmental fluctuations recorded today, showed no effect on sensory quality (Lemasson *et al.*, 2017b – see Chapter 5). In larvae, effects include developmental delays, reduced feeding activity and energy balance (Gray *et al.*, 2017), all of which may be exacerbated under multiple stressors (Parker *et al.*, 2017). Similarly, in juveniles and adults, changes to physiology and performance including increased metabolism, impaired feeding and

[2] http://www.fao.org/fishery/culturedspecies/Ostrea_edulis

calcification, and reduced condition have been reported (Ong *et al.*, 2017; Scanes *et al.*, 2017). The effects of ocean acidification are already being felt in the case of oysters (Lemasson *et al.*, 2017a), with several hatcheries experiencing significant decline in production, with large economic losses (Barton *et al.*, 2012; Barton *et al.*, 2015).

To date, relatively little attention has been given towards changes in the 'quality' of shellfish under climate change, such as sensory and nutritional aspects, despite being a critical factor in consumer's food choice (Lee *et al.*, 2008). Given that warming and acidification negatively affect the physiology of adult oysters (see Chapter 2; Lemasson et al., under review; Scanes *et al.*, 2017), it is possible that their nutritional quality will also reflect such changes. To date, only two studies have investigated changes in seafood nutritional quality under future climate scenarios, both showing that protein and lipid content is reduced (Tate *et al.*, 2017; Valles-Regino *et al.*, 2015). Therefore, not only is it important to understand how the quantity of oysters produced will vary, but also how nutritional quality may be impacted in the future.

Here, using two economically important species of oysters – *Magallana gigas* and *Ostrea edulis* – we test the effects of multi-stressors (acidification and warming) on seafood nutritional quality, to determine if climate change will affect the potential for seafood to provide important nutritional requirements in the future.

Table 4.1: Volume and associated value of a) aquaculture production (farming only) and b) overall production (aquaculture + harvest) of marine living resources for the UK, Europe, and global world in 2015 (data obtained from the FAO 2015 online query statistics). t=tonnes (1000 kg); (*)=FAO estimates; N/A=not available.

a) Aquaculture production

	UK		**Europe**		**World**	
	Quantity (t)	*Value (USD)*	*Quantity (t)*	*Value (USD)*	*Quantity (t)*	*Value (USD)*
Total Aquaculture	206 834	1 098 065 000	2 977 867	11 433 755 000	106 004 184	162 974 582 000
Mollusc	(*) 21 645	(*) 54 421 000	(*) 636 520	(*) 1 189 377 000	16 431 989	17 853 597 000
Bivalve	(*) 21 645	(*) 54 421 000	(*) 636 456	(*) 1 188 941 000	14 674 105	15 732 529 000
Oysters	(*) 1 568	(*) 6 486 000	(*) 95 019	(*) 500 321 000	5 321 737	4 094 411 000

b) Overall production

	UK		**Europe**		**World**	
	Quantity (t)	*Value (USD)*	*Quantity (t)*	*Value (USD)*	*Quantity (t)*	*Value (USD)*
Total Production	912 079	N/A	17 343 460	N/A	199 702 397	N/A
Mollusc	104 142	N/A	1 079 142	N/A	23 864 830	N/A
Bivalve	72 779	N/A	849 200	N/A	16 123 676	N/A
Oysters	(*) 1 611	N/A	(*) 96 156	N/A	5 468 565	N/A

4.2 Methods

4.2.1 Organism collection and acclimation, treatments, and mesocosm set-up

Adult Pacific oysters (*M. gigas* 112.4 ± 6.9 mm in length and weighing 285.9 ± 13.4 g) and European flat oysters (*O. edulis*: 79.4 ± 5.7 mm in length and weighing 92.8 ± 15.1 g) were wild-collected from the same low-intertidal site in Plymouth Sound, UK described in Chapter 2 in July 2015 and January 2016, respectively. Oysters were cleaned of epibionts then allowed to acclimatise in a recirculating system at ambient laboratory conditions of ~16.5°C and atmospheric pCO_2 pressure of ~400 ppm. During the acclimation period, oysters were fed *ad libitum* with a mixed algal diet (Shellfish Diet 1800, Reed Mariculture).

OAW experimental treatments

To test the effect of current and future warming and ocean acidification scenarios on the nutritional value of shellfish, oysters were exposed to three levels of pCO_2 (ambient ~400 ppm, intermediate ~750 ppm, elevated ~1000 ppm), and two temperatures (control 16.8 °C, elevated 20 °C) in an orthogonal experimental design. Scenarios are described in full in Chapter 2. Twenty-four *M. gigas* were placed in their own 3-L experimental tanks and exposed to the treatment conditions for 12 weeks (n=4 replicate oyster per treatment). 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, removing no more than 20% of the volume, and left to slowly refill with the incoming equilibrated seawater. This experiment was repeated with *O. edulis* following the same procedures, with forty-eight experimental tanks (n=8 replicate oyster per treatment) to allow sufficient soft tissues for the proximate analysis.

Experimental design and mesocosm set-up

The ocean acidification and warming system used during the experiment is a modified version of the one described by Calosi *et al.,* (2013). Briefly, each treatment consisted of a header tank (volume=80 L) of seawater, supplied from one of two sumps (16.5 °C and 20 °C), and aerated with either the ambient air pipe (pCO_2 ~400 ppm) or one of the two CO_2- enriched air pipes (~750 ppm pCO_2,

~1000 ppm pCO_2). Mixing in all header tanks was achieved using a submersible pump (Hydor Koralia Nano 900, Italy). CO_2 gas mix were obtained by slowly releasing CO_2 into two Buchner flasks where it mixed with ambient air, achieving two different levels of pCO_2, using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK) Figure 2.1). Throughout the experiment, the three CO_2 levels varied in a similar manner following natural variations in CO_2 in the ambient air and as such, take account of natural daily variability (reflected in daily pH variability, see Appendix 1). Increasingly, accounting for this variability has been suggested as a critical consideration point for climate change experimental studies (Humphreys, 2016; Reum *et al.*, 2015). CO_2 levels in the two CO_2-enriched pipes were recorded using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA) and adjusted manually to the desired level twice daily. CO_2 levels in the ambient air pipe were also recorded to monitor for the control treatments. Seawater was gravity-fed from the header tanks to each of the replicate tanks at a constant rate of ~60 mL/min. The replicate tanks were held within four larger 300 L holding trays, each sump supplying seawater to two of the holding trays, effectively creating water baths maintaining the replicate tanks at the desired temperature. Each tray held two (*M. gigas*) or four (*O. edulis*) replicates of each CO_2 levels. 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 was added and replaced on a daily basis, and 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.

Measurement of seawater parameters

Temperature, salinity, and pH were measured daily in all replicate tanks (Table 4.2). 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 (Ωca and Ωar), were calculated at the end of the experiment using CO_2 SYS (Pierrot D *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 4.2).

Table 4.2: Seawater chemistry for *Magallana gigas* and *Ostrea edulis*. Data shown are means (±sd) values. T=Temperature in °C. ppm= parts per milliom. 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	Ω_{Ar}	Ω_{Ca}
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

4.2.2 Proximate analyses

After 12 weeks exposure, oysters were manually shucked by cutting the adductor muscle with an oyster knife. The soft tissue was left to drain on a sieve for approximately 30 seconds, and the surface water was blotted with absorbent paper to remove excess moisture. The wet tissue mass (g) was recorded on an electronic balance (Mettler AE240), before being oven-dried at 105°C for 24 hours, then placed in a desiccator until reaching room temperature and reweighted.

Condition Index and Moisture

The Condition Index (CI) of each oyster was calculated following the methodology described in Chapter 2. Moisture percentage was calculated according to the following formula:

$$Moisture\ (\%) = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

The moisture content is expressed as percentage of total wet weight for each individual oysters.

Dried samples were pooled by treatment in order to provide sufficient tissue material for all analyses, then homogenised and grounded into a fine powder using a coffee grinder. Complete or partial pooling of specimen 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 method provides nutritional information at the population level. The following assays were performed in triplicate to ensure their accuracy.

Ash

Ash content was determined by adapting the Association of Official Agricultural Chemists official method (AOAC, 1995). 500mg of dried tissue powder from each treatment was placed in a dried and pre-weighted porcelain crucible. Samples were then heated in a muffle furnace at 550°C for 8 hours, and then placed in a desiccator to cool down, leaving the inorganic (ash) residue. Samples were re-weighed once reaching room temperature using an electronic balance (Mettler AE240), and ash content calculated according to the following equation:

$$Ash\ (\%) = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$ (adapted from AOAC, 1995)

Results are expressed as percentage of total wet weight.

Lipid

Lipid content of ~2g samples was determined by continuous extraction of fat with petroleum ether as a solvent using the Soxhlet method (Luque de Castro & Garcı□ a-Ayuso, 1998; Manirakiza *et al.*, 2001). Briefly, samples were added to an extraction thimble, plugged with glass wool, and inserted into a pre-weighed extraction cup with ~140mL of solvent. Extraction cups were placed in the

Soxtherm Rapid Extraction Unit (C. Gerhardt GmbH & Co. KG) where the extraction process took place. At the end of the extraction process, each cup was placed in a fume cupboard to cool down until all the remaining solvent had evaporated. Lipid content was then quantified gravimetrically using an electronic balance (Satorius L420P) and the following equation:

$$Lipids(\%) = \frac{\text{Weight of lipids}}{\text{Weight of sample}} \times 100$$

Results are expressed as percentage of wet weight.

Protein

Total protein content was determined using the Kjeldahl method (Kjeldahl, 1883) on ~150 mg samples, using an electronic balance (Oxford XB220A). Briefly, samples were transferred to borosilicate digestion tubes, and a Kjeldahl catalyst tablet (3g K_2S0_4, 105 mg $CuSO_4.5H_2O$, and 105 mg TiO_2. BDH LTD, UK) and 10 mL of 98% H_2S0_4 were added to each tube. Digestion was conducted in a Gerhardt Kjeldatherm digestion block at 105°C for 15 min (Gerhardt Laboratory Instruments, Bonn, Germany). The temperature was then raised to 225°C for a further 60 min, and was finally raised to 380°C for 45 min. The digestion block was attached to a scrubber unit (Gerhardt Turbosog unit) in which acid fumes were neutralized through 15% NaOH. The cooled samples were distilled using a Gerhardt Vapodest 50s distillation unit, where the samples were diluted with distilled water and neutralized with 37% NaOH. The liberated ammonia in the sample was then trapped into 50 mL of 4% orthoboric acid (H_3BO_3) by automatic steam distillation. Subsequently the distillate was back-titrated against 0.1 M H_2SO_4. Blank and reference (acetanilide and casein) samples with known protein content were run for validation. Protein content was calculated according to the following formula:

$$Protein\ (\%) = \frac{(\text{Sample titre} - \text{Blank titre}) \times 0.2 \times 14.007 \times 6.25}{\text{Sample weight}} \times 100$$

where:

0.2 = Normality of H_2SO_4

14.007 = Molecular weight of nitrogen

6.25 = Conversion factor for protein

Sample titre = Volume of H_2SO_4 used as titrant for the sample (mL)

Blank titre = Volume of H_2SO_4 used as titrant for the blank (mL)

Results are expressed as percentage of wet weight.

Carbohydrate (Glycogen)

Glycogen content was determined indirectly by calculating carbohydrate content using the above results for moisture, ash, lipid, and protein contents. Carbohydrate content was calculated as follows (Maclean *et al.*, 2003):

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

Where:

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

4.2.3 Energy content (calorimetry)

Caloric content was measured as gross energy content ($MJ.kg^{-1}$) and determined by bomb calorimetry using an isoperibol oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois, USA) on ~1 g samples. The ground samples were made into pellets then weighted prior to calorimetry. Pellets were then placed in a stainless steel decomposition vessel filled with oxygen (30 bar) and electrically ignited using a wire thread. The heat created by the combustion process was converted into energy, corrected for the sample weight.

4.2.4 Macro and micro-minerals

The macro-mineral contents (i. e. calcium [Ca], potassium [K], magnesium [Mg], sodium [Na]) and micro-mineral contents (i.e. copper [Cu], iron [Fe], zinc [Zn]) were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; iCAP 7000series Thermo Scientific), and the content of micro-mineral selenium [Se] was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Xseries2, Thermo Scientific), using standard protocols. Briefly, borosilicate digestion tubes (Kjeldahl digestion tube) were washed, rinsed and soaked in 10% nitric acid solution, rinsed in distilled water then oven dried before use. 100-150 mg of dried sample were weighed into borosilicate digestion tube. 10 mL of concentrated nitric acid (s. g. 1.42 (70%), Fisher

Scientific) was added to each tube. Digestion was conducted in a Gerhardt Kjeldatherm digestion block (Gerhardt Laboratory Instruments, Bonn, Germany) at 60°C for 1h. The temperature was then raised to 90°C for a further 1h, then raised to 110°C for 30 min, and finally raised to 140°C for 2h. The digestion process was performed in a scrubber unit (Gerhardt Turbosog unit) in which acid fumes were neutralized through a 15% NaOH. The cooled samples were made up to 50 mL with deionised water in volumetric flasks, then transferred into 50 mL labelled Falcon tubes for analysis (ICP-OES or IC-MS). Before use, ICP-OES and ICP-MS were calibrated using 5 standard solutions of different known concentrations (see Appendix 3). Additionally, procedural blanks and certified reference material samples (DORM-3 – dogfish muscle) were analysed to ensure the accuracy of the assay, and showed >90% recoverability.

4.2.5 Statistical analyses

Natural variations in the chemistry of the seawater used during the experiments and changes in the partial pressure of ambient air used meant that the treatments applied to each species were not consistent and resulting data could not be compared using a factorial design. Species responses were therefore analysed separately. All data were analysed using the public domain package *R* [version 3.2.5 65]. Differences were considered significant for $p < 0.05$.

Condition Index and Moisture content

All data were tested for the assumption of homogeneity of variances, and where not met, data were transformed using logarithmic or square-root transformations. Differences in CI and in Moisture content with treatment were analysed using 2-factor ANOVA with 'temperature' and 'pCO_2' as fixed factors. If significant differences were present, *post-hoc* Tukey test was performed to assess differences between treatment levels.

Proximate data, calorimetry, and minerals

As oyster tissues were homogenised (pooled) in order to provide sufficient material to perform all analysis, there were no 'true replicates' (*sensu* Hurlbert, 1984). Triplicate measures of protein, lipid, ash and mineral analysis were performed to determine within sample variability, but were pooled

(averaged) for statistical analysis to avoid Type I error. Analyses were thus performed on the means of the triplicates 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 the design, interactions between the two factors could not be assessed. When significant differences were present, *post-hoc* Tukey tests were performed to assess differences between treatment levels. Correlation between calorific content and lipid content was assessed using Pearson correlation coefficient. Calorific content and lipid content were plotted as independent variables, and a linear model was used to determine the relationship. ANOVA (single factor) was used to test the significance of the relationship.

4.3 Results

4.3.1 Condition Index

The results for the Condition Index (CI) of *M. gigas* were previously presented in Chapter 2, as well as part of the CI results for *O. edulis* (n=4 in Chapter 2, n=8 here). Briefly, only temperature had a significant effect on the condition index of *M. gigas* (Table 4.3; $p<0.01$), with CI being negatively impacted by elevated temperature (Figure 4.1a). While not significant (Table 4.3; $p=0.07$), there were clear differences between specimens of *M. gigas* culture under different temperature and pCO_2 regimes, with a trend for increasing CI with pCO_2 at control temperature, and decreasing CI with pCO_2 at elevated temperature. Mean CI of *M. gigas* decreased with increased temperature, from ~3.2 (±0.3) at ambient temperature to ~2.1 (±0.20) at elevated temperature. In contrast, the CI of *O. edulis* did not significantly vary with any of the treatments, and averaged ~2.6 (±0.1) (Figure 4.2a).

Table 4.3: Summary of two-way ANOVA results for the effects of pCO_2, Temperature, and their interaction on the Condition Index (CI) of *Magallana gigas* and *Ostrea edulis*. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded p-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas						***Ostrea edulis***					
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***	
pCO_2	2	0.261	0.131	0.204	0.81708		2	0.233	0.1165	0.139	0.871	
Temperature	1	7.867	7.867	12.298	**0.00252**	**	1	0.01	0.0104	0.012	0.912	
pCO_2:Temperature	2	3.796	1.898	2.967	0.07698	.	2	2.692	1.3459	1.608	0.216	
Residuals	18	11.515	0.64				33	27.618	0.8369			

4.3.2 Proximate analysis

Results of the proximate analysis are presented in Table 4.4 (composition) and Table 4.5 (ANOVA summary). Thereafter, results for the biochemical compositions are presented as proportions of total wet weight.

Table 4.4: Proximate composition of *Magallana gigas* and *Ostrea edulis* under six ocean acidification and warming scenarios. %WW= percentage of total wet weight. %DW= percentage of total dry weight (which therefore excludes moisture).

		Magallana gigas		***Ostrea edulis***	
		%WW	%DW	%WW	%DW
Ambient *p* CO_2 x Control temperature	**Protein**	16.6	57.9	9.9	45.6
	Carbohydrate	4.5	15.8	10.5	38.6
	Lipid	4.8	16.8	1.3	6.1
	Ash	2.7	9.4	2.1	9.7
	Moisture	71.4	-	76.2	-
Ambient *p* CO_2 x Elevated temperature	**Protein**	12.8	56.5	8.8	36.9
	Carbohydrate	4.9	21.9	9.3	47.9
	Lipid	2.7	11.8	1.6	6.8
	Ash	2.2	9.8	2	8.4
	Moisture	77.4	-	78.3	-
~750ppm *p* CO_2 x Control temperature	**Protein**	14.6	60.9	10.3	46
	Carbohydrate	4.6	19.2	10.3	38.5
	Lipid	2.9	11.9	1.3	5.9
	Ash	1.9	7.9	2.1	9.5
	Moisture	76	-	75.9	-
~750ppm *p* CO_2 x Elevated temperature	**Protein**	13.7	65.7	10.4	43
	Carbohydrate	3.3	16	8.7	42.9
	Lipid	1.4	6.7	0.9	3.7
	Ash	2.4	11.6	2.5	10.5
	Moisture	79.6	-	77.6	-
~1000ppm *p* CO_2 x Control temperature	**Protein**	12.6	56	9.7	41.3
	Carbohydrate	3.9	17.4	8.7	42.3
	Lipid	3.9	17.3	1.5	6.2
	Ash	2.1	9.3	2.4	10.2
	Moisture	77.5	-	77.7	-
~1000ppm *p* CO_2 x Elevated temperature	**Protein**	11.8	57.1	9.2	41.5
	Carbohydrate	4.3	20.6	11.1	43.8
	Lipid	2.6	12.4	1.3	6
	Ash	2.1	9.9	1.9	8.7
	Moisture	79.2	-	76.4	-

Table 4.5: Summary of two-way ANOVA results for the effects of pCO_2 and Temperature on the proximate composition of *Magallana gigas* and *Ostrea edulis*. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded p-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas						***Ostrea edulis***					
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***	
Moisture												
pCO_2	2	0.00733	0.003665	1.406	0.2708		2	0.000229	0.000115	0.207	0.814	
Temperature	1	0.00867	0.008668	3.325	0.0849	.	1	0.000836	0.000836	1.508	0.2262	
pCO_2:Temperature	2	0.00181	0.00905	0.347	0.7114		2	0.002789	0.001395	2.517	0.0928	.
Residuals	18	0.04692	0.002607				42	0.023271	0.000554			
Ash												
pCO_2	2	1.63E-05	8.16E-06	0.738	0.575		2	7.49E-06	3.74E-06	0.413	0.708	
Temperature	1	7.30E-08	7.30E-08	0.007	0.943		1	5.49E-07	5.49E-07	0.061	0.829	
Residuals	2	2.21E-05	1.11E-05				2	1.81E-05	9.07E-06			
Lipid												
pCO_2	2	2.79E-04	1.39E-04	15.09	0.0622	.	2	1.46E-05	7.31E-06	0.999	0.5	
Temperature	1	4.12E-04	4.12E-04	44.64	**0.0217**	*	1	1.22E-06	1.22E-06	0.167	0.723	
Residuals	2	1.85E-05	9.20E-06				2	1.46E-05	7.31E-06			
Protein												
pCO_2	2	6.30E-04	3.15E-04	2.351	0.298		2	1.17E-04	5.87E-05	3.688	0.213	
Temperature	1	5.59E-04	5.59E-04	4.173	0.178		1	4.03E-05	4.03E-05	2.528	0.253	
Residuals	2	2.68E-04	1.34E-04				2	3.18E-05	1.59E-05			
Carbohydrate												
pCO_2	2	7.19E-05	3.60E-05	0.718	0.582		2	1.94E-05	9.7E-06	0.073	0.932	
Temperature	1	5.46E-06	5.46E-06	0.109	0.773		1	0.000339	0.000339	2.548	0.252	
Residuals	2	1.00E-04	5.01E-05				2	0.000266	0.000133			

Moisture

Moisture – the principal component of oyster flesh – ranged between 70-80% for both species and was unaffected by temperature or pCO_2 (Table 4.4; Figure 4.1b, 4.2b).

Ash

Ash represented a very small proportion of tissue mass in both *M. gigas* and *O. edulis*, accounting for between 1.9-2.6% (Table 4.4; Figure 4.1g, 4.2g). Neither temperature nor pCO_2 affected the ash content of oysters, however the ash content of *M. gigas* appeared to decrease with increasing temperature and pCO_2, from 2.7 to 1.9% (Table 4.5; Figure 4.1c).

Protein

After moisture, protein was the second largest component in *M. gigas* and *O. edulis*, representing between 11-17% and 8-10% of the total wet weight, respectively (Table 4.4; Figure 4.1g, 4.2g). Trends suggest a possible decrease under elevated temperature and pCO_2 (Figure 4.1d), although there was no significant effect of temperature and pCO_2 on the protein content of *M. gigas* (Table 4.5). Protein content was the highest in the control temperature and ambient pCO_2 treatment (16.6%) and lowest in the elevated temperature and ~1000 ppm treatment (11.8%) (Table 4.4). No differences were apparent in *O. edulis*, irrespective of treatment (Table 4.5).

