Impacts of ocean acidification on predator – prey interactions of molluscs

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List of achievements in the candidature

Publications

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Conference proceedings

International presentations


The impact of ocean acidification on the predation of populations of the oyster *Crassostrea gigas*. 10$^{th}$ International Temperate Reef Symposium, Perth, Australia, 2014

The impact of ocean acidification on shell strength and predation of populations of the Pacific oyster *Crassostrea gigas*. Asian Pacific Aquaculture, Ho Chi Minh City, Vietnam, 2013
National presentations

Ocean acidification alters the predator-prey relationship between the oyster *Crassostrea gigas* and the whelk *Tenguella marginalba*. Australian Marine Science Association annual conference, Geelong, Australia, 2015

The effect of ocean acidification and predatory relationships on estuarine oysters. Third Annual Marine and Freshwater Student Symposium, Stradbroke Island, Australia, 2014

Populations of Pacific oysters *Crassostrea gigas* respond variably to elevated CO$_2$ and predation by *Tenguella marginalba*. Australian Marine Science Association annual conference, Canberra, Australia, 2014

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In addition to the statements above, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author

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Prof. Pauline Ross 28/02/2017

Supervisor Name Signature Date
Statement of authentication

This is to certify that to the best of my knowledge, the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged. This thesis has not been submitted for any degree or other purposes.

Signature

John Matthew Wright
Abstract

Elevations in atmospheric carbon dioxide (CO\textsubscript{2}) are anticipated to lead to the acidification of world’s oceans over the next century. Molluscs are proving to be amongst the most vulnerable phyla to a more acidic ocean. While there has been a rapid increase in the number of studies examining the effect of ocean acidification on the morphology and physiology of molluscs and other marine organisms, only a handful of studies have investigated whether species interactions will be altered.

This thesis sought to understand the fate of the predator-prey interactions between the endemic predator, the Mulberry whelk \textit{Tenguella marginalba} (Blainville, 1832) and their prey the native Sydney rock oyster \textit{Saccostrea glomerata} (Gould, 1850) and the recently introduced Pacific oyster \textit{Crassostrea gigas} (Thunberg, 1793) in a marine environment increasingly affected by elevated pCO\textsubscript{2}.

It was predicted that predator-prey relationships will be altered by exposure to elevated pCO\textsubscript{2} because:

1. The energetic costs for \textit{T. marginalba} to survive in an acidified environment will increase causing them to compensate by increasing their consumption rate of prey. A number of recent studies have suggested that increased energy demand may be met through greater food consumption which may ameliorate the negative impacts of elevated CO\textsubscript{2} (Melzner et al. 2011; Parker et al. 2012; Hettinger et al. 2013).

2. Growth and physiological defences of \textit{S. glomerata} and \textit{C. gigas} will decrease as reduced seawater calcium carbonate saturation states make shell calcification and maintenance more difficult and greater energy is required for the maintenance of acid-base balance.

3. It was also predicted that responses will vary between oyster species and within populations of oysters. Differences in response to pCO\textsubscript{2} between \textit{C. gigas} and \textit{S. glomerata} have already been demonstrated in the absence of a predator (Parker et al. 2010). Parker et al. (2010) showed that the impacts of elevated pCO\textsubscript{2} on larvae and spat of \textit{S. glomerata} were far
greater than the impacts on larvae and spat of *C. gigas*. Further, Parker et al. (2011) showed differences in the growth of family lines of *S. glomerata* under elevated \( pCO_2 \), with some family lines not affected. Here we hypothesised that the inter/species-specific differences in the responses of *C. gigas* and *S. glomerata* to elevated \( pCO_2 \) would in turn also alter their predator prey relationship under elevated \( pCO_2 \). Further, that within species differences would also be found for *C. gigas* and that these too would alter predatory prey relationships under elevated \( pCO_2 \).

To test these hypotheses, both predator and prey; *T. marginalba, C. gigas* and *S. glomerata* were exposed to ambient and elevated \( pCO_2 \) (-0.3 to -0.4 pH units) in a series of complex, orthogonal and chronic experiments in which the entire species complex (both the predator and two prey species) were exposed to elevated \( CO_2 \).

This study provided support consistent with the hypothesis that predation of oysters by *T. marginalba* will increase because exposure to elevated \( pCO_2 \) will cause an increase in the energetic costs of the predator. In general a greater number of oysters (both *C. gigas* and *S. glomerata*) were consumed by *T. marginalba* when whelks were exposed to elevated \( pCO_2 \) than when whelks were exposed to ambient \( pCO_2 \). Increased consumption of oysters under elevated \( pCO_2 \) suggested that the whelk generally increased its energetic uptake in an attempt to meet increased maintenance costs of acid-base balance and shell calcification at elevated \( pCO_2 \). An increase in consumption was at times correlated with a significant increase in the Standard Metabolic Rate (SMR) of *T. marginalba* in response to elevated \( pCO_2 \). Overall there was no long term effect of elevated \( pCO_2 \) on the growth of *T. marginalba*, but there was an increase in the strength of the shell at elevated \( pCO_2 \). In summary this study found *T. marginalba* was relatively resilient to ocean acidification. Although SMR of *T. marginalba* were increased, they were able to prevent any potential negative long term impacts of elevated \( pCO_2 \) on their shell by increasing their rates of consumption of prey. There was a positive effect of elevated \( pCO_2 \) on shell growth of small sized *C. gigas*. This response was in contrast to
previous studies which have found the impacts of elevated $pCO_2$ on shell growth to be more profound for smaller, younger oysters as they attempt to deposit their calcium carbonate shell. Small oysters may be able to invest energy into growth when exposed to elevated $pCO_2$ as a mechanism, to avoid negative impacts in oceans which are becoming more acidic. Potentially faster growth at elevated $pCO_2$ may allow them to reach a less vulnerable life stage and perhaps even earlier reproductive maturity. There was a change in the SMR of small sized $C. gigas$ held at elevated $pCO_2$ in response to $T. marginalba$. In the absence of $T. marginalba$ the SMR of $C. gigas$ reared at elevated $pCO_2$ was similar to that of the ambient controls. In the presence of the $T. marginalba$, however, the SMR of $C. gigas$ was significantly lower at ambient than elevated $pCO_2$.

This study provides some support for the second hypothesis that growth and physiological defences of $C. gigas$ will decrease as reduced seawater calcium carbonate saturation states make shell calcification and maintenance more difficult and greater energy is required for the maintenance of acid-base balance. In contrast to the response of the smaller sized $C. gigas$ in this study, the larger sized $C. gigas$ showed a significant reduction in shell growth at elevated $CO_2$ and did not display a reduction in SMR at ambient $CO_2$ when in the presence of the predator. This lack of predatory defence in the larger sized oysters may occur because 1. the metabolic output of a larger sized oyster is higher and it will still be high enough for a predator to detect if it is reduced, and/ or 2. given that their shell is larger and that $T. marginalba$ preferentially select smaller sized prey, larger $C. gigas$ they may be less concerned about predation. There was no effect of elevated $CO_2$ on the size of $S. glomerata$ with or without the presence of the predator $T. marginalba$. In addition, SMR of $S. glomerata$ was decreased under elevated $CO_2$. Unlike $C. gigas$, $S. glomerata$ did not reduce their SMR in the presence of the predator $T. marginalba$. This may suggest that $S. glomerata$ do not utilise reduced metabolic output as a defensive strategy to avoid detection by $T. marginalba$, perhaps because they have thicker, stronger shells compared to $C. gigas$. 


Triploid breeding programs were not specifically established to produce oysters resilient to elevated \( pCO_2 \), yet in this study triploid \( C. gigas \) were more resilient than diploids \( C. gigas \) to elevated \( pCO_2 \). Triploid \( C. gigas \) grew faster than diploid \( C. gigas \). Further, diploid \( C. gigas \) had a significant reduction in growth at elevated \( pCO_2 \) while the growth of triploid \( C. gigas \) was not reduced by elevated \( CO_2 \), except for shell depth. Triploid \( C. gigas \) had greater growth in shell height at elevated \( pCO_2 \). As a result, triploid \( C. gigas \) may be used to help to sustain important aquaculture industries in Australia and around the world but this will not improve the ecological resilience and sustainability of invasive \( C. gigas \). Triploid \( C. gigas \) may have a greater pool of energy available for acid-base balance and shell growth then diploid \( C. gigas \) of a similar size under elevated \( pCO_2 \) stress which was likely to contribute to their increased resilience.

This study provides evidence that alterations in predator-prey relationships will be complex.

Responses of oysters to elevated \( CO_2 \) were variable and dependent on the species, family line, ploidy and size which in some cases interacted with the presence of the whelk.

Given the large global consumption of oysters as the most abundant harvested shellfish, near-future ocean acidification was predicted to have serious consequences for the sustainability of these vital aquaculture industries. This thesis provides evidence that utilising triploid breeding programs to produce oysters which can divert a greater proportion of their energy budget into growth and acid-base balance, may be a viable option to reduce the predicted impacts of elevated \( pCO_2 \) on oyster aquaculture over this century. Preliminary evidence for selecting oyster family lines that are resilient to both elevated \( pCO_2 \) and predation suggests that this may be a challenge and more research is required to determine whether this is a feasible option to help ‘climate-proof’ aquaculture industries and oyster populations in Australia and around the world.
Chapter one
General introduction

The aim of this general introduction is to introduce climate change (1.1), the evidence for climate change (1.2) describe how ocean chemistry is changing (1.3) to create ocean acidification (1.4), the potential response of marine molluscs (1.5), the likelihood of alterations on the predator-prey interactions (1.6) and the aim, species selected and the hypotheses tested in this thesis (1.7).

1.1 Climate change

Concerns that global climate change will affect the diversity and survival of species and ecosystems have arisen over the last two decades. Global climate change is now recognised as the biggest threat to the function of Earth’s abiotic and biotic systems (IPCC 2013a). It is widely agreed within the scientific community that the Earth’s climate has varied over geologic time due to natural processes including solar variation (Ammann et al. 2007) volcanic eruptions (Crowley 2000) and El Niño – Southern Oscillation (Cobb et al. 2003). However, it is the more recent exponential increase in atmospheric carbon dioxide (CO$_2$) that is most alarming. Atmospheric concentrations of CO$_2$ are rising at a rapid rate because of the combustion of fossil fuels, deforestation and cement production (Houghton 2001; IPCC 2013a). Currently the rate of CO$_2$ emissions exceeds that of any other geological time on the planet. As a consequence of this increasing atmospheric CO$_2$, it has been suggested that oceans will acidify in a process known as ocean acidification. The rate of CO$_2$ absorption by the oceans may threaten the biodiversity and survival of marine ecosystems because many marine organisms may be unable to adapt (Dupont et al. 2010; Gazeau et al. 2013; Gattuso et al. 2015). An understanding of the effect of near-future ocean acidification on marine organisms has been difficult to predict (Pörtner et al. 2004; Dupont et al. 2010; Hendriks et al. 2010) but will be essential to improve our understanding of the severity and consequences of future change.
1.2 Evidence for climate change

Strong scientific evidence suggests the concentration of atmospheric $p$CO$_2$ now exceeds the natural levels present on earth for the past 650,000 years (Lüthi et al. 2008; IPCC 2013a). Evidence that the climate has changed has been sourced from paleontological studies (Zachos et al. 2005; Lüthi et al. 2008; Merilä and Hendry 2014), comparative sampling, physical analysis (Raven et al. 2005; Lejeusne et al. 2010; Kitidis et al. 2016) and computer modelling (Caldeira and Wickett 2005; Canadell et al. 2007; Domingues et al. 2008; Yospin et al. 2015). Anthropogenic activities such as fossil fuel burning, deforestation, agriculture, industrial production and an increase in the net human population have exponentially increased the amount of CO$_2$ released into the atmosphere (Pörtner and Reipschlager 1996; Feely et al. 2004; Raven et al. 2005; Tyrrell 2011). Atmospheric $p$CO$_2$ has increased from 280 ppm in the mid-18th century to 406 ppm recorded at Mauna Loa Observatory, Hawaii in January 2017 (NOAA 2017). Atmospheric $p$CO$_2$ has increased at an unprecedented rate faster than that which has occurred for millions of years (Turley et al. 2006; Guinotte and Fabry 2008; Tyrrell 2011). The Mauna Loa Observatory in Hawaii and historical measurement stations at the South Pole provide evidence for this exponential increase in atmospheric $p$CO$_2$ (Forbes 2014; Lin et al. 2014) with an annual increase of approximately 2 µatm in recent years (NOAA 2017). The average annual increase in atmospheric $p$CO$_2$ ranged between 0.5 and 1 µatm during the early 1950’s when records began (NOAA 2017).

1.3 Changing ocean chemistry

The oceans are the largest active sinks of anthropogenic carbon on Earth and have absorbed around one-third of the CO$_2$ released into the atmosphere (Sabine et al. 2004; Orr et al. 2005; Marshall and Thunell 2013). At first scientists believed the Earth’s oceans could absorb the excess greenhouse gas, but now scientists recognise that there will be a limit to the capacity of the oceans to absorb the CO$_2$. Out of the approximately seven billion tonnes of carbon being released globally as CO$_2$, the
oceans are absorbing around two billion tonnes each year (Feely et al. 2010; IPCC 2013a; Gruber et al. 2014).

Since the early 1800’s the oceans have absorbed an estimated 26% of atmospheric CO₂ emissions (Sabine et al. 2004; Canadell et al. 2007; Le Quéré et al. 2015), while only 43% remains in the atmosphere (Canadell et al. 2007). The remaining CO₂ has been sequestered for vegetation growth (Canadell et al. 2007) (Figure 1.1). Already the increased production of CO₂ has caused noticeable changes in oceanic environments with reductions in surface ocean pH and carbonate ion concentration (Gruber et al. 2012; Takahashi et al. 2014; Kitidis et al. 2016).

**Figure 1.1** The movement of carbon between land, atmosphere, and oceans in billions of tons of carbon in a certain year (quantities are changing over time). Yellow numbers are natural fluxes; red are human contributions in billions of tons of carbon per year. White numbers indicate stored carbon. The net uptake of carbon by the oceanic sink is on average two billion tonnes per year (Adapted from; DOE, USA, 2011).
1.4 Ocean acidification

The carbonate buffer regulates the concentration of hydrogen ions by converting $\text{CO}_2$ to bicarbonate using carbonate as the buffer. When $\text{CO}_2$ dissolves into seawater it combines with $\text{H}_2\text{O}$ to form carbonic acid ($\text{H}_2\text{CO}_3$), a weak acid which quickly dissociates to its constituents, readily losing hydrogen ions in seawater to form bicarbonate ($\text{HCO}_3^-$) ions. The ensuing free hydrogen ($\text{H}^+$) then combines with free carbonate ions ($\text{CO}_3^{2-}$) to produce further $\text{HCO}_3^-$ (Raven et al. 2005; Cao and Caldeira 2008; Buck and Folger 2009). It is anticipated that an increase in $\text{CO}_2$ will be absorbed into seawater will exceed the rate of replenishment of carbonate ions at the seawater surface in the near-future (Canadell et al. 2007). The net effect will be an increase in hydrogen and bicarbonate ions and a reduction in the concentration of carbonate ions. The resulting increase in the concentration of hydrogen will increase the acidity of the seawater (Figure 1.2).

![Figure 1.2 Comparison of the chemistry of surface seawater in the later 1800s (left) and 2100 (right). The acidity of the surface seawater had remained stable at around pH 8.2 for the most part of the past 750,000 years as the carbonate buffer system had kept the concentration of hydrogen](image-url)
ions constant. There will be fewer carbonate ions available and a reduction in the pH of surface seawater in the near-future as anthropogenic CO$_2$ exceeds the buffering capacity of carbonate. (Encyclopaedia of Britannica, 2012).

Since the start of industrialisation the pH of oceanic waters has decreased by 0.1 units (IPCC 2013b). As the pH scale is logarithmic, this equates to a 30% reduction in hydrogen ions between industrialisation and the mid-1990’s (Orr et al. 2005; Canadell et al. 2007; Doney et al. 2009). The Intergovernmental Panel on Climate Change (IPCC; 2013a) predicts that if the present CO$_2$ emission trend continues a further decrease in oceanic pH of 0.3 to 0.5 units (pH 7.9 - 7.6) will occur by the end of this century (2100) and a decrease of 0.7 to 0.77 units (pH 7.5 - 7.33) by 2300. The carbonate chemistry of the oceans, however, is variable and likely to become even more variable depending on the degree of local upwelling of cold CO$_2$ rich water. Absorption of CO$_2$ into the oceans is prolonged by an extended residence time of CO$_2$ in the atmosphere. The oceans will continue to absorb CO$_2$ from the atmosphere for many years even if all CO$_2$ emissions were stopped tomorrow (Bates et al. 2012; Le Quéré et al. 2015; Taylor et al. 2015).

An increase in seawater acidity results in a reduction in the availability of carbonate ions essential for life processes (Buck and Folger 2009; Doney et al. 2009). The combination of calcium ions, abundant in nature, and carbonate ions produces calcium carbonate, a solid structural formation (Fabry et al. 2008; Buck and Folger 2009). Current seawater conditions are slightly alkaline, around 8.1 pH units (IPCC 2013b) which provides ample carbonate ion availability for shell calcification and skeletal growth (Raven et al. 2005; Thomsen et al. 2015; Rosón et al. 2016). The saturation of calcium carbonate is negatively affected where the concentration of carbonate ions are reduced. An increase in seawater acidity will shift the abundance of this resource, the carbonate saturation horizon will decrease and the availability of carbonate ions will decrease (Carter et al. 2014; Feely et al. 2014). Calcifying marine biota exist above the saturation horizon where the calcium carbonate saturation state ($\Omega$) is >1.0. Where $\Omega <1.0$, below the saturation horizon, under saturation of
carbonate ions occurs, making it more difficult for calcifying organisms to construct their shells and skeletons.

1.5 Response of marine molluscs

One of the biggest problems in the current research agenda is how to predict the future effects on species in the context of both rapid change in physical conditions and the accompanying changes in species interactions. Marine species may adapt through evolutionary change in a future where the oceans are more acidic (Reusch 2013; Sunday et al. 2014). Evolutionary adaptation may be possible through variation of the genetic traits sensitive to $pCO_2$ (Sunday et al. 2014). There is a growing appreciation of population based assessments to determine the impact environmental change will have on a species as such variability in response may be the key to species survival in a climate changed ocean (Reusch et al., 2014, Sunday et al., 2014). These responses will be summarised in the next section. One of the biggest unknowns that remains in ocean acidification research is how species will adapt and respond to each other.

Many of the most striking consequences of ocean acidification will arise through altered species interactions (Gaylord et al. 2015). One of the most vulnerable groups of organisms to ocean acidification are molluscs (Doney et al. 2009), the phylum of focus in this thesis. Already we know that exposure of mollusc species to elevated $pCO_2$ may impact a wide range of morphological and physiological characteristics at the individual level including shell and somatic growth, protein degradation, behaviour acid-base balance, energy metabolism and survival (Gazeau et al. 2013, Parker et al. 2013). How morphological, physiological and behavioural changes will impact complex species interactions remains relatively unknown as most studies have focused on the impacts of climate change on single species.

All organisms are part of a complex network of species whose interactions determine population distribution, abundance and community structure. As summarised by Kroeker et al. (2014), “the
emergent effects of environmental change on any one species will therefore depend on its interactions with others”. Despite this knowledge, our understanding of the effects of ocean acidification on the outcomes of species interactions is very limited (Brooker et al. 2007; Gaylord et al. 2015). Predator-prey interactions play a central role in structuring natural communities (Paine 1976; Holt 1977; Ambrose 1984), and the effects of ocean acidification on predator - prey interactions could disproportionately influence community structure in the future (Kroeker et al. 2014; Gaylord et al. 2015).

1.6 Alterations to predator-prey interactions

Only a handful of studies (Table 1.1) and three review papers document alterations of predator - prey interactions in molluscs due to ocean acidification (Kroeker et al. 2014; Sanford et al. 2014; Nagelkerken and Munday 2015). Of these studies, only one focussed on the effects of $p$CO$_2$ on predator foraging and just seven used values of $p$CO$_2$ as predicted for the end of this century by the IPCC (2013a). To summarise the findings of these studies, the total energy available to calcified species must be allocated among offensive capacity (capture of prey), defences (including calcification, avoidance behaviours, and immune responses), growth, and reproduction (Figure 1.3). While many of these changes could increase per capita predation rates on calcified prey, emerging evidence suggests that ocean acidification may have complex neurological effects that impair chemoreception, learning, and other behaviours important for predator foraging (Manríquez et al. 2013; Watson et al. 2014; Manríquez et al. 2016; Glaspie et al. 2017). Ferrari et al (2010) described the interaction between predator and prey as an evolutionary arms race between the foraging ability of predators and the defences of prey. The ability of molluscs to maintain defensive capacity will become more energetically costly as they work to maintain their acid-base balance in an ocean that will continue to increase in acidity (Beniash et al. 2011; Cole et al. 2016; Waldbusser et al. 2016). The current knowledge of the effects of ocean acidification and the implications for predator foraging and prey defence will now be discussed.
Figure 1.3 Conceptual framework of the influence of ocean acidification on the pathways that determine predator-prey interactions (Kroeker et al. 2014).
Table 1.1 Summary of the direct impacts of ocean acidification on the predator-prey interactions of molluscs.

d = days, w = weeks, mo = months. When the experiment manipulated more than one factor (e.g., temperature, oxygen or salinity), the effect of ocean acidification is reported only at the ambient level of the second factor.

<table>
<thead>
<tr>
<th>Prey species</th>
<th>Predator species</th>
<th>Parameter measured</th>
<th>pH (unit decrease from ambient)</th>
<th>Impact (↑/↓/=)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.1</td>
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<td></td>
<td></td>
<td>-1.0</td>
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</table>

<table>
<thead>
<tr>
<th>Prey species</th>
<th>Predator species</th>
<th>Parameter measured</th>
<th>Predicted 2100</th>
<th>Predicted 2300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

**Gibberulus gibbosus** (stromb snail)

**Conus mamoreus** (cone shell)

1 w (Watson et al. 2013)
- Prey reaction time ↓
- Escape response ↓

2-3 w (Watson et al. 2017)
- Predator activity ↑
- Predation rate ↓

**Semibalanus balanoides** (barnacle)

**Nucella lapillus** (dogwhelk)

80 d (Harvey and Moore 2017)
- Prey quality ↓
- Predation rate ↓

**Brachidontes variabilis** (mussel)

**Thais clavigera** (muricid gastropod)

21 d (Xu et al. 2017)
- Prey foraging time ↑
- Prey handing time ↓
- Predation rate =

**Littorina littorea** (periwinkle snail)
<table>
<thead>
<tr>
<th><strong>Carcinus maenas</strong> (green crab)</th>
<th>15 d</th>
<th>(Bibby et al. 2007)</th>
<th>Predator avoidance</th>
<th>= -1.34</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Predator avoidance (+predation cue)</td>
<td>↑ -1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Respiration rate</td>
<td>= -1.34</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Respiration rate (+predation cue)</td>
<td>↓ -1.34</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Shell thickness</td>
<td>= -1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shell thickness (+predation cue)</td>
<td>↓ -1.34</td>
<td></td>
</tr>
<tr>
<td><strong>Tegula funebralis</strong> (black turban snail)</td>
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<tr>
<td><strong>Pisaster sp.</strong> (Pacific sea star)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>(Jellison et al. 2016)</td>
<td>Time in refuge</td>
<td>↑</td>
<td></td>
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<tr>
<td></td>
<td>Detect predator</td>
<td>=</td>
<td></td>
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<tr>
<td><strong>Ostrea lurida</strong> (Olympia oysters)</td>
<td></td>
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<tr>
<td><strong>Urosalpinx cinerea</strong> (Atlantic oyster drills)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(Sanford et al. 2014)</td>
<td>Drilling</td>
<td>↑</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Consumption</td>
<td>↑</td>
<td></td>
<td></td>
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<tr>
<td><strong>Concholepas concholepas</strong></td>
<td></td>
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<tr>
<td><strong>Acanthocyclus hassleri</strong> (crab)</td>
<td></td>
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</tr>
<tr>
<td>83 d</td>
<td>(Manríquez et al. 2013)</td>
<td>Calcification</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td><strong>Concholepas concholepas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mo</td>
<td>(Manríquez et al. 2014)</td>
<td>Predator detection</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predator avoidance</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Manríquez et al. 2016)</td>
<td>Shell growth</td>
<td>↓</td>
<td></td>
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<tr>
<td></td>
<td>Self righting</td>
<td>↓</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Predator escape</td>
<td>↓</td>
<td></td>
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<tr>
<td><strong>Littorina littorea</strong></td>
<td></td>
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<tr>
<td>Species</td>
<td>Feature</td>
<td>Timeframe</td>
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<td>Result 2</td>
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<td>----------------------------------</td>
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</tr>
<tr>
<td><em>Carcinus maenas</em> (green crab)</td>
<td>Shell strength</td>
<td>5 mo</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Handling time</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><em>Mytilus californianus</em> (California mussel) <em>Nucella canaliculata</em></td>
<td>Shell thickness</td>
<td>14 days</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Consumption</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><em>Brachidontes pharaonis</em> (mussel) <em>Eriphia verrucosa</em> (crab)</td>
<td>Condition index</td>
<td>4 w</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shell strength</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Handling time</td>
<td></td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Energy content</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><em>Crassostrea virginica</em> <em>Panopeus herbstii</em></td>
<td>Consumption</td>
<td>71 d</td>
<td>↓</td>
<td>↓-1.5</td>
</tr>
<tr>
<td></td>
<td>Handling time</td>
<td></td>
<td>=</td>
<td>↓-1.5</td>
</tr>
<tr>
<td></td>
<td>Prey calcification</td>
<td></td>
<td>=</td>
<td>↓-1.5</td>
</tr>
<tr>
<td></td>
<td>Prey calcification (+predator)</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predator calcification</td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td><em>Mytilus coruscus</em> (mussel) <em>Charybdis japonica</em> (crab)</td>
<td>Byssus production</td>
<td>3 d</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Byssus shedding</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Byssus length</td>
<td></td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td><em>Nucella lapillus</em> (gastropod)</td>
<td>Shell density of prey</td>
<td>14 mo</td>
<td>↓</td>
<td></td>
</tr>
</tbody>
</table>
### Metabolic rate

- **↑**

### Foraging time

- **=**

### Foraging distance

- **↑**

### Handling time

- **↑**

### Foraging cost

- **↓**

---

<table>
<thead>
<tr>
<th>Species</th>
<th>Time (h/d)</th>
<th>Trait</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilus coruscus</em> (mussel)</td>
<td>72 h</td>
<td>Byssus production</td>
<td>↓</td>
</tr>
<tr>
<td><em>Charybdis japonica</em> (Asian paddle crab)</td>
<td></td>
<td>Byssus shredding</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Byssus thickness</td>
<td>↓</td>
</tr>
<tr>
<td><em>Mya arenaria</em> (soft shell clam)</td>
<td>30 d</td>
<td>Prey responsiveness</td>
<td>↓</td>
</tr>
<tr>
<td><em>Callinectes sapidus</em> (blue crab)</td>
<td></td>
<td>Shell dissolution</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time to find prey</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prey encounters</td>
<td>↑</td>
</tr>
<tr>
<td><em>Austrocochlea porcata</em></td>
<td>95 d</td>
<td>Shell repair rate</td>
<td>↓</td>
</tr>
<tr>
<td><em>Ozius truncates</em> (durophagous crab)</td>
<td></td>
<td>Shell integrity</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Condition</td>
<td>↓</td>
</tr>
<tr>
<td><em>Subninella undulata</em> (top shell)</td>
<td>65 d</td>
<td>Shell repair rate</td>
<td>=</td>
</tr>
<tr>
<td><em>Ozius truncates</em> (durophagous crab)</td>
<td></td>
<td>Shell integrity</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Condition</td>
<td>=</td>
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</tbody>
</table>
**Physiology and metabolic function**

The number of studies which have examined the metabolic functioning of molluscs in response to elevated $pCO_2$ is rapidly expanding. We already know that living in a high-CO$_2$ world will cause an increase in the demand of energy for many molluscs including a reduction in their scope for growth (Pörtner 2008; Parker et al. 2013; Gaylord et al. 2015). This increase in the energy budget arises because in the absence of acclimation or adaptation, the cost of routine maintenance is much higher at elevated $pCO_2$. An early study on *Mytilus galloprovincialis* exposed to the upper limit predictions of carbon dioxide concentration for 2300 found a significant negative metabolic response and a three-fold increase in haemolymph pH (Michaelidis et al. 2005). Thomsen and Melzner (2010) found significantly greater metabolic respiration of the Mediterranean mussel, *Mytilus edulis*, in moderately elevated $pCO_2$ as the species attempted to compensate homeostasis. Using carbon dioxide concentrations well beyond levels predicted for 2100, other mollusc species have also shown a similar negative response in extracellular acidosis, e.g. crab, *Necor puber* (Spicer et al. 2007) and the oyster, *Crassostrea gigas* (Lannig et al. 2010).

Increased energetic demands due the physiological effects of ocean acidification could increase predation rates (Gooding et al. 2009) as predators attempt to regulate internal acid base balance in elevated $pCO_2$ conditions (Ishimatsu et al. 2008) and maintain their energy budget. A number of studies have suggested that supply of adequate food may ameliorate the negative impacts of elevated $pCO_2$ (Clements 2016; Cole et al. 2016; Ramajo et al. 2016). For example, juvenile king scallop, *Pecten maximus*, were able to maintain adequate feeding efficiency to meet the energetic requirements following three months exposure to elevated $pCO_2$ (750, 1150 ppm) when the availability of food was high (Sanders et al. 2013). Supply of an adequate supply of food may also benefit prey. Kroeker et al. (2016) found that when food availability was high the effect of low pH on the growth and calcification of the shell and subsequent risk of predation of the mussel, *Mytilus californianus* was low compared to when food was limited.
In episodes of more extreme stress the food supply available to an animal may not be adequate to meet the requirements of an increased energy demand. When this occurs and compensation of homeostasis is not possible, species, including both predator and prey may enter a state of metabolic depression in an effort to conserve energy and prolong survival (Pörtner 2008). In one of the only studies on the effect of ocean acidification on molluscan predators, the resting oxygen consumption of the predatory gastropod *Nucella lapillus* decreased gradually with increase in $pCO_2$. This was coupled with a decrease in the distance spent foraging (Queirós et al. 2015). Similarly for the dogwhelk *Nucella lapillus* energy gained from barnacle prey, *Semibalanus balanoides*, may not have been sufficient to meet an increase in metabolic demand as predation rates had reduced to the level of starvation, even when prey were abundant (Harvey and Moore 2017). The muricid gastropod, *Thais clavigera*, was found to prefer large over small mussel, *Brachidonte variabilis*, and increased foraging time following exposure to elevated $pCO_2$ (950 μatm) compared to ambient $pCO_2$ (380 μatm) (Xu et al. 2017). Ocean acidification may increase the number and duration of unsuccessful predation attempts as mollusc predators source, handle and consume prey (Gaylord et al. 2015). The capacity of predatory molluscs to take advantage of the physiological and metabolic changes of prey may be hindered by ocean acidification.

*Calcification and morphology*

A general increase in the metabolic costs of all species, including both predators and prey could impact populations through basic energy constraints including the calcification, growth, strength and composition of the shell within the IPCC (2013a) predicted pH range for 2300 (Ross et al. 2011; Byrne and Przeslawski 2013; Gazeau et al. 2013; Parker et al. 2013; Azevedo et al. 2015; Harvey et al. 2016). As many predator-prey interactions are size structured (Paine 1976), a reduction in predator size could reduce the preferred size of prey (Hughes 1985). Reduced predator size could reduce the size of prey that a predator is able to successfully capture and consume (Dayton 1971; Paine 1976) or increase its handling time of prey. Among calcified predators, feeding modes may provide insight
into which types of predators may be more vulnerable to ocean acidification. For example, the success of predatory gastropods that use their chitinous radulae and acidic secretions to bore a hole in shells of prey may be less affected by acidification because it is independent of their own shell thickness or strength (Amaral et al. 2012a; Sanford et al. 2014).

The classical foraging model of ecology predicts that predators preferentially target prey which would provide the greatest energetic gain (Kelley 1991; Thomas and Day 1995; Gosselin and Chia 1996). Predators must consider if the energy gained from a predation attempt will provide sufficient return on their investment. Consequently, prey with high energy content that can be captured and processed more easily are generally favoured by predators. For example, the cannibalistic gastropods, *Lunatia heros* and *Polinices duplicatus*, selected size-specific prey which would provide sufficient energy to outweigh the time it takes and energy required to break through a shell and consume the flesh (Kelley 1991). Boreholes by the muricid gastropod, *Haustrum baileyanum*, have been found to be concentrated around the thickest part of the shell of the abalone *Haliotis rubra*. This area is also where the energy rich flesh of the muscle is attached to the shell, suggesting the energetic gain for *H. baileyanum* outweighs energy required to access the flesh (Thomas and Day 1995). The size, defences of prey and the location of drill site will influence the energetic benefits of prey to predators and may determine the success rate of predation attempts (Thomas and Day 1995).

Many mollusc prey display inducible defensive responses in the presence of predators (Kats and Dill 1998; Wisenden 2000; Luttbeg and Trussell 2013; Hollander and Bourdeau 2016). Calcified prey allocate a large amount of energy to shell growth as a defence against predators (Palmer 1992). For example, the mussel *M. edulis* increased the thickness and mass of the shell in areas where predation risk by crabs was high (Leonard et al. 1999), had thicker shell lips when exposed to waterborne predator cues (Smith and Jennings 2000), and the ability to partition energy to defences relative to the type of predator when held with sea stars or crabs (Lowen et al. 2013). One of the
first studies to look at predator mediated change on bivalves found a reduction in the growth of the shell of the clam *Mercenaria mercenaria* in the presence of the whelk *Busycon carica*, even with no direct contact with the whelk (Nakaoka 2000). Similarly in the eastern oyster *Crassostrea virginica*, the weight and thickness of the shell was significantly greater in the presence of the oyster drill, *Urosalpinx cinerea* (Lord and Whitlatch 2012). There was also an increase in the length and secretion rate of byssus threads of the mussel *Mytilus coruscus* that were exposed to predatory cues of the crab *Charybdis japonica* (Li et al. 2015). The production of the enzymes required for the deposition of calcium carbonate will be energetically costly for marine calcifiers as the oceans become more acidic and the availability calcium carbonate ions are reduced (Dupont and Thorndyke 2009).

A number of studies have found some prey defences to be susceptible to ocean acidification. For example, the lateral length of the shell of the intertidal snail *Littorina obtusta* was significantly shortened after 27 days held in seawater with an elevated pH of 0.6 units (Ellis et al. 2009). Changes to prey morphology will increase their risk of being dislodged, crushed or drilled and consumed by a predator. Defensive mechanisms of molluscs may indeed be impaired as oceans acidify. For example Bibby et al. (2007) found the periwinkle snail *Littorina obtusata* which increased thickness of their shell in response to the predator *Carcinus maenas*, but was unable to do this when exposed to elevated $pCO_2$ (1.3 pH) for 15 days. The snails did, however, increase their avoidance behaviour at elevated $pCO_2$, which may partly compensate for their lack of morphological defence (Bibby et al. 2007). Similarly reduced shell strength of *Littorina littorea* was associated with reduced handling time by the predatory green crab *Carcinus maenas* when exposed to reduced pH -0.5 (Landes and Zimmer 2012).

Similar negative consequences have been found for the anti-predator response of bivalves. For mussels, byssus threads of *M. coruscus* were thinner and weaker (Sui et al. 2015), grew slower and
shorter after exposure to elevated pCO$_2$ (Li et al. 2015). The shell strength of prey mussel *Brachidontes pharaonis* was 65% weaker following exposure to a reduced pH (pH 7.5) for four weeks (Dupont et al. 2015). Consequently the prey handling time by the predatory crab *Eriphia verrucosa* was reduced by 27% (Dupont et al. 2015).

Studies have also found severe impacts of ocean acidification on the calcified defences of oysters. Shells of the pearl oyster *Pinctada fucata* were up to 27% weaker and begun to dissolve when held in seawater with elevated levels of acidity (pH 7.8 and 7.6) (Welladsen et al. 2010). Furthermore, the Atlantic oyster drill *Urosalpinx cinerea* consumed 48% more juveniles of the Olympia oyster *Ostrea lurida* from a high pCO$_2$ treatment (1000 µatm) than those from ambient (500 µatm) conditions and this increase in predation was correlated with a 29-40% decrease in the size of *O. lurida* from the elevated treatment (Sanford et al. 2014). Calcified shells and skeletal structure are particularly susceptible to environmental stress and can provide an indicator of the effect of ocean acidification and predation. Any impact on the ability of sessile molluscs like bivalves to calcify and maintain structural morphology may limit their ability to defend against predators.

*Behaviour*

Some mollusc species have behavioural responses that allow them to avoid and escape predators. For example, the herbivorous snail *Tegula funebralis* has exhibited a strong flee response to the presence of the sea star predators *Pisaster ochraceus* and *Leptasterias* sp. (Morgan et al. 2016). There are now a number of studies which have found changes in the behaviour of molluscs following exposure to elevated levels of pCO$_2$. Studies have found changes in the clapping performance of adult *Pecten maximus* (Schalkhausser et al. 2013), burrowing activity of adult *Mercenaria mercenaria* (Green et al. 2013) and juvenile *Mya arenaria* (Clements and Hunt 2014), and feeding activity of *Concholepas concholepas* and *Perumytilus purpuratus* (Vargas et al. 2015).
Alterations to mollusc behaviour can impact on the ability of prey to respond to predation risk. For example, the anti-predator defences of the snail *T. funebralis* was disrupted following exposure to elevated $p$CO$_2$ (pH -0.9) with a 50% reduction in time individuals spend in refuge locations, but there was no disruption to the ability of the snail to detect the predator cue of *Pisaster* sp. (Jellison et al. 2016). Manriquez et al. (2013) found an increase in self-righting behaviour of *Concholepas concholepas* exposed to the crab predator *Acanthocyclus hassleri* even under elevated (706 and 1036 µatm) compared to ambient $p$CO$_2$ (388 µatm) and this was complemented with no effect of CO$_2$ on calcification or growth.

Watson et al. (2014) found the stromb snail, *Gibberulus gibbosus*, exposed to elevated $p$CO$_2$ of 961 µatm for one week had a slower reaction time and an altered escape trajectory in the presence of a predator, the cone shell *Conus mamoreus*. This change in escape response following exposure to elevated $p$CO$_2$ was attributed to impaired decision making of the prey possibly because of interference with the function of neurotransmitter receptors (Watson et al. 2014). Heightened prey vulnerability and resultant targeting of a prey species by a predator may alter the stability and character of consumer–prey dynamics (Gaylord et al. 2015).

1.7 Aims and hypotheses of this thesis

As the worlds’ oceans become more acidic, bivalve molluscs are proving to be amongst the most vulnerable phyla that may struggle to acclimate (Figure 1.4). While there has been a rapid increase in the number of studies examining the effect of ocean acidification on molluscs and other marine organisms, only a handful of studies have investigated species interactions and potential alterations to species relationships caused by ocean acidification. The aim of this thesis is to measure alterations in species relationships, specifically the predator-prey interactions of the native Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the non indigenous Pacific oyster *Crassostrea gigas* (Thunberg, 1793) and the endemic whelk muricid whelk *Tenguella marginalba* (Blainville,
1832). These ecologically and economically important molluscs provide a novel and dynamic species complex in which to measure any alteration in morphological, physiological and predation responses to elevated pCO₂. Already it is known that the Pacific oyster is more robust that the Sydney rock oyster to elevated pCO₂ (Parker et al. 2010). Here we combine this difference in sensitivity to elevated pCO₂ between oyster species with the whelk to determine any alterations in dynamic predator-prey interactions. Sessile marine mollusc species lack an escape response and are therefore at high risk of predation. We predict that predation may increase during exposure to elevated pCO₂ because: 1) energetic costs are increased in the endemic T. marginalba causing them to increase their consumption rate of prey; and/or 2) morphological and physiological defences of oysters are reduced as more energy is directed toward maintenance of acid-base balance away from shell calcification. To test these hypotheses, we expose both prey and predator, C. gigas, S. glomerata and T. marginalba respectively to ambient and elevated pCO₂ (-0.3 to -0.4 pH units) and focus on the variation in morphological and physiological responses in natural and commercial populations that may lead to acclimation. As ocean change is gradual and will have a severe impact on both predator and prey, quantitative experiments lasting greater than two months were used to simulate chronic exposure and investigate the foraging ability of predators and the induced defences of prey of mollusc species to ocean acidification.

The aims and hypotheses addressed in this thesis are described in four chapters:

**Chapter Two** characterises the predation by T. marginalba primarily on the introduced Pacific oyster, Crassostrea gigas. It was important to first identify the predatory behavior of T. marginalba and secondly identify environmental variables which may influence this predatory behavior. It was hypothesized that if 1) T. marginalba will be able to detect prey then the time for T. marginalba to self-right would decrease and the speed of movement would increase in the presence of prey; 2) T. marginalba would have greater preference for smaller prey and 3) that emersion and illumination
patterns would dictate predation attempts as *T. marginalba* may seek shelter during unfavourable conditions to prevent desiccation.

**Chapter Three** investigates the response of the native Sydney rock oyster *S. glomerata* and invasive Pacific oyster *C. gigas* to predation following exposure to levels of *pCO₂* predicted by the IPCC (2013a) for the end of this century. It was predicted that predation of oysters by *T. marginalba* will increase during exposure to elevated *pCO₂* because: 1) energetic costs are increased in *T. marginalba* causing them to increase their consumption rate of prey; and/or 2) or morphological defences of oysters are reduced as more energy is directed to maintain acid-base balance and away from shell calcification.

**Chapter Four** investigates the variation in response within natural populations of *C. gigas* to elevated *pCO₂*. There is evidence that marine species may adapt through evolutionary change in a future where the oceans are more acidic (Reusch 2013; Sunday et al. 2014). Observations of variation in genetic traits sensitive to *pCO₂* suggest evolutionary adaptation is possible (Sunday et al. 2014). It was predicted that differences in the response to elevated *pCO₂* would be present in specific family lines; with some family lines being more resilient to elevated *pCO₂* and predation.

**Chapter Five** compares the response of commercially farmed diploid and triploid types of *C. gigas* to elevated *pCO₂* found in south-eastern Australian estuaries. It was predicted that triploid oysters will be more robust to predation and ocean acidification because they allocate less energy to reproduction and have potentially more energy for growth and shell calcification.
Chapter Two

Predation by the endemic whelk *Tenguella marginalba* on the invasive Pacific oyster *Crassostrea gigas*

2.1 Abstract

The endemic Mulberry whelk, *Tenguella marginalba*, is common on eastern Australian intertidal rocky shores where it preys on oysters. The introduced Pacific oyster *Crassostrea gigas*, found within the natural range of *T. marginalba*, is a potential prey for the whelk. In experiments designed to better understand predation of *C. gigas* we found that adult *T. marginalba* detected *C. gigas* and increased movement in the presence of oyster prey. *T. marginalba* showed a preference for smaller *C. gigas*, but consumed oysters up to 60 mm in shell height (dorsal to ventral margin). Whelks used their radula to consume and drill holes in oysters, 0.68 ± 0.09 mm in diameter, most frequently central to the pericardial cavity on the upper right valve. Predation rates were greatest when predator and prey were both submerged but were unaffected by diurnal cycle. When offered *C. gigas*, Sydney rock oysters (*Saccostrea glomerata*) and mussels (*Trichomya hirsuta*) concurrently, whelks displayed no preference among prey. With a comparatively greater proportion of *C. gigas* in NSW found in the lower to mid tidal range compared to the native *S. glomerata*, *T. marginalba* may consume proportionally more *C. gigas* than *S. glomerata*. If the rate of predation outweighs the rate of recruitment and the morphological growth of *C. gigas*, *T. marginalba* may provide top down control on this introduced species.
2.2 Introduction

The muricid whelk *Tenguella marginalba* is common on the low and mid intertidal rocky shores from Queensland to northern Victoria on the east coast of Australia, and on islands in the Indo-Pacific (Moran 1985; Fairweather 1988). The distribution and abundance of *T. marginalba* is patchy and variable within and among shores. Major influences of its distribution and abundance are emersion (Moran 1985), wave exposure (Meyer and O’Gower 1963; Moran 1985), competitor abundance (Moran 1985) and the availability of suitable prey species (Fairweather 1988). *T. marginalba* has been found to avoid areas of the shore with strong wave exposure (Meyer and O’Gower 1963) and favour the edges of pools (O’Gower and Meyer 1971) and crevices (Moran 1985; Coulson et al. 2011). *T. marginalba* are more common in low shore habitat where prey availability is greatest (Moran 1985). Growing to 35 mm in size, *T. marginalba* consumes a diverse range of prey such as barnacles, limpets and oysters (Fairweather et al. 1984; Moran et al. 1984) and is sufficiently common within its range to be able to influence ecological interactions on local shores (Moran et al. 1984).

*T. marginalba* is considered a generalist predator, with its diet varying in response to prey availability (Fairweather and Underwood 1991), but with preferences for particular prey species and sizes (Fairweather 1985). *T. marginalba* uses a variety of feeding modes, including drilling using its radula, dislodging prey or prizing open barnacle valves and bivalve shells with its muscular foot (Fairweather et al. 1984; Hughes 1985). To feed on bivalves such as oysters, *T. marginalba* most commonly uses its radula which leaves a characteristic drill-hole. Factors affecting the rate of feeding are complex and include predator size, prey size, and prey density. For example, Moran (1985) found predation of adult barnacles was dependent on the size of *T. marginalba*. Larger *T. marginalba* (15 mm aperture length) fed on adult surf barnacles *Tesseropora rosea* 4.2 times faster than smaller individuals (12 mm); but predator size was not a factor in the consumption of juvenile
barnacles. *T. marginalba* also ate juvenile limpets *Patelloida latistrigata* 1.4 times faster than adult limpets. Overall the predation rate of *T. marginalba* increased with prey density (Moran 1985).

Other non-prey-dependent factors affect predation. Environmental influences may determine the success of attempts by *T. marginalba* to forage and consume suitable prey. Predation by *T. marginalba* can be localised on rocky shores with whelks foraging over relatively short distances from shelter (Moran et al. 1984; Moran 1985). *T. marginalba* also feed on prey over several tidal cycles, putting them at risk of exposure to high temperature and desiccation. In unfavourable environmental conditions, whelks will stop foraging and seek aggregated shelter (Moran 1985; Seed 1993). There is evidence that *T. marginalba* can also forage during periods of long submersion and seek shelter during periods of air exposure at low tide (Moran 1985).

The native Sydney rock oyster *Saccostrea glomerata* is one of the most abundant prey species on rocky shores within the tidal range of *T. marginalba* (Fairweather et al. 1984). Drill-holes characteristic of *T. marginalba* predation are common in the shells of dead *S. glomerata* and *T. marginalba* has been found to completely consume entire populations of *S. glomerata* at all levels on the shore (Fairweather et al. 1984). In recent years the introduced Pacific oyster, *Crassostrea gigas* has increased significantly in numbers on rocky shores offering *T. marginalba* an alternative prey species (Hedge and Johnston 2014; Scanes et al. 2016). In laboratory experiments where *T. marginalba* had no choice between prey species, *S. glomerata* and *C. gigas* were consumed at the same rate (Wilkie and Bishop 2013), but on commercial oyster farms, *T. marginalba* has been found to selectively feed on *C. gigas* (Rodley 2010). Ecologically, this is important as the potential for non-native species to proliferate to pest status can depend on top-down control by native predators (Wilkie and Bishop 2013). If *T. marginalba* prefer *C. gigas*, then there is less risk of *C. gigas* displacing native species of oysters, such as *S. glomerata*, assuming consumption rates are sufficient to control population growth and geographic spread has occurred elsewhere (Herbert et al. 2016).
C. gigas is now of considerable commercial and ecological significance in New South Wales (NSW) (O’Connor and Dove 2009), but little is known about the relationship between T. marginalba and introduced C. gigas, particularly how predation varies as a function of major environmental variables. The aim of this study was to better describe the predation of C. gigas by T. marginalba to inform both managers and oyster farmers and to establish basal knowledge required to further investigate the impacts of climate change on the interactions between predatory whelks and this introduced oyster species. It was important to first identify the predatory behavior of T. marginalba and secondly identify environmental variables which may influence this predatory behavior. It was hypothesized that if 1) T. marginalba will be able to detect prey then the time for T. marginalba to self-right would decrease and the speed of movement would increase in the presence of prey; 2) T. marginalba would have greater preference for smaller prey and 3) that emersion and illumination patterns would dictate predation attempts as T. marginalba may seek shelter during unfavourable conditions to prevent desiccation.

2.3 Materials and methods

For all experiments, adult T. marginalba (shell height range 17-24 mm; mean ± S.E. 20.81 ± 0.29 mm) were hand collected on the low tide from intertidal rocky shores at Boat Harbour (32°47’S, 152°06’E) and Anna Bay (32°47’S, 152°04’E) Port Stephens NSW, Australia. Oysters (S. glomerata and C. gigas) were provided by a commercial oyster farm in Port Stephens, while mussels Trichomya hirsuta were collected from oyster farm infrastructure.

Initially, T. marginalba and oysters and mussels were held in separate recirculating tank systems for a minimum two week acclimation period at the NSW Department of Primary Industries, Port Stephens Fisheries Institute. During acclimation, T. marginalba were fed C. gigas and S. glomerata that were similar in size to the experimental oysters. Consumed oysters were replaced daily. Whelks were starved for four days prior to each predation experiment, a period chosen on the basis of
previous trials to increase predatory drive without affecting overall physiological condition. Oysters used during the trials were fed a mixed diet of three algal species; *Chaetoceros muelleri, Tisochrysis lutea* and *Pavlova lutheri*. For acclimation and for each experiment, seawater was maintained at ambient summer temperature (mean ± S.E. 23.5 ± 0.5 °C), salinity (mean ± S.E. 33.2 ± 0.5 ppt) as recorded in Tilligerry Creek, Port Stephens NSW, Australia (32°45′S, 152°03′E). With the exception of the diurnal predation experiment, the shells of oysters that had been consumed were removed daily and replaced with a live oyster of a similar size. During the diurnal experiment, consumed oysters were replaced every 12 h.