Lipids

The proportion of lipids in *M. gigas* was significantly negatively impacted by temperature (Table 4.5; $p<0.05$) but not pCO_2 (Table 4.4; $p=0.06$), despite clear reductions in average lipids at ~750 ppm in both control and elevated temperature treatments (Figure 4.1e, g). Lipids decreasing from 4.8% in the control temperature and ambient pCO_2 treatment, to 1.4% in the elevated temperature and ~750 ppm treatment (Table 4.4).

Lipids represented the smallest proportion in *O. edulis* and accounted only for 0.8-1.6% (Table 4.4; Figure 4.2g). There were evident differences between ambient and intermediate (~750 ppm) pCO_2 at

elevated temperature, with lipids dropping down to 0.8% (Table 4.4; Figure 4.2e,g), although these were not statistically significant (Table 4.5).

Carbohydrates

Elevated temperature and pCO_2 appeared to negatively impact the carbohydrate proportion in *M. gigas*. This dropped from 4.9% at control temperature and ambient pCO_2 conditions to 3.3% at elevated temperature and intermediate (~750 ppm) pCO_2 (Table 4.4; Figure 4.1f,g), albeit not significantly. Carbohydrates were present in large proportions in *O. edulis* averaging 9.0%, irrespective of treatments (Table 4.4, Table 4.5, Figure 4.2f,g).

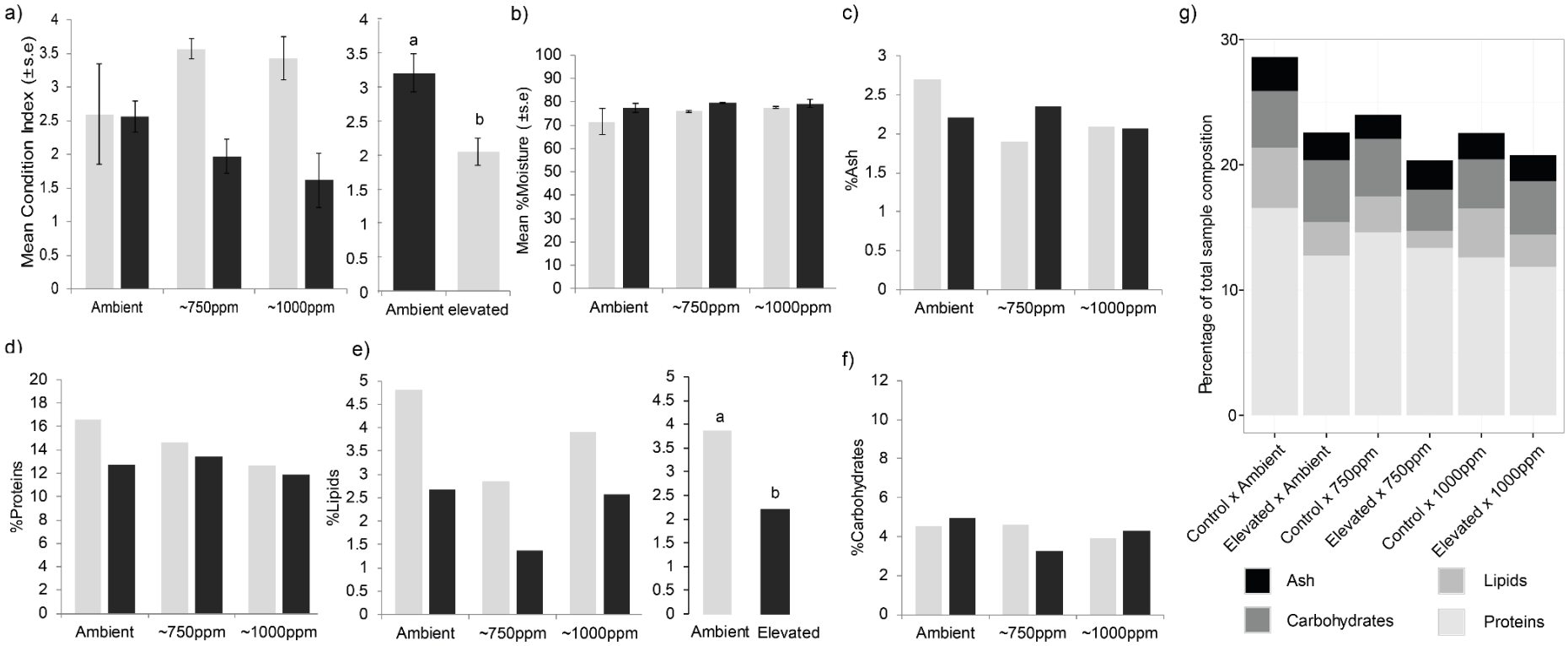

Figure 4.1: Variations in a) the Condition Index (CI), b-f) the proximate composition (as log% of total wet weight), and g) the relative composition of proximate components present in *Magallana gigas* across temperature and pCO_2 treatments. ppm= part per million; J= Joules; DW= dry weight; s.e= standard error. Light grey = control temperature. Dark grey= elevated temperature. Treatments that do not share a letter are significantly different. a-b) n=4 per treatment. c-f) The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bar were obtained. g) Moisture, excluded from this graph, represents the remaining portion, making the total composition of 100%.

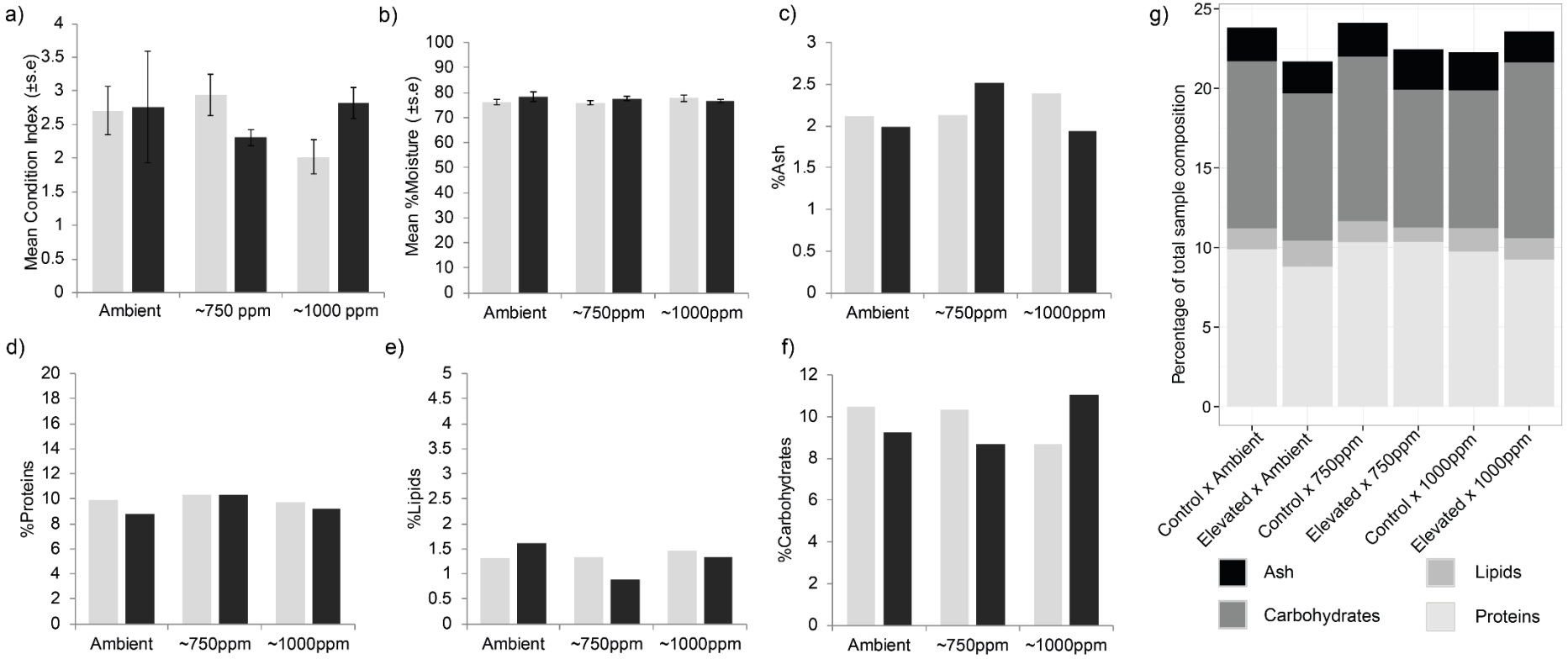

Figure 4.2: Variations in a) the Condition Index (CI), b-f) the proximate composition (as % of total wet weight), and g) the relative composition of proximate components present in *Ostrea edulis* across temperature and pCO_2 treatments. ppm= part per million; DW= dry weight; s.e= standard error. Light grey = control temperature. Dark grey= elevated temperature. Treatments that do not share a letter are significantly different. a-b) n=8 per treatment. c-f) The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bar were obtained. g) Moisture, excluded from this graph, represents the remaining portion, making the total composition of 100%.

4.3.3 Calorimetry

There were clear decreases in the calorific content of *M. gigas* with Temperature and pCO_2, although not significantly (Table 4.6), from 20.97 MJ/kgDW at control temperature and ambient pCO_2 to 18.41 MJ/kgDW at elevated temperature and intermediate pCO_2 (~750 ppm) (Figure 4.3a). The calorific value of *O. edulis* averaged 17.63 MJ/kgDW, irrespective of treatment (Figure 4.3b). For both species, the calorific value of oysters were significantly positively correlated with their lipid content) (Figure 4.3).

Table 4.6: Summary of two-way ANOVA results for the effects of pCO_2 and Temperature on the calorific content of *Magallana gigas* and *Ostrea edulis*. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded p-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas						***Ostrea edulis***				
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***
pCO_2	2	0.829	0.4145	1.62	0.3817		2	0.0632	0.031598	1.914	0.343
Temperature	1	2.4695	2.4695	9.65	0.0899	.	1	0.00895	0.008947	0.542	0.538
Residuals	2	0.5118	0.2559				2	0.03302	0.016512		

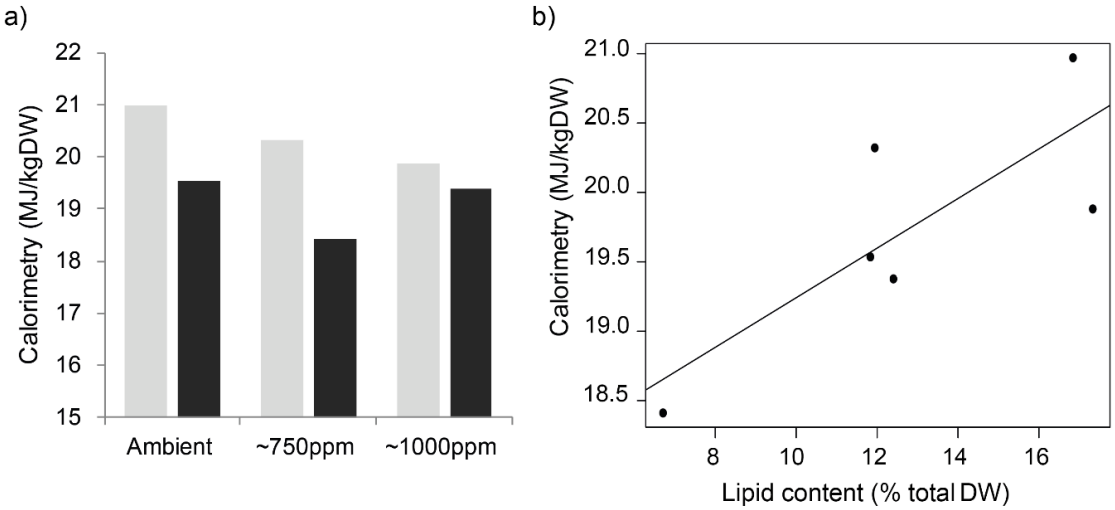

Figure 4.3: Variations in the calorific content of oysters across temperature and pCO_2 treatments (left), and relationship between lipid content (as % of total oyster DW) and calorific content of oysters (right), for a) *Magallana gigas* (cor=0.800; y=0.1787x +17.4540; R^2=0.6354; p<0.05) and b) *Ostrea edulis* (cor=0.812; y= 0.10869x +17.00149; R^2= 0.5742; p<0.05). ppm= part per million; Light grey = control temperature. Dark grey= elevated temperature. The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bar were obtained. J= Joules. Values are per kg of oyster dry weight (DW)

4.3.4 Trace elements

Results for the mineral composition are presented in Table 4.7 (composition) and Table 4.8 (ANOVA summary). Thereafter, results for the mineral content are as proportions of total wet weight. Marked differences in the mineral contents of oysters between treatments were apparent (Figure 4.5, Figure 4.6), despite lack of statistical significance (Table 4.7, Table 4.8; with the exception of K with pCO_2 in *O. edulis*, $p<0.05$).

Calcium (Ca)

There was a 55% decrease in Ca content in *M. gigas* at elevated pCO_2 levels (~750 ppm and ~1000 ppm), irrespective of temperature, compared to the ambient pCO_2 treatment. In *O. edulis*, Ca content increased but only at the most stressful treatment (elevated temperature and ~1000 ppm pCO_2) by ~37%.

Copper (Cu)

In *M. gigas*, compared to the control temperature and ambient pCO_2 treatment, Cu content increased by ~212% in the elevated temperature and ~750 ppm treatment, and by ~126% in the ~1000 ppm treatment, irrespective of temperature. Increased pCO_2 and elevated temperature led to a~45% decrease in *O. edulis* Cu content, but not synergistically.

Iron (Fe)

Iron content of *M. gigas* decreased with elevated temperature, but not synergistically with pCO_2, with a ~72% reduction between the control temperature and ambient pCO_2 treatment and all other treatments. In contrast, the effects of temperature and pCO_2 in *O. edulis* were more complex. While elevated temperature led to increases by ~15% and ~34% at ambient and ~750 ppm pCO_2 respectively, at control temperature, elevated pCO_2 led to reductions in Fe content by up to ~19%, and combined elevated temperature and ~1000 ppm pCO_2 led to further reductions of ~22%.

Potassium (K)

Potassium content in *M. gigas* followed a decreasing trend with increased stress from temperature and pCO_2, displaying a ~34% reduction between the control temperature and ambient pCO_2 treatment and the ~1000 ppm pCO_2 and elevated temperature treatment. Elevated temperature and pCO_2 led to reductions in K content of *O. edulis* by ~6%.

Magnesium (Mg) and Sodium (Na)

For both species, trends in Mg and Na contents were similar and showed complex changes with treatments. In *M. gigas*, elevated pCO_2 led to reductions in Mg and Na contents at the control temperature by up to ~35% and ~33% at ~750 ppm pCO_2, respectively. It is apparent that the effect of temperature was dependent on the pCO_2 level, with marked reductions at ambient pCO_2 (Mg: ~40%; Na: ~33%), important increases at ~750 ppm pCO_2 (Mg: ~43%; Na: ~77%), but without clear effects at ~1000 ppm pCO_2. In *O. edulis*, only limited changes in the contents of Mg and Na with increased pCO_2 at control temperature were apparent, increasing by ~17% and ~22%, between ambient and ~1000 ppm pCO_2 respectively.

Zinc (Zn)

Zinc content followed a similar trend to Cu in *M. gigas*, with a ~136% increase in the elevated temperature and ~750 ppm treatment, and a ~72% increase in the ~1000 ppm treatment, irrespective of temperature. In contrast, Zn content of *O. edulis* was negatively affected by the treatments, and decreased by ~42% under elevated temperature and by ~16% under elevated pCO_2. Interestingly, combined pCO_2 and temperature did not reduce Zn content further, but elevated pCO_2 seemed to attenuate the negative effect of temperature, with only a ~17% reduction.

Selenium (Se)

Elevated temperature and pCO_2 reduced Se content in *M. gigas* by up to ~46%. Similar trends but with smaller amplitudes were observed in *O. edulis*, with a ~27% reduction with temperature, and a ~6% reduction with elevated pCO_2 (indifferently of temperature).

Table 4.7: 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. WW= percentage of total wet weight. DW= percentage of total dry weight. 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	
		DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW
Ostrea edulis	Ambient x Control	6578.7	1567.2	823.7	196.2	203.3	48.4	1164.4	277.4	2291.7	546	15729.2	3747	4522.2	1077.3	1106.7	263.6
	~750ppm x Control	7079.2	1535.5	586	127.1	181.3	39.3	1167.8	253.3	2290.4	496.8	15629.5	3390	3978.5	862.9	1073.5	232.8
	~1000ppm x Control	5611.6	1353.5	427.1	103	174.2	42	1122.5	270.7	2640	636.7	18954.5	4571.7	3897.3	940	1045.3	252.1
	Ambient x Elevated	5647	1267.3	390.4	87.6	248.9	55.9	1147	257.4	2233.8	501.3	15611.1	3503.5	2779.5	623.8	855.7	192
	~750ppm x Elevated	7005.8	1560.6	498.9	111.1	290.7	64.8	1161.7	258.8	2422	539.5	17247.7	3842.1	3949.1	879.7	1149.8	256.1
	~1000ppm x Elevated	9084.3	2142.2	480.8	113.4	160.4	37.8	1094.3	258	2313.1	545.5	15573.8	3672.5	3808.6	898.1	1055.2	248.8
Magallana gigas	Ambient x Control	4371	1250	226.2	64.7	718.6	205.5	1126.5	322.1	2962.3	847.1	16178.3	4626.3	1574.5	450.3	1878.2	537.1
	~750ppm x Control	2182.2	492.8	265.8	60	158.1	35.7	1187.1	268.1	2450.7	553.5	13734.3	3101.7	1793	404.9	1386.5	313.1
	~1000ppm x Control	2577.8	618.1	621.3	149	225	54	1127	270.2	2822	676.7	15894.9	3811.5	3124.5	749.2	1917	459.7
	Ambient x Elevated	6924.2	1409.6	454.3	92.5	366.9	74.7	1076.6	219.2	2503.4	509.6	15282.9	3111.3	2784.1	566.8	1222.2	248.8
	~750ppm x Elevated	2416.2	544.5	896.4	202	255.9	57.7	1155.7	260.4	3521.5	793.6	24301.9	5476.6	4718	1063.2	1372.9	309.4
	~1000ppm x Elevated	3463.1	719.1	691	143.5	334.2	69.4	1031.4	214.2	2934.8	609.5	19113.1	3969.1	3844.2	798.3	1512.3	314

Table 4.8: Summary of two-way ANOVA results for the effects of pCO_2 and Temperature on the mineral content of *Magallana gigas* and *Ostrea edulis*. Ca=calcium; Cu=copper; Fe=Iron; K=potassium; Mg=magnesium; Na=sodium; Zn=zinc; Se=selenium. Df= degrees of freedom. SS= sum of squares. MS= mean squares. Bolded p-values denote significant effects at * ≤0.05, **≤0.01, ***≤0.001.

	Magallana gigas						***Ostrea edulis***					
	df	**SS**	**MS**	**F**	***p***		**df**	**SS**	**MS**	**F**	***p***	
Ca												
pCO_2	2	1.24E+07	6.21E+06	8.682	0.103		2	1.66E+06	8.28E+05	0.304	0.767	
Temperature	1	2.25E+06	2.25E+06	3.142	0.218		1	1.01E+06	1.01E+06	0.372	0.604	
Residuals	2	1.43E+06	7.15E+05				2	5.45E+06	2.73E+06			
Cu												
pCO_2	2	1.09E+05	5.45E+04	1.303	0.434		2	2.36E+04	1.18E+04	0.376	0.727	
Temperature	1	1.44E+05	1.44E+05	3.435	0.205		1	3.63E+04	3.63E+04	1.157	0.395	
Residuals	2	8.36E+04	4.18E+04				2	6.28E+04	3.14E+04			
Fe												
pCO_2	2	1.25E+05	6.24E+04	1.806	0.356		2	5.52E+03	2.76E+03	1.456	0.407	
Temperature	1	3.50E+03	3.50E+03	0.101	0.78		1	3.33E+03	3.33E+03	1.754	0.316	
Residuals	2	6.91E+04	3.46E+04				2	3.79E+03	1.90E+03			
K												
pCO_2	2	9.26E+03	4.63E+03	8.512	0.1051		2	3661	1830.4	29.882	**0.0324**	*
Temperature	1	5.22E+03	5.22E+03	9.597	0.0903	.	1	448	447.9	7.313	0.1139	
Residuals	2	1.09E+03	5.44E+02				2	123	61.3			
Mg												
pCO_2	2	6.46E+04	3.23E+04	0.108	0.902		2	4.60E+04	2.30E+04	0.866	0.536	
Temperature	1	8.96E+04	8.76E+04	0.293	0.642		1	1.07E+04	1.07E+04	0.402	0.591	
Residuals	2	5.97E+05	2.99E+05				2	5.31E+04	2.65E+04			
Na												
pCO_2	2	1.08E+07	5.42E+06	0.321	0.757		2	2.54E+06	1.27E+06	0.395	0.717	
Temperature	1	2.77E+07	2.77E+07	1.942	0.329		1	5.89E+05	5.89E+05	0.182	0.71	
Residuals	2	3.37E+07	1.69E+07				2	6.44E+06	3.22E+06			
Zn												
pCO_2	2	1.94E+06	9.71E+05	1.448	0.408		2	100694	50347	0.106	0.904	
Temperature	1	3.93E+06	3.93E+06	5.857	0.137		1	577148	577148	1.22	0.384	
Residuals	2	1.34E+06	6.71E+05				2	945761	472881			
Se												
pCO_2	2	1.12E+05	5.61E+04	1.071	0.483		2	17037	8518	0.569	0.637	
Temperature	1	1.92E+05	1.92E+05	3.671	0.195		1	4530	4530	0.303	0.637	
Residuals	2	1.05E+05	5.24E+04				2	29941	14970			

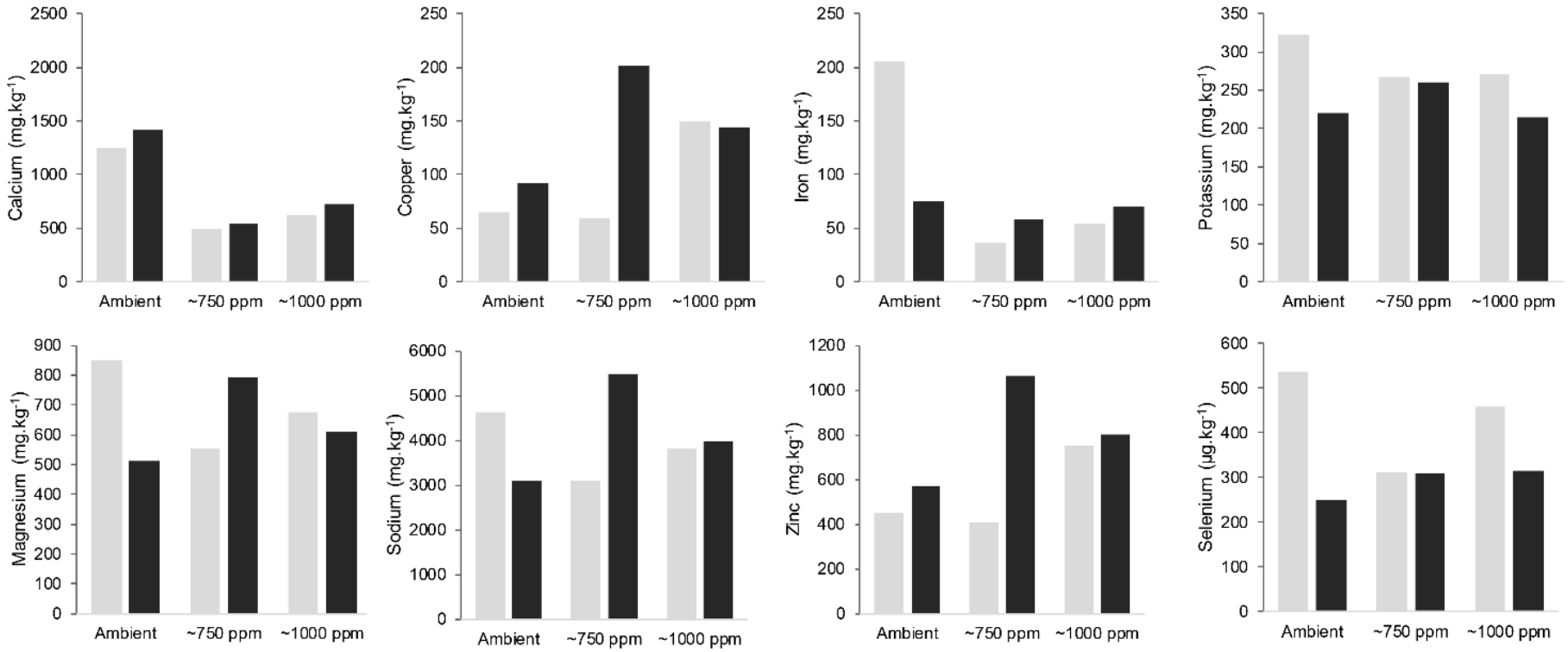

Figure 4.4: Variations in the mineral contents present in *Magallana gigas* across temperature and pCO_2 treatments. ppm= part per million; Light grey = control temperature. Dark grey= elevated temperature. The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bar were obtained. Values are per kg of oyster wet weight.

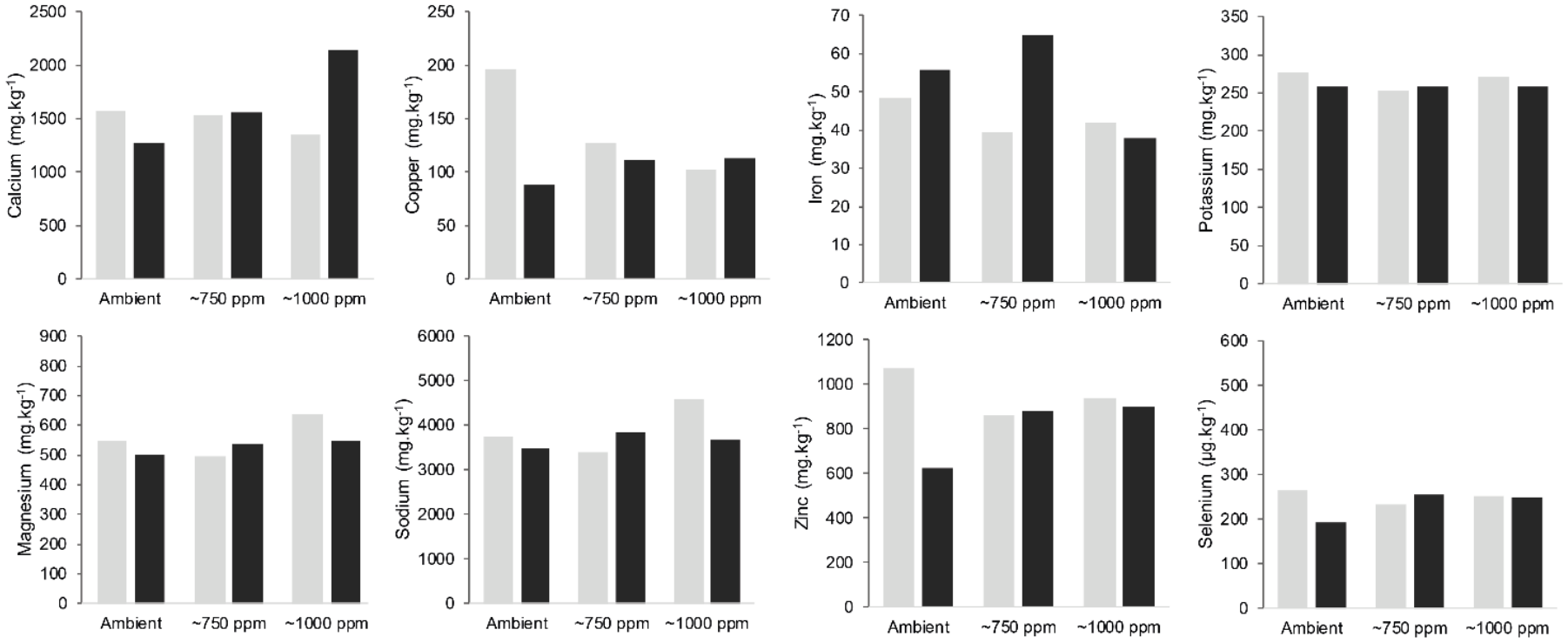

Figure 4.5: Variations in the mineral contents present in *Ostrea edulis* across temperature and pCO_2 treatments. ppm= part per million; Light grey = control temperature. Dark grey= elevated temperature. The value for each treatment represents the mean of the three procedural replicates of the pooled samples, therefore no error bar were obtained. Values are per kg of oyster wet weight.

4.4 Discussion

Societies' ability to feed the ever-growing human population is a major on-going concern, particularly as climate change is already negatively impacting food production from both terrestrial and marine environments (Brander *et al.*, 2017; UNEP, 2010). Mollusc aquaculture is increasingly recognised as a solution, not only due to the high nutritional value of marine molluscs, but also as the production cost is relatively low, especially when compared to other fish and shellfish species. Here, following exposure to temperature and pCO_2 levels predicted for 2050 and 2100, species-specific variations in the nutritional properties of two commercially important oyster species indicate impoverished nutritional quality. Both *O. edulis* and *M. gigas* underwent important changes to their biochemical composition, and particularly *M. gigas* contained lower lipid, carbohydrate, and protein levels, but higher contaminant concentration, which holds concerns for both future food security and future food safety.

4.4.1 Condition Index

Condition indices (CI) are widely used tools in aquaculture to evaluate the overall physiological status and health of bivalves, and are adopted in foreign trade as a criterion to select specimens of the highest quality (Knights, 2012; Orban *et al.*, 2006; Orban *et al.*, 2002). CI reflect the ability of bivalves to withstand stress (Marin *et al.*, 2003), and are correlated with the meat yield. Stressful environmental conditions requiring significant energetic expenditure result in low CI in bivalves (Orban *et al.*, 2002).

The CI data were previously presented and discussed in more detail in Chapter 2. But briefly, the CI of *M. gigas* was negatively impacted by elevated temperature but not elevated pCO_2, whereas the CI of *O. edulis* was unaffected by any of the treatment conditions, suggesting that the two species did not experience or respond to environmental stress in the same way. Chapter 2 demonstrated that the CI of the two oyster species reflected their respective physiological responses to OAW. Results showed *M. gigas* increased its metabolic rate under elevated temperatures – therefore its energetic expenditure – while its feeding rate was reduced under ~750 ppm pCO_2. In contrast, *O. edulis* did not appear importantly affected by OAW and was able to sustain its feeding rate. Declines in CI usually suggest

depletion of energy reserves, leading to changes in the biochemical composition and nutritional characteristics.

The proximate composition (moisture, ash, protein, lipid, carbohydrate) provide an overview of an organism's energetic resources, and along with the mineral composition, contribute to its nutritional value for consumption (see EFSA NDA Panel, 2014; Tate *et al.*, 2017).

4.4.2 Moisture content

Water constitutes the major part of oysters (Asha *et al.*, 2014), and is linked to juiciness, which is an important sensory trait of oysters and influences their acceptability (Cruz-Romero *et al.*, 2004). Here, moisture content averaged between 70-80% of total oyster content for both *M. gigas* and *O. edulis*, which are within the range of values found in the literature for bivalves (Asha *et al.*, 2014; Azpeitia *et al.*, 2017). The absence of change in the moisture content of either *M. gigas* or *O. edulis* recorded here when exposed to OAW conditions was also observed in *Turbo militaris* (Ab Lah, 2017). In contrast, the moisture content of the whelk *D. orbita* increased with elevated pCO_2 (at ambient temperature only) (Tate *et al.*, 2017) likely in response to changes in the proportion of lipid, protein and sodium.

4.4.3 Energetic reserves

By influencing organisms' physiology and metabolic responses, environmental conditions, and therefore OAW, can dictate the accumulation and depletion of energetic reserves in oysters. Protein, lipids, and carbohydrates constitute the main energy storages in bivalves, which all hold important physiological function, for instance towards gametogenesis and reproduction (Dridi *et al.*, 2007).

Carbohydrates

Carbohydrate, in the form of glycogen, is thought to be the energy reserve present in the highest quantity in bivalves and supplies energy to sustain routine metabolic processes (Anacleto *et al.*, 2014, and references therein). Here, the carbohydrate content of *M. gigas* was reduced under OAW, particularly at ~750 ppm pCO_2 and elevated temperature. Declines 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). Carbohydrate content was high in *O. edulis* (>8.5% wet weight) compared to *M. gigas* (<5% wet weight). A study by Anacleto *et al.,* (2014) on clams also found that the native species displayed higher glycogen content than invasive species, which was associated with a metabolic adaptive strategy to cope with environmental changes. Unlike in *M. gigas*, the carbohydrate content of *O. edulis* was not impacted by OAW, suggesting that it was not experiencing physiological stress and importantly using its energetic reserves. 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 their survival in the long term, which is already apparent from its reduced CI.

Proteins

Proteins supply structural elements and have a crucial role in the catalysis of metabolic reactions. Under stress, bivalves can use protein fractions once lipids and carbohydrates have been depleted (Barber & Blake, 1985). Given the importance of protein to the human diet and thus, an appropriate measure of food security, it is crucial to consider how protein content in seafood may change in the future. *M. gigas* and *O. edulis* were both high in protein with *M. gigas* yielding higher protein content – 11-17% of *M. gigas* wet mass; 8-10% of *O. edulis* wet mass. In both species, there was some decline in protein content under OAW conditions, but the relative protein content of *M. gigas* remained slightly higher than that of *O. edulis*. Decline in protein content under OAW has been previously recorded in the whelk *Dicathais orbita,* with a 54% reduction (Tate *et al.*, 2017). Given the reduced protein content in seafood under OAW, sustaining a level of protein supply needed as part of a healthy and nutritious human diet would require to harvest and provide seafood in greater quantities, which might become problematic for societies.

Lipids

The high amount of lipids in molluscs, low proportion of saturated fatty acids (SFA) and high proportion of polyunsaturated fatty acids (PUFA – including Ω-3), exert numerous health benefits (Sprague *et al.*, 2017). Here, *M. gigas* contained high levels of lipids (~1.4-4.8% wet weight), and correspond with the range reported elsewhere in the literature (Cochet *et al.*, 2015; Pogoda *et al.*, 2013;

Shpigel *et al.*, 1992). In contrast, *O. edulis* used in this study were relatively poor in crude lipids (~0.8-1.6% wet weight), and poorer than values reported elsewhere (Pogoda *et al.*, 2013).

OAW scenarios led to a decrease in lipid content in both species, although this effect was more pronounced in *M. gigas* (see also Pogoda *et al.*, 2013). Reductions were particularly notable at ~750 ppm pCO_2, for both species where lipids were reduced from 4.8% to 1.4% in *M. gigas*, and form 1.6% to 0.8% in *O. edulis*. Reductions in total lipid content and changes to fatty acid composition (including decreases in PUFA) under OAW have been recorded in other molluscs including *D. orbita* (Tate *et al.*, 2017; Valles-Regino *et al.*, 2015) and could be due to differential deposition and use of the various energy stores between the two species (Child & Laing, 1998; Pogoda *et al.*, 2013). Lipids are the most efficiently used energy source in oyster, providing higher energy for metabolic activities than the same amount of carbohydrates or proteins (Pogoda *et al.*, 2013), and is highlighted here by the positive relationship between lipid content and calorific content.

Although carbohydrates, such as glycogen, are argued to generally be the preferred source of energy for oysters, species-specific preferences may exist (see discussion in Pogoda *et al.*, 2013). Previously, it has been suggested that *O. edulis* preferentially use lipids whereas *M. gigas* use proteins as their principal energy source for metabolic activity when subjected to food limitation (Child & Laing, 1998). Here, both oyster species when exposed to OAW readily used lipids and carbohydrates as energetic reserves, but to a lesser extent by *O. edulis*. Depletions of energetic reserves were indeed particularly apparent for *M. gigas*, with additional reductions in proteins reflected in the reduced CI and calorific content, especially at intermediate pCO_2 level. The differential use of energetic reserves by oysters is therefore likely a consequence of the differential physiological stress endured when exposed to OAW conditions (detailed in Chapter 2). Given the importance of protein, lipid, and carbohydrate in human diet, changes in their proportions under OAW will alter the nutritional value of *M. gigas* in the future, more so than for *O. edulis*.

4.4.4 Inorganic mineral composition

Seafood quality also varies based on the proportion (total ash) and composition (essential macro- and micro-minerals) of inorganic minerals. Minerals are an essential component of a healthy diet in humans (EFSA NDA Panel, 2014). Here, the two oyster species displayed similar ash content (~1.9-2.6%), which importantly, was unimpacted by OAW. In fact, a modest increase in ash content in *O. edulis* under elevated pCO_2 might indicate a process of mineral accumulation within the tissue, further discussed below.

Although nutritionists used to focus on macronutrients – such as Ca and Mg which are beneficial for teeth and bones (Lambert *et al.*, 2017) – there is now an increased understanding of the key dietary benefits of micronutrients (vitamins and trace minerals) (FAO, 2016). For instance, K-rich foods are considered particularly healthy, Se promotes the immune system and reduces oxidative stress in tissue (Rayman, 2000), and Zn and Fe are critical for stamina and disease resistance (Knez *et al.*, 2017; Solomons & Schümann, 2017). Moreover, micronutrient deficiencies afflict an enormous proportion of the population. For instance, over 2 billion people are diagnosed as iron-deficient, and an estimated 800 000 children die every year from zinc deficiency (FAO, 2016).

In this study, large differences in the quantity of macro and micro-nutrients between *M. gigas* and *O. edulis* were evident. Notably, *M. gigas* exposed to the current climate scenario were high in Mg, Fe, and Se when compared to *O. edulis*. In contrast, although relatively poor in those minerals, *O. edulis* had a high Zn content. The values presented here for macro-minerals (Na, K, Mg, Ca) are similar to those of Orban *et al.*, (2004) for *M. gigas* in the lagoon of Venice. Similarly, values for some of the micro-minerals (Fe, Se) were within the range described for other oyster species (Se: Cantillo, 1998; Fe: Diaz Rizo *et al.*, 2010; Fe, Se: Orban *et al.*, 2004). However, concentrations of Cu and Zn in this study were significantly higher than commonly found in literature (Cantillo, 1998). Copper in the marine environment if often derived from antifouling paints, while zinc can originate from anti-corrosion coatings and urban road run-off (tyre wear) (Councell *et al.,* 2004). High Cu and Zn contents have been described for oysters living in contaminated locations associated with mining and harbour activities (Diaz Rizo *et al.*, 2010; Frias-Espericueta *et al.*, 2009). In this instance, the high

concentrations may be explained by the fact that Plymouth Sound – the location of oyster collection for this study – is known for being a contaminated site due to the region's important mining history (Knights *et al.*, 2016), with Cu and Zn levels reaching 13-200 $\mu g.L^{-1}$ and 13-910 $\mu g.L^{-1}$, respectively, in pore waters (Langston *et al.*, 2003).

Here, exposure to OAW conditions led to species-specific changes in the concentration of those minerals, which hold both nutritional and safety implications. Indeed, while some trace minerals are considered essential for invertebrates physiological functioning, others need to be excreted to avoid toxic effects from accumulation (see Bray *et al.*, 2015). In particular, the reductions in Ca, Fe, and Se content in *M. gigas* exposed to OAW to levels similar or lower than *O. edulis* represent an impoverishment of its nutritional value. Additionally, although present in high quantities in both oyster species, the accumulation of Cu in *M. gigas,* in particular under OAW, constitutes a food safety concern for future production. Other trace metals, such as arsenic, copper and lead, can become toxic in high concentrations. Since bivalves are filter feeders, they readily accumulate metals present in the surrounding waters into their edible tissue (Bray *et al.*, 2015; Lu *et al.*, 2017; Raposo *et al.*, 2009). This process can be modulated by ocean acidification, for instance, enhancing the bioaccumulation of Cu and cadmium in oysters (Belivermiş *et al.*, 2015; Götze *et al.*, 2014; Hawkins & Sokolova, 2017; Ivanina *et al.*, 2015; Ivanina *et al.*, 2016). If accumulation of metals reaches levels beyond the accepted thresholds, the safety of seafood consumption may be threatened (Lu *et al.*, 2017).

4.4.5 Implications for food security and aquaculture management

The results suggest that oysters are likely to become less nutritious in the near future, with reduced proteins, lipids, energetic value, as well as changes to their essential mineral contents.

Islands and countries with little agricultural land rely on wild-caught seafood and aquaculture for their protein intakes, and should these changes become widespread, then the nutritional benefits of seafood as food and for human health may be compromised (Cooley *et al.*, 2012; Ding *et al.*, 2017). Given the need to look for additional and sustainable sources of proteins, an expansion and re-evaluation of the aquaculture industry appear inevitable. Diversifying the target species and

promoting those currently under-utilized may supplement the industry with 'novel' sources of protein. In order to optimise the protein supply and secure socio-economic benefits of mollusc aquaculture, research need to focus on selecting aquaculture species that are resilient to future climate conditions, and able to retain their beneficial nutritional properties (Cooley *et al.*, 2012).

For western populations who rely more heavily on land agriculture for their source of protein, a reduction in the nutritional quality of oysters may not quickly be recognised by consumers, but may pose a significant economic threat to the UK aquaculture industry. *Magallana gigas* and *O. edulis* are two important aquaculture species in the UK, although *M. gigas* currently represents >90% of the production (Humphreys *et al.*, 2014). Our results suggest *M. gigas* is at higher risk of reduction in nutritional quality than its native counterpart *O. edulis* under future OAW scenarios. Because the biochemical composition can also dictate meat appearance, aroma, taste and texture (Cochet *et al.*, 2015; Fratini *et al.*, 2013), any changes occurring because of OAW can impact on the sensory quality (Lemasson *et al.*, 2017b, investigated in Chapter 5), and therefore influence the consumer appeal for the product, reducing the demand for it and depressing its economic value (Cooley *et al.*, 2012). These findings might therefore hold important implications for the UK aquaculture industry, who 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.

Chapter 5: Sensory qualities of oysters unaltered by a short exposure to combined elevated pCO_2 and temperature

"Every cloud has a silver lining"

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5 Sensory qualities of oysters unaltered by a short exposure to combined elevated pCO_2 and temperature

Abstract:

Reliance on the marine environment for the provision of food is ever-increasing, but future climate change threatens production. Despite this concern, the impact on seafood quality and success of the seafood industry is unknown. Using a short-term study, we test these concerns using a major aquaculture species – *Magallana gigas* – exposing them to three acidification and warming scenarios: 1) ambient pCO_2 (~400 ppm) & control temperature (15°C) 2) ambient pCO_2 (~400 ppm) & elevated temperature (20°C), 3) elevated pCO_2 (~1000 ppm) & elevated temperature (20°C). Oyster quality was assessed by scoring appearance, aroma, taste, and overall acceptability. A panel of five experts was asked to score nine oysters – three from each treatment – according to agreed criteria. Results indicate that these levels of acidification and warming did not significantly alter the sensory properties of *M. gigas*, and notably the overall acceptability remained unchanged. Non-statistically supported trends suggest that several sensory attributes – *opacity, mouthfeel, aspect of meat, shininess, meat resistance, meat texture,* and *creamines*s – may improve under acidification and warming scenarios. These findings can be considered positive for the future of the aquaculture and food sectors. From this chapter, it appears that *Magallana gigas* therefore can be expected to remain a key species for food security that can retain its valuable attributes under climate change, although this seems to contradict the physiological and ecological findings of Chapters 2-4.

5.1 Introduction

Seafood represents a significant contribution to the provision of animal protein globally – approximatively 17% of the global population animal protein intake in 2013 was from fish consumption (FAO, 2016). If the world population is to reach between 9.6 and 12.3 billion by the year 2100 as predicted (Gerland *et al.*, 2014), demand for animal protein is unlikely to be met by terrestrial farming. Instead, increasing reliance on the marine environment for the provision of proteins and other products is expected (Cooley *et al.*, 2012; Delgado, 2003).