**Initial observations**

To assess if the presence of prey would influence the behavior of *T. marginalba*, the self-righting time and speed of movement of *T. marginalba* was calculated under three sets of conditions using footage recorded using video cameras (GoPro). In experiment one, *T. marginalba* (n = 11) were placed upside down and fully submerged on the floor of a 20 L plastic tank and the time taken for each whelk to self-right was recorded. After being ‘rested’ for 30 min to negate any handling effects, the distance and direction that each *T. marginalba* travelled was then recorded for 10 min. This was repeated three times with a total of 33 whelks. In experiment two, the previous procedure was repeated; however, on this occasion *C. gigas* had been placed in the corners of three replicate tanks to gauge the impact of the presence of prey on *T. marginalba* (n = 11) behaviour. In experiment three, *T. marginalba* (n = 11) were offered a choice of prey and no prey. Prey included *C. gigas, S. glomerata* or *T. hirsuta* each randomly assigned to a corner of the tank (n = 3) with the fourth corner with no prey.

To further assess the ability of *T. marginalba* to detect food, 20 whelks were placed at the entrance of a plexiglass chamber with two connecting channels arranged in a ‘Y’ shape. Each channel was 40 cm in length. Using an adapted method from (Manríquez et al. 2013), one channel contained *C.*
gigas, obscured from the view of the whelks, while the other channel was empty. Water was pumped from a reservoir and entered the top of each channel and flowed toward the base of the chambers containing the whelks, where it drained to waste. The behaviour of T. marginalba was recorded using video cameras (GoPro) for 24 h and repeated twelve times. The Y-maze was cleaned using fresh water to remove any residue and filled with filtered (5 µm) seawater between each repeat. A whelk was determined as having made a ‘choice’ of channels once it was within 5 cm of the end of a particular channel and had remained in the channel. The number of T. marginalba that moved between channels was also recorded.

Predation of C. gigas

To determine if T. marginalba select oyster prey based on size, ten C. gigas from each of three size (height) classes, small (10 to 30 mm), medium (30 to 50 mm), and medium to large (50 to 60 mm) were placed in one of three replicate 20 L recirculating tanks, each holding 20 T. marginalba. Observations of the number of T. marginalba attached to oysters, including those in the process of drilling through the shell and consuming flesh were recorded for each size class and the number of oysters consumed was recorded after 24 and 84 h of the same tank of 20 T. marginalba. Total meat weight of oysters consumed whelk⁻¹ day⁻¹ for each size class was estimated using an average dry flesh mass of small= 0.2478 g, medium = 0.346 g and medium to large = 0.493 g C. gigas, calculated using a standard curve constructed from data collected during Wright et al. (2014) and subsequent studies for hatchery produced C. gigas from the same farming location.

To determine whether T. marginalba drilled in a specific area of the shell of C. gigas, the left and right valve were notionally divided into five regions and observations of the location of predation were recorded. A study published by Thomas and Day (1995) demonstrated that drill holes from a muricid gastropod Haustrum baileyanum were concentrated to the shell area that was directly above the adductor muscle of the abalone Haliotis rubra. The shell was divided to distinguish 1) the
area of shell directly above the adductor muscle and pericardial cavity, 2) new shell growth along the ventral margin, 3) older shell growth along the dorsal margin and hinge, 4) the anterior and 5) posterior of the shell divided through a centre line (Figure 2.1). The location and the valve (left or right) of each drill hole were recorded. The diameter of 20 randomly selected drill holes was measured under a microscope (Leica) with an eyepiece micrometer to determine conformity with the diameter of the radula of *T. marginalba*

![Diagram](image)

**Figure 2.1** External morphology of the shell of *C. gigas* divided into five regions; 1) central and above the pericardial cavity, 2) ventral, 3) dorsal, 4) anterior and 5) posterior for the assessment of the location of *T. marginalba* drill holes.

**Impacts of emersion and illumination on *C. gigas* predation**

To investigate the potential impact of immersion and emersion on the selection of prey, 30 *C. gigas* were placed on the base of three replicate 20 L tanks that had been tilted at 15° to approximate the
overall slope of the rocky shores of NSW (range of slope of NSW rocky shores; 4 – 23°, Lathlean et al. 2015). A continuous flow of seawater was supplied using a purpose built sprinkler system attached to the top of each tank. A stand pipe was used to ensure sufficient seawater was maintained to cover half the floor of the tank. Oysters were positioned so that half of the oysters were completely submerged and the other half were completely out of the water. Twenty whelks were added to the centre of each replicate tank at the water-line. The number of *T. marginalba* submerged or not was recorded after 1 h and again after 24 h and the number of oysters consumed in the two halves of the tank was recorded after 24 h. Oysters were only recorded as consumed once greater than 80% of flesh had been removed. This trial was paired with a no choice experiment where predation rates under submerged and emersed conditions were compared. In this experiment, 20 *T. marginalba* were placed into six replicate 20 L tanks containing 30 oysters. Seawater was supplied to both submerged and emersed tanks through a sprinkler system attached to the top of each tank. In three tanks the oysters were completely submerged using a stand pipe to increase the water level, while the oysters in the remaining tanks were not submerged. The number of oysters consumed from each tank was recorded after 24 h.

To investigate the potential for diurnal predation patterns, 20 *T. marginalba* and 30 oysters were submerged in each of three replicate 20 L tanks and exposed to a 12 h light and 12 h dark cycle for 84 h, beginning with a 12 h light exposure. The number of oysters consumed following each successive 12 h cycle was recorded. This experiment was then repeated beginning with a 12 h dark cycle.

*Statistical analyses*

To determine any significant differences on the behaviour, biology, and predation of *T. marginalba* data were analysed using a two-factor analysis of variance. The GMAV 5 for Windows (Underwood Underwood et al. 2002) analysis package was used to analyse differences among treatments.
Heterogeneity of the variances was assessed by Cochran’s test and as there were no significant differences the data was not transformed. A significance level of $P < 0.05$ was used for all analyses. Any difference in means was detected by Student Newman Kuels (SNK) test on each parameter (Sokal and Rohlf 1995). The data were tested for normality using a series of Kolmogorov-Smirnov tests. All data had a normal distribution.

2.4 Results

Observations of predation

There was no effect of the presence of $C. gigas$ on the self-righting time (ANOVA, $F(1,60) = 0.76$, $P > 0.05$). On average ($\pm$ S.E.) it took whelks $2.92 \pm 0.69$ minutes to right themselves in the absence of prey and $3.85 \pm 0.81$ minutes to right themselves when prey were present. The mean rate of movement of $T. marginalba$ was however significantly faster (mean $\pm$ S.E. $22.9 \pm 1.26$ mm min$^{-1}$) when $C. gigas$ were present than when no oysters were in the tanks (mean $\pm$ S.E. $15.7 \pm 0.58$ mm min$^{-1}$ ANOVA, $F(1,60) = 25.52$, $P < 0.05$), assuming each whelk had moved independent of other whelks.

There was no preferential selection of prey when $T. marginalba$ were offered a choice of $C. gigas$, $S. glomerata$ or $T. hirsuta$, however, significantly fewer whelks moved toward the corner of the tank with no food (ANOVA, $F(3,15) = 3.36$, $P < 0.05$, SNK $C. gigas = S. glomerata = T. hirsuta >$ no food).

Whelks moved rapidly in Y-channels; 84% of $T. marginalba$ moved into the channel with $C. gigas$ within 3 h, and remained in association with the oysters for the duration of the trail. Those $T. marginalba$ that entered the channel without food continued to explore the channel and 55% of the whelks that had entered the food-free channel eventually moved into the channel with $C. gigas$. After 24 h, over 92% (mean $\pm$ S.E. $18.50 \pm 0.70$ whelks) of the whelks had selected the chamber containing $C. gigas$. 
**Predation of C. gigas**

During the first 24 h, on average 50% of *T. marginalba* actively sought out and associated with *C. gigas* with no apparent effect of the size class of oysters present (ANOVA, F, (2,6) = 0.02, P > 0.05). The number of oysters consumed, however, differed significantly as whelks consumed more small (mean ± S.E. 0.03 ± 0.01 day⁻¹) and medium (mean ± S.E. 0.04 ± 0.01 day⁻¹) size oysters than medium to large oysters (mean ± S.E. 0.01 ± 0.01 day⁻¹; ANOVA, F (2,6) = 12.3, P < 0.05; SNK: small = medium > medium to large). On a dry flesh mass basis, *T. marginalba* also consumed significantly more small and medium size oysters than medium to larger oysters (ANOVA, F (2,6) = 6.57, P < 0.05; SNK: small = medium > medium to large).

All oysters consumed by *T. marginalba* were consumed by drilling through the shell. The mean (± S.E.) size of holes was 0.68 ± 0.09 mm in diameter. Distinct preferences were evident in the specific area of the valve drilled. The majority of drill holes (64%) were in the right or upper valve (76%) central to the pericardial cavity and the least number of drill holes were found posterior (3%). In total (84%) of drill holes were found on the dorsal valve central to the pericardial cavity. When oysters were drilled through the left or lower valve there was no significant preference in the location of the drill hole.

**Impacts of emersion and illumination on C. gigas predation**

After 1 h in tanks with both submerged and emersed oysters, 67% of *T. marginalba* were associated with submerged oysters. This behaviour was broadly consistent with that observed after 24 h when (75%) of whelks were associated with submerged oysters. Significantly more oysters were consumed when whelks were submerged (mean ± S.E. 0.20 ± 0.04 per whelk) than emersed (mean ± S.E. 0.01 ± 0.00 per whelk; ANOVA, F (1,22) = 22.42, P < 0.05). Similarly, in the no choice experiment significantly more *T. marginalba* consumed oysters when submerged in water compared to unsubmerged (ANOVA, F (1,6) = 16.68, P < 0.05). There was no significant difference in the number
of oysters consumed by T. marginalba between light (mean ± S.E. 5.33 ± 0.51) or dark conditions (mean ± S.E. 4.67 ± 0.51) after 84 h irrespective of whether the diurnal cycle started in the light or dark.

2.5 Discussion

Although the ranges of T. marginalba and C. gigas have only recently overlapped, T. marginalba demonstrated that it recognises C. gigas as a potential prey item. While some T. marginabla behaviour, such as self-righting speed, was not influenced by presence of C. gigas, possibly because it is more likely to be a defensive response, other behaviours were clearly affected. When C. gigas were present T. marginalba were found to move at a greater rate even without a direct line of sight, than when C. gigas were not present in the experimental chambers. Although not assessed in this study, movement patterns of whelks may not have been independent as whelks may have been influenced by the movement patterns or scents of other individuals (Fairweather 1988). Typical of a generalist predator, T. marginalba did not differentiate between prey species and fed on C. gigas, S. glomerata and T. hirsuta equally. While it is not clear what mechanism T. marginalba uses to detect prey, they may recognize general prey signals such as vibration in the water column, chemical, ammonia and fecal odour signals (Kats and Dill 1998; Wisenden 2000).

Despite being capable of a range of predatory behaviors, predation observed in this study was exclusively achieved by drilling, with the majority of drill holes dorsal and central to the pericardial cavity on the right upper valve. The whelks may be attracted toward the pulsating heart, or alternatively, T. marginalba may prefer drilling over the pericardial cavity as this area is in close proximity to the energy rich adductor muscle. The adductor muscle scar was the most frequently drilled site of cultured oysters Crassostrea virginica and the wild mussels Mytilus edulis and Geukensia demissa by the predatory Atlantic oyster drill Urosalpinx cinerea and veined Rapa whelk Rapana venosa (Harding et al. 2007). Similarly, the gastropod Hastrum baileyanum drilled over the muscle tissue of the abalone Haliotis rubra, even though this region has the thickest part of the shell
and required a longer period of time to penetrate. It is presumed that the benefits of the energy rich muscle tissue outweigh the cost of the extended drill time in this area of the valve; the pericardial region being the thickest part of the valve (Thomas and Day 1995). The benefit of the energy reward of the adductor muscle may outweigh the time taken by the whelk to drill and consume the oyster in this area.

Overall *T. marginalba* consumed a greater number and dry flesh mass of small and medium than medium to large sized *C. gigas*, despite handling a variety of oyster sizes prior to consumption. Smaller oysters have thinner shells which allow *T. marginalba* to more quickly access and consume the smaller mass. Other studies have found strong correlations between size of prey and the rate of predation by *T. marginalba* (Fairweather et al. 1984; Moran et al. 1984; Moran 1985; Seed 1993). Similar to this study, some studies have found that larger oysters are less frequently a source of prey for *T. marginalba* (Fairweather and Underwood 1983; Taylor 1990; Tan et al. 2003; Amaral et al. 2012a). In this study, *T. marginalba* adults were 20.81 ± 0.29 mm in size, specimens however, can reach 35mm (Murphy 2015). Further studies are needed with a broader size range of whelks to further determine the relationships between predator and prey size (McQuaid 1985; Moran 1985; Wilkie and Bishop 2013). Smaller predators typically prefer smaller prey because of their limited capacity to handle large prey, however, *T. marginabla* were often observed attempting to prey upon larger *C. gigas*.

Immersion influenced whelk predation. *T. marginalba* more often consumed submerged rather than emerged oysters. During low tides and high air temperatures *T. marginalba* may be at risk of desiccation and accordingly need to balance the energy gained from consuming prey against the risk of physical harm. The energy gained from eating an oyster must outweigh the time and energy taken to break through a shell and consume prey. Favourable conditions enable the whelk to effectively source food and return maximum gain on investment. When conditions are unfavourable a trade-off must be made between foraging and shelter. Surveys of the prevalence of *C. gigas* and *S. glomerata*
in Port Stephens have found *C. gigas* to be more abundant at low and mid-tidal rather than high shore elevations (Krassoi 2001; Bishop et al. 2010), potentially with greater exposure to *T. marginalba* predation.

Given these results and patterns on how *T. marginalba* consumes prey it is expected that unfavourable conditions would slow or decrease the ability of whelks to consume prey. Here, we have described the predatory behaviour of the whelk *T. marginalba* on the introduced oyster *C. gigas*. *C. gigas* has already invaded 66 countries and displaced native oysters in NW Europe (Diederich et al. 2005; Troost 2010), America (Escapa et al. 2004) and New Zealand (Dinamani 1974). However, further work is required to determine how other factors, particularly those facing our future climate, will impact on the ability of whelks to predate. Any disruption to this ability may have detrimental consequences to intertidal rock shore ecology. Top down control through predation may prevent *C. gigas* from proliferating to invasive species status, however, there remains a need to further elucidate the effect predation may actually have on the population of prey. Consideration must be given to the population growth of the prey which could negate any negative effect of predation. Future studies should incorporate a dynamic population growth model. The effect of predation on oysters may be exacerbated in the future as ocean acidification may reduce the morphological defence of mollusc prey and increase the energetic demands of predators.
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Chapter 3

Ocean acidification alters the predator-prey interactions between the oysters

*Saccostrea glomerata* and *Crassostrea gigas* and the whelk *Tenguella marginalba*

3.1 Abstract

As the world’s oceans acidify, bivalve molluscs will be vulnerable and ecological processes such as predator-prey interactions are predicted to be altered. This chapter tested whether the predatory whelk *Tenguella marginalba* could induce defensive responses in the native Sydney rock oyster *Saccostrea glomerata* and the invasive Pacific oyster *Crassostrea gigas* and whether under elevated pCO$_2$ this response was impaired. A two month trial was run in which oysters were held alone at ambient or reduced pH or with *T. marginalba* at ambient and reduced pH. The shell morphology, standard metabolic rate (SMR) of oysters and whelks and the consumption of oysters was measured. *C. gigas* were overall significantly larger at elevated than ambient pCO$_2$, but significantly smaller in size when held with *T. marginalba*. In contrast, there was no effect of pCO$_2$ or *T. marginalba* on the size of *S. glomerata*. Overall, the SMR of *S. glomerata* was lower but for *C. gigas* greater at elevated than ambient pCO$_2$. *T. marginalba* increased their SMR and initially increased consumption of both *S. glomerata* and *C. gigas* at elevated pCO$_2$. There was a significant decrease in the SMR of *C. gigas*, but not *S. glomerata* when they were exposed to waterborne chemical cues released from *T. marginalba* at ambient pCO$_2$. There was no change in SMR of *C. gigas* in response to cues under elevated pCO$_2$. *C. gigas* may have the ability to enter a state of low metabolic activity and reduce the output of
metabolites to prevent being detected by whelks at ambient $pCO_2$, whereas $S.\ glomerata$ may have greater morphological defences to alleviate predation. Under elevated $pCO_2$ the ability of $C.\ gigas$ to regulate metabolic rate and response to predatory cues was lost, possibly in favour of metabolic maintenance as the oysters attempt to meet the higher energy demand required to sustain homeostasis.

3.2 Introduction

The unprecedented increase in the concentration of carbon dioxide ($CO_2$) of the world’s oceans over this century, known as ocean acidification will impact marine organisms (IPCC 2013a). It is predicted that shelled mollusc species will be amongst the most sensitive marine phyla (Parker et al. 2013). Already we know that ocean acidification impacts the morphology and physiology of molluscs including shell and somatic growth (Melzner et al. 2011; Wright et al. 2014), acid-base balance, energy metabolism (Green et al. 2009; Lischka et al. 2010; Range et al. 2011) immune responses (Bibby et al. 2008) and protein degradation (Michaelidis et al. 2005). We know less about how ocean acidification and the consequential alterations in morphology and physiology impact on ecological processes such as predator-prey interactions.

Many molluscs are known to have the ability to detect and respond to predators (Li et al. 2015; Queirós et al. 2015; Jellison et al. 2016) by altering their morphological, physiological, behavioural and chemical outputs, which reduce predation risk (Leonard et al. 1999; Smith and Jennings 2000; Lord and Whitlatch 2012; Wang et al. 2013; Wright et al. 2014). For sessile molluscs, escape in the form of locomotion is not an option in response to predators. Therefore, sessile molluscs are known to alter shell morphology and metabolic output to increase defence and prevent detection by predators. For example, the mussel, $Mytilus\ edulis$ increased the thickness and mass of the shell in areas of the shore where predation risk by crabs was high (Leonard et al. 1999) and developed thicker shell margins when exposed to waterborne predator cues (Smith and Jennings 2000).
Similarly the weight and thickness of the shell of the Eastern oyster, *Crassostrea virginica* was significantly greater in the presence of the oyster drill, *Urosalpinx cinerea* (Lord and Whitlatch 2012).

Mollusc species can alter their metabolic output in the presence of a predator, possibly an induced response to avoid detection by predators. For example, the green-lipped mussel, *Perna viridis* decreased scope for growth in response to cues released by the predatory crab *Thalamita danae* when it fed on conspecifics. When the predator did not feed on conspecifics, chemical cues did not alter scope for growth (Wang et al. 2013). Sanford (2014) postulated that an observed increase in the rate of predation by Atlantic oyster drills *Urosalpinx cinerea* on the Olympia oyster *Ostrea lurida* following exposure to elevated pCO$_2$ could be due to the metabolic output of the oyster, which can be greater during episodes of unfavourable environmental conditions (Lesser 2016). An earlier study found *U. cinerea* preferentially selected oysters with a greater metabolic rate (Blake 1960).

Only a handful of studies have investigated alterations to predator-prey interactions in molluscs caused by the impact of ocean acidification. All studies done to date report alterations in predator avoidance and defence mechanisms following exposure to elevated pCO$_2$ and in some cases increased predation (Bibby et al. 2007; Manríquez et al. 2013; Watson et al. 2014; Wright et al. 2014; Li et al. 2015; Queirós et al. 2015; Sui et al. 2015; Jellison et al. 2016; Manríquez et al. 2016). One of the first studies by Bibby et al. (2007) found the periwinkle snail, *Littorina obtusata* increased the thickness of their shell in response to the predator *Carcinus maenas*, when held under ambient pCO$_2$, but was unable to do this when exposed to a much greater level of pCO$_2$ (pH 6.6) for 15 days. Similar changes have been found in the behavioural defences of molluscs following exposure to elevated pCO$_2$. Watson et al. (2014) found the stromb snail, *Gibberulus gibbosus* exposed to elevated pCO$_2$ of 961 µatm for one week had a slower reaction time and an altered escape trajectory in the presence of a predator, the cone shell, *Conus mamoreus* compared to ambient pCO$_2$. This change in escape response following exposure to elevated pCO$_2$ was attributed to impaired decision making of the prey possibly because of interference with the function of neurotransmitter receptors.
(Watson et al. 2014). Watson et al. (2014) also found that exciting these neurotransmitter receptors with gabazine restored the anti-predator behaviour of G. gibbosus exposed to elevated $pCO_2$. Gabazine acts as a GABA antagonist in some invertebrate nervous systems, suggesting an interference with neurotransmitter function at elevated $pCO_2$. Changes in defensive responses such as those reported for L. obtusata and G. gibbosus may increase the likelihood of predation of molluscs.

Here we examine the impact of ocean acidification on the predatory-prey interaction of two species of oyster common in eastern Australia with the endemic predatory whelk Tenguella marginalba. It was hypothesised that predation of the native Sydney rock oyster Saccostrea glomerata and the invasive Pacific oyster Crassostrea gigas by T. marginalba would increase during exposure to elevated $pCO_2$ because: 1) energetic costs are increased in T. marginalba requiring them to increase their consumption rate of prey; and/or 2) S. glomerata and C. gigas may no longer be able to reduce their metabolic rate, making them more easily detected by the predator. A lowered metabolic rate is an inducible defence mechanism that has been observed in other mollusc species in the presence of a predator under ambient $pCO_2$ (Bourdeau 2009; Bourdeau 2012; Wang et al. 2013). A second experiment tested for this inducible defence using chemical cues released from T. marginalba (predator cue), S. glomerata/C. gigas (conspecific cue) or both cues, and measuring their metabolic responses. We hypothesised that this inducible defence mechanism may be impaired at elevated $pCO_2$ because of alterations in the ability of prey to detect or respond to the chemical cues.

### 3.3 Material and Methods

**Collection and feeding of Saccostrea glomerata, Crassostrea gigas and Tenguella marginalba**

S. glomerata were spawned at the Port Stephens Fisheries Institute, New South Wales in 2014 and transferred as spat to Cromarty Bay, Tilligerry Creek, Port Stephens NSW Australia (32°45’S, 151°58’E, Figure 3.1) where they were grown-out to juveniles (mean individual mass 6.34 ± S.E 0.01
g) and held in aquaculture baskets (Seapa 600, volume 15 L gauge 3 mm) on an intertidal long-line system. The adult parent stock were sourced from commercial oyster growing locations of New South Wales, Australia. Diploid *S. glomerata* were selected for this study because they are most representative of farmed stocks in New South Wales (NSW; Parker et al. 2011).

*C. gigas* were spawned at a commercial hatchery in Tasmania (mean individual mass ± S.E. 0.45 ± 0.02 g). Although *C. gigas* were sourced from a single location, it is however likely that the oysters used in this study are representative of those in Australia as hatchery reared and wild type have low genetic diversity and variation in allele frequency is minor (1%) in Australia (English et al. 2000). Due to seasonal restrictions in the availability of oysters of a comparable size, a direct comparison of *S. glomerata* and *C. gigas* was not possible in this trial. Data of each species of oyster was analysed independently and standardisation to dry flesh mass was used as a point of comparison.

![Map of the Port Stephen’s estuary and its location in NSW and the Australian continent.](image)

**Figure 3.1** Map of the Port Stephen’s estuary and its location in NSW and the Australian continent.

Adapted from Google Earth
Adult *T. marginalba* (mean mass ± S.E. 1.75 ± 0.04 g) were collected from intertidal rocky shores at Boat Harbour (32°47’S, 152°06’E) Port Stephens NSW, Australia. All animals were transferred to holding tanks at NSW Department of Primary Industries, Port Stephens Fisheries Institute, Taylors Beach NSW, Australia. Although minimal all biofouling, mainly juvenile *S. glomerata* and *C. gigas*, adhered on oysters was removed by shucking and excess mud removed by high-pressure cleaning (Figure 3.2; Dove and O’Connor 2007). This was done to prevent excessive alterations in the water quality and the availability of food during the experiment. Throughout all experiments, the oysters were fed a mixed algal diet consisting of *Chaetoceros muelleri*, *Tisochrysis lutea* and *Pavlova lutheri* at a concentration of $1.19 \times 10^8$ cells oyster$^{-1}$ day$^{-1}$ for the duration of the experiment. *T. marginalba* were fed *C. gigas* that were similar in size and at a similar density to the experimental oysters. Consumed oysters were replaced daily. A complete water change was conducted on each tank every second day to maintain water quality throughout all experiments.