The 'Blue Revolution' – the emergence of aquaculture as an important alternative to meat production from land agricultural activity – is thought by many to be a solution to securing food provision in the future (Barange *et al.*, 2014; Naylor *et al.*, 2000; Tacon & Metian, 2013). In 2015, total aquaculture production volume was estimated at over 106 million tonnes and valued in excess of US$162.9 billion; ~15% of this total is attributed to molluscan aquaculture (over 16 million tonnes; worth over US$18 billion) (FAO data[1]). In comparison to other seafood species, the production cost of many molluscs is relatively low, making them ideal for aquaculture, yet several species are often seen as luxury items (e.g. mussels, scallops, and oysters) and attract high market values. However, demographic characteristics, social representation, and psychological aspects play important roles in consumer perception and selection of luxury products (Debucquet *et al.*, 2012), and any negative perceptions can lead to products being ignored by consumers (Danenberg & Mueller, 2011; Kow *et al.*, 2008; Liu *et al.*, 2006).

Due to pressure on natural fish stocks from overfishing and habitat destruction (Macura *et al.*, 2016; McCauley *et al.*, 2015; Molfese *et al.*, 2014), a switch to more sustainable aquaculture practices, such as mollusc production, is timely. However, there is increasing concern over the fate of seafood harvesting and production under future climate scenarios (Cooley *et al.*, 2015; Ekstrom *et al.*, 2015; Lloret *et al.*, 2016). To date, the main scientific focus has been on the biomass produced, with much of the evidence suggesting aquaculture production will be negatively impacted by climate change due to increased larvae and juvenile mortality, and changes in shell development and calcification rates (Guo *et al.*, 2015; Hettinger *et al.*, 2012). Yet environmental stressors, such as acidification and warming, can also influence the physiology of marine organisms and in turn affect the meat quality (Borderias & Sanchez-Alonso, 2011), which is an important measure of seafood quality and a major trait that determines consumer choice. The sustainability of the industry is, in part, driven by consumer demand. It is therefore crucial to detect the potential factors (e.g. seafood quality) that may negatively or positively affect public perception and consumer choice regarding molluscs, such that the aquaculture sector is better positioned to make informed decisions regarding the choice of cultured species and respond to consumer demand.

[1]http://www.fao.org/figis/

Seafood products are known for their high nutritional value, and are key to human health and well-being (Lloret *et al.*, 2016). Oysters, especially, are consumed around the world and are a good example of highly nutritious seafood. They are considered a delicacy in many developed countries. For instance in France, oysters are a festive delicacy consumed on Christmas Day and New Year's Day (Debucquet *et al.*, 2012). In the UK, they are also associated with special occasions, and are the central point of several seasonal events (e.g. Whitstable Oyster Festival, Falmouth Oyster Festival, North East Oyster Festival). It was estimated that in 2015 throughout the globe, over 5 million tonnes of oysters were produced, worth in excess of US$4 billion, of which 1600 tonnes was produced in the UK, worth in excess of US$6 million FAO data[1]. The biological effects of ocean acidification and warming on individuals and the production industry are already well documented (Lemasson *et al.*, 2017a), with several hatcheries experiencing significant decline in production and considerable economic losses (Barton *et al.*, 2012; Barton *et al.*, 2015). However, less attention has been given to the quality of shellfish, including aesthetic aspects, taste, and nutritional properties, despite being a critical factor in consumer's food choice (Lee *et al.*, 2008). To date, only one study has looked at the effects of ocean acidification on the eating quality of seafood, and showed that future climate scenarios negatively affect the quality of northern shrimps (*Pandalus borealis*) by altering their sensory quality (taste and texture) (Dupont *et al.*, 2014). Given that the physiology of adult oysters is negatively impacted by warming and acidification (Scanes *et al.*, 2017, but see Chapter 2), it is possible that their sensory quality will reflect such changes.

The quality of a food product can be assessed using a number of measurable parameters, such as Condition Index (Orban *et al.*, 2006), chemical composition and microbial growth (Aaraas *et al.*, 2004; Cao *et al.*, 2009; Khan *et al.*, 2005), but is also linked to sensory parameters that are essential to consumer preference (Brennan & Kuri, 2002). Sensory parameters, such as appearance, aroma, and taste, have been used to assess seafood freshness, shelf-life and acceptability (Azpeitia *et al.*, 2017; Liu *et al.*, 2010; Šimat *et al.*, 2012), and can be assessed using descriptive sensory analysis (Lawless & Heymann, 2010; Stone *et al.*, 2004). This technique is considered an accurate quality predictor (Chang *et al.*, 1998; Reineccius, 1991), and has been used extensively for oyster quality assessments (Aaraas *et*

al., 2004; Buzin *et al.*, 2011; Cao *et al.*, 2009; Cochet *et al.*, 2015), but requires the use of trained or expert panellists to conduct the assessment (Lawless & Heymann, 2010; Stone *et al.*, 2004).

Here, we evaluate changes to the sensory properties of the Pacific oyster *Magallana gigas* using descriptive sensory analysis, to determine the effects of exposure to acidification and warming conditions on consumer perception.

5.2 Methods

5.2.1 Experimental design and set-up

Here, Pacific oysters (*Magallana gigas*) under two years of age and of UK market size (10-12cm long and 80-100g) were obtained from Menai Oysters and Mussels Ltd. (Llanfairpwllgwyngyll, Wales, UK) and exposed to ocean acidification and warming scenarios predicted for the UK coastline for 2100 (temperature estimates based on the medium emission scenario IPCC SRES A1B and UKCP09 predictions[2]; pCO_2 estimates based on predictions by the Marine Ecosystem Evolution in a Changing Environment (MEECE)[3] for 2080-2099). Two levels of pCO_2 (ambient ~400 ppm, elevated ~1000 ppm) and two temperatures (control 15°C, elevated 20°C) were used to create three distinct climate scenarios (Figure 5.1). The first treatment, hereafter referred to as AC_T, replicated the current climate (ambient pCO_2 & control temperature). The other two treatments corresponded to future scenarios of ocean warming and acidification; one simulating warming alone, hereafter referred to as AE_T (ambient pCO_2 & elevated temperature), due to local geological conditions allowing the buffering of acidification (such as the occurrence of limestone), and the other simulating acidification and warming simultaneously, hereafter referred to as EE_T (elevated pCO_2 & elevated temperature) (Humphreys, 2016). No 'acidification alone' treatment was used, as it does not reflect a predicted (realistic) scenario for the region (see discussions in Humphreys, 2016; Reum *et al.*, 2015).

Three replicate tanks (33x20x19cm) filled with 9L of filtered seawater were established per treatment, with each tank containing five randomly allocated individual oysters of similar age and size class.

[2] http://ukclimateprojections.metoffice.gov.uk/23223
[3] http://www.meece.eu/datasets.html

Oysters were exposed to treatment conditions for 5 days, as longer exposure duration while unfed would have caused physiological stress from starvation and confounded the effects of the treatment. Each replicate tank was cleaned and a third of the water partially renewed daily, with either seawater or deionized water as needed to maintain stable salinity levels. Oxygen concentration was monitored to insure adequate aeration (air saturation >90%). Throughout the duration of the experiment, oysters were subjected to a 12h light/12h darkness cycle. Oysters were not fed, so as not to alter the natural taste of the Menai variety attributed to the site-specific seawater characteristics (Krijnen, S. Menai Oysters and Mussels Ltd. pers. communication).

Replicate tanks were aerated with either ambient air (pCO_2 ~400 ppm) or CO_2-enriched air (pCO_2 ~1000 ppm). CO_2-enriched air was created by a controlled release of CO_2 into a Buchner flask using a multistage CO_2 regulator (EN ISO 7291; GCE, Worksop, UK) and mixed with ambient air until a concentration of ~1000 ppm pCO_2 was achieved. The CO_2 level in the ambient and CO_2-enriched pipes were recorded using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA). Tanks were maintained at control (~15°C) and elevated (20°C) temperatures using controlled-temperature rooms.

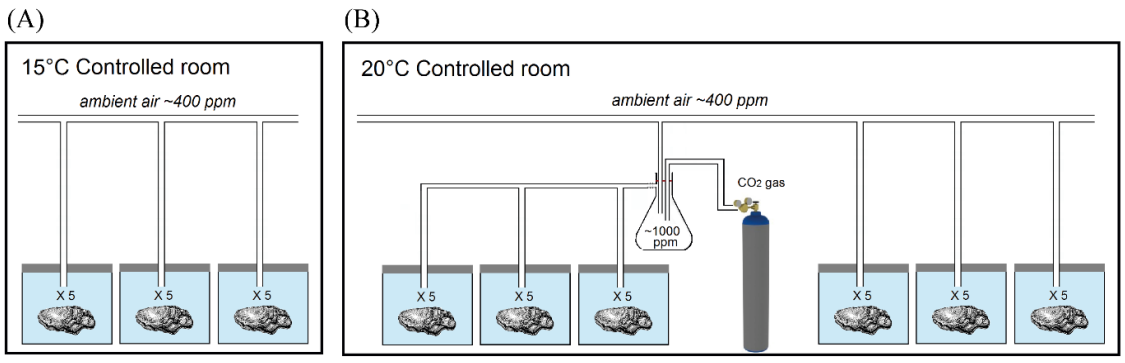


Figure 5.1: Experimental design used to expose oysters to three acidification and warming treatments. (A) temperature-controlled room kept at 15°C and holding oysters supplied with ambient air at ~400 ppm. (B) temperature-controlled room kept at 20°C and holding oysters supplied with ambient air at ~400 ppm and oysters supplied with ~1000 ppm pCO_2. Each tank contained 5 oysters, one for each of the 5 panellists, such that there were 15 oysters per experimental treatment, and 9 oysters per panellist.

5.2.2 Measurements of seawater parameters

At least once a day throughout the exposure duration, temperature, salinity, and pH were measured in all replicate tanks (Table 5.1.). Salinity was measured using a handheld refractometer (D&D The Aquarium Solution Ltd, Ilford, UK). Temperature was 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) after calibration with NIST traceable buffers. 125 mL water samples were taken once on day 5 for Total Alkalinity (A_T) measurements from each of the replicate tanks, and directly analysed by automatic Gran titration (Titralab AT1000 © Hach Company/Hach Lange GmbH). Partial pressure of carbon dioxide (pCO_2), and saturation states of calcite and aragonite (Ωca and Ωar), were calculated at the end of the experiment (Table 5.1.), 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 5.1: Physical and chemical characteristics of seawater in the three experimental treatments for *Magallana gigas* (presented as mean values over the duration of the experiment ± standard deviation (s.d.)). The ambient pCO_2 was set at ~400 ppm, the elevated pCO_2 at ~1000 ppm. Control temperature was set at 15°C, the elevated temperature at 20°C. T= Temperature (°C); A_T= Total Alkalinity (μmol/kg SW); S= Salinity; Ωar= saturation state of aragonite, Ωca= saturation state of calcite.

Treatment (pCO_2 × Temperature)	Measured				Calculated		
	pH	T	A_T	S	pCO_2	Ωca	Ωar
Ambient × Control	7.94 ±0.01	15.07 ±0.16	1797.31 ±0.02	33.56 ±0.18	349.65 ±5.45	2.76 ±0.03	1.77 ±0.02
Ambient × Elevated	7.91 ±0.01	19.39 ±0.10	1689.02 ±0.01	33.06 ±0.21	396.66 ±7.80	2.52 ±0.03	1.63 ±0.02
Elevated × Elevated	7.63 ±0.01	20.29 ±0.16	1737.63 ±0.03	33.17 ±0.22	949.21 ±44.61	1.37 ±0.05	0.89 ±0.03

5.2.3 Development of sensory terms and evaluation method

This study was carried out in accordance with the recommendations of the University of Plymouth's policy on the Ethical Principles for Research Involving Human Participants, and approved by the University of Plymouth Research Ethics Committee, with written informed consent from all subjects.

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Plymouth Research Ethics Committee. A sensory panel was selected according to criteria IV.4.1.2 (Codex Standards, 1999) and consisted of five panellists (A-E), each of them expert in the field of food sensory evaluation, oyster production, or oyster meroir – the effect of seawater environment on the taste and texture of oysters – and was trained during group sessions following standard methods (ISO 6658, 1985). A focus group activity aimed to: (i) develop the attributes and descriptors to be used in the descriptive sensory analysis, (ii) obtain a consensus on their definition, and (iii) develop a standardised method of evaluation. The panellists agreed on three major categories of attributes: *Appearance*, *Aroma*, and *Degustation*. Each main category comprised several attributes (see Table 5.2.), whilst *Overall Acceptability* was also chosen as a key attribute, adding up to 17 attributes in total. The panel agreed on the following method of evaluation. First, the appearance of the meat was visually assessed and scores for attributes including *shell-to-meat ratio*, *aspect*, *shininess*, and *opacity* of the meat were recorded. Then, the oyster (in its shell and liquor) was brought to the nose to assess the aroma, *with liquor* and *without liquor*. Finally, the degustation was assessed in four steps: liquor (*saltiness* and *depth*), meat texture (*resistance, juiciness, texture)*, meat taste and flavour *(sweetness, creaminess, meatiness*), and aftertaste (*aftertaste intensity* and *mouthfeel*). Panellists were required to score each attribute using a 4 or 5-point scale semantically anchored with descriptors on the extremes and the midpoint. Each oyster was then finally scored for overall acceptability (likeness) using a hedonic scale (Table 5.2.). The scales were linked to demerit scales ranging from 0 (optimum score) to 3 (least favourable score) (Table 5.2.).

Table 5.2: Assessment categories and attributes selected during the focus session and used for the sensory analysis, along with the extreme descriptors. Each category was scored on a seven-point scale (0; 0.5; 1; 1.5; 2; 2.5; 3) except for "*Overall acceptability*" which was on a five-point hedonic scale. (Optimum, scored 0; Least desirable, scored 3).

Category	Sub-Category	Attribute	Optimum Descriptor (Score 0)	Least Desirable Descriptor (Score 3)
Appearance	**Meat**	*Shell to meat ratio*	Perfect shell:meat ratio (50:50)	Too much/too little meat
		Aspect of meat	Very plump	Deflated/not plump
		Shininess of meat	Shiny and bright coloured	Dull
		Opacity of meat	Very opaque	Very translucent
Aroma	-	*With liquor*	Strong sea breeze aroma	Ammonia/hydrogen sulphide
		Without liquor	Strong earthy/beachy aroma	Ammonia/hydrogen sulphide
Degustation	**Liquor**	*Liquor saltiness*	Good/acceptable saltiness	Too salty/Too bland
		Liquor depth/breadth	Good/acceptable intensity/depth	Too intense/Not flavoured
	Meat Texture	*Resistance of first bite*	Good resistance/acceptable 'bite'	Too resistant and hard to chew/too mushy
		Juiciness after first bite	Very juicy	Very dry
		Texture	Gelatinous/good 'bite'	Fibrous
	Taste and Flavour	*Sweetness*	Very sweet	No sweetness
		Creaminess	Very creamy	No creaminess
		Meatiness	Very meaty	No meatiness
	After-taste and Mouthfeel	*After-taste*	Very intense and lingering	Not perceptible/Quickly fading
		Mouthfeel	No perceptible bitterness	Very bitter
Overall acceptability	-	*Likeness*	Very pleasant	Very unpleasant

5.2.4 Sample preparation and presentation

All oysters were blind-coded by assigning them a randomly generated 3-digit number. At the end of the exposure period, oysters were brought to the Food and Nutrition Unit at Plymouth University, and prepared for tasting by the same highly experienced practitioner. They were manually shucked by cutting the adductor muscle with an oyster knife after 1h at room temperature. The meat was left inside the shell, with the liquor, taking care to avoid spillage. The oysters were individually labelled and presented to each panellist on aluminium trays. Each panellist (5) was presented with an oyster from each replicate tank (9 oysters in total per panellist; see Figure 5.1). The order of presentation

was randomized across panellists. The assessments were carried out in individual partitioned booths, as recommended in standard methods (ISO 6658, 1985).

5.2.5 Statistical analyses

Data for each attribute were analysed using two-way nested Analysis of Variance, with "Treatment" as a fixed factor, and "Panellist" as a random factor nested within "Treatment". This type of model is commonly used for analysis of sensory studies data (Aaraas *et al.*, 2004; Buzin *et al.*, 2011; Cochet *et al.*, 2015). Homogeneity of variance was checked using Cochran's *C* test. When data did not meet this assumption, they were transformed using ln(x+1). Where significant differences were present, *post-hoc* Student-Newman-Keuls (SNK) multiple comparison of means was performed to determine which treatment levels differed. All data were analysed using the GMAV 5 software (University of Sydney, Australia).

5.3 Results

The sensory attributes of oysters were not significantly impacted by the treatment alone ($p>0.05$ in all instances) (Figure 5.2 and Figure 5.3). However, there were significant differences in the scores allocated to five of the attributes between panellists within treatment levels: *shell-to-meat ratio* ($F_{12,44} = 3.39$; $p<0.001$), *opacity* ($F_{12,44} = 2.10$; $p<0.05$), *aroma without liquor* ($F_{12,44} = 2.54$; $p<0.05$), *aftertaste* ($F_{12,44} = 3.01$; $p<0.01$), and *mouthfeel* ($F_{12,44} = 4.41$; $p<0.001$) (Figure 5.4).

While panellists agreed on the *shell-to-meat ratio* score in the AE_T treatment, there were large variations in scoring between panellists in the AC_T and EE_T treatments (Figure 5.4). Moreover, panellists agreed on the *opacity of the meat* score for oysters in the AC_T and AE_T treatments, whereas there were large variations in scoring in the EE_T treatment. There was an apparent improving trend in the *opacity* of oysters with stressful treatment (as depicted by a decreasing average score from ~1 (somewhat *opaque)* to ~0.5 *(very opaque),* although this was not statistically significant (Figure 5.4). One panellist scored the *aroma without liquor* differently than all four others in the AC_T treatment, but they all agreed on scores in the AE_T and EE_T treatments. There was an apparent trend for worsened aroma with stressful treatment, as depicted by an increasing average score from ~1.2 (distinctive earthy/beachy

aroma) to ~1.5 (lack of aroma), albeit not statistically significant (Figure 5.4). One panellist also scored the *aftertaste* differently than the other assessors in the AC_T and AE_T treatments, whereas no differences between panellists were observed in the EE_T treatment. Finally, there were significant differences in the scoring of the *mouthfeel* between panellists across all three treatments. *Mouthfeel* scores followed an apparent improving trend with stressful treatment, from ~1.1 (neutral/pleasant) to ~0.7 (very pleasant), although this trend was not statistically significant (Figure 5.4).

Several other attributes appeared to be improving (decreasing score) with the level of stress applied, albeit not in a statistically significant manner, including *aspect of meat*, *shininess*, m*eat resistance*, *meat texture*, and c*reaminess* (Figure 5.2).

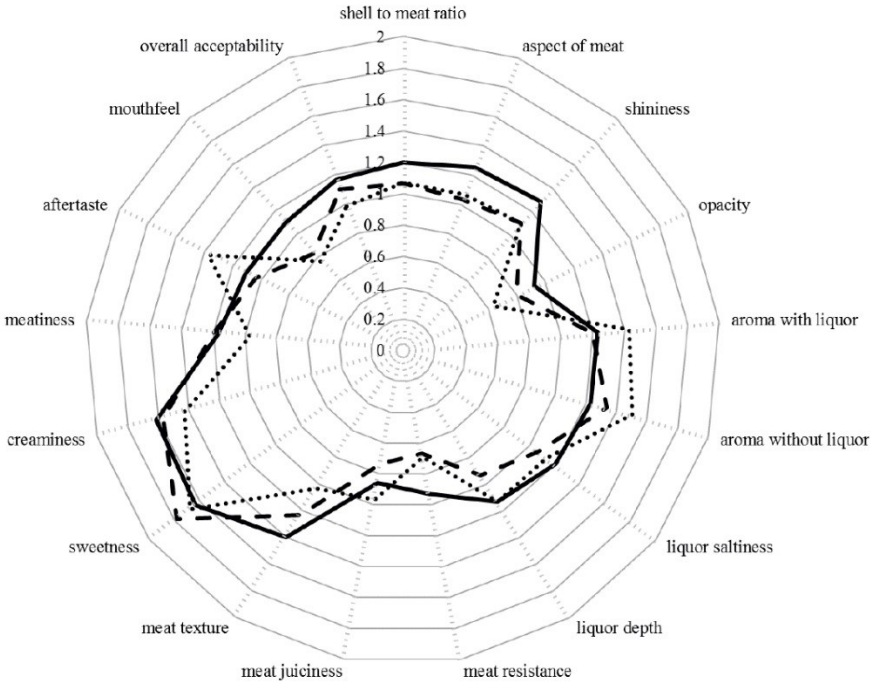


Figure 5.2: Mean score attributed by the panellists to each of the 17 oyster attributes (n = 9 oysters per treatment). Continuous line: Ambient pCO_2 & control temperature; Dashed line: Ambient pCO_2 & elevated temperature; Dotted line: Elevated pCO_2 & elevated temperature. The scales ranged from 0 (optimum score 0) to 3 (least favourable score).