**Figure 3.2** An individual *S. glomerata* with substantial by-catch

Experiment 1: Shell morphology and physiology of *C. gigas* in response to *T. marginalba*

Once collected, *S. glomerata, C. gigas* and *T. marginalba* were initially held for two weeks in separate aquaria within a mean (± S.E.) temperature range of 21.6 – 22.1 °C (± 0.07, n = 12). A recent study investigating the effect of acclimatisation on *S. glomerata* found an equivalent period of time was required before a stabilisation of haemocyte and phenoloxidase enzyme activity occurred which
resulted from stress-induced impacts of transportation (Thompson et al. 2012). S. glomerata (102 oysters tank\(^{-1}\)) and C. gigas (560 oysters tank\(^{-1}\)) were then equally and randomly divided among six 750 L experimental tanks. Similarly, T. marginalba were equally and randomly divided into two separate sets of twelve 750 L tanks (72 whelks tank\(^{-1}\)). One set of six tanks holding T. marginalba was assigned to the experiment using S. glomerata and the other set of six tanks holding T. marginalba were assigned to the experiment using C. gigas.

The tank systems were randomly allocated positions within a climate-controlled laboratory using a random number table, where the tank numbers corresponded with a location within the facility. The seawater was sourced from Shelley Beach, Shoal Bay, (32°43’08”S, 152°10’16”E) and Little Beach, Nelson Bay (32°72’S, 152°07’E), NSW. Seawater was transported by water truck to the Port Stephens Fisheries Institute where it was filtered through successive 10 µm and 1 µm nominal filters. After settling in large on-site fibreglass tanks for one week the seawater was pumped into the experimental systems at each water exchange following filtration through another set of 1 µm nominal filters.

The S. glomerata, C. gigas and T. marginalba 750 L tanks were divided among two pH treatments, three tanks for each species of oyster and two sets of six tanks for T. marginalba at ambient pH of 8.02 (± 0.01; 481 µatm) as measured in Tilligerry Creek, Port Stephens. Another set of three tanks for each species of oyster and two sets of six tanks for T. marginalba maintained filtered seawater at a reduced pH of 7.74 (± 0.01; 909 µatm). The value was consistent with intermediate pCO\(_2\) scenario projections for the year 2100 (IPCC 2013a). The reduced pH level was maintained by direct diffusion of carbon dioxide and was monitored by a microprocessor in each of the replicate tanks (Aqua Medic pH computer set; accuracy ± 0.01). Under cooler temperatures the physiological behaviour of oysters would have been reduced to a state of low metabolic function (Dove and O’Connor 2007), and subsequently the water temperature of the winter period (16°C) was not used in this study. The pH, salinity and temperature (maintained at 22 °C ± 0.05) were measured in each 750 L tank daily.
with a hand-held probe (Palin test Waterproof 800 meter). Total alkalinity (TA) was measured using a Gran-titration (mean TA = 2297 ± S. E. 38 µmol kg⁻¹; Gran 1952). Titrations were conducted weekly by manual titration following the methods of Riebesell et al. (2010). A pCO₂ system calculation program (CO₂ sys) developed by Lewis and Wallace (1998) was used to calculate the pCO₂, dissolved inorganic carbon, aragonite and calcite saturation state corresponding to the experimental pH levels, using the dissociation constants of Mehrbach et al. (1973) (for seawater physicochemical conditions see Table 3.1). These pCO₂ levels and control systems were used for both Experiment one and two.

**Table 3.1** Seawater physiochemical conditions of the experimental tanks during the exposure of a) *S. glomerata* and b) *C. gigas* to ambient or elevated pCO₂.

<table>
<thead>
<tr>
<th>Species/Treatment</th>
<th>Salinity (psu)</th>
<th>Temperature (°C)</th>
<th>pH NBS</th>
<th>TA (µmol kg⁻¹)</th>
<th>pCO₂ (µatm)</th>
<th>DIC (µmol/kg⁻¹)</th>
<th>Ω_{aragonite}</th>
<th>Ω_{calcite}</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. glomerata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient pCO₂</td>
<td>33.42 ± 0.15</td>
<td>21.82 ± 0.13</td>
<td>8.02</td>
<td>1799.8</td>
<td>481</td>
<td>1643.5</td>
<td>1.72</td>
<td>2.64</td>
</tr>
<tr>
<td>Elevated pCO₂</td>
<td>33.38 ± 0.11</td>
<td>21.84 ± 0.10</td>
<td>7.74</td>
<td>1657.78</td>
<td>909</td>
<td>1593.9</td>
<td>0.90</td>
<td>1.38</td>
</tr>
<tr>
<td>C. gigas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient pCO₂</td>
<td>33.36 ± 0.11</td>
<td>21.73 ± 0.08</td>
<td>8.02</td>
<td>1799.8</td>
<td>481</td>
<td>1643.5</td>
<td>1.72</td>
<td>2.64</td>
</tr>
<tr>
<td>Elevated pCO₂</td>
<td>33.50 ± 0.10</td>
<td>21.68 ± 0.08</td>
<td>7.74</td>
<td>1657.78</td>
<td>909</td>
<td>1593.9</td>
<td>0.90</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Values for pCO₂, Ω_{aragonite} and Ω_{calcite} calculated from salinity, temperature, pHNBS and TA.

TA, total alkalinity; DIC, dissolved inorganic carbon. Salinity, temperature, pHNBS and TA ± S.E.

To determine whether changes in predation relating to pCO₂ treatments were due to effects on the whelk *T. marginalba* or the oysters *S. glomerata* and *C. gigas* a two month reduced factorial experiment was run. Oysters and whelks were kept in their aforementioned six separate tanks and exposed to ambient (481 µatm) and elevated (909 µatm) pCO₂ for six weeks. After six weeks, a
reciprocal cross of the oysters and whelks was conducted. Half of the S. glomerata or C. gigas that had been held at ambient pCO$_2$ were removed and divided equally across six tanks (separate set of six tanks for each species of oyster) of T. marginalba; three of which were held at ambient pCO$_2$ and three were held at elevated pCO$_2$. Likewise, half the S. glomerata or C. gigas that had been held at elevated pCO$_2$ were removed and divided across the remaining six tanks that held T. marginalba. The remaining half of the oysters from the ambient and elevated treatments stayed in their respective ambient or elevated treatment without whelks. S. glomerata and C. gigas were held in separate tanks for the duration of the experiment. This created six treatments for each oyster species, S. glomerata or C. gigas alone at 1) ambient or 2) elevated pCO$_2$, 3) S. glomerata or C. gigas kept for 6 weeks at ambient pCO$_2$ + T. marginalba at ambient pCO$_2$, 4) S. glomerata or C. gigas kept for 6 weeks at ambient pCO$_2$ + T. marginalba at elevated pCO$_2$, 5) S. glomerata or C. gigas kept for 6 weeks at elevated pCO$_2$ + T. marginalba at ambient pCO$_2$, 6) S. glomerata or C. gigas kept for 6 weeks at elevated pCO$_2$ + T. marginalba at elevated pCO$_2$. A fully orthogonal cross was not possible because T. marginalba cannot be kept without oysters. These treatments were maintained for a further 10 days for S. glomerata and 17 days for C. gigas.

Measurement of shell size, whole mass and strength

At the completion of the period where oysters and whelks were kept together, the shell height (dorsal to ventral margin) and length (posterior to anterior margin) of 30 S. glomerata or 32 C. gigas were measured from the four treatments (n=3) that contained oysters that had not been placed into a new pCO$_2$ treatment after 6 weeks of exposure (i.e. oysters alone at 1) ambient or 2) elevated pCO$_2$, 3) oysters kept for 6 weeks at ambient pCO$_2$ + T. marginalba at ambient pCO$_2$, 6) kept for 6 weeks at elevated pCO$_2$ + T. marginalba at elevated pCO$_2$). Similarly, the height and length of the aperture of 19 T. marginalba from the two treatments that contained whelks, and the oysters were not transferred to a new treatment (treatments 3, 5) were measured to the nearest mm using vernier callipers (Mitutoyo, Digimatic vernier callipers, precision ± 0.01 mm). Whole mass (shell and
flesh) of ten *S. glomerata*, 16 *C. gigas* and six *T. marginalba* from each replicate was recorded using a precision balance (Mettler Toledo, XPE303S, precision ± 0.001 g). At this time the compression strength of 16 *C. gigas* and six *T. marginalba* from each tank was also assessed for the capacity of the shell to resist an axially directed pushing force (Instron 5960 dual column universal testing system, Centre for Infrastructure Engineering, Western Sydney University). Shell compression strength was expressed as Newtons of force at failure standardised to shell height using methods adapted from Currey (1976) and Zuschin and Stanton (2001). Briefly, each shell was placed on a flat surfaced load cell with pressure applied to the centre of the upper surface of the lower valve of *C. gigas* and upper surface of the shell of *T. marginalba* at a constant rate until failure. An Instron universal testing machine applied pressure at a rate of four mm min⁻¹ (Welladsen et al. 2010) through a pinhead of three mm diameter (Neo and Todd 2011). The pressure and point of maximum load was monitored and recorded by electronic graphing instruments and computer software (PT Global). The compression strength of the shell of *S. glomerata* was not assessed in this trial.

**Measurement of metabolic rate**

The standard metabolic rate (SMR) of 16 *S. glomerata* or 16 *C. gigas* from each tank of the four treatments that contained oysters that had not been placed into a new pCO₂ treatment after 6 weeks of exposure (treatments 1, 2, 3 and 6) and six *T. marginalba* from each treatment containing whelks (n = 3) was measured using a closed respiratory system using the methodology of Parker et al. (2012) at the end of the experiment. Briefly, individuals were gently placed into a sealed 500 mL chamber containing filtered (2 µm) seawater at the respective pH level for each treatment to monitor the rate of oxygen depletion. An organism remained in the chamber until the oxygen level had reduced by 20 % from the beginning concentration (organisms were in the chamber for approximately two hours). All measurements were made using a fibre-optic probe (PreSens fibre optic dipping probe DP PsT3) sealed in the chambers. The probes were calibrated with a two-point
calibration (0 and 100%). The probes were connected to a four-Channel Minisensor Oxygen Meter (PreSens OXY-4 mini) which measured the oxygen uptake of each specimen.

Oysters and whelks were starved for the 24 h prior to the measurement of SMR. Measurements of SMR were standardised to the dry mass of the tissue of each individual measured. The change in oxygen concentration of a “blank” chamber containing filtered seawater was measured concurrently to organisms for each treatment level. The temperature within each chamber was maintained at 22 °C using a constant temperature water bath (Labec). SMR was calculated as the percentage of oxygen decline, expressed in mg of O₂ over time that organisms were actively respiring;

$$\text{SMR h}^{-1} = \left( \frac{\text{Vol} \times \Delta V_{\text{meas}}}{\Delta t} - \text{Blank} \right) \times W_b^{-1}$$

Where;

$\text{Vol}$ = volume of the respirometry chamber minus the volume of the oyster

$\Delta V_{\text{meas}}$ = change in the oxygen concentration of the seawater

$\Delta t$ = time over which the measurement was made

Blank = the rate of change measured concurrently in a chamber with no organism

$W_b^{-1}$ = dry tissue mass tissue of the individual organism

**Measurement of T. marginalba consumption**

The number of *S. glomerata* or *C. gigas* consumed by *T. marginalba* held at ambient and elevated $p\text{CO}_2$ was recorded each day that oysters and whelks were together. The mean number of oysters consumed was identified by the presence of a drill hole in the shell after the oyster flesh had been consumed. Drilling was the likely method of predation throughout the experiment as the shell of all oysters consumed had a drill-hole characteristic of the size of the radula of *T. marginalba* as reported in chapter two of this thesis. Consumed oysters were removed from the experimental tanks once counted and replaced with oysters which had been similarly exposed to maintain overall
consistency in the food supply to whelks. Total meat weight of oysters consumed whelk$^{-1}$ day$^{-1}$ for each size class was estimated using an average dry flesh mass of *S. glomerata* = 0.300 g and *C. gigas* = 0.041 g, calculated using a standard curve constructed from data collected during SMR calculation.

**Experiment 2: Physiological responses of *S. glomerata* and *C. gigas* to chemical cues from *T. marginalba***

A second experiment was conducted to determine whether *S. glomerata* and *C. gigas* responded to chemical cues released by *T. marginalba*, and whether elevated pCO$_2$ impaired this response. The SMR of a separate set of juvenile *S. glomerata* (mean mass ± S.E. 4.64 ± 0.06 g) and *C. gigas* (mean mass ± S.E. 4.15 ± 0.09 g) was measured in response to a series of waterborne cues. Oysters were exposed to either ambient or elevated pCO$_2$ for 3 weeks in six 750 L tanks (n=3) using the pCO$_2$ controllers and levels described above for experiment one. There was a complete set of six tanks for each species of oyster. The SMR of eight *S. glomerata* and eight *C. gigas* from each independent tank (n = 3) was assessed in response to exposure to a conspecific alarm cue (homogenised oyster tissue), predator kairomones from live *T. marginalba* and dead homogenised *T. marginalba* tissue and a mixed cue (50:50 alarm cue : predator kairomones made from live and dead tissue). The SMR measurements were taken for each oyster at the pCO$_2$ levels that the oyster had been kept at for the previous three weeks. Alarm cues are chemical cues released by conspecifics after injury. An alarm cue was produced by homogenising 20 mL of *S. glomerata/C. gigas* tissue in 20 L of seawater. Prey have been shown to display defence responses when exposed to the kairomones released by small volumes of live or crushed predators (Schoepner and Relyea 2005). Predator kairomones from live predators were obtained from a subsample of 20 L of seawater which held 30 *T. marginalba* actively feeding for 24 h. Kairomones from dead predators were obtained by homogenising *T. marginalba* tissue and mixing through seawater at a concentration of 20 mL of homogenised tissue in 20 L of filtered seawater. The “mixed” cue was made by mixing the Kairomones solutions made from live
and dead *T. marginalba* in a 50:50 ratio. All four cue solutions were prepared directly prior to the measurement of the SMR of *C. gigas*.

**Measurement of SMR of oysters with cue exposure**

Closed respirometry was used to determine SMR as described above for experiment one. Individual oysters were placed into closed respirometry chambers containing filtered sea water set at the $pCO_2$ level of their treatment, and the resting SMR of each oyster determined prior to introduction of the cue. The chambers were then flushed with seawater and filled with either with one of the four cues (alarm, dead predator, live predator, mixed) or a filtered seawater control. Once the SMR of an oyster had been measured with a cue, the oyster was removed, the chamber was washed by scrubbing with a sponge and seawater, and a new oyster was then placed in the chamber to be measured. This process was repeated until all oysters had been measured during exposure to each of the four cues and the control filtered seawater. Previous studies have shown that only a small amount of cue is needed to elicit a defensive response in bivalves, therefore the cues in this instance were added in excess (eg. Jacobsen and Stabell 2004). All SMR measurements were recorded until the oxygen within the chamber had decreased by 20% as described for experiment 1. The temperature within each chamber was maintained at 22 °C using a constant water bath (Labec).

Dry flesh mass of each oyster was calculated using visible-near infrared reflectance spectroscopy. Near infrared reflectance spectroscopy (NIRS) has recently been adapted for the analysis of oyster moisture content (Brown et al. 2012). Brown et al. (2012) outlined the process of NIRS in detail describing it as a financial and time generous alternative to traditional laboratory methods with improved reproducibility and potential for growth in its current application. In order to analyse moisture content of individual oysters, the wet flesh mass was determined immediately after shucking and recorded using a calibrated electronic scientific balance. Oysters were immediately placed in a -80°C freezer to ensure no further loss of moisture would occur as oysters have a
tendency to quickly denature once shucked at room temperature. The NIRS process was
implemented and moisture content was obtained as a percentage of mass.

The moisture data was converted to dry flesh mass by the equation;

\[
\text{Dry flesh mass} = \text{wfm} - (\text{mc} \times \text{wfm})
\]

Where;

\( \text{wfm} = \) wet flesh mass as measured

\( \text{mc} = \) moisture content as determined by NIR analysis as a fraction

Statistical analyses

Experiment 1

To determine any significant differences in the SMR, compression strength and size (length, height, mass) of \( S. \ glomerata \) and \( C. \ gigas \), data were analysed independently for each species of oyster using a three-factor analyses of variance (ANOVA) where the first factor was the \( pCO_2 \) treatment (2 levels; ambient, 481 µatm and elevated, 909 µatm) and the second factor “presence of \( T. \ marginalba \)” (2 levels, present and absent), both factors were fixed and orthogonal. The third factor was “tank” which was random and nested in “\( pCO_2 \)” and “presence of \( T. \ marginalba \)”. The length, height, and strength of shells and SMR of \( T. \ marginalba \) was analysed with a single factor ANOVA, where \( pCO_2 \) (2 levels; ambient, 481 µatm and elevated, 909 µatm) was the single factor. As there were no significant interaction of \( pCO_2 \) with tanks the variance of tanks were pooled for each treatment following the procedure outlined by Winer et al (1991) with a residual of \( \alpha= 0.25 \).

To determine any significant differences in the number of oysters consumed by whelks, data were analysed using a two-factor ANOVA after 10 days for \( S. \ glomerata \) and at two time points for \( C. \ gigas \); after 10 and 17 days. For this analysis, the first factor was the \( pCO_2 \) conditions that oysters were held in for 6 weeks (2 levels; ambient, 481 µatm and elevated, 909 µatm), and the second
factor was the $pCO_2$ conditions (2 levels; ambient, 481 µatm and elevated, 909 µatm) that they were transferred into with whelks. The two factors were fixed and orthogonal. A second analysis was performed to allow a comparison of the consumption between the two species of oyster using the calculated values of the total meat weight consumed. Only the data of $S. glomerata$ or $C. gigas$ kept for 6 weeks at ambient $pCO_2 + T. marginalba$ at ambient $pCO_2$ and $S. glomerata$ or $C. gigas$ kept for 6 weeks at elevated $pCO_2 + T. marginalba$ at elevated $pCO_2$ were used in this analysis. The first factor was the species of oyster (2 levels; $S. glomerata$ and $C. gigas$), the second factor was the $pCO_2$ conditions (2 levels; ambient, 481 µatm and elevated, 909 µatm).

Experiment 2

Data from the second experiment were analysed with a four factor ANOVA. The first factor was species (2 level; $S. glomerata$ and $C. gigas$), the second factor was $pCO_2$ (2 levels; ambient, 481 µatm and elevated, 901 µatm), the third factor was “cue” (5 levels; alarm, dead predator, live predator, mixed, and control). The first three factors were fixed and orthogonal, and the fourth factor “Tank” was random nested in the “species”, “$pCO_2$” and “cue” factors.

In all analyses, the “tank” factor was found to be not significant ($P > 0.05$) and was therefore pooled for each treatment, following the procedure outlined by Winer et al. (1991) with a residual of $\alpha = 0.25$. All data analyses were completed using GMAV-5 for Windows (Underwood et al. 2002). Heterogeneity of variances were assessed by Cochran’s test, which indicated that all tests satisfied the assumption of heterogeneity of variances and no transformations were required ($P < 0.05$). Significant differences among means were detected by Student Newman Keuls (SNK) tests on significant main effects and interactions of interest (Sokal and Rohlf 1995).
3.4 Results

Experiment 1

*Shell morphology and physiology of S. glomerata*

There was no significant effect of elevated $p$CO$_2$ or *T. marginalba* on the shell height, length or whole mass of *S. glomerata*. The SMR of *S. glomerata* was significantly lower at elevated than ambient $p$CO$_2$ (Tables 3.2 and 3.3, Figure 3.3).

**Table 3.2** Analysis of variance of shell height, length, whole mass ($n = 90$) of *S. glomerata* in response to ambient and elevated carbon dioxide ($p$CO$_2$, 481, 909 µatm) treatments with and without *T. marginalba* (Wh) for 17 days. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Shell height</th>
<th>Shell length</th>
<th>Whole Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>$F$</td>
</tr>
<tr>
<td>$p$CO$_2$</td>
<td>1</td>
<td>5.21</td>
<td>0.29</td>
</tr>
<tr>
<td>Wh</td>
<td>1</td>
<td>4.41</td>
<td>0.25</td>
</tr>
<tr>
<td>$p$CO$_2$ x Wh</td>
<td>1</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td>RES</td>
<td>116</td>
<td>17.94</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table 3.3 Analysis of variance of SMR ($n = 48$), of *S. glomerata* in response to ambient and elevated carbon dioxide ($pCO_2$, 481, 909 µatm) treatments with and without *T. marginalba* (Wh) for 17 days. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pCO_2$</td>
<td>1</td>
<td>454.30</td>
<td>18.96</td>
<td>***</td>
</tr>
<tr>
<td>Wh</td>
<td>1</td>
<td>11.97</td>
<td>0.50</td>
<td>ns</td>
</tr>
<tr>
<td>$pCO_2$ x Wh</td>
<td>1</td>
<td>81.77</td>
<td>3.41</td>
<td>ns</td>
</tr>
<tr>
<td>RES</td>
<td>116</td>
<td>26.26</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
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</tbody>
</table>

Figure 3.3 The mean SMR of *S. glomerata* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without *T. marginalba* for 17 days (with standard error bars).

*Shell morphology and physiology of *C. gigas***

At ambient $pCO_2$ the shell length of *C. gigas* was significantly less when held in tanks with *T. marginalba* than *C. gigas* held without *T. marginalba* as seen by the significant $pCO_2$ x whelk interaction (Table 3.4, Figure 3.4). *C. gigas* were significantly smaller in shell height when held with
T. marginalba compared to when held without T. marginalba, irrespective of pCO$_2$ treatment (Table 3.4, Figure 3.4). There was no significant effect of the presence of T. marginalba on the shell mass, shell strength or SMR of C. gigas (Table 3.4, Figure 3.4). C. gigas grown under elevated pCO$_2$ were, however, larger in shell height and length and heavier in mass compared to C. gigas grown under ambient pCO$_2$ (Table 3.4, Figure 3.4). In contrast, the shell compression strength of C. gigas was significantly less at elevated compared to ambient pCO$_2$ (Table 3.5, Figure 3.4).

There was a significant effect of T. marginalba on the SMR of C. gigas at ambient pCO$_2$, however this effect was reduced or absent at elevated pCO$_2$. When T. marginalba were present, the SMR of C. gigas were significantly lower at ambient than elevated pCO$_2$, but there was no difference in the SMR of C. gigas at either ambient or elevated pCO$_2$ when T. marginalba were absent as determined from the significant interaction. (Table 3.5, Figure 3.4).

**Table 3.4** Analysis of variance of shell height, length, whole mass ($n = 96$) of C. gigas in response to ambient and elevated carbon dioxide (pCO$_2$, 481, 909 µatm) treatments with and without T. marginalba (Wh) for 17 days. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Shell height</th>
<th>Shell length</th>
<th>Whole mass</th>
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<tr>
<td>pCO$_2$</td>
<td>1</td>
<td>272.53</td>
<td>9.23</td>
</tr>
<tr>
<td>Wh</td>
<td>1</td>
<td>411.47</td>
<td>13.94</td>
</tr>
<tr>
<td>pCO$_2$ x Wh</td>
<td>1</td>
<td>20.86</td>
<td>0.71</td>
</tr>
<tr>
<td>RES</td>
<td>380</td>
<td>29.52</td>
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</tr>
<tr>
<td>Total</td>
<td>383</td>
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<td></td>
</tr>
<tr>
<td>481 &lt; 909 µatm; With Wh &lt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNK without Wh</td>
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</tbody>
</table>

481 µatm: with < without T. marginalba 481 < 909 µatm
Table 3.5 Analysis of variance of compression strength and SMR (n = 48), of C. gigas in response to ambient and elevated carbon dioxide (pCO₂, 481, 909 µatm) treatments with and without T. marginalba (Wh) for 17 days. Significance level is indicated by asterisks, * P < 0.05; ** P < 0.01; *** P < 0.001; ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO₂</td>
<td>1</td>
<td>4.28</td>
<td>6.39</td>
<td>*</td>
<td>272.53</td>
<td>17.37</td>
<td>***</td>
</tr>
<tr>
<td>Wh</td>
<td>1</td>
<td>0.19</td>
<td>0.30</td>
<td>ns</td>
<td>411.47</td>
<td>0.18</td>
<td>ns</td>
</tr>
<tr>
<td>pCO₂ x Wh</td>
<td>1</td>
<td>0.09</td>
<td>0.14</td>
<td>ns</td>
<td>20.86</td>
<td>2.94</td>
<td>*</td>
</tr>
<tr>
<td>RES</td>
<td>188</td>
<td>0.66</td>
<td></td>
<td></td>
<td>29.52</td>
<td></td>
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<td>Total</td>
<td>191</td>
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</tr>
</tbody>
</table>

SNK 481 > 909 µatm

Without Wh: 481 = 909 µatm

With Wh: 481 < 909 µatm
Figure 3.4 The mean shell a) height b) length, c) whole mass and d) compression strength of the shell and e) SMR of *C. gigas* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without *T. marginalba* for 17 days (with standard error bars).
Shell morphology and physiology of *T. marginalba*

There was no effect of $p$CO$_2$ on the height (ANOVA, $F_{(1,383)} = 0.32$, $P > 0.05$), length of aperture (ANOVA, $F_{(1,383)} = 3.25$, $P > 0.05$), mass (ANOVA, $F_{(1,383)} = 0.32$, $P > 0.05$) or compression strength of the shell (ANOVA, $F_{(1,191)} = 0.79$, $P > 0.05$) of *T. marginalba*. There was a significant increase in the SMR of *T. marginalba* at elevated compared to ambient $p$CO$_2$ (ANOVA, $F_{(1,134)} = 17.57$, $P < 0.05$, Figure 3.5).