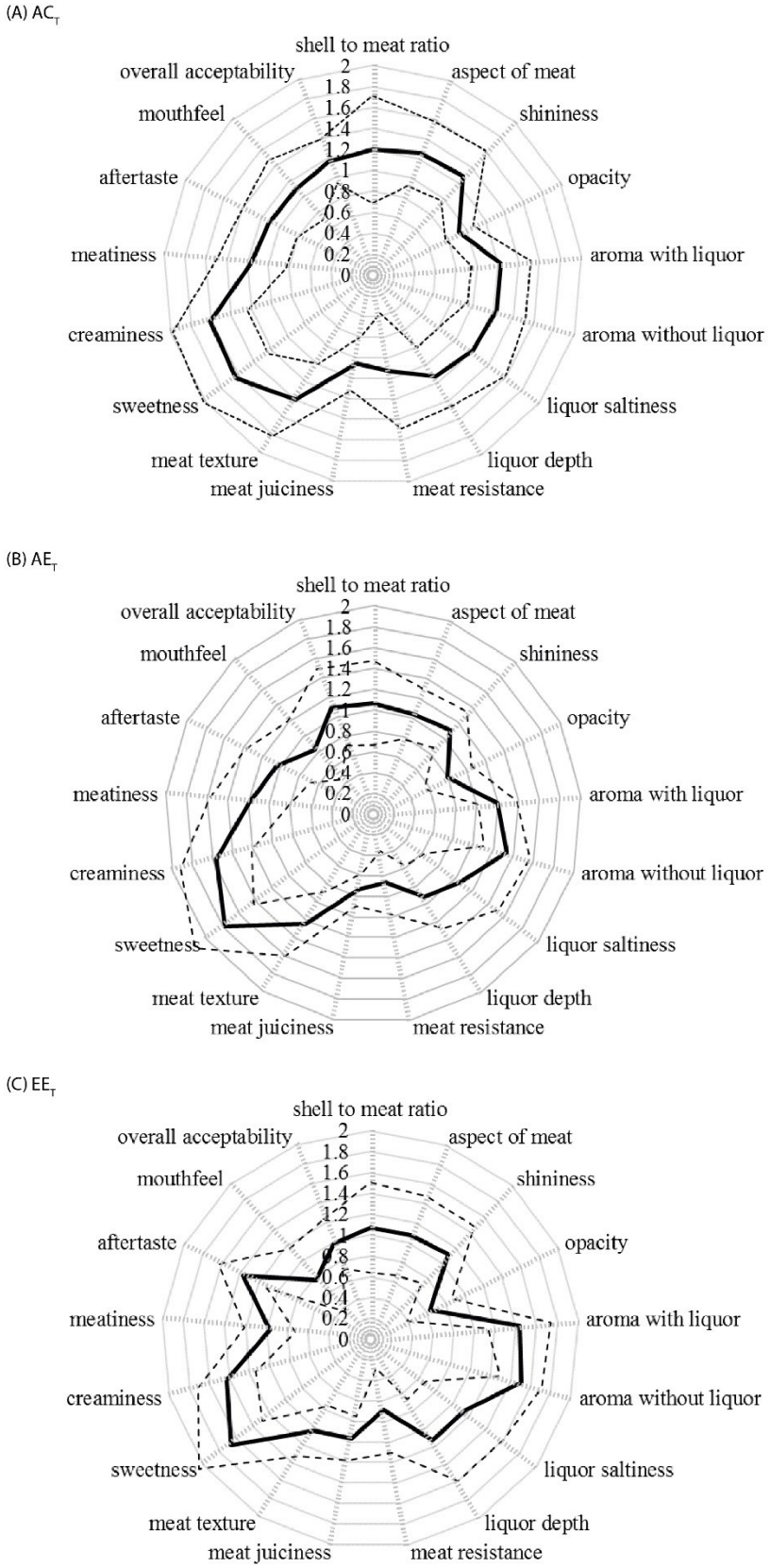


Figure 5.3: Score attributed by the panellists to each of the 17 oyster attributes. (A) Ambient pCO_2 & control temperature (AC_T), (B) Ambient pCO_2 & elevated temperature (AE_T), (C) Elevated pCO_2 & elevated temperature (EE_T). Mean (n=9) scores (continuous line) with confidence interval (dotted lines), on scales ranging from 0 (optimum score) to 3 (least favourable score).

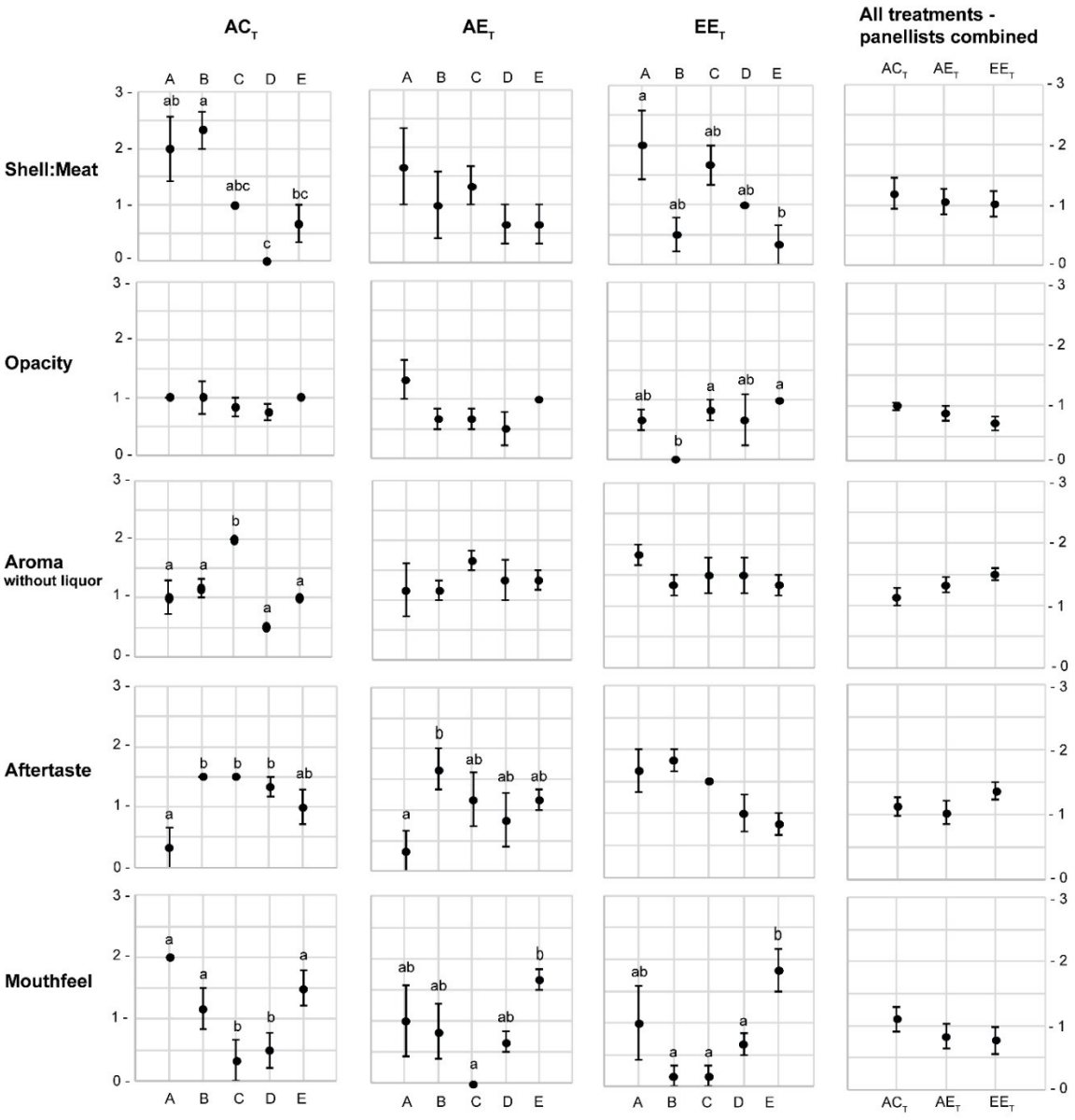


Figure 5.4: Mean score (± S.E.) of the attributes that displayed significant interaction between 'panellist' and 'treatment'. Letters A -- E represent the five panellists. AC_T = ambient pCO_2 and control temperature; AE_T = ambient pCO_2 and control temperature; EE_T = elevated pCO_2 and elevated temperature. All attribute scores ranged from 0 to 3 (0 representing the optimum score, and 3 the least favourable score). Treatment levels that do not share a letter are significantly different.

5.4 Discussion

We investigated whether the sensory quality of Pacific oyster *Magallana gigas* would be affected by short-term exposure to seawater acidification and warming conditions. Sensory and cognitive perceptions have been recognised as key elements dictating consumer's attitudes towards seafood (Carlucci *et al.*, 2015) and changes in environmental conditions have been predicted to negatively alter their taste and quality as a result of physiological stress (Borderias & Sanchez-Alonso, 2011; Dupont *et al.*, 2014). Our work revealed that acidification and warming conditions did not significantly alter the sensory properties of *M. gigas* after a short exposure. The mean scores attributed by the panel to each of the 17 attributes was neither statistically positively nor negatively affected by the treatments. Notably, the overall acceptability of oysters remained unchanged to a panel of seafood experts, with mean scores between 1 and 1.2, which corresponded to 'pleasant'. Moreover, non-statistically supported trends suggest that several sensory attributes may improve under acidification and warming scenarios.

Admittedly, the growth phase of oysters is much longer than the exposure duration used in this study, and previous environmental history during growth can have an impact on the final product characteristics. Physiological stress arising from changes in environmental conditions has been shown to reduce the quality and affect the sensory properties in other seafood species through physical, morphological, and biochemical ways (Borderias & Sanchez-Alonso, 2011; Dupont *et al.*, 2014). Many species take months to acclimate to stressful conditions, if they acclimate at all, and thus it is possible that changes to the taste and sensory properties can only be discerned during that initial acclimation period. Although no changes were found in this relatively short exposure study, acidification and warming has been shown to induce important physiological stress in oysters over longer exposure duration (Scanes *et al.*, 2017, see Chapter 2). While we concede that this limited exposure may not be sufficient to induce significant long-term physiological changes, some mechanisms of physiological responses, such as protein synthesis, oxygen supply, and acid-base regulation, are also involved in short-term exposures, but may not be detectable nor detrimental (Gazeau *et al.*, 2007; Pörtner, 2008). The scenario used here reflects transient warming and acidification events – thought to increase and intensify in the future (Fischer & Knutti, 2015; Helmuth *et al.*, 2014; Stocker *et al.*, 2013) – occurring

due to natural temporal variability in seawater conditions (Artioli *et al.*, 2014; Guyondet *et al.*, 2014; Hauri *et al.*, 2013), but also events such as heat waves and coastal upwellings, rather than more prolonged and continuous environmental conditions (Ghedini *et al.*, 2015). Such events have been shown to yield disproportionally high ecological change, and are exacerbated by additional local disturbances (Ghedini *et al.*, 2015, and references therein; Wernberg *et al.*, 2013). Thus, transient warming and acidification events have the potential to harm wild and aquaculture stocks, particularly those located near anthropogenically disturbed coastlines, if they occur close to the time of harvest. Studies have shown that pre-harvest conditions – even of relatively short-duration, known as "finishing" – can hold important implications for the quality of the final product by altering oysters physiology and consequently yielding different biochemical make-up, influencing the sensory attributes (Cruz-Romero *et al.*, 2004; Fratini *et al.*, 2013). Some of those sensory traits may or may not be detectable by panellists and may not demerit the overall acceptability of the product.

As explained by Cochet *et al.*, (2015), the use of descriptive sensory techniques for oysters can be difficult considering that oysters reared under the same conditions display a wide range of different sensory profiles (Aaraas *et al.*, 2004), making it difficult to decipher sensory changes due to experimental treatment. The batch of oysters used for our experiment was of consistent age and size class, however there were slight natural morphological variations between individuals, which could have had an influence on their sensory profiles. During training, panellists were aware of organism-to-organism variability, and how that could play a role in the eating experience. The extrinsic and intrinsic cues that determine eating quality are complex. The adopted methodology, including panel selection, training and consensus, is expected to focus the measurements on intrinsic sensory attributes. Consumers can be expected to have a wide range of sensory attribute perception thresholds – due to age, gender, genetic makeup, previously acquired responsiveness – and inclusion criteria for this small panel did not discriminate for these. Our study revealed significant differences in absolute scores given to several attributes between panellists, which suggests variability in the initial perception of certain attributes by panellists, however, the magnitude of changes between treatments was perceived similarly by the panellists. Such variability, which could be due to a number of factors such as experience, gender, or tasting technique, would occur naturally within the population of

consumers. While the sensory panel does not necessarily represent the expectations for the whole consumer population, these findings are useful to predict acceptability; in relation to consumption predictions over long periods of time, it could be expected that context, socio-demographic factors and product presentation would influence oyster acceptability (Debucquet *et al.*, 2012).

The sensory properties of oysters have been linked to various quality attributes of the meat. For instance, taste and flavour profile are linked to the proximate composition and the type of amino acids they contain, while aroma is associated with fatty acids and diet composition, and texture, mouthfeel, chewiness and juiciness are linked to glycogen content, fat content, and meat pH (see references in Cochet *et al.*, 2015). It is little understood how climate change and other changes to the marine environment will affect the quality of seafood through changes in their proximate composition and nutritional characteristics. To our knowledge, only three studies to date have investigated this matter (Ab Lah, 2017; Tate *et al.*, 2017). A study on the turban snail *Turbo militaris* (Ab Lah, 2017) did not detect any changes in nutritional properties (protein, lipids, minerals, fatty acids) with combined warming and acidification, whereas two studies on the edible dogwhelk *Dicathais orbita* (Tate *et al.*, 2017; Valles-Regino *et al.*, 2015) revealed that the nutritional properties (protein and lipids) were negatively affected by similar conditions. The findings of these three studies are in agreement with our findings from Chapter 4 on the changes in the nutritional properties of oysters. Results from Chapter 4 show a decrease in protein, lipid and carbohydrate content in *M. gigas* and *Ostrea edulis* under warming and acidification. Although these studies are not directly comparable to the one presented here due to differences in exposure duration (Ab Lah, 2017, :38 days ; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015, :35 days; Chapter 4: 12 weeks), it is possible that these longer-term physiological changes – in the form of nutritional alterations – could lead to decreased sensory quality of these species. Negative changes to the nutritional and sensory quality of seafood species, such as *D. orbita*, *M. gigas* and *O. edulis,* may in turn influence consumer preferences by altering their perception as healthy and appealing food choices.

Our study showed that only limited negative alterations to the sensory profile of oysters take place under acidification and warming scenarios, and even revealed improving trends (albeit non

statistically significant) for their opacity, mouthfeel, aspect of meat, shininess, meat resistance, meat texture, and creaminess. Therefore, if the outcomes of this short-exposure remain valid over longer-term OAW, oysters are still likely to represent a key species for future food security, bringing together a degree of resilience to climate change, whilst retaining nutritional value and taste. Although oysters are celebrated throughout the UK during various festivals (e.g: Whitstable Oyster Festival, Falmouth Oyster Festival, North East Oyster Festival), the perception that it is a niche luxury food product or not a tasty food choice (Danenberg & Mueller, 2011; Kow *et al.*, 2008; Liu *et al.*, 2006) is strong. An important factor in the reluctance to purchase and eat seafood is the perceived taste and smell (Carlucci *et al.*, 2015). In our study, there was a trend for worsened Aroma in oysters, which could have negative implications for their public perception and purchase. However, taste and perception are thought to be suggestible and likely to evolve through time, particularly when it comes to health-promoting foods (Urala & Lähteenmäki, 2007). As such, promoting oysters as a tasty and healthy food could improve perception encouraging consumption and changes in purchase behaviour, thereby shifting the source of dietary protein towards sustainable marine proteins.

5.5 Conclusion and future directions

Unlike findings for northern shrimps (Dupont *et al.*, 2014), it appears that the sensory quality of oysters may not be negatively impacted. Further research into other mollusc species may provide similar results, and lead to informed shifts in aquaculture species choice. This in turn will offer consumers a wider range of sustainable marine protein sources, whose sensory quality may revealed to be unlikely altered in the future. We concede that the exposure durations to the treatment conditions between the two studies are not directly comparable (3 weeks for the northern shrimps, compared to 5 days for the oysters), nevertheless our study provides a valuable base for future studies to build upon.

As recommended by Dupont *et al.*, (2014), future research towards food security should be solution-oriented, with targets of not only quantity, but also quality and sustainability. Molluscs and bivalves in particular, constitute promising species that should be further investigated. Studies looking into

changes in their nutritional properties (i.e. proximate composition, lipid profile, amino acids) should be undertaken in order to obtain a more complete picture of their quality in the future.

A limitation of our study is the short duration of exposure to future environmental conditions; changes to the sensory profile of oysters may require considerably more time. Our experimental scenario reflects abrupt and transient warming and acidification events, which are expected to increase and intensify in the future, rather than a chronic perturbation (Ghedini *et al.*, 2015). When investigating factors that may influence the eating quality of oysters, over a defined but limited time scale, it is confirmed that acceptability is not significantly affected by the tested scenarios. At the time of writing, the time required for any changes in sensory profile to occur is unknown and future studies should determine over what time scale, if any, changes occur. In the absence of other studies, no significant change in eating quality of oysters as observed in this study, and particularly the absence of negative effects on the sensory attributes, can be considered positive for the future of the aquaculture and food sectors. While production numbers may decrease because of acidification and warming (Barton *et al.*, 2015), these initial results suggest that the quality and demand for this important aquaculture product may well remain.

Chapter 6: General Discussion

"To see the wood for the trees"

Chapter 6: General Discussion

Ocean acidification and warming have been shown to affect a wide range of marine organisms globally (bivalves: Ong *et al.*, 2017; fish: Koenigstein *et al.*, 2017; gastropods: Leung *et al.*, 2017a, Wang *et al.*, 2017; macroalgae: Sampaio *et al.*, 2017; see also Poloczanska *et al.*, 2016), and impact whole assemblages and ecosystems (Brown *et al.*, 2017; Kroeker *et al.*, 2011). Many of the species experiencing negative biological effects provide valuable ecosystem services, including water purification (e.g. bivalves) and food security (e.g. bivalves, gastropods, fish), yet it is unclear how these negative biological effects will affect ecosystems and influence ecosystem services provision. Investigations into the loss of ecosystem services associated with ocean acidification and warming remain largely absent from the current literature, despite the increased prevalence of ecosystem-based approaches in environmental legislation and management (Knights *et al.*, 2014; Knights *et al.*, 2013).

6.1 Thesis aim

This thesis aimed to address this gap by appraising the scope and nature of the consequences of ocean acidification and warming on important shellfish species, using a multidisciplinary approach (see schematic Fig. 6.1). Specifically, the effects of ocean acidification and warming expected for the mid- to end- century on the physiology of two UK oyster species were investigated, and the responses linked to the functioning of oyster reefs and the provision of associated ecosystem services (Fig. 6.1). Additional aims included: 1) assessing differences in responses to ocean acidification and warming between an invasive and a native species and differences in their abilities to provide ecosystem services, and 2) informing species management and aquaculture practices to secure the delivery of those ecosystem services in the future, with a focus on the UK.

To achieve these goals, I assessed the responses of individuals of two species of oysters that are ecologically and commercially important in the UK – the native European Flat oyster *Ostrea edulis*, and the non-native Pacific oyster *Magallana gigas*. To test these questions, I used relatively long-term exposures (3 months) of individuals to a range of ocean acidification and warming scenarios representative of conditions predicted for 2050 and 2100.

6.2 Thesis background and rationale

Oysters, including *O. edulis*, and *M. gigas,* provide numerous ecosystem services, including but not limited to: improvement of water quality, reef formation, and food provision (Herbert *et al.*, 2016). Although *O. edulis* was historically highly abundant, the dramatic declines in stocks led to its designation as a protected species in the UK, with active conservation and restoration efforts (Coolen, 2017). In contrast, *M. gigas,* which was introduced for aquaculture purposes in response to the decline of *O. edulis*, today has formed wild populations on UK and Irish shores where it is considered invasive, with a number of management measures aiming at preventing further proliferation of *M. gigas* or its eradication (discussed below in section 6.4.3). The conflicting aims of conserving protected habitats and species, while maintaining ecosystem services and securing sustainable socio-economics benefits from aquaculture have led to debates around the status of *M. gigas* as an invasive non-native species and the need to eradicate it (section 6.4.3). However, it was unclear how added pressure from ocean acidification and warming would affect populations of *M. gigas* and *O. edulis* and what the consequences for ecological functioning and provisioning of ecosystem services would be.

Ocean acidification is a threat to oysters, which was the focus of an extensive review in Chapter 1. The aim of this review was to highlight and link the biological and ecological impacts of ocean acidification on oysters to the provision of associated ecosystem services. To do so, I assessed the effects of ocean acidification on individual life history stages (planktic larvae, juveniles, and adults), populations and ecosystem-level responses. I then reviewed the range of ecosystem services provided by oyster reefs, including an assessment of their economic value and associated metrics. I concluded by considering how impacts at the organismal-level can affect ecosystem services provision. I showed that negative impacts affect multiple life-stages, and highlighted that future studies should consider the combination of multiple stressors and not solely the effects of ocean acidification. It was apparent that the current research focus is heavily oriented towards early life stages and multi-generational studies (Przeslawski *et al.*, 2015). In contrast, research on adult oysters and established reefs is relatively sparse due to the general assumption that they are robust to ocean acidification and

warming, and as they are long-lived, they are unlikely to adapt to near-future ocean acidification and warming (Melzner *et al.*, 2009).

Given the clear negative impacts on oysters revealed in Chapter 1 (adult oysters display both decreased growth and calcification rates, while larval oysters show stunted growth, developmental abnormalities, and increased mortality), both *M. gigas* and *O. edulis* were expected to be negatively-impacted by ocean acidification and warming. Because non-native and invasive species are often considered able to withstand greater stress than their native counterparts (Stachowicz *et al.*, 2002), it was expected that *M. gigas* would be more robust to ocean acidification and warming. Furthermore, since *M. gigas* may be able to provide similar ecological functions and ecosystem services as *O. edulis* (Herbert *et al.*, 2012; Zwerschke *et al.*, 2016), arguably attempts to eradicate it may be unwise if it can provide ecological redundancy and support the sustained delivery of ES.