![Figure 3.5](image_url)

*Figure 3.5* The mean SMR of *T. marginalba* at ambient and elevated carbon dioxide (481, 909 µatm) treatments after transfer to tanks with oysters at ambient and elevated carbon dioxide (481, 909 µatm) for a further 17 days (with standard error bars).

Predation of *S. glomerata* by *T. marginalba*

*T. marginalba* acclimated at elevated $p$CO$_2$ consumed significantly more *S. glomerata* than *T. marginalba* acclimated at ambient $p$CO$_2$, irrespective of the level of $p$CO$_2$ exposure of the prey (Table 3.6, Figure 3.6).

**Table 3.6** Analysis of variance of the mean daily number of *S. glomerata* consumed by *T. marginalba* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments; $n = 3$. Significance
level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect.

Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S. glomerata$ (Oy)</td>
<td>1</td>
<td>1.33</td>
<td>0.25</td>
<td>ns</td>
</tr>
<tr>
<td>$T. marginalba$ (Wh)</td>
<td>1</td>
<td>33.33</td>
<td>6.25</td>
<td>*</td>
</tr>
<tr>
<td>Oy x Wh</td>
<td>1</td>
<td>0.33</td>
<td>0.06</td>
<td>ns</td>
</tr>
<tr>
<td>RES</td>
<td>8</td>
<td>5.33</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SNK**

$T. marginalba$: 909 < 481µatm

**Figure 3.6** The mean daily number of $S. glomerata$ consumed by each $T. marginalba$ after 10 days following exposure to ambient and elevated carbon dioxide (481, 909 µatm; with standard error bars).
**Predation of C. gigas by T. marginalba**

Ten days after oysters and whelks were placed together in tanks, *T. marginalba* consumed significantly more *C. gigas* when the oysters had been held at elevated CO$_2$ for 6 weeks and the whelks themselves had also been kept at elevated CO$_2$. This effect was no longer present after 17 days (Table 3.7, Figure 3.7).

**Table 3.7** Analysis of variance of the mean daily number of *C. gigas* consumed by *T. marginalba* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments; $n = 3$. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect.

Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. gigas</em> (Oy)</td>
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<td>12.00</td>
<td>0.50</td>
<td>ns</td>
<td></td>
<td>56.33</td>
<td>3.71</td>
<td>ns</td>
</tr>
<tr>
<td><em>T. marginalba</em> (Wh)</td>
<td>1</td>
<td>21.33</td>
<td>0.89</td>
<td>ns</td>
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<td>21.33</td>
<td>1.41</td>
<td>ns</td>
</tr>
<tr>
<td>Oy x Wh</td>
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<td>133.33</td>
<td>5.57</td>
<td>*</td>
<td></td>
<td>48.00</td>
<td>3.16</td>
<td>ns</td>
</tr>
<tr>
<td>RES</td>
<td>8</td>
<td>23.92</td>
<td></td>
<td></td>
<td></td>
<td>15.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$T. marginalba$ held at 909 µatm:

**SNK**

*C. gigas* 909 > *C. gigas* 481 µatm
Figure 3.7 The mean daily number of *C. gigas* consumed by each *T. marginalba* after a) 10 and b) 17 days following exposure to ambient and elevated carbon dioxide (481, 909 µatm; with standard error bars).

Dry flesh mass consumed by *T. marginalba*

On a dry flesh mass (DFM) basis, *T. marginalba* consumed significantly more *S. glomerata* than *C. gigas*, particularly *T. marginalba* exposed to elevated pCO$_2$ as seen by the significant interaction (ANOVA, F $(1,16) = 5.39, P < 0.05$; Figure 3.8).
Figure 3.8 The mean daily mass of dry flesh of *S. glomerata* and *C. gigas* consumed by each *T. marginalba* after 10 days following exposure to ambient and elevated carbon dioxide (481, 909 µatm; with standard error bars).

Experiment 2

*Physiological responses of S. glomerata and C. gigas to chemical cues from T. marginalba*

At ambient pCO₂, when exposed to water treated with predator kairomones (live *T. marginalba* or dead homogenised *T. marginalba*) the standard metabolic rate of *C. gigas* was significantly less than the control, alarm and mixed cue treatments (ANOVA, Cue x pCO₂ interaction, F(4,64) = 6.00, P < 0.05; SNK - at ambient pCO₂: live *T. marginalba*, dead homogenised *T. marginalba* < control, alarm, mixed cue treatments; Figure 3.9) but there was no significant difference in the SMR of *C. gigas* among any treatments when exposed to elevated pCO₂ (Figure 3.9). Overall there was no effect of pCO₂ on the SMR of *S. glomerata* to any of the chemical cues.
Figure 3.9 The mean SMR of *S. glomerata* and *C. gigas* in response to alarm and predator cues at ambient and elevated carbon dioxide (481, 909 µatm; with standard error bars).

### 3.5 Discussion

This study found more *S. glomerata* and *C. gigas* were consumed by *T. marginalba* held at elevated pCO₂ than *T. marginalba* held at ambient pCO₂ after ten days. This finding was consistent with changes observed to the morphology and SMR of both species of prey and *T. marginalba*. Exposure of *T. marginalba* to elevated pCO₂ also led to a significant increase in their SMR, supporting our first hypothesis; that under elevated pCO₂ energetic costs are increased in *T. marginalba* which may have triggered them to increase their consumption of prey. Furthermore, under elevated pCO₂ the shells of *C. gigas* were weaker and metabolic rate was unaltered in the presence of a cue from predators; a result that supported our second hypothesis. These findings show that elevated pCO₂ affect both the whelk and oyster which may result in greater predation.

Numerous studies have shown a similar increase in the SMR of mollusc species following exposure to elevated pCO₂. This has been attributed to an increase in the cost of maintaining acid-base balance.
and calcification (Pörtner et al. 2004; Cummings et al. 2011; Parker et al. 2012). It should be noted, however that not all bivalves display an increase in SMR following exposure to ocean acidification. In fact, metabolic depression has been documented in a number of species (Michaelidis et al. 2005; Liu and He 2012; Navarro et al. 2012; Schalkhausser et al. 2013; Lesser 2016). This has been attributed to an inability of these species to compensate for extracellular acidosis however, in some cases this may have been due to different pH levels and exposure times that have been used (Michaelidis et al. 2005; Lesser 2016). It has been suggested that if food supply and consumption are adequate to sustain the increased energy demand caused during exposure to elevated $p\text{CO}_2$, then some mollusc species will not experience negative impacts. For example in the mussel *M. edulis*, exposure to elevated $p\text{CO}_2$ led to a diversion of energy from maintenance of shell to maintenance of somatic mass when food concentrations were low. When food concentrations were high, however, these negative impacts were ameliorated (Melzner et al. 2011). Here, exposure of *T. marginalba* to elevated $p\text{CO}_2$ had no significant effect on their shell morphology that could be measured with our equipment. This may suggest that any changes in shell growth were subtle, and the increase in consumption rate of *S. glomerata* and *C. gigas* may have been enough to sustain the increase in energy demand.

Analysis of the SMR of similar sized oysters showed that under ambient $p\text{CO}_2$ conditions, *C. gigas*, but not *S. glomerata*, reduced their SMR in the presence of live *T. marginalba* and chemical cues released from *T. marginalba*. A reduced metabolic output has been found in the green-lipped mussel *P. viridis*. It was suggested that reduced metabolic output is a defensive mechanism, reducing the risk of being detected by the predator (Wang et al. 2013). A number of other studies have found that greater metabolic output increases predation risk (Blake 1960; Pratt 1974; Trussell and Nicklin 2002; Bourdeau 2009; Bourdeau 2012). One of the earliest studies to investigate the relationship between SMR and predation found that the Atlantic oyster drill *Urosalpinx cinerea* consumed less prey (*Modiolus demissus* and *C. virginica*) when the metabolic rate of the prey was low (Blake 1960). In sessile species which lack an escape response, the ability to avoid detection by a
predator is a vital defensive mechanism. This will be particularly true over this century as ocean acidification weakens the shells of many mollusc species (Parker et al. 2013). Although C. gigas were larger and weighed more at elevated compared to ambient pCO$_2$, the compression strength of their shell was less at elevated pCO$_2$. For C. gigas, a reduction of metabolic output may provide a defensive mechanism at ambient pCO$_2$, but following exposure to elevated pCO$_2$ this capacity was lost. This result supported our second hypothesis; that C. gigas may no longer be able to reduce their metabolic rate, making them easier to detect by the predator under elevated pCO$_2$. It is unknown if the inability to alter SMR is because of an inability to detect cue or because of increased energy required. It was assumed that T. marginalba can more easily detect the normal level of respiration (or some cue associated with respiration) at elevated pCO$_2$, than the reduced respiration of C. gigas at ambient pCO$_2$. This is the first study to show that ocean acidification may make C. gigas more ‘visible’ to predators.

The inability of molluscs to respond to predation risk when exposed to elevated pCO$_2$ has been shown in previous studies (Bibby et al. 2007; Watson et al. 2014; Wright et al. 2014). For example, the shell strength of the mussel Brachidontes pharaonis was 65% weaker following exposure to elevated pCO$_2$. Consequently the prey handling time by the predatory crab Eriphia verrucosa was reduced by 27% (Dupont et al. 2015). Increases in the thickness of the shell of Littorina littorea were observed in a previous study by (Bibby et al. 2008) in the presence of a predator (Carcinus maenas) chemical cue compared to the control (no cue). However, it was found that the prey did not have a thickened shell after exposure to predator cues at elevated pCO$_2$. The authors attribute the loss of induced defences to a reduction in the rate of oxygen uptake when exposed to the combined stress of ocean acidification and the predatory cue (Bibby et al. 2008), which may suggest snails had entered a hypometabolic state (Pörtner et al. 2004; Michaelidis et al. 2005). Further, the black turban snail Tegula funebralis had a strong flight response when the predatory crab Thalamita danae was present, however this induced defence behaviour was lost with increased time spent in refuge when exposed to elevated pCO$_2$ (Wang et al. 2013). More recent studies have found elevated
pCO$_2$ did not affect the ability of *T. funebralis* (Jellison et al. 2016) or *Mytilus coruscus* (Sui et al. 2015) to detect a predator, although, there were significant effects on their anti-predatory defence including a reduction in growth and the time spent in refuge.

In aquatic environments chemical cues are one of the most effective ways to detect predators (Chivers and Smith 1998; Jacobsen and Stabell 2004; Ferrari et al. 2010; Ferrari et al. 2011). For example *T. funebralis* exhibited a strong flee response to the presence of the sea star predators *Pisaster ochraceus* and *Leptasterias* sp. (Morgan et al. 2016). Similarly, the presence of cue of the crab *Charybdis japonica* induced an anti-predator response increase in byssus length and secretion rate in the mussel *Mytilus coruscus* (Li et al. 2015).

There are at least two possible explanations for the lack of change in the SMR of *C. gigas* at elevated pCO$_2$ in the presence of *T. marginalba* or cues associated with its presence. First, the energetic cost of maintaining calcification and acid-base balance during exposure to elevated pCO$_2$ may be too great, preventing the oysters from reducing their metabolic rate (Green et al. 2009; Lischka et al. 2010; Range et al. 2011). Second, exposure to elevated pCO$_2$ may impair the ability of *C. gigas* to detect the presence of the predator. In the snail *Gibberulus gibbosus*, exposure to elevated pCO$_2$ led to slower reaction time and altered escape trajectory in the presence of the predator *Conus mamoreus* (Watson et al. 2014). The authors attributed this response to impaired neurotransmitter receptors of *G. gibbosus* to detect chemical cues released from the predator when exposed to elevated pCO$_2$.

*S. glomerata* may have alternate strategies to a reduction in SMR to defend itself against predation at ambient pCO$_2$. Although not directly assessed in this study, among larger oysters, *S. glomerata* have a stronger shell relative to size than *C. gigas* (Wilkie and Bishop 2013). Thickened shells have been shown to be one of the most reliable defences for prey against predators (Kroeker et al. 2014; Gaylord et al. 2015). Although in this study *C. gigas* and *S. glomerata* were not of a comparable size, it should be noted that differences in the underlying composition and matrix structure of the shell
would likely influence the strength of the shell. As yet there has been no study that has compared the shell composition and structure of *C. gigas* and *S. glomerata*. In regards to *C. gigas*, naïve prey that lack evolutionary history with predators may suffer heavy predation because they exhibit ineffective antipredator responses to novel predators (Sih et al. 2010). Further, predators could instead suffer from a novelty disadvantage because they are also naïve to their new prey. For example, the Japanese oyster drill *Ocinebrina inornata* introduced in the U.S.A. was found to prefer invasive *C. gigas* over native oysters (Buhle and Ruesink 2009). In a study looking at the effect of pCO$_2$ on predation, the predatory dogwhelk *Nucella lapillus* consumed a greater number of the native mussel *Mytilus galloprovincialis* than the non-indigenous mussel *Xenostrobus* secures after being held in mesocosms treated with pCO$_2$ for three weeks (Gestoso et al. 2015). Wilkie and Bishop (2013) found that in the absence of shell strength differences among smaller oysters, *T. marginalba* preferentially consumed native over non-native oysters. The preference of predators for sympatric prey may reflect learned specialization for the dominant prey in their natural range. Some caution is required when comparing the consumption of *C. gigas* and *S. glomerata* in this study. *S. glomerata* used in experiment one were medium sized (mean ± SE 6.34 ± 0.10 g), while *C. gigas* were small sized (mean ± SE 0.45 ±0.02). Smaller sized *S. glomerata* were not available, but may have displayed similar defensive mechanisms as the smaller sized *C. gigas*.

There has been considerable research attention on the physiological impacts of ocean acidification on marine molluscs. There still remains a paucity of information, however, on the effect of environmental change on species interactions. The aim of this study was to better understand predator-prey interactions of an endemic whelk and two of its oyster prey in a high pCO$_2$ world and to determine whether increased predation of oysters by the whelk *T. marginalba* occurred because of impacts on the predator or the prey. Given the age and size difference between the oysters used in this study, *S. glomerata* were juveniles and *C. gigas* were spat, the ability to directly analyse differences in response and predation between the two species of oyster was confounded. *S. glomerata* may have greater morphological defence, while *C. gigas* may respond to the presence of
a generalist predator by reducing metabolic outputs, which would ultimately place greater strain on energetic demands and may disadvantage the species. A comparison was made assessing the response of a secondary set of similar sized oysters to chemical cues. This study enabled a comparison of the response of two species of oyster found in eastern Australia. This study found increased predation at elevated pCO$_2$ because 1, energy, and thus food, requirements of the predator maybe increased following exposure to elevated pCO$_2$; 2, for _C. gigas_, shells were weakened by elevated pCO$_2$ and 3, oysters become more ‘visible’, with increased energetic needs and a higher SMR in the presence of the predator. Further studies are required to better understand if this is a response of spat or a species specific response. In particular, an assessment of the strength of shells of _S. glomerata_ at elevated pCO$_2$ may support the findings of this study that increased predation at elevated is due, in part, to morphological changes of prey. This study provides further evidence that in a future acidified ocean _S. glomerata_ and _C. gigas_, internationally important aquaculture species will be increasingly vulnerable to predation.
Chapter Four

Populations of Pacific oysters *Crassostrea gigas* respond variably to elevated $p\text{CO}_2$ and predation by *Tenguella marginalba*

4.1 Abstract

Ocean acidification is anticipated to decrease calcification and increase dissolution of shelled molluscs. Molluscs with thinner and weaker shells may be more susceptible to predation, but not all studies have measured negative responses of molluscs to elevated $p\text{CO}_2$. Recent studies measuring the response of molluscs, have found greater variability at the population level than first anticipated. Here we investigate the impact of acidification on the predatory whelk *Tenguella marginalba* on genetically distinct subpopulations of the Pacific oyster, *Crassostrea gigas*. Whelks and eight family lines of *C. gigas* were separately exposed to ambient (387 µatm) and elevated (1108 µatm) $p\text{CO}_2$ for six weeks. Following this period *T. marginalba* was transferred into tanks with oysters at ambient and elevated CO$_2$ for 17 days. Shell growth on average was 63% less at elevated compared to ambient $p\text{CO}_2$, but this response was variable among family lines. There were differences in shell compression strength, depth and mass among family lines, with sometimes a $p\text{CO}_2 \times$ family line interaction, but this was not consistent across family lines. Shell growth and shell compression of *T. marginalba* decreased during the experiment being significantly less at elevated compared to ambient $p\text{CO}_2$. After 10 days, *T. marginalba* consumed significantly more oysters regardless of whether *C. gigas* had been exposed to ambient or elevated $p\text{CO}_2$, but this was not dependent on the
family line and the effect was not significant after 17 days. Our study found an increase in predation following exposure of the predator to near-future predicted levels of estuarine pCO₂.

4.2 Introduction

Atmospheric concentrations of carbon dioxide (CO₂) are rising at a rapid rate because of the combustion of fossil fuels (Chapman 1977; Houghton 2001; Solomon 2007). Since the early 1800’s the oceans have absorbed an estimated 30% of atmospheric carbon emissions (Sabine et al. 2004; Canadell et al. 2007). The International Panel on Climate Change (IPCC 2007) predicts that if the present CO₂ emission trend continues a further decrease in oceanic pH of 0.3 to 0.5 units (pH 7.4-7.9) will occur by the end of this century (2100). Already the increased production of CO₂ has caused noticeable changes in oceanic environments with reductions in surface ocean pH and carbonate ion concentration (Chapman 1977; Feely et al. 2004; Orr et al. 2005). Of particular concern is the impact of alterations of seawater chemistry on marine calcifying organisms. Molluscs are predicted to be one of the most sensitive marine groups which will find it more difficult to deposit their calcium carbonate (CaCO₃) shells and survive in a climate changed ocean (Walter 1977; Townend and Pethick 2002; Cooley et al. 2011; Narita et al. 2012).

In recent decades there have been severe declines in mollusc populations which in part may be due to ocean acidification (Feely et al. 2010; Crim et al. 2011; Barton et al. 2012). Studies simulating an acidifying ocean, reveal negative correlations between elevated pCO₂/reduced saturation state of aragonite (Ω), calcification and growth in a number of economically and ecologically significant molluscs including the oysters Crassostrea virginica (Ries et al. 2009) Saccostrea glomerata and Crassostrea gigas (Parker et al. 2009; Parker et al. 2010; Parker et al. 2012) and gastropods such as the whelk Urosalpinx cinerea, periwinkle Littorina littorina and conch Stombus alatus (Ries et al. 2009). Reduced growth and calcification can reduce shell strength and individual molluscs with weaker shells are more susceptible to predation (Kent 1981; Sommer et al. 1999; Zuschin 2001;
Buschbaum et al. 2007; Amaral et al. 2012b). Even among molluscs like *L. littorea*, that can develop heavily calcified and mechanically stronger shells in the presence of predators, exposure to elevated $pCO_2$ (pH 6.45) still reduces net calcification and causes a significant decline in their shell thickness (Bibby et al. 2007), counteracting their ability to resist predation.

In a recent study using oysters collected from areas affected by acid sulphate soils, the strength and size of the shell of the Sydney rock oyster, *Saccostrea glomerata* was correlated with predation by the gastropod *Tenguella marginalba* (Amaral et al. 2012a). On average, the gastropod consumed significantly more oysters from locations with increased seawater acidity (pH < 5) than oysters from control locations (pH 6.8). The shells of the oysters from the acidified sites were significantly weaker than the shells of the oysters from the control locations (Amaral et al. 2012a).

Potentially the impacts of reduced predator defence in molluscs may also be exacerbated by increased predatory activity. Most recent studies have found increases in the standard metabolic rate and energy demand of molluscs exposed to elevated $pCO_2$ (Beniash et al. 2010; Lannig et al. 2010; Cummings et al. 2011; Parker et al. 2012). A number of studies have suggested that increased energy demand may be met by marine organisms, including molluscs, through greater food consumption, which may ameliorate the negative impacts of elevated $pCO_2$ (Melzner et al. 2009; Parker et al. 2012; Hettinger et al. 2013).

It is unlikely, however, that ocean acidification and flow on impacts such as increased predation will act uniformly across populations of molluscs. Although there have been in general negative structural and functional responses of molluscs to elevated $pCO_2$, there has been variability at the level of population in this response. For example, there was no effect of elevated $pCO_2$ in two out of ten pair-mated family lines of the Sydney rock oyster *Saccostrea glomerata* (Parker et al. 2011). Parker et al. (2011) also found that the spat of oyster populations selected for fast growth had greater growth than wild populations in response to elevated $pCO_2$. Similarly Waldbusser et al. (2011) found significant variability in the dissolution of shells among populations of the Eastern...
oyster *Crassostrea virginica* at elevated pCO$_2$ (pH 7.67, 7.38 and 7.17). Such variability in response by oysters and other molluscs may be the key to species survival in a climate changed ocean (Sunday et al. 2014; Reusch 2014).

Here, we measure the variability in the response of the family lines of the Pacific oyster *C. gigas* and the response of one of its predators the muricid whelk *T. marginalba* to elevated pCO$_2$. The aim of this study was to assess the impact of ocean acidification on the i) calcification of family lines of *C. gigas*, ii) the predation rates of *T. marginalba* reared under near-future climate scenarios and any impact on iii) SMR. We hypothesised that i) calcification would vary among family lines, ii) predation would increase under elevated pCO$_2$ and that this (iii) rate of predation would vary among family lines of *C. gigas* due to variation in shell morphology.

### 4.3 Method

**Organism**

The Pacific oyster *C. gigas* is a keystone species found in shallow and subtidal estuarine environments and provides an important food source for marine invertebrates and larval fish, habitat stabilisation and biological filtration of estuarine environments (Shpigel and Blaylock 1991; Reise et al. 2006; Kochmann et al. 2008; Markert et al. 2010). *C. gigas* contribute 32% of the total value of global aquaculture production which is worth in excess of three billion USD annually. Any impact of ocean acidification on the production of *C. gigas* may have a significant impact on ecosystem function and the production of protein across the globe.

**Collection and feeding of Crassostrea gigas and Tenguella marginalba**

Eight pair-mated family lines of *C. gigas* were randomly selected from a pool of 42 families held at Tilligerry Creek, Port Stephens NSW Australia (32°45’S, 151°58’E). A breeding program was established for *C. gigas* to enhance the production output of the species from the Australian
hatchery-based industry. Six single-pair crosses of genetically diverse and unrelated individuals were established in the summer of 1997/98 from a single male-female pair. Oysters were selected for inclusion in the breeding program originally based on their meat yield, shell growth rate and shape of shell (Ward et al. 2000). For selection methods used in the breeding program see Ward et al. (2000).

Adults of the predatory whelk *T. marginalba* were collected from intertidal rocky shores at Boat Harbour (32°47'S, 152°06'E) and Anna Bay (32°47'S, 152°04'E), Port Stephens NSW, Australia. Twelve month old juveniles of the eight family lines and wild-caught adult *T. marginalba* were transferred to holding tanks at NSW Department of Primary Industries, Port Stephens Fisheries Research Institute, Taylors Beach NSW, Australia. Holding tanks were comprised of a 40 L tray supported above a 750 L reservoir. Seawater was collected from Little Beach (152°07'E, 32°72'S), Nelson Bay NSW Australia and was filtered through one, five and 10 µm nominal filters prior to delivery to the hatchery. Seawater was maintained at 22°C and pumped constantly from the reservoir to the tray at a flow of three L min⁻¹ and allowed to overflow back to the reservoir. Water in the reservoir was completely exchanged every two days during acclimation and experimentation. *C. gigas* was fed an algal diet of *Chaetoceros muelleri, Tisochrysis lutea* and *Pavlova lutheri* at a concentration of 1.19 x 10⁸ cells oyster⁻¹ day⁻¹, for the duration of the experiment. *T. marginalba* was fed a mixed diet of *C. gigas* and Sydney rock oyster *Saccostrea glomerata* that were similar in size to *C. gigas* used in the experiment.

**Experimental set-up**

*C. gigas* and *T. marginalba* were acclimated for two weeks in separate aquaria systems. *C. gigas* of each family line were then divided equally at random among six experimental systems (70 oysters family line⁻¹ tank⁻¹: mean mass 1.20 ± S.E 0.24 g). Similarly, *T. marginalba* were divided equally at random into another independent set of six systems (64 whelks tank⁻¹ mean mass 2.85 ± S.E 0.38 g). Two pH levels were used in this study; a current atmospheric pCO₂ level of pH 8.2 and an elevated
level of pH 7.8. Experimental treatments were randomly assigned as described in chapter 3. The elevated $p$CO$_2$ treatment was set-up using independent supplies of seawater that were maintained by direct diffusion of CO$_2$ in each of the replicate tanks and were monitored by microprocessors (Aqua Medic pH computer set; accuracy ± 0.01). The pH levels were determined and maintained to give a $p$CO$_2$ value of approximately 387 µatm and 1108 µatm respectively in line with current values and projections for the year 2100 (IPCC 2007).

The acidity of the 750 L tanks were gradually increased to the desired experimental condition (either pH 8.2 or pH 7.8). The $p$CO$_2$ of the seawater was maintained by direct diffusion of carbon dioxide and monitored by microprocessors (Aqua Medic pH computer set; accuracy ± 0.01). pH and temperature were measured in each 750 L tank daily with a hand-held probe (Palin test Waterproof 800 meter). The pH probe was calibrated each day using a three point calibration with automatic temperature compensation (NBS pH 4, 7 and 9) and a Cresol Purple test (AquaMedic). Temperature was maintained at 22°C (± 0.09) in a climate-controlled laboratory with thermostatically controlled immersion heaters (Weinu). Total alkalinity (TA) was measured using a Gran-titration (mean TA = 2297 ± S.E. 38 µmol kg$^{-1}$; Gran 1952). Titrations were conducted weekly by manual titration following the methods of Riebesell et al. (2010). A CO$_2$ system calculation program (CO$_2$ sys) developed by Lewis and Wallace (1998) was used to calculate the pH corresponding to the desired $p$CO$_2$ of the treatment levels, using the dissociation constants of Mehrbach et al. (1973) (for seawater physiochemical conditions see Table 4.1).

**Table 4.1** Seawater physiochemical conditions during the eight week exposure experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salinity psu</th>
<th>Temp. °C</th>
<th>pH$_{NBS}$</th>
<th>Total alkalinity µmol kg$^{-1}$</th>
<th>$p$CO$_2$ µatm</th>
<th>DIC µmol/kg$^{-1}$</th>
<th>Ω$_{aragonite}$</th>
<th>Ω$_{calcite}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient $p$CO$_2$</td>
<td>32 ± 0.10</td>
<td>22 ± 0.10</td>
<td>8.2 ± 0.01</td>
<td>2297 ± 38</td>
<td>387</td>
<td>2032.7</td>
<td>3.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Elevated $p$CO$_2$</td>
<td>32 ± 0.10</td>
<td>22 ± 0.10</td>
<td>7.8 ± 0.01</td>
<td>2297 ± 38</td>
<td>1108</td>
<td>2206.2</td>
<td>1.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Values for CO$_2$, Ω$_{aragonite}$ and Ω$_{calcite}$ calculated from salinity, temperature, pH$_{NBS}$ and TA.

Salinity, temperature, pH$_{NBS}$, total alkalinity, Ω$_{aragonite}$ and Ω$_{calcite}$ ± SE. $n = 18$. 85
Oysters and whelks were kept in their aforementioned six separate tanks and exposed to ambient (481 µatm) and elevated (909 µatm) \( pCO_2 \) for six weeks. After six weeks, half of \( T. \) marginalba from each tank were moved to a corresponding second tank so that there were six tanks of \( T. \) marginalba at ambient \( pCO_2 \) and six tank of \( T. \) marginalba at elevated \( pCO_2 \). Half of the \( C. \) gigas that had been held at ambient \( pCO_2 \) were removed and divided equally across six tanks of \( T. \) marginalba at ambient \( pCO_2 \) three of which were held at ambient \( pCO_2 \) and three were held at elevated \( pCO_2 \). Likewise, half the \( C. \) gigas that had been held at elevated \( pCO_2 \) were removed and divided across six tanks with \( T. \) marginalba at elevated \( pCO_2 \), three of which were held at ambient \( pCO_2 \) and three were held at elevated \( pCO_2 \). The remaining half of the oysters from the ambient and elevated treatments stayed in their respective ambient or elevated treatment without whelks. Each family line was held in each tank and separated by mesh inserted into each holding tank. This created six treatments, \( C. \) gigas alone at 1) ambient or 2) elevated \( pCO_2 \), 3) \( C. \) gigas kept for 6 weeks at ambient \( pCO_2 + T. \) marginalba at ambient \( pCO_2 \), 4) \( C. \) gigas kept for 6 weeks at ambient \( pCO_2 + T. \) marginalba at elevated \( pCO_2 \), 5) \( C. \) gigas kept for 6 weeks at elevated \( pCO_2 \) + \( T. \) marginalba at ambient \( pCO_2 \), 6) \( C. \) gigas kept for 6 weeks at elevated \( pCO_2 \) + \( T. \) marginalba at elevated \( pCO_2 \) (Figure 4.1). These treatments were maintained for a further 17 days.

\[ n = 8 \text{ family lines} \]

![Figure 4.1 Schematic of experimental design for the predation experiment (10/6/13 – 27/6/13).](image-url)
Measurement of shell size, mass and strength

To determine the specific growth and compression strength of shells of family lines of *C. gigas*, the shell was separated from the flesh using an oyster knife at two time-points, after four and eight weeks of CO₂ exposure. The shell growth of *C. gigas* was determined by measuring the height (dorsal to ventral margin) and depth (depth of the left valve) of the shell of 8 oysters per family line from each tank of the two treatments that contained oysters that had not been placed into a new pCO₂ treatment after 6 weeks of exposure (i.e. oysters alone at ambient or elevated pCO₂). Similarly, the height and length of the aperture of 6 *T. marginalba* from each tank of the two treatments that contained whelks and oysters that were not transferred between CO₂ treatments were measured to the nearest mm using vernier callipers (Mitutoyo, Digimatic vernier callipers, precision ± 0.01 mm). The shell mass of the left valve of eight *C. gigas* of each family line and shell of six *T. marginalba* from each tank were measured using an electronic balance (Mettler Toledo, XPE303S, precision ± 0.001 g). The change in shell height, depth and shell mass was calculated by subtracting the mean starting size of each family line of *C. gigas* from the mean size after four and eight weeks of exposure in each of three tanks (n=3) in ambient or elevated pCO₂ treatments. The same was done for *T. marginalba* (n = 3). The compression strength of three *C. gigas* of each family line per tank (n = 3) from the two treatments that contained oysters that had not been placed into a new pCO₂ treatment after 6 weeks of exposure (i.e. oysters alone at ambient or elevated pCO₂) and twelve *T. marginalba* per tank (n = 3) from the two treatments that contained whelks and oysters that were not transferred between CO₂ treatments were assessed as the capacity of the shell to resist an axially directed pushing force using methods adapted from Currey (1976) and Zuschin and Stanton (2001) The yield strength of the shell was determined as the ability to resist compression pressures, and was expressed by Newtons of Force at the point of maximum fracture.
Measurement of metabolic rate

The standard metabolic rate of eight *C. gigas* of each family line per tank (n = 3) from the two treatments that contained oysters that had not been placed into a new *pCO₂* treatment after 6 weeks of exposure (i.e. oysters alone at ambient or elevated *pCO₂*) and 19 wild *T. marginalba* per tank (n = 3) from the two treatments that contained whelks oysters that were not transferred between *CO₂* treatments were measured using a closed respiratory system using the methodology of Parker et al. (2012).

Briefly, individuals were gently placed into a sealed 500 mL chamber containing filtered (2 µm) seawater at the respective pH level for each treatment to monitor the rate of oxygen depletion. Organisms remained in the chamber until oxygen was reduced by 20% from the beginning concentration (organisms were in chamber for approximately two hours for each individual). All measurements were made using a fibre-optic probe (PreSens fibre optic dipping probe DP PsT3) sealed in the chambers. The probes were calibrated with a two-point calibration (0 and 100%). The probes were connected to a four-Channel Minisensor Oxygen Meter (PreSens OXY-4 mini) which measured the oxygen uptake of each specimen.

Oysters and whelks were starved for the 24 h prior to the measurement of SMR. Measurements of SMR were standardised to the dry mass of the tissue of each individual measured. The change in oxygen concentration of a “blank” chamber containing filtered seawater was measured concurrently to organisms for each treatment level. The temperature within each chamber was maintained at 22 °C using a constant temperature water bath (Labec). SMR was calculated as the percentage of oxygen decline, expressed in mg of O₂ over time that organisms were actively respiring;
$$\text{SMR h}^{-1} = \left( \frac{\text{Vol} \times \Delta V_{\text{meas}}}{\Delta t} - \text{Blank} \right) \times W_b^{-1}$$

Where;

$\text{Vol} = \text{volume of the respirometry chamber minus the volume of the oyster}$

$\Delta V_{\text{meas}} = \text{change in the oxygen concentration of the seawater}$

$\Delta t = \text{time over which the measurement was made}$

$\text{Blank} = \text{the rate of change measured concurrently in a chamber with no organism}$

$W_b^{-1} = \text{dry tissue mass tissue of the individual organism}$

**Measurement of T. marginalba consumption**

The number of oysters consumed by *T. marginalba* held at ambient and elevated $p\text{CO}_2$ was recorded each day that oysters and whelks were together. The mean number of oysters consumed was identified by the presence of a drill hole in the shell after the oyster flesh had been consumed. Consumed oysters were removed from the experimental tanks once counted and replaced with oysters which had been similarly exposed to maintain overall consistency in the food supply to whelks. The number of oysters from each family line consumed was recorded each day for 17 days with results analysed following 10 and 17 days.

**Statistical analyses**

To determine any significant differences in the change in shell height, depth or mass among family lines of *C. gigas*, data were analysed using a two-factor analysis of variance where level of ‘$p\text{CO}_2$’ and ‘family line’ were fixed factors and tanks were used as replicates. It was not possible to analyse tanks as a nested factor as the mean size of the animals of each family line in each tank was used to calculate change in size. Individual growth measurements were not possible as oysters were not individually tagged. In later experiments oysters were individually tagged and the growth of individual oysters were calculated. The SMR of *C. gigas* were analysed as two-factor ANOVA where
level of ‘pCO₂’ and ‘family line’ were fixed factors. Data of tanks was pooled for each treatment. The effect of pCO₂ on the growth, strength and SMR of T. marginalba was analysed using single factor analysis of variance, where pCO₂ was the single factor. Tanks were pooled as there were no significant differences in either strength of SMR of whelks among the replicate tanks. The number of C. gigas consumed by T. marginalba was analysed using a three factor analysis of variance after 10 and 17 days. The first factor was the pCO₂ conditions that oysters were held in for 6 weeks, the second factor was the pCO₂ conditions that they were transferred into with whelks and the third factor was ‘family line’. All factors were orthogonal and fixed. The GMAV 5 for Windows (Underwood et al. 2002) analysis package was used to analyse differences among treatments and family lines of C. gigas. Heterogeneity of the variances was assessed by Cochran’s test with no significant differences among variances. A significance level of P < 0.05 was used for all analyses. Any difference in means was detected by Student Newman Kuels (SNK) test on each parameter (Sokal and Rohlf 1995).

4.4 Results

Response among family lines of C. gigas

Growth, mass, standard metabolic rate and strength

Pair-mated family lines of C. gigas differed significantly in the growth (height, depth, mass) and shell strength (Table 4.2; Figure 4.2). Oysters from family lines 39 and 59 had the greatest growth in shell height, while those from family line one had the least growth in shell depth after both four and eight weeks at ambient pCO₂ (Table 4.2 Figure 4.2 a-d). Although the growth of family line 59 was the greatest observed, their change in mass was least (Figure 4.2 a-b, e-f). This may indicate allocation of energy resources into shell rather than somatic growth which may vary among lines.
Overall growth of *C. gigas* at ambient $p\text{CO}_2$ (mean ± SE 2.69 ± 0.46 mm mean ± SE) was 63% greater than at elevated $p\text{CO}_2$ (mean ± SE 1.61 ± 0.37 mm) after eight weeks (Table 4.2 Figure 4.2 a-b) but the effect on shell depth was dependent on the family line. The greatest shell depth was at elevated $p\text{CO}_2$ in family line one, as shown by the significant $p\text{CO}_2 \times \text{Fa}$ interaction after four weeks (Table 4.2 Figure 4.2 c-d). The mass of shells of *C. gigas* varied dependent on the family line after four but not eight weeks of exposure. Only family line 20 was consistently affected by elevated $p\text{CO}_2$ and there was no difference in the mass between treatments after eight weeks of exposure (Table 4.2 Figure 4.3 e-f).

Significant differences in compression strength were detected between family lines (Table 4.2 Fig. 4.3). Overall the compression strength of *C. gigas* increased over time and on average, the shell of *C. gigas* was 24% stronger at elevated $p\text{CO}_2$ (mean ± SE 3.41 ± 0.33 N mm$^{-1}$) compared to ambient $p\text{CO}_2$ (mean ± SE 2.75 ± 0.25 mm$^{-1}$), but despite this no significant difference was detected as a function of $p\text{CO}_2$ after four or eight weeks of exposure (Table 4.2, Figure 4.3). Differences in compression strength of shell were still apparent between family lines at the end of the trial. The compression strength of the shell of family 30 was significantly greater than most other family lines (Table 4.2, Figure 4.3).
Table 4.2 Analysis of variance of the mean growth and strength of family lines (Fa) of *C. gigas* in response to ambient and elevated carbon dioxide ($p$CO$_2$, 387 µatm, 1108 µatm) treatments after 4 (28/4/13 – 26/5/13) and 8 (28/4/13 – 23/6/13) weeks; n = 3. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns = non-significant effect. Cochran’s test = ns.

<table>
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<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
<th>MS</th>
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<th>$P$</th>
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<td>0.07</td>
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<td>14.08</td>
<td>5.94</td>
<td>*</td>
<td>0.07</td>
<td>0.34</td>
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<td>ns</td>
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<tr>
<td>Fa</td>
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<td>5.00</td>
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<td>*</td>
<td>12.74</td>
<td>5.38</td>
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<td>µatm</td>
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</tr>
<tr>
<td>Fa 59 &gt; 1, 17, 20, 45, 47</td>
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<td>1108</td>
<td>µatm</td>
<td>: 47 &gt; 1</td>
<td>1, 17, 45, 47</td>
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<td>Fa 39 &gt; 1, 20</td>
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<td>&lt; 1108</td>
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<td>SNK</td>
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<td>µatm</td>
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<td>108 µatm</td>
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<td>1,17,20,39,47</td>
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<td>47</td>
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<td></td>
<td>20,39</td>
<td>47</td>
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</table>
Figure 4.2 The mean growth, depth and mass of family lines of C. gigas in response to ambient and elevated carbon dioxide (387 µatm, 1108 µatm) treatments after a) four weeks and b) eight weeks (with standard error bars; 28/4/13 – 23/6/13). n = 24
Figure 4.3 The mean compression strength (N mm⁻¹) of the shell of family lines of C. gigas in response to ambient and elevated carbon dioxide (387 µatm, 1108 µatm) treatments after a) four weeks and b) eight weeks; n = 3 (with standard error bars).

There was a trend for greater standard metabolic rate of family lines one, 17, 20 and 30 at elevated than ambient $pCO_2$, however, family lines 39 and 47 had greater standard metabolic rate at ambient than elevated $pCO_2$ at four weeks (Table 4.3, Figure 4.4). There was no effect of $pCO_2$ on the family lines after eight weeks with no significant differences among family lines (Table 4.3).
**Table 4.3** Analysis of variance of the mean standard metabolic rate of family lines of *C. gigas* in response to ambient and elevated $pCO_2$ (387 µatm, 1108 µatm) treatments after 4 (28/4/13 – 26/5/13) and 8 (28/4/13 – 23/6/13) weeks; n = 24. Significance level is indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
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<td></td>
<td>MS</td>
<td>$F$</td>
<td>$P$</td>
<td>MS</td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
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<td>0.00</td>
<td>ns</td>
<td>13.99</td>
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<td>ns</td>
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<td>**</td>
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<tr>
<td></td>
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<td>FL 39, 47: 387 &gt; 1108 µatm</td>
<td></td>
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<td></td>
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<tr>
<td>SNK</td>
<td>387 µatm: 47 &gt; 1</td>
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</table>
Figure 4.4 The mean standard metabolic rate ($g \cdot O_2 \cdot g^{-1} \cdot dry\ tissue\ mass\ h^{-1}$) of the shell of *C. gigas* in response to ambient and elevated carbon dioxide (387 µatm, 1108 µatm) treatments after a) four weeks and b) eight weeks; $n = 3$
Overall, there was a significant decrease in the growth of *T. marginalba* exposed to elevated (mean ± S.E. height -2.17 ± 0.22 mm; length of aperture -1.24 ± 0.5 mm) compared to ambient *pCO₂* seawater (mean ± S.E. height -1.27 ± 0.09 mm; length of aperture -1.01 ± 0.12 mm) after four weeks (height, ANOVA, F (1,4) = 3.36, P < 0.05; length of aperture, ANOVA, F (1,4) = 10.68, P < 0.05; SNK: 387 > 1108 µatm), but not after eight weeks (height, ANOVA, F (1,4) = 0.06, P > 0.05; length of aperture, ANOVA, F (1,4) = 0.14, P > 0.05). In contrast to *C. gigas*, the compression strength of *T. marginalba* decreased over the experiment. Further, the compression strength of *T. marginalba* was significantly less at ambient compared to elevated *CO₂* treatments at four (ANOVA, F (1,20) = 6.92, P < 0.05; SNK: 387 < 1108 µatm) and eight weeks (ANOVA, F (1,20) = 7.92, P < 0.05; SNK: 387 < 1108 µatm). Similarly, there was a significantly greater standard metabolic rate of *T. marginalba* at ambient than elevated *pCO₂* at eight (ANOVA, F (1,20) = 4.48, P < 0.05), but not four weeks (height, ANOVA, F (1,20) = 0.15, P > 0.05).

**Effect of *pCO₂* on predation**

Overall, significantly more *C. gigas* were consumed by *T. marginalba* at elevated (mean ± S.E. height 0.54 ± 0.09) compared to ambient *pCO₂* (mean ± S.E. height 0.83 ± 0.13) after 10, but not 17 days (Table 4.4 Fig. 4.6 a-b). The significant increase in predation after 10 days was independent of whether the oysters had been held at ambient or elevated *pCO₂*. *T. marginalba* consumed some family lines more than others at 10 (MS = 1.83, Df = 7, F =3.58, P < 0.05; Table 4.4 Fig. 4.5 a) and 17 days (MS = 2.14, Df = 7, F =2.41, P < 0.05; Table 4.4 Fig. 4.5 b), however there was no relationship between the family lines consumed by *T. marginalba* and those affected by elevated *pCO₂* (Table 4.4).
Table 4.4 Analysis of variance of the mean predation in response to ambient and elevated carbon dioxide (387 µatm, 1108 µatm) treatments after 10 (10/6/13 – 20/6/13) and 17 (10/6/13 – 27/6/13) days; n = 3. Significance level is indicated by asterisks, * P < 0.05; ** P < 0.01; *** P < 0.001; ns = non-significant effect. Cochran’s test = ns.

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<td>MS</td>
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<td>4.00</td>
<td>*</td>
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<td>Total</td>
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</tr>
<tr>
<td>SNK</td>
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<td>387 &lt; 1108 µatm</td>
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</table>
Figure 4.5 The mean number of oysters consumed in response to ambient and elevated carbon dioxide (387 µatm, 1108 µatm) treatments after a) 10 and b) 17 days; n = 3 (with standard error bars). The top line indicates the initial $p$CO$_2$ exposure of *T. marginalba*. The bottom line indicates the level of $p$CO$_2$ of the tanks *T. marginalba* were transferred to for the predation stage.
4.5 Discussion

Effect of pCO₂ on growth, strength and standard metabolic rate

The response of *C. gigas* to pCO₂ varied among family lines. Overall, we found oysters were 63% shorter at elevated pCO₂ after eight weeks exposure. Similar to this study Parker et al. (2011) found an overall significant reduction in the lateral growth of pair-mated family lines of *C. gigas* to elevated pCO₂ than ambient pCO₂ with significant variation among family lines. Although Parker et al. (2011) study exposed *C. gigas* for a shorter period, experiments on bivalves and oysters using both long and short exposure times have recorded decreased shell mass and length at elevated pCO₂. For example, an 11 week exposure of post-metamorphosed juveniles of *C. virginica* and an eight week exposure of mussels *M. edulis* caused a significant reduction in shell mass and length and shell hardness and fracture resistance for the oyster and mussel, respectively (Thomsen and Melzner 2010). Early stage larvae and spat are perhaps the most vulnerable life stage of *C. gigas* with reductions in size being a commonly reported response of exposure of *C. gigas* to elevated pCO₂ (Kurihara et al. 2007). Most studies suggest calcification of molluscs is limited by a reduced capacity to regulate pH at the site of calcium carbonate secretion (see review Parker et al. 2013). In our study, *C. gigas* may not have been able to maintain a sufficient rate of calcification as seen by the reduced shell growth at elevated pCO₂.

In this study, initially there was a 19% increase in shell strength of *C. gigas* exposed to elevated pCO₂ but this pattern did not persist to the end of the experiment where the shell strength of oysters at elevated and ambient treatments was similar. An increase in calcification under elevated pCO₂ has been found in only a handful of studies (Miller et al. 2009; Ries et al. 2009; Range et al. 2011). For example Ries et al. (2009) found greater calcification of the limpet *Crepidula formicate* following exposure to elevated pCO₂ of 605 and 903 ppm. The authors attributed this increase in calcification to the ability of some molluscs to maintain localised pH levels thus allowing an increase in the
dissolved organic carbon at the site of calcification (Ries et al. 2009). Other studies have found a 26 and 27% reduction in shell strength of the pearl oyster _Pinctada fucata_ following exposure to pH 7.8 and 7.6 and signs of shell dissolution at pH 7.6 after 28 days (Welladsen et al. 2010). The compression strength of adult _C. gigas_ reported in our study was between 2.75-3.41 N mm⁻¹. It is difficult to compare the shell strength of _C. gigas_ in our study to those obtained in earlier studies because compression strength of shells has been measured in a variety of ways. Taylor and Layman, 1972 found the mean (± SE) compression strength of _C. gigas_ to be 6.28 ± 0.98 N mm⁻¹ (Taylor and Layman 1972) while adult _P. fuctata_ had a compression strength of 190 ± 200 kN at elevated pCO₂ (pH 7.8) compared to 260 ± 200 kN at ambient pCO₂ (mean ± SE) following a 28 day exposure. Although there was no size provided the height of shells at the start of the experiment was on average 53.7 ± 2.6 mm (Welladsen et al. 2010).

Measurements of oxygen consumption by different tissues, however, may have changed (Lannig et al. 2010) reflecting altered energy allocation while the net effect of whole body SMR remained the same. This is in contrast to other studies which have found over a longer period of exposure (20 weeks) an increase in the SMR of juvenile oysters _C. virginica_ at elevated pCO₂ of 3,523 µatm (pH - 0.7) (Beniash et al. 2010). The increase in SMR was accompanied by slower shell and somatic growth.

Overall, the growth of shell height of _C. gigas_ decreased at elevated pCO₂ as did the depth in some family lines. However, contrary to predictions that the shell mass and compression strength would decrease and the standard metabolic rate of molluscs would increase (Beniash et al. 2010; Welladsen et al. 2010; Gaylord et al. 2011; Dickinson et al. 2012) our study found no effect of elevated pCO₂ on the shell mass strength or standard metabolic rate of _C. gigas_. The exception to this was family line 20 which had stronger shells at elevated than ambient pCO₂ after four and eight weeks and a significant reduction at elevated pCO₂ of the standard metabolic rate after eight weeks. Some populations of mollusc may be more robust than others. While most studies report a decline
in standard metabolic rate of molluscs, the standard metabolic rate of a Northern European population of *C. gigas* was found to increase only when the oysters were exposed to the combined effects of elevated $p$CO$_2$ and elevated temperature (Lannig et al. 2010).

It has been suggested that, although, there is usually an overall negative effect of elevated $p$CO$_2$, within species there may exist some populations which may resist the effects of climate change (Foo et al. 2012; Reusch 2014). Similar to our study, a recent study found variation in the morphological response of embryos of the sea urchin *Centrostephanus rodgersii* among family lines following exposure to near-future predicted climate scenarios (Foo et al. 2012). A recent review on the current knowledge of the adaptive potential of marine species has concluded that genetic variation and phenotypic plasticity among populations was high (Reusch 2014) and as such adaptive potential of species to survive in a climate change world may also be high.

For *T. marginalba*, our study found a significant reduction in shell growth, shell compression strength and a 49% decrease in the standard metabolic rate when exposed to elevated $p$CO$_2$. It has been suggested that slower somatic growth may be the result of a greater proportion of an organism’s energy being diverted from growth to basal metabolism due to a higher cost of homeostasis (Beniash et al. 2010; Thomsen et al. 2010; Dickinson et al. 2012) affecting the immune response (Bibby et al. 2007; Beesley et al. 2008; Bibby et al. 2008) and reproduction which may be directly caused by a redistribution of energy (Wood et al. 2008).