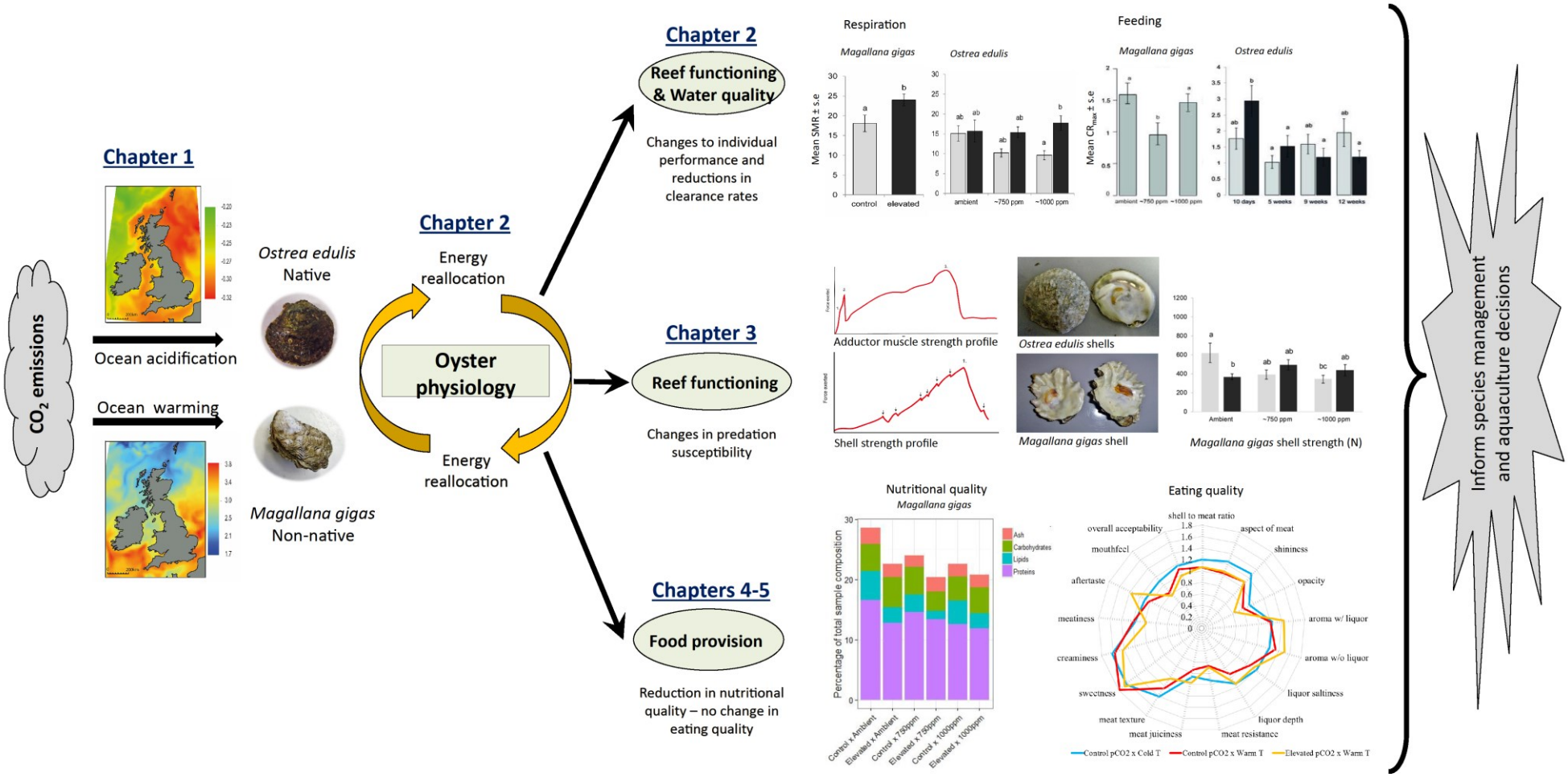


Figure 6.1: General schematic of chapter objectives and holistic approach of this thesis. From left to right: background context of ocean acidification and warming due to CO_2 emissions (Chapter 1), with a focus on the UK; effects of ocean acidification and warming on the physiological processes and energy reallocations in native and non-native oyster species (Chapter 2), reflected in reef functioning and water quality (Chapter 2), reef functioning and community interactions (Chapter 3), and provision of a key ecosystem service – food provision (Chapters 4 and 5). These findings are expected to have direct applications for society through species management and aquaculture practices.

6.3 Synopsis of data chapters

In Chapter 2, I demonstrated the occurrence of sub-lethal effects of OAW on adult oysters, reflecting the degree of physiological susceptibility, and countering the general assumption that this life-history stage is robust to environmental change. Surprisingly, *M. gigas* experienced a greater degree of stress than *O. edulis*, displaying both increased Standard Metabolic Rate by over 43% and reduced feeding by up to 40% (in the form of Clearance Rate), reflected in poorer Condition Indices (Fig 6.1).

In Chapter 3, I provided evidence that the physiological changes experienced by individual oysters held important implications for the functioning of the reefs (Fig 6.1). Exposure to ocean acidification and warming conditions led to changes in predation resistance by impacting on muscle and shell strength. Again, *M. gigas* appeared to undergo more pronounced changes: 52% increase in muscle strength, but 44% weakening in shell strength, under ocean acidification and warming. These changes are expected to alter its susceptibility to predators and influence community level interactions.

In Chapter 4, I assessed the direct implications of altered oyster physiology under OAW on the provision of an important ecosystem services: sea food. Both *O. edulis* and *M. gigas* underwent important changes to their biochemical composition, with trends for impoverished nutritional quality. In particular, the flesh of *M. gigas* contained lower lipid, carbohydrate, and protein levels, but higher contaminant concentration (copper); this change holds concerns for both future food security and future food safety. It was apparent that the physiological stress experienced by oysters discussed in Chapter 2, led to significant energy reallocation from somatic growth to metabolism by depleting protein, lipid and carbohydrate reserves (Chapter 4), at the detriment of their nutritional quality (Fig 6.1).

In the final chapter (Chapter 5), I established that short-term exposure to ocean acidification and warming may not negatively affect the eating quality of oysters (Fig 6.1). For this study, I focused solely on *M. gigas* as it became evident throughout the thesis that *M. gigas* was impacted more greatly by ocean acidification and warming than *O. edulis*. Moreover, it is a major aquaculture species globally, and dominates the UK market over *O. edulis*. Therefore, changes to its consumer appeal may yield

critical social and economic consequences. The study revealed an absence of negative effects on various aspects of the eating quality (appearance, aroma, texture, taste, and overall acceptability), which are considered positive for the aquaculture sector and the ability of shellfish to secure future food provision.

6.4 Implications of physiological responses for ecosystem services provision, species management and aquaculture practices

It is clear from the work presented in this thesis that the impacts of ocean acidification and warming on oysters are multifaceted and occurring at multiple scales and levels of organisation. Although it is not always straightforward to link biological and physiological responses to ecosystem function and ecosystem services provisions (Le Quesne & Pinnegar, 2012) below I attempt to put the findings into perspective.

6.4.1 Physiological responses

Animal performance and fitness provide indications of their degree of tolerance *vs* sensitivity to a particular perturbation. Performance and fitness can be depicted by a suite of indicators: aerobic scope, locomotory scope, reproduction output, calcification and somatic growth (Melzner *et al.*, 2009). To obtain a truly holistic assessment of an organism's energetic budget, all the indicators of performance and fitness that are described above should ideally be integrated. Here, I assessed aerobic scope using Standard Metabolic Rates, which reflects acute "real time" stress (Chapter 2), and somatic growth using Condition Indices, which incorporates reallocation of energetic stores over longer time and perhaps better reflects overall energetic fluctuations (Chapters 2 and 3). However, I did not assess reproduction, and calcification was indirectly assessed using shell strength (Chapter 3) (locomotory scope is only limited in sessile organisms). In terms of energy budget, responses may be more easily detected using long-term budget metrics such as Condition Indices (Melzner *et al.*, 2009).

Chapter 1 shed light on the limited information available regarding the response of adult oysters to ocean acidification and warming, consequence of the general assumption that they are robust to environmental change. However, it is apparent from this work that sub-lethal effects can occur and

yield important consequences to individual performance, depicted by several metrics of fitness (Chapters 2-4). It was apparent that both species of oyster responded differently to potentially stressful ocean acidification and warming conditions. Contrary to expectations, invasive *M. gigas* experienced a higher level of stress than the native *O. edulis*, which was consistently observed across chapters. In Chapter 2, it was shown that *M. gigas* experienced an increased Standard Metabolic Rate, and reduced Clearance Rate and Condition Index, which likely led to reallocation of energetic reserves, yet *O. edulis* remained somewhat unimpacted. Additionally, while the muscle and shell strength of *O. edulis* remained unaffected by the conditions, *M. gigas* experienced a 52% increase in muscle strength and a 44% weakening in shell strength (Chapter 3). I showed in Chapter 4 that both species readily use energetic reserves when exposed to ocean acidification and warming. However, *O. edulis* was seemingly less impacted and depletions of energetic reserves was particularly apparent for *M. gigas*. This difference is likely a consequence of the higher metabolic stress endured by *M. gigas* compared to *O. edulis* when exposed to ocean acidification and warming conditions, demonstrated in Chapter 2. A reallocation of energetic resources, for instance, from somatic growth to calcification, is documented for several molluscs under ocean acidification and warming; a process which may allow a degree of tolerance to abiotic stress (Gray *et al.*, 2017; Ong *et al.*, 2017).

As mentioned above, invasive *M. gigas* was less tolerant to changes in temperature and acidification conditions than the native *O. edulis*, In Australia, exotic and native oyster species have also been shown to display markedly different tolerance to abiotic environmental conditions, with native oysters able to withstand harsher environmental conditions, such as those experienced in the high intertidal, that exotic oysters were unable to tolerate (Krassoi *et al.*, 2008). This allowed the native species to be released from competition at the highest intertidal level, whereas the exotic species invaded the middle and lower intertidal levels, where it was better able to cope with the environmental conditions. In the UK, at locations where both *O. edulis* and *M. gigas* co-occur, such as sites in Plymouth Sound, higher tolerance of *O. edulis* over *M. gigas* of ocean acidification and warming may allow its persistence in the future and hinder potential phase shifts from native to non-native dominated environments, similar to those observed in the Wadden Sea (Diederich, 2005; Kochmann *et al.*, 2008; Nehls *et al.*, 2009).

It must be noted that responses were not always consistent across experiments. Specifically, there were variations in the effects of ocean acidification and warming on the Condition Index of *M. gigas* between Chapter 2 and Chapter 3. It is likely that variation in the carbonate chemistry and different feeding regimes between the two experiments caused such differences in Condition Index, given their important in calcifiers tolerance to ocean acidification and warming (see the Discussion section in Chapter 3; Ramajo *et al.*, 2016; Waldbusser *et al.*, 2014). Filter-feeding molluscs are particularly good at efficiently utilizing available food, and reallocating energy between reproduction, somatic growth, or calcification according to changes environmental conditions (Irisarri *et al.*, 2015; Melzner *et al.*, 2011), which reinforces the idea that species physiological responses to ocean acidification and warming are highly dependent on environment-specific biological and geochemical conditions.

The various changes observed in these experiments – Condition Index, shell and muscle strength, biochemical composition – may well be the consequence of altered physiological processes resulting in energy reallocation and trade-offs between the various processes (metabolism, maintenance, reproduction [not investigated here], calcification). Further research using molecular, cellular and other new research tools such as -omics (Freitas *et al.*, 2017; Goncalves *et al.*, 2017) may provide additional insights into physiological responses and help decipher the mechanisms behind tolerance and acclimatisation to ocean acidification and warming observed here.

6.4.2 Changes to reef function and ecosystem service provision

Changes to reef function

Ocean acidification and warming can yield important direct negative biological and physiological impacts, but it is argued that their expression at the population and ecosystem levels is of higher societal concern (Le Quesne & Pinnegar, 2012). Recently, climate change has been shown to affect biogenic habitats and their complexity, which could lead to important consequences for ecosystem structure and functioning (Fabricius *et al.*, 2014; Sunday *et al.*, 2016). Biogenic reefs experiencing important changes may differ in their functioning (Coen & Luckenbach, 2000; Fabricius *et al.*, 2014), therefore poorer performance and condition of *M. gigas* individuals under warming and acidification

(Chapter 2), in addition to the likely changes in predator-prey interactions from altered muscle and shell strengths (Chapter 3), may threaten the maintenance and functioning of *M. gigas* reefs. Understanding changes to ecosystem functioning is important in order to predict decreases in, or even losses of, provision of ecosystem services (Coen *et al.*, 2007; Coen & Luckenbach, 2000, see also Chapter 1).

Changes to ecosystem service provision: water quality

Oysters are important bio-filters that improve water quality and affect nutrient cycling (Chapter 1). Reductions in clearance rates of oysters under ocean acidification and warming (Chapter 2) are especially concerning and may have important ecological impacts by limiting their ability to improve water quality. Oyster beds consisting in majority of *M. gigas*, might see their surrounding water quality diminish, which is particularly concerning given the expanding distribution of *M. gigas* populations on UK shores. Several other bivalve species display reduced clearance rates under warming and/or acidification conditions (Ong *et al.*, 2017; Speights *et al.*, 2017; Vargas *et al.*, 2015). As their filtration rates might be negatively impacted, concerns have been expressed regarding the fate of waste bioremediation service by mussels under ocean acidification (Broszeit *et al.*, 2015). Consequently, bivalves may not be a viable choice of bioregulator for water management in polluted and eutrophic areas of the UK, such as the Liverpool Docks (Bohn *et al.*, 2013). Altogether, these findings suggest that bivalves, including oysters, may not provide the same level of water quality in the future. As detailed in Chapter 1, impoverished water condition can affect the provision of other ecosystem services, such as the facilitation of submerged vegetation (and their associated ecosystem services) and recreational activities, which ultimately rely on environments of high water quality. However, only partial information regarding water quality can be inferred from clearance (filtration) rates measured in the laboratory. Laboratory-based estimates of clearance rates do not incorporate aspects such as natural particle load, hydro-dynamisms, and reef size, which can influence ecosystem-level variations in water quality (Coen & Luckenbach, 2000). Additional information incorporating aspects of water quality such as nutrient load, turbidity, pathogens is needed in order to fully appreciate the extent of change to water quality and safety.

Changes to ecosystem service provision: food security

A major aim of this thesis was to assess the ability of marine species to provide food security in the future under ocean acidification and warming. Oysters are considered highly nutritious, being natural sources of proteins, lipids, fatty acids, vitamins, and minerals (detailed in Chapter 4). Global oyster production has been estimated to exceed 5.4 million tonnes in 2015, worth in excess of US$4 billion (Chapter 4). Thus, food provision is arguably the most socially and economically important ecosystem service provided by oysters. However, if oysters are susceptible to ocean acidification and warming conditions, continued provision of these services might be put in jeopardy. Harvest and aquaculture productions under ocean acidification and warming depend on the physiological condition of individuals and the state of the wider ecological setting (indirect effects of ocean acidification and warming on the ecosystem) (Le Quesne & Pinnegar, 2012). Molluscan aquaculture, in particular, has been shown to be at high risks from ocean acidification and warming (Branch *et al.*, 2013; Cooley & Doney, 2009). For instance, production numbers are expected to be reduced if the high larval mortality and developmental delays (amongst other – see Chapter 1), already experienced for oyster producers elsewhere (e.g. Barton *et al.*, 2015), come to fruition here in the UK.

I show here that oysters, and *M. gigas* especially, may become less nutritious in the near future due to reductions in proteins, lipids, energetic value, and due to important changes to their essential mineral contents (Chapter 4). These nutritional traits contribute to the 'sensory profile' of oysters, and thus their reductions under ocean acidification and warming may lead to diminished eating (sensory) quality. However, the eating quality of *M. gigas* may remain unaltered (Chapter 5), which was in contrast with findings from Dupont et al., (2014) showing that the taste and texture of the northern shrimp *Pandalus borealis* deteriorate when exposed to ocean acidification conditions. There were some differences between these studies. Here, I assessed changes in sensory profile after transient exposure to ocean acidification and warming (5 days) which incorporated daily fluctuations in pCO_2, whereas the study by Dupont et al., (2014) spanned over three weeks and used static pCO_2 (pH) values. Short-lived extreme climatic events and environmental variability are predicted to increase in occurrence and intensify in the future (Ghedini *et al.*, 2015; Wernberg *et al.*, 2013), and may be at least equally as important as static change in global average conditions. This work (Chapters 4 and 5), combined with

the findings of recent studies on other molluscs (Ab Lah, 2017; Dupont *et al.*, 2014; Tate *et al.*, 2017; Valles-Regino *et al.*, 2015), shows that, by influencing product quality and appeal and consumer demand, changes to the nutritional and sensory qualities of molluscs under climate change can impact the aquaculture sector in the future. Further research is needed to identify which species may see their fitness decline, and which are robust to climate change. In order to secure future food provision, health benefits, and economic revenue, the UK aquaculture industry might need to reconsider its management strategy in the future (Fernandes *et al.*, 2017; Jennings *et al.*, 2016), and encourage the production and consumption of *O. edulis,* in addition to the already popular *M. gigas*.

6.4.3 Implications for species management and reef conservation

In order to optimise the ecological, social and economic benefits available from the marine environment in the future, species and ecosystem conservation and management need to integrate their vulnerability to climate change and the predicted changes to ecosystem services provision (Henson *et al.*, 2017). In view of the findings of this thesis, it is clear that the risks from ocean acidification and warming on oysters and oyster reefs are species-specific; in the UK, introduced *M. gigas* may be more vulnerable than native *O. edulis*. To secure benefits and minimise costs related to the management of introduced species, these findings could be integrated into the current management and conservation measures in place for these species and the reefs they can form.

Shellfish reefs are often considered to be amongst the most endangered coastal habitat in Europe, yet receive some of the lowest degree of protection (Airoldi & Beck, 2007) despite constituting a "Reef" feature under the Habitat Directive Annex I. In particular, native oyster reefs around the UK are in critically poor condition, with over 90% considered lost or functionally extinct (Beck *et al.*, 2011). However, wild beds of native oysters remain around the Thames Estuary, in the Solent, the River Fal, and the west coast of Scotland and Ireland (Airoldi & Beck, 2007). *Ostrea edulis* is protected under the UK Biodiversity Action Plan with a specific action plan in place, yet harvest is still allowed. Nevertheless, current restoration efforts towards *O. edulis* aim to recover reef function and ecosystem services (Harding *et al.*, 2016; Shelmerdine & Leslie, 2009; Woolmer *et al.*, 2011). Given the findings of this work, those should be continued if we are to secure ES, particularly in the event that *M. gigas*

populations collapse and are no longer able to provide ecological redundancy. Careful choice of location for future *O. edulis* restoration and conservation is crucial, and must consider a multitude of parameters associated with environmental, geological and hydrological conditions, such as embayment type, elevation, substrate type, but also disease free/pest free sites (Filgueira *et al.*, 2016; Sawusdee, 2015). Establishing marine protected sites comprising native oyster beds, such as Blackwater, Crouch, Roach and Colne Estuaries (Marine Conservation Zone [MCZ]), Chesil Beach and Stennis Ledges (MCZ), and Loch Sween (Nature Conservation Marine Protected Area) (JNCC MPA mapper[1]), could be an effective tool towards *O. edulis* conservation, given that marine reserves have been suggested to help mitigate and promote adaptation to climate change (Roberts *et al.*, 2017).

The extent of *M. gigas* is predicted to expand northward due to higher larval recruitment and increased habitat suitability driven by climate change (Rinde *et al.*, 2016; Thomas *et al.*, 2016; Townhill *et al.*, 2017). This is of particular concern given that this species is commonly thought to have considerable impacts on ecosystems and industries, and thus represents an important 'cost' to society (Herbert *et al.*, 2016; Townhill *et al.*, 2017). *Magallana gigas* was shown to have negative ecological impacts in the Wadden Sea, by competing for food and causing a shift in abundance of dominant associated species compared to native mussel beds (Kochmann *et al.*, 2008). Negative socio-economic impacts linked to the spread of wild *M. gigas* settlements include injury to the public from oyster shells, expensive maintenance of navigational channels for recreational craft, aesthetic issues, and trophic competition with commercial shellfish farming (Herbert et al., 2016). *Magallana gigas* is considered 'High impact list' species under UK Technical Advisory Group and of 'medium risk' by the GB Non-Native Species Secretariat. A number of costly management measures aimed at eradicating *M. gigas* and preventing its further proliferation along UK coasts are currently in use, including mechanical removal and the use of sterile triploids in aquaculture (Herbert *et al.*, 2016). Yet, at several European sites, *M. gigas* was shown to promote biodiversity and the maintenance of beds of *Mytilus edulis*, without overlapping with native oysters nor interfering with indigenous bivalve abundances, and even provide useful service such as coastal protection (Markert *et al.*, 2010; Nehls *et al.*, 2009; Walles *et al.*, 2015; Zwerschke *et al.*, 2016). Additionally, in parts of Europe, *M. gigas* reefs are the subject of guided

[1] http://jncc.defra.gov.uk/page-5201

walks for visitors to collect wild oysters (see Herbert *et al.,* 2016), and valuable opportunities could arise in the UK from fishing and hand-collection in the wild as a means of managing stock at locations where it is abundant (Herbert *et al.*, 2012), and thus provides an economic and societal benefit.

In light of the findings of this work, in parts of the UK eradication of *M. gigas* might occur slowly – but naturally – over time from long-term consequences of sub-lethal effects from ocean acidification and warming described in previous sections. As such, there might be an argument for reconsidering the money and time currently invested into eradication efforts. The validity of eradicating non-native species over naturalising them is often debated (Epstein, 2017; Epstein & Smale, 2017; Townhill *et al.*, 2017), and it is suggested that ecological concerns should be considered in equilibrium with society's needs, particularly when non-native species are not drivers of negative ecological change and are able to deliver desirable ESs (Ruesink *et al.*, 2005). Eradication of non-native and invasive species represents a financial burden and is not always feasible, therefore 'cost' of eradication against 'value' of potential benefits emanating from these species must be carefully considered in order to optimise the ecological, social and economic benefits available from the marine environment.

6.5 Limitations

This thesis work was entirely conducted in a laboratory, and consequently it incorporates limitations inherent to laboratory-based studies.

6.5.1 Laboratory-based vs in situ ocean acidification and warming experiments

Laboratory experiments hold the advantage that environmental conditions can be controlled, but fail to recreate the complex natural living environment of marine organisms. As such, research pursued in 'natural laboratories' – naturally acidified environments [CO_2 seeps, underwater volcanoes] – are now promoted in order to obtain a more accurate *in situ* representation of long-term community response and ecosystem change under ocean acidification and warming (Smith, 2016).

These natural environments hold the advantage that organisms have been exposed to the conditions over a much longer period – sometimes life-long exposure – than that possible to achieve in

laboratory studies. The logistic time constraints placed on laboratory-based experiment may limit the occurrence of long-term effects, plastic acclimation, and trans-generational adaptation, particularly in the case of long-lived specimens like oysters. Nevertheless, the past few years have seen efforts towards laboratory-research incorporating trans-generational and long-term exposure to multi-stressors (Chakravarti *et al.*, 2016; Parker *et al.*, 2015; Rodríguez-Romero *et al.*, 2015; Rühl *et al.*, 2017), efforts that must be pursued in the future. The 12-week exposure period adopted in Chapters 2, 3 & 4 is generally accepted as being of sufficient duration to distinguish between shock responses and acclimation, and is generally longer than the majority of published studies on ocean acidification and warming. In Chapter 5, such long exposures were not possible due to logistic considerations. The relatively short exposure and results may not reflect changes to the sensory profile over longer duration exposures (weeks or months) to ocean acidification and warming conditions. Predictions and current records of global change, however, show an intensification of short-term extreme climatic events and environmental variability (Ghedini *et al.*, 2015; Wernberg *et al.*, 2013). As such, experimental designs that reflect transient and short-term punctuations in environmental conditions (e.g. increased temperature or ocean acidification) are increasing recognised as valuable and further research incorporating these fluctuations are needed (Boulais *et al.*, 2017; Mangan *et al.*, 2017).

Finally, the mesocosm set-up at Plymouth University laboratory fully immersed the oysters throughout the exposure duration to the experimental treatments, and as such did not incorporate the natural conditions they experience in the field. These coastal organisms were collected from an intertidal site (see Chapter 2, section 2.2.1), and as such experienced important natural fluctuations in seawater levels and environmental conditions (e.g: salinity, temperature, pH), seasonally and on a daily basis (Beaumann *et al.*, 2015). The relative uniformity of the conditions experienced by the oysters in the mesocosm set-up is uncharacteristic of coastal environments, and as such the responses observed may not have accurately reflected the responses of intertidal organisms to future changes in environmental conditions (see discussion in Mangan *et al.*, 2017). Importantly, responses may have been underestimated, as a recent study demonstrated that living in naturally variable environment, with fluctuating pCO_2 levels for instance, is more energetically expensive than living in static conditions, such as those experienced here by the oysters in the mesocosm (Mangan *et al.*, 2017).

6.5.2 Statistical effects

The laboratory system mimicking climate change set-up at Plymouth University, used throughout this thesis work, has a defined carrying capacity, beyond which the stability and safety of the abiotic conditions experienced by organisms is no longer optimum. This essentially limited the number of individuals that could be exposed at one time, constraining the number of replication – "n" – per treatment. The absence of statistical differences in biological responses sometimes observed in this work (Chapters 2, 3 & 4) may derive from low statistical power, as well as the variability in individual responses by oysters within a treatment. Nevertheless, studies focusing on effect size of biological responses provide useful information and should not be disregarded (Cornwall & Hurd, 2015).

6.5.3 Single ontogenic stage

The importance of the larval stages of marine invertebrates has been widely recognised in the past few years, with increasing amount of evidence that they are particularly sensitive to ocean acidification and warming (Byrne & Przeslawski, 2013, discussed in Chapter 1). It is conspicuous that considerable work has been done on the early-life stages of oysters (reviewed in Chapter 1), but little on post-settlement stages. A gap in knowledge regarding adult performance was apparent despite their importance in population maintenance and reef functioning, which led me to focus this work on that single ontogenic stage. In order to understand the overall population outcome, it is essential to investigate the effects of environmental stress on all life stages of an organism as well as trans-generational effects. Yet the number of studies taking into consideration more than one life-stage, or spanning over multiple generations, remains limited. The logistic difficulties associated with running these laboratory-based scenario experiments over multiple life stages or generations, particularly for oysters that are relatively long-lived, constitute a considerable limitation to the comprehensive understanding of ocean acidification and warming effects on whole populations.

6.6 Future research directions

If we are to predict changes to ecosystem services and their implications for society in the future, emphasis should be put on linking biological and ecological effects to the processes behind ecosystem services provision. While in this instance I focused on food provision, and only partially investigated

water quality, the possible effects of ocean acidification and warming on other ecosystem services, such as coastline stabilisation and protection from erosion, were not assessed. Understanding the possible alterations to the provision of ecosystem services can support decision-making towards sustainable management of the marine environment (Henson *et al.*, 2017; Knights *et al.*, 2014), and provide guidance towards taking decisive steps towards "climate proofing" societies and secure those valuable ecosystem services.

6.7 Conclusion

This thesis moved beyond the biological implications of ocean acidification and warming and bridged the gap to ecosystem services assessments, making links between an organism's physiological responses, changes to ecosystem function, and outcomes for ecosystem services provision. It is apparent that oyster reefs in the UK, and particularly *M. gigas* reefs, are facing serious risks from ocean acidification and warming due to direct lethal and sub-lethal effects on individuals, with consequences for community interactions and ecosystem functioning. The provision of valuable ecosystem services, such as water quality and food provision, are expected to change as a result of the biological and ecological effects of ocean acidification and warming on oysters. These effects are likely to cascade into the anthropogenic socio-economic sphere and as such, future research efforts should be solution oriented to prevent significant social and financial repercussions

Appendices

The following can be found in the appendices:

Appendix 1: Accompanying Chapter 2 and 4

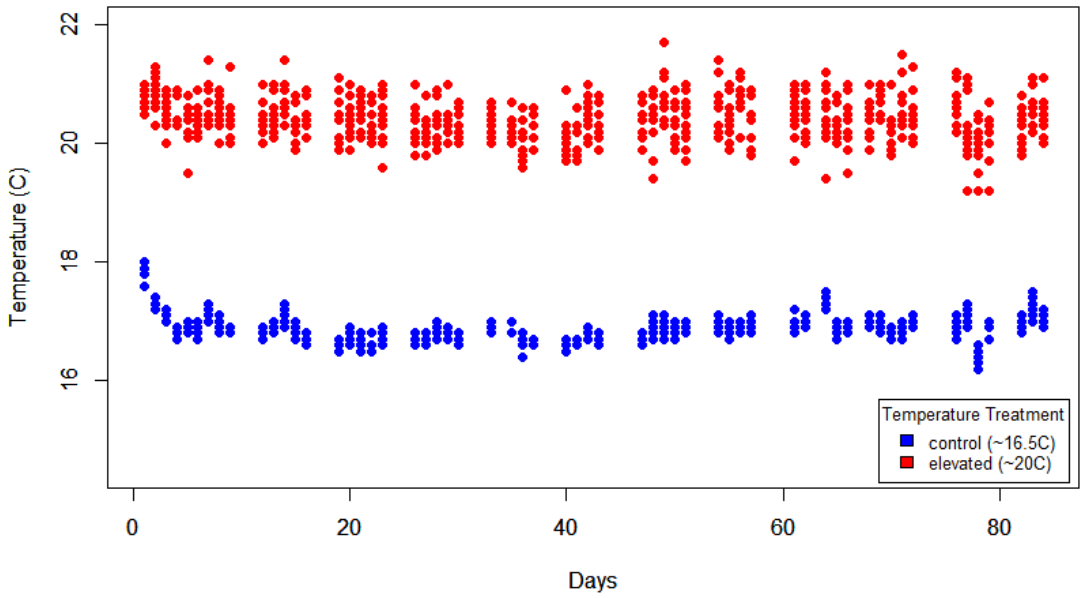


Figure A1.1: Variation in temperature within the mesocosm set-up throughout 3-month exposure of *Magallana gigas* exposed to two temperature levels: control (~16.5°C); elevated (~20°C) - used in Chapter 2 and Chapter 4.

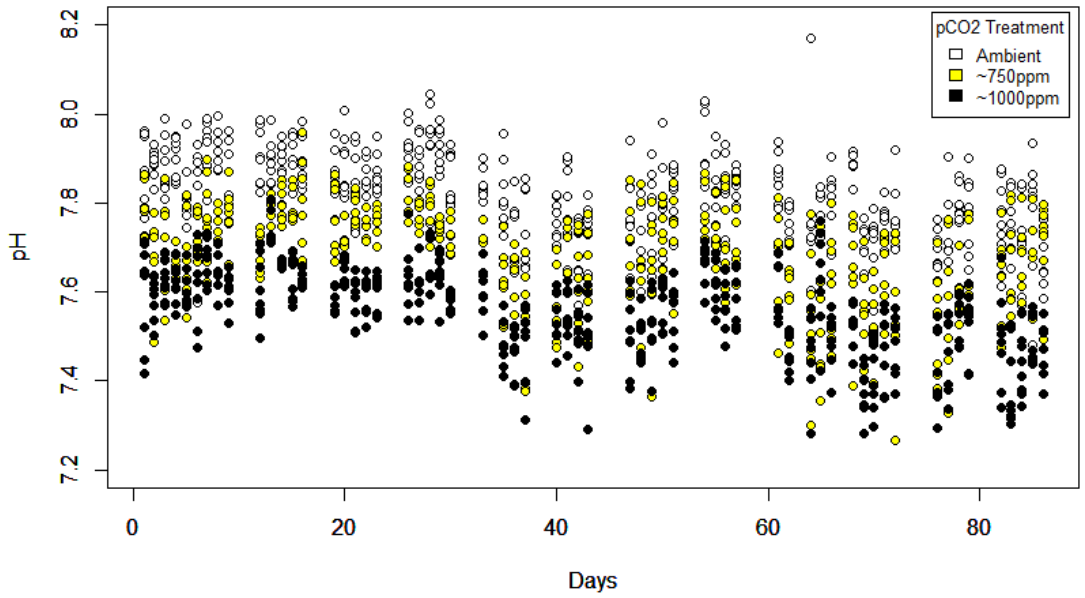


Figure A1.2: Variation in pH within the mesocosm set-up throughout 3-month exposure of *Magallana gigas edulis* to three pCO_2 levels: Ambient (~400 ppm), ~750 ppm, ~1000 ppm - used in Chapter 2 and Chapter 4.

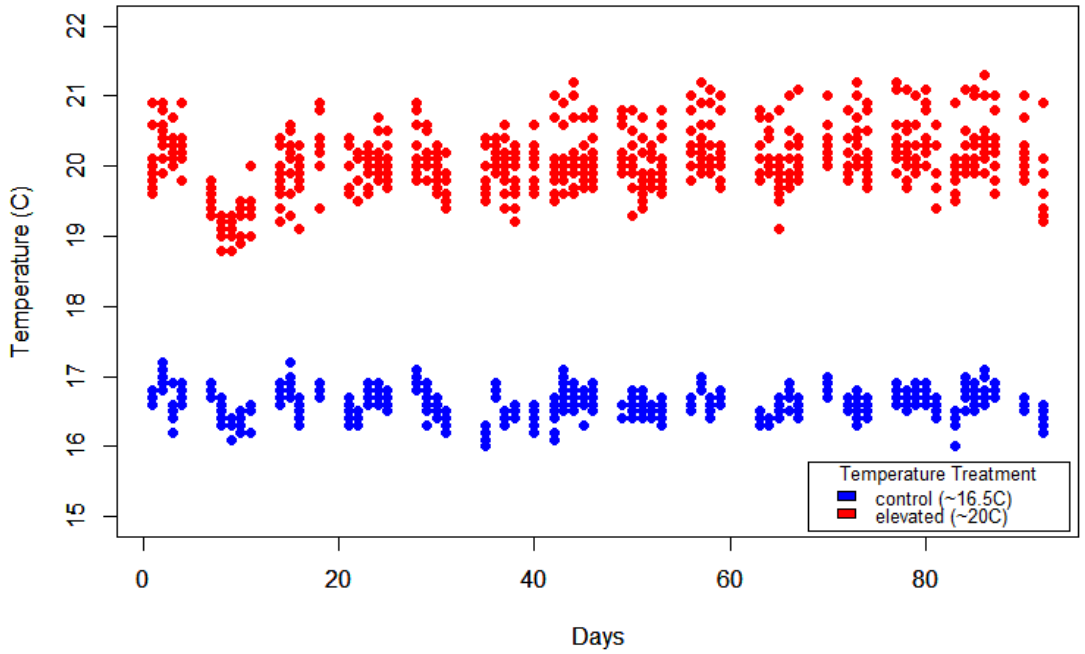


Figure A2.3: Variation in temperature within the mesocosm set-up throughout 3-month exposure of *Ostrea edulis* exposed to two temperature levels: control (~16.5°C); elevated (~20°C) - used in Chapter 2 and Chapter 4.

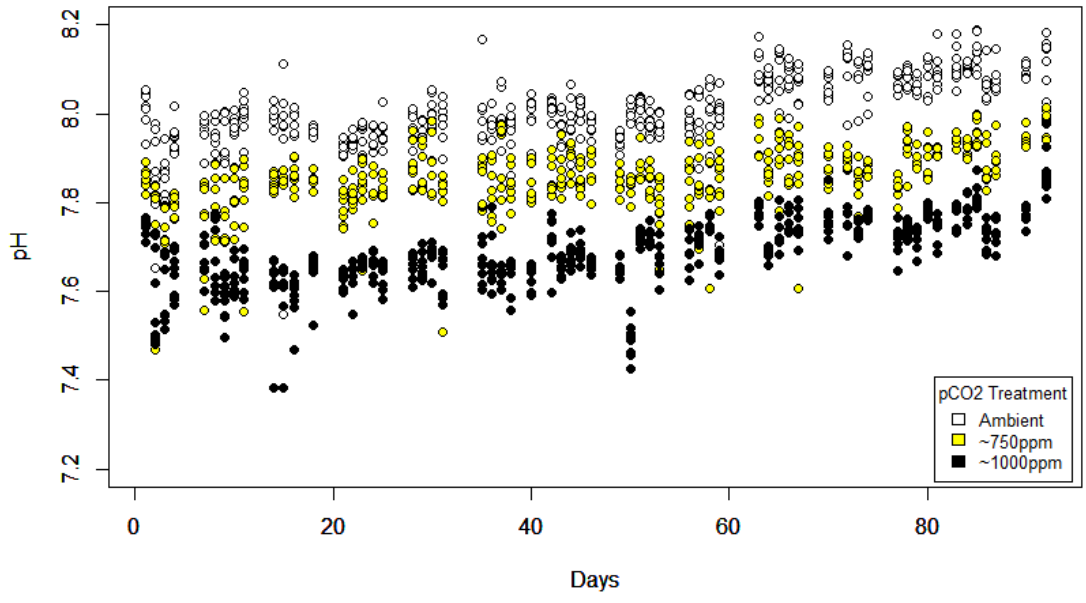
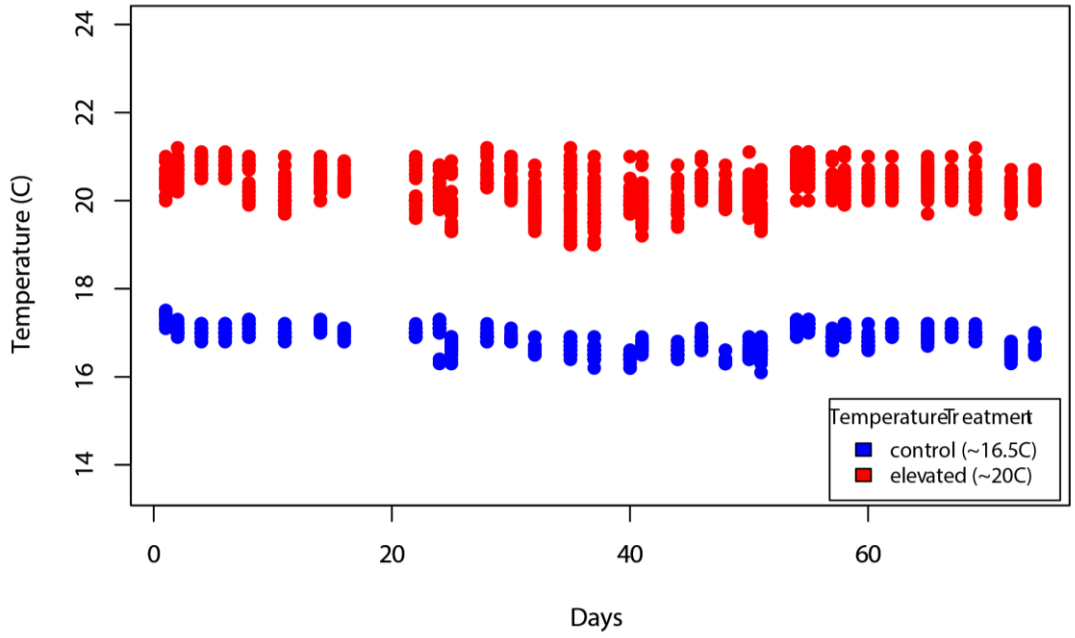


Figure A1.4: Variation in pH within the mesocosm set-up throughout 3-month exposure of *Ostrea edulis* to three pCO_2 levels: Ambient (~400 ppm), ~750 ppm, ~1000 ppm - used in Chapter 2 and Chapter 4.

Appendix 2: Accompanying Chapter 3



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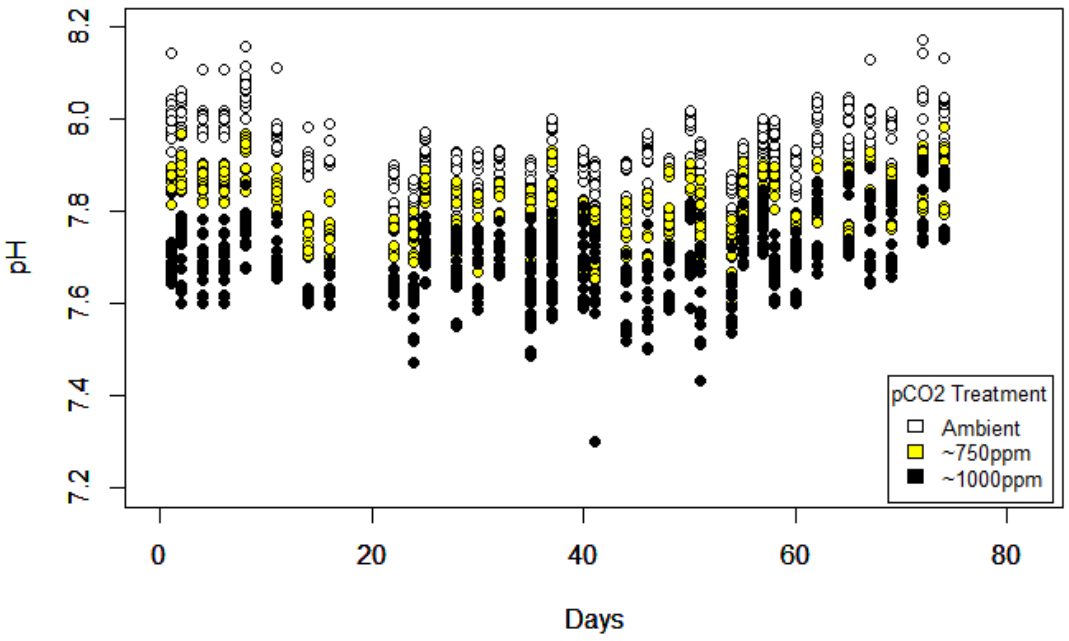

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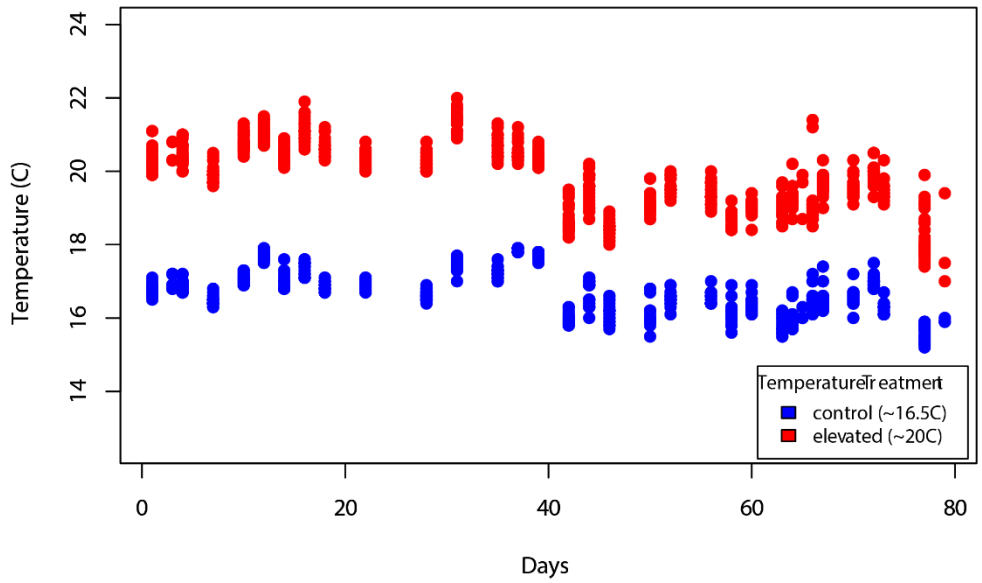

Figure A2.3: Variation in temperature within the mesocosm set-up throughout 3-month exposure of *Ostrea edulis* exposed to two temperature levels: control (~16.5°C); elevated (~20°C) - used in Chapter 3.

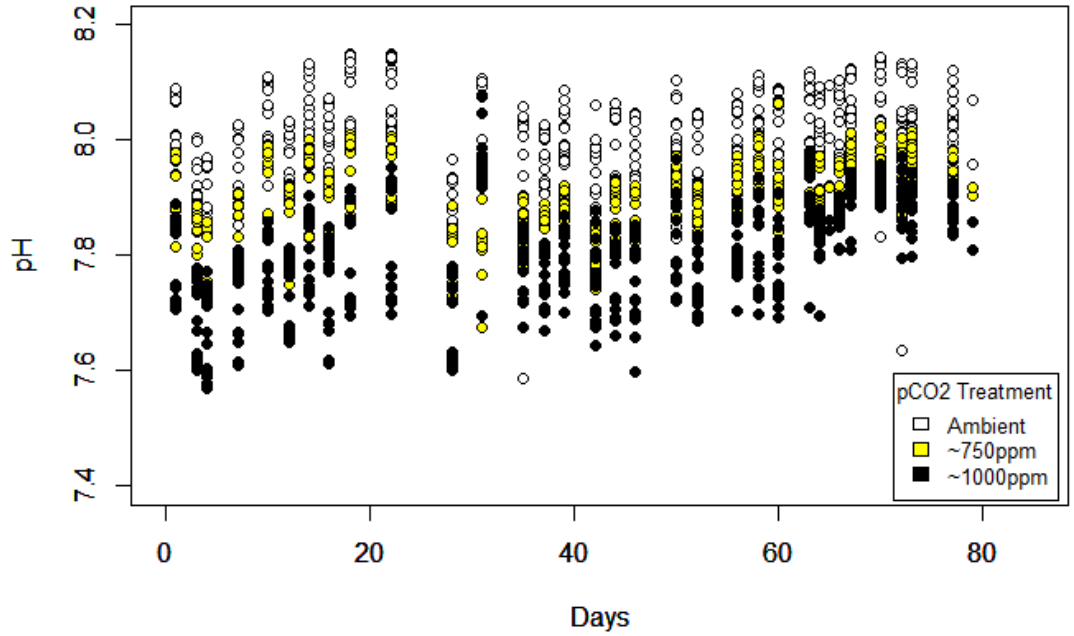

Figure A2.4: Variation in pH within the mesocosm set-up throughout 3-month exposure of *Ostrea edulis* to three pCO_2 levels: Ambient (~400 ppm), ~750 ppm, ~1000 ppm - used in Chapter 3.