*Effect of $p$CO$_2$ on predation*

Although there was preferential consumption of family lines, there was no evidence this was affected by $p$CO$_2$ treatment. Overall, there were more oysters consumed by *T. marginalba* at elevated compared to ambient $p$CO$_2$ after 10, but not 17 days of the experiment. *T. marginalba* at elevated $p$CO$_2$ consumed 54% more *C. gigas* regardless of whether *C. gigas* had been exposed to ambient or elevated $p$CO$_2$. Studies which have investigated the effect of $p$CO$_2$ on predation have
found increased risk of predation under elevated $p$CO$_2$ correlated with a significant decline in shell depth and a reduction in net calcification after 15 days of exposure to elevated $p$CO$_2$ (Bibby et al. 2007; Cooley et al. 2011). Unlike these studies, our study found decreased shell strength of the predator, *T. marginalba*, but no decrease in the shell strength of the prey *C. gigas*. Our findings are also in contrast to Amaral et al. (2012a) who found predation of *S. glomerata* was greater from acidified locations compared to control locations because *T. marginalba* required less time to bore through the shell of weaker oysters (Amaral et al. 2012a). In our study the shell of *C. gigas* was 24% stronger at elevated $p$CO$_2$ (mean ± SE 3.41 ± 0.33) compared to ambient $p$CO$_2$ (mean ± SE 2.75 ± 0.25) after four weeks, but after eight weeks of exposure, there was no difference in shell strength except in one family line (20) which had weaker shells. The shell strength of molluscs has been found to correspond with the rate that predators are able to break through the shell and access flesh. The shell strength of the mussel *Brachidontes pharaonis* was 65% weaker following exposure to a reduced pH (pH 7.5) for four weeks. Consequently the prey handling time by the predatory crab *Eriphia verrucosa* was reduced by 27% (Dupont et al. 2015). Amaral et al. (2012a) found *T. marginalba* consumed 67% more *S. glomerata* (12 month old) that were sourced from acidified locations (pH 6.52 - 6.98, mean ± SE 1.7 ± 0.2 oysters per whelk) compared to control locations (pH 7.72 – 7.92, mean ± SE 1.0 ± 0.1 oysters per whelk) after 10 days. In our study where the pH was greater (pH 8.2), there was less consumption with 54% more oysters consumed by *T. marginalba* at elevated $p$CO$_2$.

Other studies have found no effect of $p$CO$_2$ on the depth of Olympia oysters *Ostrea lurida*, although the size of oysters were substantially smaller from elevated (pH 7.80) than ambient $p$CO$_2$ (pH 8.09) (Sanford et al. 2014). Reduced size was directly correlated with a 20% increase in predation of oysters under elevated compared to ambient $p$CO$_2$. Further still, the predatory whelk *Urosalpinx cinera* preferentially selected smaller oysters and this selection was accentuated under elevated $p$CO$_2$ (Sanford et al. 2014). An assessment of the development of *Mytilus californianus* found the integrity, strength and size of the shell were significantly reduced in larvae reared at $p$CO$_2$ (Gaylord
et al. 2011). Such alterations to shell morphology may leave the larvae more susceptible to drilling by predators (Gaylord et al. 2011). Although we found a significant reduction in the growth C. gigas at elevated than ambient pCO₂ after eight weeks, there was no difference in the number of oysters consumed between oysters exposed to ambient or elevated pCO₂. Although we found T. marginalba consumed significantly more oysters at elevated pCO₂ irrespective of whether C. gigas had been held at ambient or elevated pCO₂ at 10 days, by 17 days this effect had disappeared. Although we did not find an increase in the standard metabolic rate of the predator T. marginalba, a number of recent studies have suggested that increased energy demand may be met through greater food consumption which may ameliorate the negative impacts of elevated pCO₂ (Melzner et al. 2011; Parker et al. 2012; Hettinger et al. 2013).

Conclusion

Given that oysters are the most abundant harvested shellfish globally (He et al. 2002), climate change may impact on the sustainability of our aquaculture industries. Research attention is required on the interactions between marine organisms to better understand how ecologically and economically important marine species will respond to a climate changed ocean (Halldórsson et al. 2005; Neuberger-Cywiak et al. 2007; Poloczanska et al. 2007). Against expectations, this study found increased shell strength in the prey and reduced shell strength in the predator. Increased consumption of oysters by whelks occurred whether oysters were exposed to ambient or elevated pCO₂, with some family lines being more susceptible to predation irrespective of pCO₂.

There is now a growing appreciation of population based assessments to determine the impact environmental change will have on a species. Further work is required to determine the consistency and reasons for this increase in predation under future climate scenarios. Such experiments are required if we are to understand complex predator-prey interactions and their interaction with other stressors already confronting marine organisms. Our current lack of understanding limits our
capacity to predict how marine organisms will acclimatise and adapt to ocean acidification (Kroeker et al. 2010; Parker et al. 2012) at a time when the rate of acidification exceeds that experienced by marine organisms and habitats than at any other time on the planet (Caldeira and Wickett 2003). It can be hypothesised that if climate change continues as predicted, there will be adverse impacts on economically and ecologically significant species including C. gigas, however our study finds there may be hope. Species will survive through populations which attribute favourable genetic potential or plasticity among populations. Species with tolerant populations may have a greater rate of survival and spread under future climate scenarios.
Chapter Five

Responses of diploid and triploid Pacific oysters *Crassostrea gigas* to 
elevated $pCO_2$

5.1 Abstract

The world’s population depends on a reliable source of protein. In the hope of contributing to the demand for protein, genetic tools have been applied to the production of oysters to increase production capacity. In Australia the production of Pacific oysters *Crassostrea gigas*, has been enhanced through selective breeding and via the production of triploids. Triploids are oysters that possess three sets of chromosomes and do not commit as much of their energy budget to gonad maturation and reproduction. Triploids are usually sterile and invest more energy to shell formation and biomineralisation then diploids. Already the results in this thesis have shown that there will be greater energetic demands on oysters as oceans become more acidic and that there are differences in responses among selectively bred family lines of the Pacific oyster *Crassostrea gigas* to elevated $pCO_2$. Whether differences in responses of family lines reflect differences in partitioning of energy to somatic and reproductive processes is unknown. It was predicted that triploid oysters will be more resilient to elevated $pCO_2$ and predation than diploid oysters because triploids have more energy available for shell growth and biomineralisation because energy is diverted away from reproductive conditioning.

As predicted triploid *C. gigas* grew faster than diploid *C. gigas* and while diploid *C. gigas* had a significant reduction in growth when exposed to elevated $pCO_2$ there was almost no effect of elevated $pCO_2$ on the growth of triploids. The SMR of triploid *C. gigas* was greater with *T. marginalba* at ambient then elevated $pCO_2$. For diploid *C. gigas*, SMR was greater with than without *T. marginalba* at ambient but not elevated $pCO_2$. This study found that triploids were more resilient to
elevated CO$_2$ than diploids and there was no increased rate of predation due to elevated CO$_2$ or oyster type. Triploid oysters may be more resilient to pCO$_2$ because heterozygosity provides a greater rate of feeding and energy allocation to shell growth and biomineralisation rather than reproduction. For aquaculture the production of triploid oysters will provide some protection against the impacts of ocean acidification.

5.2 Introduction

As the world’s population heads towards 11.2 billion (United Nations medium fertility expectation for 2100; United Nations 2015) and climate change threatens marine ecosystems, the security of aquacultural and food resources has become an area of significant concern (FAO 2016). The demand for agriculture products will increase by 50% over the next century (Bruinsma 2003; Godfray et al. 2010; Bodirsky et al. 2015; FAO 2016). Such an increase will require a shift toward intensification of sustainable food systems.

An increase in aquacultural yields may provide a potential solution to world population growth. Already aquaculture production has increased on average by 17% each year and now accounts for 35% of all seafood produced from 16.7 million tons in 1990 to 51 million tons in 2002 (FAO 2014). Growth in aquaculture production has been faster for fed species than for non-fed species (eg. carp and filter-feeders), even though production of non-fed species may be more beneficial in terms of food security and the environment. There has been considerable investment into the production of non-fed species which accounted for 31% of global aquaculture in 2014 (FAO 2016) with advances in technology and genetic selection.

Oysters represent 23% of the world’s aquaculture production and the total global value of shellfish is expected to increase to 100 billion USD by 2100 (Narita et al. 2012; FAO 2016). Oysters have one of the highest return on investment and are less reliant on feed than cultivated marine fish (Bourre and Paquotte 2008; Cooley et al. 2012; Gjedrem et al. 2012).
In developing counties, already struggling with limited infrastructure, oysters provide a sustainable source of protein to improve national food security and nutrition in those regions. Edible oysters were one of the top five seafood species produced during 2013-14 with an increase in value of $14 million during the ten years prior (FAO 2011). Cultivation of oysters has the potential to ameliorate the increasing demand for food. In a future where oceans are becoming more acidic, it is important to assess the response of aquacultural resources so that the oyster industry can be sustained as we work toward a “climate-smart food system” that is more resilient to ocean acidification influences on food security. The impacts of ocean acidification will have many effects on the global food equation, both for supply and demand, and could potentially slow down or reverse progress toward a world without hunger.

Selective breeding of oysters has been achieved through a number of successful breeding programs. For example, the Sydney rock oyster *Saccostrea glomerata* has been selected to be both faster growing and resilient to diseases such as QX and winter mortality (O’Connor et al. 2014). Suspecting that faster growing oysters may also be similarly resilient to elevated pCO₂, Parker et al. (Parker et al. 2011) found that family lines of selected oysters varied in their response to elevated pCO₂. While oysters overall had slower growth at elevated pCO₂, some family lines were less affected and some were capable of growing at the rate expected for non-selected oysters under elevated pCO₂ conditions (Parker et al. 2011). Wright et al. (2014) also found significantly different sensitivities to elevated pCO₂ were dependent on the family line of the Pacific oyster *Crassostrea gigas* under investigation. Likewise, some genotypes of the sea urchin *Centrostephanus rodgersii* performed well in high temperatures and low pH (Foo et al. 2012).

Differences in responses or lack of sensitivity to elevated pCO₂ by oysters may be the ability of some family lines to allocate greater energy to growth. It is thought that there will be a higher energetic cost for oysters living in a high pCO₂ world and diversion of energy away from reproduction and shell biomineralisation (Shirayama and Thornton 2005; Berge et al. 2006; Green et
Some oysters are genetically altered to allocate less energy to reproduction and more to shell growth and biomineralisation (Maguire et al. 1995; Cox et al. 1996; Garnier-Géré et al. 2002; Wang et al. 2002; Nell and Perkins 2005; Kingsley-Smith et al. 2009; O’Connor and Dove 2009). Triploids oysters possess three sets of chromosomes and are usually sterile (Hawkins et al. 1994). Triploid oysters in contrast to normal diploid oysters (2n) do not commit as much of their energy budget to gonad maturation and are much less likely to reproduce (Cox et al. 1996). The main benefits of triploids are faster rates of growth and higher meat yields. Whether triploid oysters will be also resilient to elevated pCO₂ is unknown.

In Australia, there are two types of C. gigas exploited for aquaculture: diploids (2n) and triploids (3n). A triploid C. gigas breeding program was established in the mid 1990’s to enhance the production output of C. gigas from the Australian hatchery-based industry at a time when there was a need to improve product quality and reduce production costs (Ward et al. 2000; Nell 2002; Nell and Perkins 2005). In Australia, triploid C. gigas are produced by tetraploidy and incorporate selection for disease resistance. Triploid C. gigas can grow 31 - 81% faster than diploids (Maguire et al. 1995) because of reduced expenditure of energy toward reproductive conditioning (Hawkins et al. 1994). Pacific oysters are the most commercially exploited species of shellfish in the world. Economically, C. gigas has the highest production by tonnage of any mollusc with a global value in excess of three billion USD annually (FAO 2011).

The aim of this study was to compare the response of diploid and triploid C. gigas, to elevated pCO₂ and predation by Tenguella marginalba. As in previous chapters, T. marginalba was predicted to have a greater SMR and consume more oysters at elevated than ambient pCO₂. It was predicted that triploid oysters will be more resilient to elevated pCO₂ and predation than diploid oysters because triploids have more energy available for shell growth biomineralisation because energy is diverted away from reproductive conditioning. Triploid oysters may be able to maintain homeostasis more
readily than diploid oysters and be more resilient to predators in an ocean which in the next 100 years will become more acidic.

5.3 Methods

Collection of Crassostrea gigas and Tenguella marginalba

Diploid and triploid C. gigas were spawned in a hatchery in Tasmania and transferred as spat to Tilligerry Creek, Port Stephens NSW Australia (32°45’S, 151°58’E) where they were grown-out to juveniles in aquaculture baskets (Seapa 600, volume 15 L gauge 3 mm) on an intertidal long-line system. Triploid oysters were produced from tetraploids created by commercial hatcheries. Adult T. marginalba were collected from intertidal rocky shores at Boat Harbour (32°47’S, 152°06’E) and Anna Bay (32°47’S, 152°04’E) Port Stephens NSW, Australia. All animals were transferred to tanks at NSW Department of Primary Industries, Port Stephens Fisheries Institute, Taylors Beach NSW, Australia. A full change of water occurred every second day for the duration of the experiment.

Experimental set-up

C. gigas and T. marginalba were initially held for two weeks in separate aquaria within a temperature range of 21.8 – 22.3 °C (± 0.06, n = 12). Diploid (30 oysters tank⁻¹ mean height ± S.E. 31.39 ± 0.59 mm, mean mass ± S.E. 10.15 ± 0.24 g) and triploid (30 oysters tank⁻¹ mean height ± S.E. 31.81 ± 0.49 mm, mean mass ± S.E. 6.12 ± 0.45 g) C. gigas were then equally and randomly divided among six 750 L experimental tanks. The diploid and triploid oysters were kept in a separate and independent set of six tanks. Similarly, T. marginalba were equally and randomly divided into another separate set of six 750 L tanks (64 whelks tank⁻¹ mean height ± S.E. 19.60 ± 0.28 mm, mean mass 1.62 ± S.E 0.52 g). C. gigas were fed a mixed algal diet consisting of Chaetoceros muelleri, Tisochrysis lutea and Pavlova lutheri at a concentration of 1.82 x 10⁹ cells oyster⁻¹ day⁻¹, for the
duration of the experiment. *T. marginalba* were fed *C. gigas* that were similar in size to the experimental oysters. Consumed oysters were replaced daily.

The *C. gigas* and *T. marginalba* 750 L tanks were divided among two pH treatments. Species were held in three tanks at ambient pH of 8.07 (± 0.03; 481 µatm) as measured in Tilligerry Creek, Port Stephens and a reduced pH 7.80 (± 0.02; 909 µatm) (6 tanks in total) in line with intermediate pCO₂ scenario projections for the year 2100 (IPCC 2013a). The reduced pH treatment was maintained in each of the replicate tanks by direct diffusion of carbon dioxide and monitored by microprocessors (Aqua Medic pH computer set; accuracy ± 0.01). pH, salinity and temperature (maintained at 22 °C ± 0.05) were measured in each 750 L tank daily with a hand-held probe (Palin test Waterproof 800 meter). Total alkalinity (TA) was measured using a Gran-titration (Gran 1952) after each water change. A CO₂ system calculation program (CO₂ sys) developed by Lewis and Wallace (1998) was used to calculate the pCO₂, dissolved inorganic carbon and the aragonite and calcite saturation state corresponding to the experimental pH levels, using the dissociation constants of Mehrbach et al. (1973) (for seawater physicochemical conditions see Table 5.1).

**Table 5.1** Seawater physicochemical conditions during the exposure experiments of *C. gigas*.

<table>
<thead>
<tr>
<th>Treatment pCO₂</th>
<th>Salinity psu</th>
<th>Temp. °C</th>
<th>pH NBS</th>
<th>TA μmol kg⁻¹</th>
<th>pCO₂ µatm</th>
<th>DIC µmol/kg⁻¹</th>
<th>Ω aragonite</th>
<th>Ω calcite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>31.21</td>
<td>22.05 ± 0.17</td>
<td>8.07 ± 0.03</td>
<td>1993 ± 13</td>
<td>481</td>
<td>1674.2</td>
<td>2.05</td>
<td>3.16</td>
</tr>
<tr>
<td>Elevated</td>
<td>31.85</td>
<td>22.04 ± 0.10</td>
<td>7.80 ± 0.02</td>
<td>1821 ± 16</td>
<td>909</td>
<td>1707.2</td>
<td>1.14</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Values for pCO₂, Ω aragonite and Ω calcite calculated from salinity, temperature, pH NBS and TA.

TA, total alkalinity DIC, dissolved inorganic carbon; Salinity, temperature, pH NBS and TA ± SE.

**Measurement of shell size and whole mass**

Oysters and whelks were kept in their aforementioned separate tanks and exposed to ambient (481 µatm) and elevated (909 µatm) pCO₂ for six weeks. To determine if triploid *C. gigas* were more
resilient to elevated $pCO_2$ and predation by *T. marginalba*, a reciprocal cross of the oysters and whelks was conducted similar to that described in chapter 3. Half of the diploid and half of the triploid *C. gigas* that had been held at ambient $pCO_2$ were removed and divided equally across the six tanks of *T. marginalba*; three of which were held at ambient pH and three were held at elevated pH. Likewise, half of the diploid and half of the triploid *C. gigas* that had been held at elevated $pCO_2$ were removed and divided across the six tanks that held *T. marginalba*. The oysters from different $pCO_2$ treatments were kept separately in the same tanks using a four compartment holding basket. The remaining half of the oysters from the ambient and elevated treatments stayed in their respective ambient or elevated treatment without whelks. The diploid and triploid oysters were kept separately in independent tanks. This created six treatments, diploid and triploid *C. gigas* alone at 1) ambient or 2) elevated $pCO_2$, 3) diploid and triploid *C. gigas* kept for 6 weeks at ambient $pCO_2 + T. marginalba$ at ambient $pCO_2$, 4) diploid and triploid *C. gigas* kept for 6 weeks at ambient $pCO_2 + T. marginalba$ at elevated $pCO_2$, 5) diploid and triploid *C. gigas* kept for 6 weeks at elevated $pCO_2 + T. marginalba$ at ambient $pCO_2$, 6) diploid and triploid *C. gigas* kept for 6 weeks at elevated $pCO_2 + T. marginalba$ at elevated $pCO_2$. These treatments were maintained for a further 15 days until the end of the two month experiment.
Figure 5.1 Schematic of the experimental design. *T. marginalba* and *C. gigas* (diploid and triploid) were held for six weeks in separate 750 L tanks, after which a reciprocal cross was performed to create six treatments (n=3 reps per treatment; numbered 1-6) where *C. gigas* from both ambient (481 µatm) and elevated (909 µatm) pCO$_2$ were transferred to tanks with *T. marginalba* at both ambient (481 µatm) and elevated (909 µatm) pCO$_2$. *C. gigas* were also held on their own without whelks at ambient (481 µatm) and elevated (909 µatm) pCO$_2$. These six treatments remained for 15 days, during which consumption of oysters was measured daily. Asterisks indicate the tanks that were excluded from the oyster shell size, strength and SMR measurements.
To estimate growth, the shell height (posterior to anterior margin), length (dorsal to ventral margin) and depth of 12 diploid and 12 triploid *C. gigas* were measured from the four treatments (n=3) that contained oysters that had not been placed into a new pCO\(_2\) treatment after 6 weeks of exposure (i.e. diploid and triploid *C. gigas* alone at 1) ambient or 2) elevated pCO\(_2\), 3) diploid and triploid *C. gigas* kept for 6 weeks at ambient pCO\(_2\) + *T. marginalba* at ambient pCO\(_2\) and 6) diploid and triploid *C. gigas* kept for 6 weeks at elevated pCO\(_2\) + *T. marginalba* at elevated pCO\(_2\)). Specific growth of shell height, length and depth was calculated by subtracting the mean starting size of diploid and triploid *C. gigas* from the mean size of diploid and triploid *C. gigas* recorded from the four treatments (n=3) that contained oysters that had not been placed into a new pCO\(_2\) treatment after the 15 day period where the oysters and whelks were held together. The height, length of the aperture and shell depth of 36 *T. marginalba* from the four treatments that contained whelks, including the two treatments with oysters that were transferred to a new treatment (treatments 3, 4, 5 and 6) was measured to the nearest millimetre using vernier callipers (Mitutoyo, Digimatic vernier callipers, precision ± 0.01 mm). Whole mass (shell and flesh) of 12 *C. gigas* from the four treatments (n=3) that contained oysters that had not been placed into a new pCO\(_2\) treatment and six *T. marginalba* from the four treatments that contained whelks was recorded using a precision balance (Mettler Toledo, XPE303S, precision ± 0.001 g).

*Measurement of metabolic rate*

The standard metabolic rate (SMR) of 4 diploid and 4 triploid *C. gigas* (12 of each oyster type per treatment) and 6 *T. marginalba* from each tank (18 per treatment) was measured using a closed respiratory system using the methodology of Parker et al. (2012) at the end of the experiment. Briefly, after individuals were starved for 24 h they were sealed with filtered seawater (2 µm filters) in air-tight respirometry chambers designed to monitor depletion of oxygen over time. All measurements were made at the pH level for each treatment using a fibre-optic probe (PreSens fibre optic dipping probe DP PsT3) sealed in the chambers. The probes were calibrated with a two-point
calibration (0 and 100%). The probes were connected to a four-Channel Minisensor Oxygen Meter (PreSens) which measured the oxygen uptake of each specimen. SMR was calculated as the percentage of oxygen decline (from 100 to 80%), expressed in moles of O\textsubscript{2} over time after Parker et al. (2011);

\[
\text{SMR h}^{-1} = \left( \frac{\text{Vol} \times \Delta V_{\text{meas}}}{\Delta t} - \text{Blank} \right) \times W_{b}^{-1}
\]

Where;

- Vol = volume of the respirometry chamber minus the volume of the oyster
- \(\Delta V_{\text{meas}}\) = change in the oxygen concentration of the seawater
- \(\Delta t\) = time over which the measurement was made
- Blank = the rate of change measured concurrently in a chamber with no organism
- \(W_{b}^{-1}\) = dry tissue mass tissue of the individual organism

Measurement of T. marginalba consumption

The number of C. gigas consumed by T. marginalba was recorded each day for the 15 days that oysters and whelks were together. The mean number of oysters consumed whelk\(^{-1}\) day\(^{-1}\) was calculated after 10 and 15 days. Total meat weight of oysters consumed whelk\(^{-1}\) day\(^{-1}\) was also calculated using an average dry flesh mass of a subset of each oyster type following the experiment. There was no effect of oyster type (ANOVA, F (1,112) = 2.87, P > 0.05) or CO\(_2\) (ANOVA, F (1,112) = 1.77, P > 0.05) on the dry flesh weight of C. gigas. Drilling was the likely method of predation throughout the experiment as the shell of all oysters consumed had a drill-hole characteristic of the size of the radula of T. marginalba (see chapter 2). The consumed oysters were removed from the experimental tanks once counted and replaced with oysters which had been similarly exposed to maintain overall consistency in the food supply to whelks.
To determine any significant differences in the shell growth and SMR of diploid and triploid C. gigas, data was analysed using a four-factor analysis of variance where the first factor was the “oyster type” (2 levels, diploid and triploid), the second factor was “pCO₂ treatment” (2 levels; ambient, 481 µatm and elevated, 909 µatm) and the third factor was “presence of T. marginalba” (2 levels; with and without T. marginalba) which were all fixed and orthogonal. The fourth factor was “tank” (3 levels; n = 3) which was random and nested in “pCO₂” and “presence of T. marginalba”. To determine any significant effect of pCO₂ on the size and SMR of T. marginalba a three-factor analysis of variance was used where the first factor was the pCO₂ treatment of the whelks (2 levels; ambient, 481 µatm and elevated, 901 µatm). The second factor was the level of pCO₂ that the oysters were originally exposed to before being transferred to the tanks with T. marginalba (2 levels, ambient, 481 µatm and elevated, 909 µatm). Factors one and two were orthogonal and fixed. The third factor was “tank” (3 levels; n = 3) which was nested in the first two factors. As there were no significant interaction of pCO₂ with tanks the variance of tanks were pooled for each treatment following the procedure outlined by Winer et al (1991) with a residual of α= 0.25. To determine any significant differences in consumption of oysters by whelks, data were analysed at two time points (10 and 15 days) using a three-factor ANOVA. For this analysis, the first factor was “oyster type” (2 levels, diploid and triploid), the second factor was the pCO₂ conditions of the oysters (2 levels; ambient, 481 µatm and elevated, 909 µatm), the third factor was the pCO₂ conditions of the whelks (2 levels; ambient, 481 µatm and elevated, 909 µatm). GMAV 5 for Windows (Underwood et al. 2002) was used to analyse differences among treatments of C. gigas and T. marginalba. Heterogeneity of the variances was assessed by Cochran’s test and a significance level of P < 0.05 was used for all analyses. No data transformation was required. Any difference in means was detected by Student Newman Kuels (SNK) test on each parameter (Sokal and Rohlf 1995).
5.4 Results

Shell morphology and physiology of diploid and triploid *C. gigas* in response to pCO$_2$ and *T. marginalba*

Diploid *C. gigas* had significantly less growth in shell height, length and mass than triploid *C. gigas* as seen by the significant interaction. Exposure to elevated pCO$_2$ significantly reduced the growth of the shell height and length of diploid oysters. For triploid oysters, there was a significant increase in growth of the shell height (but not shell length) following exposure to elevated pCO$_2$. Exposure to elevated pCO$_2$ significantly reduced the growth of the shell depth of oysters when held with *T. marginalba*. There was also negative effect of *T. marginalba* on the growth of the shell depth of diploid oysters, but a positive effect on the shell depth of triploid oysters (Table 5.2). *T. marginalba* had effect no effect on the size (shell height, length) or mass of diploids. In triploids, however, growth of the shell height, depth and their whole mass (but not length) was greater in the presence of *T. marginalba* compared to when *T. marginalba* were not present (Table 5.2, Figure 5.2).