Appendix 3: Accompanying Chapter 4

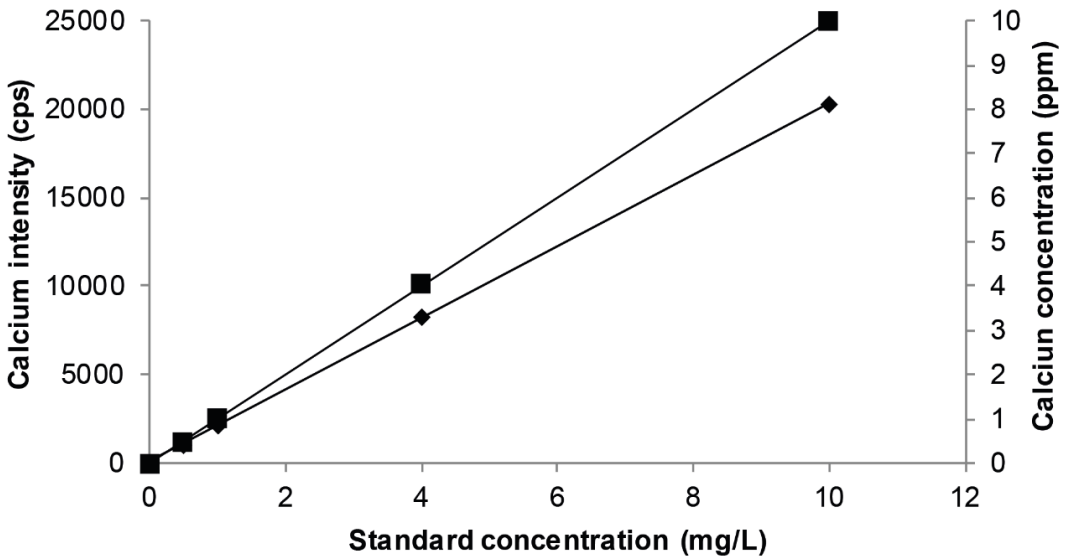

Figure A3.1: Calibration curves of Calcium, using five standard solutions of known concentrations (0; 0.5; 1; 4; 10 mg/L). ♦ Calcium intensity: y=2018.1x+125.78, R^2=1, cps=count per second; ■: Calcium concentration: y= 0.9993x +0.005, R^2=1, ppm=part per million.

Appendix 4: Additional Pilot Study

Preferential parasitism of *Ostrea edulis* over *Magallana gigas* by *Polydora sp.* in Plymouth Sound, UK

Introduction

Parasites are increasingly recognised as important structural components of marine communities (Firth *et al.*, 2017). They modulate the ecology and biogeography of their host-species, but also indirectly shape the whole community via coincidental effects on the habitat and ecological interactions, particularly in the case of an ecosystem-engineer host (Mouritsen & Poulin, 2002). Taxa affected by parasitism include mammals, teleosts (Hoberg & Brooks, 2008), birds, molluscs and crustaceans (Mouritsen & Poulin, 2002).

Bivalves are known to be hosts for various parasites that live in their mantles or within their shells (Blake & Evans, 1973; Carroll *et al.*, 2015; Sato-Okoshi *et al.*, 2012). Parasites, by definition, are harmful to their host to a certain degree. Negative effects can vary from minor metabolic changes to more important soft tissue damages (Mouritsen & Poulin, 2002). The deleterious effects of Polydorid worms on bivalve host species can vary with the intensity of infestation, but usually lead to physiological deficiencies (Chambon *et al.*, 2007), reduced condition (Ambariyanto & Seed, 1991; Chambon *et al.*, 2007; Riascos *et al.*, 2008), and reduced shell strength (Bergman *et al.*, 1982; Kent, 1981; Korringa, 1951).

This in turn affects important ecological and biological processes, such as predator-prey interactions (Ambariyanto & Seed, 1991) and behaviour (Riascos *et al.*, 2008). Polydorid parasitism is not only an issue for wild populations, but also for aquaculture (Blake & Evans, 1973; Royer *et al.*, 2006; Simon, 2011), causing mud blisters (Blake & Evans, 1973; Korringa, 1952), affecting sensory and aesthetic parameters, and general reduction in product quality (Korringa, 1951), with negative economic repercussions by reducing the half-shell value.

The native European flat oyster – *Ostrea edulis* – and the non-native invasive Pacific oyster – *Magallana gigas* – are two ecosystem engineers (sensu Jones *et al.*, 1996) present in the UK, providing numerous

ecosystem services, such as reef formation, erosion control, improvement of water quality and food provision (Herbert *et al.*, 2012). Although historically *O. edulis* was highly abundant in the UK (Orton, 1937), their continued decline has led to protection and extensive restoration programs (Woolmer *et al.*, 2011). In contrast, *M. gigas* continues to spread polewards facilitated by the warming of sea surface waters (Rinde *et al.*, 2016; Thomas *et al.*, 2016; Townhill *et al.*, 2017). In the Wadden Sea, a gradual shift from native mussel to invasive oyster beds has been recorded since its introduction in 1986 (Kochmann *et al.*, 2008), yet recent studies have shown that both species can co-exist without detrimental ecosystem impacts (Buschbaum *et al.*, 2016; Reise *et al.*, 2017). In Plymouth Sound UK, mixed oyster beds of both *O. edulis* and *M. gigas* occur, but *M. gigas* are increasingly prevalent (pers. observations). Although it is likely that some level of competition for space and resources takes place, the ecological impact of *M. gigas* on *O. edulis* remains unclear and may not alter associated assemblages (Zwerschke *et al.*, 2016).

*Polydora ciliata,*a parasitic worm affecting shellfish*,* has long been known to occur in the South-West of the UK (Kent, 1977) and in Plymouth waters, due to the occurrence of Devonian limestone, a choice settlement substrate for the species (Dorsett, 1961). Oysters found along Plymouth Sound shores present signs of parasitism, by Polydorid worms (pers. observations), with a preference for *O. edulis* as the host species. To date, the rate and prevalence of parasitism have not been reported, but based on observations, may account for reductions in survival and abundance of *O. edulis*. If this is true, the physiological consequences of differential parasitism between the two species of oysters could explain the abundance pattern observed, by slowing the recovery of *O. edulis* and facilitating the spread of *M. gigas*, with important ecological and economic outcomes.

Here, we investigate the host association of *Polydora ciliata* between *Ostrea edulis* and *Crassostrea gigas*, and examine if oyster size (proxy for age) and shell strength are affected.

Methods

Both species were collected at low tide on several occasions through 2015-2016, from a low-intertidal site in Plymouth Sound (50°23'29.95"N, 004°13'16.77"W).

Individuals were destructively sampled to assess the degree of parasitism by macroscopic examination of the inside and outside of the valves. Oyster parasitism was recorded as being either *uninfected* (no visible sign of infection), *infected low-level* (less than 10 visible burrows), or *infected high-level* (more than 10 visible burrows) (Figure A4.1).

The maximum length of each individual was measured to the nearest millimetre using Vernier callipers (Mitutoya, Japan). Each individual was categorized as being either 'small' or 'large'.
A subsample of individuals from each species (n=6 for *M. gigas;* n=8 for *O. edulis*) were randomly selected and the mechanical strength of their left valve measured using a vertical compressive force applied to the shell using by force transducer (Instron Testing System, Instron, USA. See detailed methodology in Chapter 3).

Differences in the level of worm infection between species and size classes were assessed using 4x3 contingency table and X^2 test of association. Differences in shell strength between species were assessed using a two-sample Mann–Whitney U-test, after checking for assumptions of normality of distribution and homogeneity of variances. All analyses were performed using the public domain package *R* [version 3.3.1].

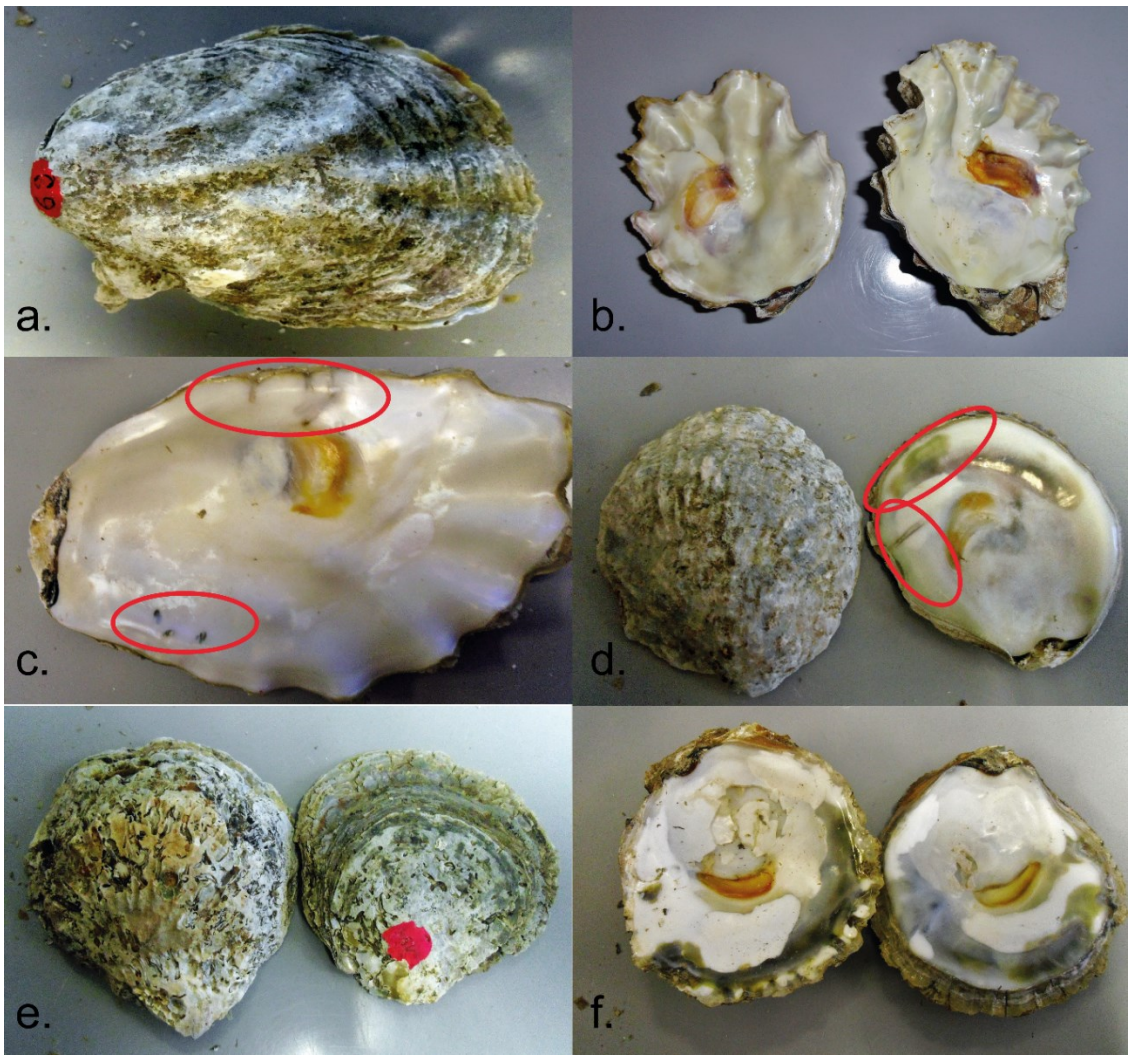

Figure *A4.*1: a) Outside view of the uninfected shell of a large Magallana gigas. b) Inside view of uninfected shells of a small M. gigas. c) Inside view of the shell of a large M. gigas with low level of worm infection, depicted by the presence of burrows (red circles). d) Outside and inside views of the shell of a large Ostrea edulis with low-level of worm infection manifested by burrows and mud blisters (red circles). e) Outside view and inside view f) of a large O. edulis displaying high-level of worm infection.

Results

Species comparison

In total, 72 shells of *Magallana gigas* and 80 shells of *Ostrea edulis* were analysed. There were significant differences in the degree of infection between species, with 19% and 99% of *M. gigas* and *O. edulis* displaying parasitism by *Polydora ciliata*, respectively (Fig A4.2). *O. edulis* was statistically more infected by the worm than *M. gigas* (Table A4.1a, Table A4.2a).

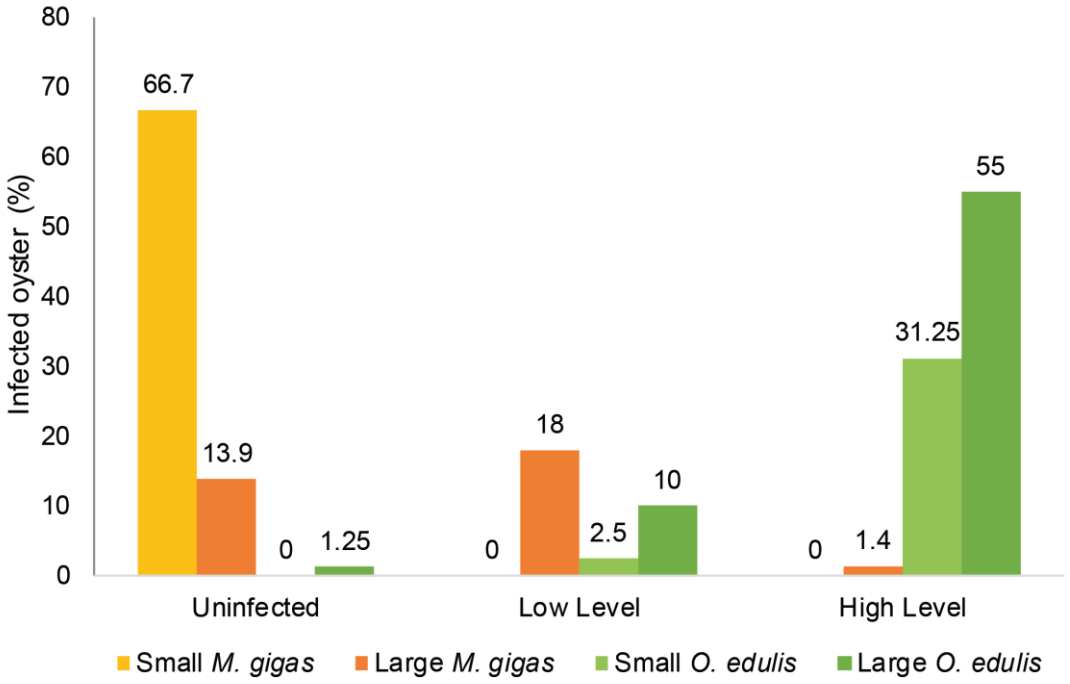

Figure *A4*.2: Percentage of oysters being infected by the parasitic worm Polydora ciliata in small and large size classes of Magallana gigas and Ostrea edulis.

Table *A4*.1: Recorded counts of oyster shells for each infection level a) by species, b) by size class within each species. In brackets are expected values.

a)

	No Infection		Low Level		High Level		Total
Magallana gigas	58	*(27.9)*	13	*(10.9)*	1	*(33.2)*	72
Ostrea edulis	1	*(31.1)*	10	*(12.1)*	69	*(36.8)*	80

b)

		No Infection		Low Level		High Level		Total
Magallana gigas	Small	48	*(38.7)*	0	*(8.67)*	0	*(0.667)*	48
	Large	10	*(19.3)*	13	*(4.33)*	1	*(0.333)*	24
Ostrea edulis	Small	0	*(0.34)*	2	*(3.38)*	25	*(23.3)*	27
	Large	1	*(0.66)*	8	*(6.62)*	44	*(45.7)*	53

Table *A4*.2: Summary of the χ^2 analyses results for a) species comparisons and b) size class within each species comparisons.

a)

Species: *Magallana gigas* vs *Ostrea edulis*

χ2	df	*P*	Outcome
121.43	2	<0.001	*Ostrea edulis* > *Magallana gigas* ***

b)

Size class: Small vs Large

	χ2	df	*P*	Outcome
Magallana gigas	34.759	2	<0.001	Large>Small***
Ostrea edulis	1.5451	2	0.4618	Large=Small

*** p<0.001

Size class comparison within species

For *M. gigas*, all oysters had a lower level of infection than expected, especially for small oysters (Table A4.1b, Table A4.2b). Relatively few individuals (<20%) were infected, with only large oysters showing levels of infection (14 out of 72) (Fig A4.2). In contrast, significantly more *O. edulis* than expected exhibited high levels of infection (86%), with 31% of small and 55% of large oysters, irrespective of size (Table A4.1b, Table A4.2b, Fig A4.2).

Shell strength

As 100% of *O. edulis* shells selected were infected, and 100% of *M. gigas* shells were uninfected, we could not assess the effect of worm infection on shell strength by species, but only the difference in shell strength between infected *O. edulis* and uninfected *M. gigas*. There was no statistically significant difference in shell strength between oyster species (W=42, p=1). The shells of *M. gigas* had a mean strength of ~619.3N ±102, and the shells of *O. edulis* had a mean strength of ~663.1N ±138 (Figure A4.3).

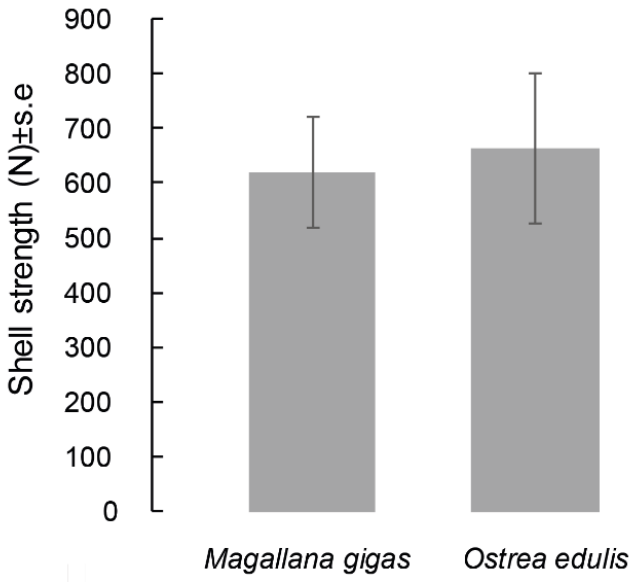

Figure *A4*.3: Strength of the lower valve of shells of Magallana gigas (n=6) and Ostrea edulis (n=8), as means ± standard error (s.e). N=newton.

Discussion

By examining the shells of small and large specimen of *M. gigas* and *O. edulis*, this study aimed to investigate the host preference of *P. ciliata*, and assess the physiological implications of infection. Our results provide evidence of preferential infection of oyster species by *P. ciliata*, in contrast to other studies suggesting no host specificity by that most shell-boring polydorid worms (Simon, 2011). Nevertheless, other studies have demonstrated preferences of *Polydora* worms for some oyster species (Calvo *et al.*, 2000; Calvo *et al.*, 1999). Specifically, the studies by Calvo *et al.*,(2000; 1999) demonstrated that infection prevalence and intensity was higher in the native oyster *Crassostrea virginica* compared to the introduced *Crassostrea ariakensis* and *M. gigas,* which appears in agreement with our findings.

The mechanisms behind infestation are complex, and traits dictating host preferences are still unclear. Infection by *Polydora* worms begin with the settlement of a planktonic larvae onto the oyster shell, which after reaching sexual maturity is able to multiply and colonize the shell (Blake & Evans, 1973). Due to the gregarious nature of the larvae, species-specific chemical cues from the shells are likely to influence settlement and host preference (Blake & Evans, 1973). Shell aspects such as surface roughness, area available for colonisation, and thickness, which vary amongst species, are also important factors for invertebrate larval settlement choice. While not assessed here, thinner shells

have been shown to be a cause of higher susceptible to infection, with infected oysters also displaying more important physiological impacts (Bishop & Hooper, 2005; Calvo *et al.*, 1999). The shell morphologies of *O. edulis* and *M. gigas* are quite distinct (Hu *et al.*, 1993):*M. gigas* possess a thick frilled shell, usually elongated with prominent ribs, whereas *O. edulis* has a thinner rounded shell with obvious concentric flat scales. It is therefore possible that *O. edulis* possess specific shell characteristics that favour the settlement and colonisation of *P. ciliata*.

Whereas there were no differences in infection with oyster size in *O. edulis*, here– *M. gigas* were linked with higher infection rate. This is consistent with the general assumption that infection can be related to the size of the organisms, with bigger organisms displaying higher levels of infection (Ambariyanto & Seed, 1991; Riascos *et al.*, 2008; Royer *et al.*, 2006). This is often explained by both an increase in shell surface for colonization with growth, and a longer exposure to the parasite with age, therefore increasing the probability of infection.

Although not the direct cause of mortality, infection can eventually lead to death through sub-lethal effects. Infection can bring about important negative physiological consequence, such as reduction in flesh weight and condition index, decrease in reproductive output, and lowering of the immune system (Royer *et al.*, 2006). Reduction in shell strength as a consequence of infection was recorded in *Placopecten magellanicus* and *Mytilus edulis*, and was linked to increased predation susceptibility (Bergman *et al.*, 1982; Kent, 1981).Here despite infection, the shell strength of infected *O. edulis* was similar to that of uninfected *M. gigas*. Because nearly 100% of *O. edulis* specimen collected were infected, we could not compare the strength of infected vs uninfected oysters. Therefore, we cannot reject the possibility that infection might have affected *O. edulis* shell strength and altered predator-prey interactions.

Conclusion

The differential infection by *P. ciliata* between *O. edulis* and *M. gigas* observed here, with *M. gigas* apparently more resistant to infection, holds potential implications for species competition and dominance, and their respective population maintenance. It is not impossible that the heavy infection

of *O. edulis* by *P. ciliata* induces other physiological consequences not assessed here that can influence its long-term resilience and survival, and lead to much lower abundances than *M. gigas*.

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