There was a significant interaction between oyster type, level of pCO$_2$ exposure of the oyster and whether oysters were held with or without *T. marginalba*. Overall, the SMR of diploid *C. gigas* were significantly greater than triploid *C. gigas*. The SMR of triploid *C. gigas* was greater with *T. marginalba* at ambient then elevated pCO$_2$ but there was no significant effect of pCO$_2$ on the SMR of triploid *C. gigas* without *T. marginalba*. For diploid *C. gigas*, SMR was greater with than without *T. marginalba* at ambient but not elevated pCO$_2$ (Table 5.3, Figure 5.3).
Table 5.2 Analysis of variance of the growth in shell height, length, depth and whole mass of diploid and triploid (Ty) C. gigas in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without T. marginalba (Wh) for 15 days. n = 3. Significance level is indicated by asterisks, * P < 0.05; ** P < 0.01; *** P < 0.001; ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<td>16.85</td>
<td>17.06</td>
<td>*</td>
<td>1.66</td>
<td>6.36</td>
<td>*</td>
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<tr>
<td>CO₂</td>
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<td>10.31</td>
<td>12.22</td>
<td>*</td>
<td>12.61</td>
<td>12.76</td>
<td>*</td>
<td>8.23</td>
<td>31.45</td>
<td>*</td>
</tr>
<tr>
<td>Wh</td>
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<td>8.00</td>
<td>*</td>
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<td>0.81</td>
<td>ns</td>
<td>0.05</td>
<td>0.18</td>
<td>ns</td>
</tr>
<tr>
<td>Ty x CO₂</td>
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<td>80.14</td>
<td>*</td>
<td>12.19</td>
<td>12.34</td>
<td>*</td>
<td>0.35</td>
<td>1.33</td>
<td>ns</td>
</tr>
<tr>
<td>Ty x Wh</td>
<td>1</td>
<td>20.28</td>
<td>24.05</td>
<td>*</td>
<td>1.29</td>
<td>1.30</td>
<td>ns</td>
<td>3.54</td>
<td>13.53</td>
<td>*</td>
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<tr>
<td>CO₂ x Wh</td>
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<td>1.98</td>
<td>2.35</td>
<td>ns</td>
<td>0.59</td>
<td>0.60</td>
<td>ns</td>
<td>3.66</td>
<td>13.98</td>
<td>*</td>
</tr>
<tr>
<td>Ty x CO₂ x Wh</td>
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<td>0.02</td>
<td>ns</td>
<td>0.03</td>
<td>0.10</td>
<td>ns</td>
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<tr>
<td>RES</td>
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<td>0.99</td>
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<td></td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>4.80</td>
<td></td>
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</tr>
</tbody>
</table>

SNK

Ty x pCO₂
Ambient and elevated: diploid < triploid
Diploid: ambient > elevated
Triploid: ambient < elevated

Ty x Wh
Wh with and without:
diploid < triploid
Diploid: Wh with = without
Triploid: Wh with > without

Ty x pCO₂
Ambient: diploid = triploid
Elevated: diploid < triploid
Diploid: Wh with < without
Triploid: Wh with > without

Ty x Wh
Wh with and without: diploid < triploid
Diploid: Wh with = without
Triploid: Wh with > without

pCO₂ x Wh
Wh with: ambient > elevated
Wh without: ambient = elevated
Ambient and elevated: Wh with > without
Figure 5.2 The mean growth of the shell a) height, b) length, c) depth and whole mass of diploid and triploid *C. gigas* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without *T. marginalba* for 15 days (with standard error bars).
Table 5.3 Analysis of variance of the mean SMR of diploid and triploid *C. gigas* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without *T. marginalba* for 15 days. \( n = 12 \). Significance level is indicated by asterisks, * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \); ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster type (Ty)</td>
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<td>2.23</td>
<td>7.36</td>
<td>*</td>
</tr>
<tr>
<td>( pCO_2 )</td>
<td>1</td>
<td>2.24</td>
<td>7.38</td>
<td>*</td>
</tr>
<tr>
<td>Whelk (Wh)</td>
<td>1</td>
<td>0.41</td>
<td>1.35</td>
<td>ns</td>
</tr>
<tr>
<td>Ty x ( pCO_2 )</td>
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<td>1.56</td>
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<tr>
<td>( pCO_2 ) x Wh</td>
<td>1</td>
<td>0.01</td>
<td>0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Ty x ( pCO_2 ) x Wh</td>
<td>1</td>
<td>1.24</td>
<td>4.09</td>
<td>*</td>
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<tr>
<td>RES</td>
<td>88</td>
<td>0.30</td>
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<tr>
<td>Total</td>
<td>95</td>
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</table>

Diploid x Wh with and without: ambient = elevated
Triploid x Wh with: ambient = elevated
Triploid x Wh without: ambient > elevated

**SNK**
- Diploid x ambient: Wh with > without
- Diploid x elevated: Wh with = without
- Triploid x ambient and elevated: Wh with = without
Figure 5.3 The mean SMR of diploid and triploid *C. gigas* in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments with and without *T. marginalba* for 15 days (with standard error bars).

*Shell morphology and physiology of *T. marginalba* in response to pCO₂*

Overall, there was no effect of exposure to pCO₂ or *C. gigas* type on the shell height, length of aperture, depth, mass or SMR of *T. marginalba* (Table 5.4).
Table 5.4 Analysis of variance of the shell height, length of aperture, Shell depth, whole mass (n = 36) and SMR (n = 18) of T. marginalba in response to ambient and elevated carbon dioxide (pCO$_2$, 481, 909 µatm) treatments with C. gigas that were transferred from either ambient or elevated carbon dioxide (Oy). n = 36. ns = non-significant effect. Cochran’s test = ns.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
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<th>P</th>
<th>df</th>
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<td>1.92</td>
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<td></td>
<td>0.00</td>
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</tr>
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<td>RES</td>
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<td></td>
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<tr>
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</table>

T. marginalba predation of diploid and triploid C. gigas in response to pCO$_2$

Overall, there was no significant difference in the number of diploid or triploid C. gigas consumed by T. marginalba after ten and 15 days. Further, there was no effect of the exposure of C. gigas or T. marginalba to elevated pCO$_2$ (Table 5.5, Figure 5.4).

On a dry flesh mass basis, there was also no significant difference in the number of diploid or triploid C. gigas consumed by T. marginalba after ten (ANOVA, F (1,16) = 2.17, P > 0.05) and 15 days (ANOVA, F (1,16) = 1.85, P > 0.05). There was also no effect of the exposure of C. gigas (ANOVA, after ten days, F (1,16) = 1.85, P > 0.05; after 15 days ANOVA, F (1,16) = 0.74, P > 0.05), or T. marginalba to elevated pCO$_2$ (ANOVA, after ten days, F (1,16) = 0.09, P > 0.05; after 15 days ANOVA, F (1,16) = 0.23, P > 0.05).
Table 5.5 Analysis of variance of the mean number of diploid and triploid C. gigas consumed by T. marginalba in response to ambient and elevated carbon dioxide (481, 909 µatm) treatments after eight weeks; *n* = 3. ns = non-significant effect. Cochran’s test = ns.

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<th>P</th>
<th>After 15 days</th>
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<td>After 10 days</td>
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<td>1.22</td>
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<td>0.14</td>
<td>ns</td>
<td>1.50</td>
<td>0.30</td>
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<td>2.67</td>
<td>0.53</td>
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<td>ns</td>
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5.5 Discussion

Triploid breeding programs were not specifically established to produce oysters resilient to elevated pCO$_2$, yet in this study triploid *C. gigas* were found to be more resilient than diploid *C. gigas* to elevated pCO$_2$. The increase in shell height and length of triploid *C. gigas* were significantly greater than diploid oysters at both ambient and elevated pCO$_2$. Further, there was a significant increase in the shell height and a significant decrease in the shell depth of triploid oysters held at elevated CO$_2$. In contrast, exposure of the diploid oysters to elevated pCO$_2$ led to a significant reduction in all shell
properties measured (height, length and depth). There was no significant effect of *T. marginalba* on the SMR of triploids. For diploid *C. gigas*, SMR was significantly greater with than without *T. marginalba* at ambient but not elevated $pCO_2$.

Most comparative studies agree that mollusc populations used for aquaculture are inherently more resistant to environmental change than wild stocks (Carlsson et al. 2006; Li et al. 2009; Dove et al. 2013; Troell et al. 2014; Hargrove et al. 2015; Thompson et al. 2015). Aquaculture can potentially enhance the resilience to climate stressors as breeding programs inherently favour individuals that are generally more resilient to environmental stress. For example, Parker et al. (2011) found that the shell growth of newly metamorphosed spat of a wild population of the Sydney rock oyster *Saccostrea glomerata* was reduced by 64% following a four day exposure to elevated $pCO_2$, but was only decreased by 25% in a selectivity bred population of the species (developed by aquaculture for faster growth and resistance to disease). Proteomic analysis revealed higher enzymatic activity of selectivity bred than wild populations of *S. glomerata* following exposure to a viral disease (Newton et al. 2004). Wild stocks may not perform as well as populations used by aquaculture as aquaculture stocks are inherently selected from the front runners of the gene pool (Ward et al. 2000).

The observed resilience to elevated $pCO_2$ in the size of triploid *C. gigas* may be attributed to a phenomenon that has been referred to as polyploid gigantism (Guo et al. 1996; Wang et al. 2002; McCarthy et al. 2015). Gigantism refers to greater growth and an increase of size of select individuals of a species. Three hypotheses have been proposed, attributing triploid gigantism to sterility, increased heterozygosity, or cell size. In contrast to diploid oysters, triploid oysters rarely spawn and consequently invest more energy into growth, which generally leads to faster growth, even under elevated $pCO_2$ (Maguire et al. 1995; McCarthy et al. 2015).

Many studies have found that molluscs will have thinner or weaker shells as calcification in an acidified ocean because it becomes more energetically costly to sustain shell biomineralisation and organisms partition energy to other essential life processes including the maintenance of acid-base
homeostasis. For example, the shell of the pearl oyster *Pinctada fucata* was susceptible to acidification with a 26 and 27% reduction in shell strength following exposure to pH 7.8 and 7.6 respectively and signs of dissolution at pH 7.6 in comparison to the controls (Welladsen et al. 2010). Further, there was a decrease in shell calcification of *Crassostrea virginica* (Dodd et al. 2015) and *Mya arenaria* (Glaspie et al. 2017) following exposure to elevated pCO$_2$. In this study most measurements of the shell growth (height and length) of triploid oysters were more robust to elevated pCO$_2$ then diploid oysters, likely due to the ability of triploid oysters to allocate more energy toward calcification. Kesarcodi-Watson et al. (2001) found diploid oysters fed on an optimum diet invested the majority of their energy to the development of gonad tissue, while triploid oysters invested energy into growth. Shpigel (1992) found greater carbohydrate and protein content of triploid than diploid oyster, suggesting reduced reproductive effort provided triploid oysters with an energetic advantage.

The stress tolerance of a species is suggested to be linked to its energy budget. Sokolova (2012) argued that limitations of both the amount of available energy and the rates of its acquisition and metabolic conversions result in tradeoffs between basal maintenance of a stressed organism and energy costs of fitness-related functions such as reproduction, development and growth and can set a limit to the tolerance of a broad range of environmental stressors. Although studies are limited, triploid *C. gigas* have been found to be more resilient than diploids to a number of environmental stressors including summer mortality (Gagnaire et al. 2006), elevated temperature (Shpigel et al. 1992). Further, triploid *C. gigas* show a 31 - 81% increase in growth rate when compared to diploids, with increased growth rates more pronounced under favourable environmental conditions (Goulletquer et al. 1996; McCarthy et al. 2015). Hawkins et al. (1994) state the faster rate of growth of triploid *C. gigas* is a result of reduced energetic expenditure compared to diploids, a factor of investment of energy into reproductive conditioning by diploids.
Some studies have suggested that ocean acidification may reduce the feeding of molluscs. Heterozygosity in fact may allow greater feeding in triploid than diploid oysters as they have a greater filtration capacity. The greater feeding rate of triploid oysters may be in part due to the large surface area of their gills. This high rate of feeding is likely to compensate for the extra nutrient requirements in polyploidy, which may explain the resilient response of triploid oysters to elevated pCO$_2$ (McCarthy et al. 2015). Polyploidy gigantism of triploid bivalves has been attributed to increased cell volume (Guo and Allen 1994). Larger cells typically require more nutrients to grow and divide. In our experiment, triploid C. gigas were fed an optimum diet which would support enlarged cells. In estuaries, however, where food is known to be limited there may be no difference in the growth of diploid or triploid oysters as cell size polyploidy gigantism becomes unsupported (Goulletquer et al. 1996; Nell 2002).

In previous experiments in this thesis, diploid oysters were less resilient to ocean acidification. In chapters three and four, juvenile diploid oysters were consumed at a greater rate at elevated than ambient pCO$_2$. However in this experiment there was no significant change in predation at elevated pCO$_2$ for either diploids or triploids. As discussed in chapter two, studies have found strong correlations between size of prey and the rate of predation by T. marginalba (Fairweather and Underwood 1983; Moran 1985; Seed 1993). The muricid gastropod Lepsiella paivae was found to have a near monotonic diet consisting of juveniles (<13mm shell length) of the bivalve Katelysia scalarina (Morton 2005). Oysters used in this experiment were $10.15 \pm 0.24$g (diploids) and $6.12 \pm 0.45$g (triploids) in size. The increased handling time to consume the larger oysters may have reduced the effect of pCO$_2$ on the whelk’s feeding rates so that the effect was not significant in this case.

There was no significant difference in the number of oysters consumed by T. marginalba. This contrasts with the findings of chapter three of this thesis where T. marginalba consumed significantly more oysters that had a greater SMR. In that chapter it was proposed that oysters may
have the ability to regulate metabolic output at ambient $pCO_2$ to avoid being detected by predators, but at elevated $pCO_2$ this ability was reduced as oysters increased SMR to maintain homeostasis. The oysters used in chapter three were smaller in size (mean individual mass 0.45 ± S.E 0.02 g) than those used in this chapter. Reponses to predation have been found in several studies to be size dependent. Smaller and younger individuals of the Atlantic salmon *Salmo salar* (Blanchet et al. 2007) and Fathead minnows *Pimephales promelas* (Pollock et al. 2006) responded with greater intensity to predator odour than larger and older individual. Large individuals may be less fearful of predation risk than smaller individuals (Ferrari et al. 2010). Here, *C. gigas* had a slightly lower SMR per gram of dry flesh weight at ambient $pCO_2$ without *T. marginalba* than that of smaller *C. gigas* used in chapters three (mean ± S.E. 6.32 ± 0.29 g$^{-1}$ O$_2$·s$^{-1}$) and four (mean ± S.E. 5.82 ± 1.10 g$^{-1}$ O$_2$·s$^{-1}$), however the volume of metabolic output would be greater given their overall greater mass. It was hypothesised that in the presence of *T. marginalba*, *C. gigas* would reduce their SMR in an attempt to reduce the risk of attracting the predator. Perhaps large *C. gigas* do not reduce SMR to a level below which *T. marginalba* can detect as was found for the smaller *C. gigas* in chapter 3. It could be that large oysters do not regulate their SMR in the presence of *T. marginalba* and subsequently there was no difference in the number of large diploid or triploid *C. gigas* consumed by *T. marginalba*.

Although triploid *C. gigas* respond more favourably than diploid *C. gigas*, triploids oysters do not put much energy into reproduction and do not usually produce offspring (Hawkins et al. 1994). Following parental exposure to elevated $pCO_2$ Parker et al. (2015) found carry over effects to offspring were very important in adaptive capacity of Sydney rock oysters *S. glomerata*. Larvae from parents exposed to elevated $pCO_2$ had lower mortality and faster rate of development than larvae from non-exposed parents. As triploids cannot produce substantial offspring they remain only a short term solution to climate proof the aquaculture industries.
This study is important because it provides evidence that triploid oysters will be more resilient in an ocean which is becoming more acidic. This information is important for an aquaculture industry facing a range of current and future environmental stressors including which oysters to farm which perform better in certain environmental conditions. It assists managers to make informed decisions and maximise potential. This study does, however, confirm that diploid *C. gigas* will have reduced shell growth and because triploid oysters do not put substantial energy into reproduction, they are at best a short term solution.
Chapter 6

General discussion

6.1 Overview

This thesis sought to understand the fate of the predator-prey interactions between the endemic predator, the Mulberry whelk *Tenguella marginalba* and their prey the native Sydney rock oyster *Saccostrea glomerata* and the recently introduced Pacific oyster *Crassostrea gigas* in a marine environment increasingly affected by elevated $p$CO$_2$.

Most recent studies suggest the impacts of ocean acidification on predator-prey interactions will be deleterious both on the foraging ability of molluscan invertebrate predators and the defences of molluscan prey (Kroeker et al. 2014; Harvey and Moore 2017; Watson et al. 2017; Xu et al. 2017). This thesis set out to experimentally investigate how predator-prey relationships will be affected by elevated $p$CO$_2$.

It was predicted that predator-prey relationships will be altered by exposure to elevated $p$CO$_2$ because:

1. The energetic costs for *T. marginalba* to survive in an acidified environment will increase causing them to compensate by increasing their consumption rate of prey;

2. Growth and physiological defences of *S. glomerata* and *C. gigas* will decrease as reduced seawater calcium carbonate saturation states make shell calcification and maintenance more difficult and greater energy is required for the maintenance of acid-base balance.

3. It was also predicted that responses will vary between oyster species and within populations of oysters. Differences in response to $p$CO$_2$ between *C. gigas* and *S. glomerata* have already been demonstrated in the absence of a predator (Parker et al. 2010). Parker et al. (2010) showed that the impacts of elevated $p$CO$_2$ on larvae and spat of *S. glomerata* were far
greater than the impacts on larvae and spat of *C. gigas*. Further, Parker et al. (2011) showed differences in the growth of family lines of *S. glomerata* under elevated $pCO_2$, with some family lines not affected. Here it was hypothesised that the inter/species-specific differences in the responses of *C. gigas* and *S. glomerata* to elevated $pCO_2$ would in turn also alter their predator prey relationship under elevated $pCO_2$. Further, that within species differences would also be found for *C. gigas* and that these too would alter predatory prey relationships under elevated $pCO_2$.

To test these hypotheses, both predator and prey; *T. marginalba, C. gigas* and *S. glomerata* were exposed to ambient and elevated $pCO_2$ (-0.3 to -0.4 pH units) in a series of complex, fully orthogonal and chronic experiments in which the entire species complex (both the predator and two prey species) were exposed to elevated $pCO_2$.

### 6.2 Model and hypothesis 1

This study provides support for the hypothesis that predation of oysters by *T. marginalba* will increase because exposure to elevated $pCO_2$ will cause an increase in energetic costs of the predator. In general a greater number of oysters (both *C. gigas* and *S. glomerata*) were consumed by *T. marginalba* when whelks were exposed at elevated $pCO_2$ than when whelks were exposed to ambient $pCO_2$. *T. marginalba* also had a preference to consume smaller sized *C. gigas* as calculated by grams per mass consumed (mean mass of *C. gigas* ± S.E. 0.45 ± 0.02 g Chapter 3, 1.20 ± 0.24 g Chapter 4) but not larger sized *C. gigas* (Chapter 5; mean mass ± S.E. diploid 10.15 ± 0.24 g). It may be more energy demanding for whelks to consume large *C. gigas* because they have thicker, stronger shells that are more difficult to crush or drill through these more robust shells (Buschbaum et al. 2007; Ferrari et al. 2010; Hedge and Johnston 2014; Weerman et al. 2014). Not only was there a preference for *T. marginalba* to consume *C. gigas* which were smaller in size, whelks had a preference to drill in the location of the pericardial cavity, directly above the heart. This may provide
whelks with direct entry into the adductor muscle which is known to be high in energy (Thomas and Day 1995).

Increased consumption of oysters under elevated $p$CO$_2$ suggests that the whelk generally increased its energetic uptake in an attempt to meet increased maintenance costs of acid-base balance and shell calcification at elevated $p$CO$_2$ (Chapter 3; Table 6.1). The initial increase in consumption of oysters by *T. marginalba*, did not last, perhaps because *T. marginalba* may have consumed sufficient prey to meet their later energetic requirements. A number of studies have suggested that supply of adequate food may ameliorate the negative impacts of elevated $p$CO$_2$ (Melzner et al. 2009; Parker et al. 2012; Hettinger et al. 2013; Melatunan et al. 2013; Vargas et al. 2013; Pansch et al. 2014; Clements 2016; Cole et al. 2016; Ramajo et al. 2016). For example, Melzner et al. (Melzner et al. 2011) found negative impacts of elevated $p$CO$_2$ on the calcification and shell dissolution of the mussel *Mytilus edulis* when food supply was low. But these negative impacts were ameliorated when food supply was high. Predatory molluscs under stress are known to increase their consumption of food and/or preferentially target prey with greater energetic content to maximise net energetic benefits (Gosselin and Chia 1996). If a specialist predator consumes only small individuals of one species which can be captured and processed more easily, the predator might increase its per capita consumption to maintain energy intake (Sanford et al. 2014). Non-specialist predators, by contrast, may expand their dietary breadth or exclude a formerly preferred prey if the abundance or energetic value of the preferred prey is not sufficient (Sanford et al. 2014). Such mechanisms enable individuals to maintain life functions including acid-base balance and growth, an energetically costly process under near future scenarios of ocean acidification (Sanders et al. 2013; Thomsen et al. 2013; Mackenzie et al. 2014; Pansch et al. 2014). In this study *T. marginalba* was considered a generalist predator (Fairweather and Underwood 1991). Whether it can change preference for prey species under elevated $p$CO$_2$ was not investigated here but maybe an area for future research.
An increase in consumption, was at times correlated with a significant increase in the SMR of *T. marginalba* in response to elevated $pCO_2$ in Chapter 3, but there was no effect on the SMR of *T. marginalba* in Chapters 4 and 5 in response to elevated $pCO_2$ (Table 6.1). Studies have shown an increase in the SMR of mollusc species in response to elevated $pCO_2$ as molluscs attempt to maintain homeostasis (energy toward growth, reproduction) and acid-base balance (Pörtner et al. 2004; Cummings et al. 2011; Parker et al. 2012). Surprisingly, elevated $pCO_2$ had little to no long term impact on the growth of *T. marginalba* which were already adults and a lack of deleterious response to elevated $pCO_2$ on calcifying species has been found in other studies (Ross et al. 2011; Parker et al. 2013). For example, in a laboratory experiment, eight out of 18 calcifying species were resilient to exposure to elevated $pCO_2$ for 60 days (Ries et al. 2009). There was also no effect of elevated $pCO_2$ on the calcification of the bivalve clam *Ruditapes decussatus* (Range et al. 2011) and mussel *Mytilus edulis* (Ries et al. 2009). Almost all other studies have found decreased calcification in gastropods exposed to elevated $pCO_2$ (Doney et al. 2009; Ross et al. 2011; Parker et al. 2013) including *Littorina littorea* (Bibby et al. 2007) and *Nucella lapillus* (Ruhl et al. 2017)

Overall, there was no long term effect of elevated $pCO_2$ on the growth of *T. marginalba*, but there was an increase in the strength of the shell at elevated $pCO_2$ (Table 6.1). Other studies which have investigated the effects of elevated $pCO_2$ on mollusc predators have found their foraging ability and the consumption of mollusc prey to be decreased. The ability of the dogwhelk *Nucella lapillus* to forage was restricted and the rate of predation was reduced to a level of starvation even when prey *Semibalanus balanoides* were abundant when dogwhelks and barnacles were exposed to elevated $pCO_2$, (Harvey and Moore 2017). Similar limitations were found on the foraging ability of the predatory gastropod *Nucella lapillus* (Queirós et al. 2015), predatory cone snail *Conus marmoreus* (Watson et al. 2017), the muricid gastropod *Thais clavigera* (Xu et al. 2017) and mud crabs *Panopeus herbstii* (Dodd et al. 2015) with a significant decrease in foraging distance, increase in foraging time and a reduction in capture success of prey following exposure to concentrations of $pCO_2$ predicted by the end of this century. *T. marginalba* in this study was exposed to elevated $pCO_2$ for eight
weeks, double the amount of time used in the experiments of Watson et al. (2017) and Xu et al. (2017) who exposed gastropods to elevated $p$CO$_2$ for two-three weeks and four weeks, respectively. The reduction in predation found in Watson et al. (2017) and Xu et al. (2017) may reflect an acute shock to gastropods. Xu et al. (2017) explained that elevated $p$CO$_2$ increased searching time by whelks and as a result they took longer to find the prey possibly also because of the weakening of chemosensory response to prey cues. i.e. their ability to locate prey was impaired. Whelks in this study were slow to consume prey. On average $T$. marginalba consumed 0.003 to 0.009 grams (ambient $p$CO$_2$) and 0.003 to 0.013 grams (elevated $p$CO$_2$) of dry flesh per whelk each day. Similar to this study, Amaral et al. (Amaral et al. 2012a) found that whelks consumed significantly more oysters from locations with decreased pH (pH < 5) from locations acidified from acid sulfate soils. They concluded this was because Sydney rock oysters also had thinner weaker shells. In this study whelks exposed to elevated $p$CO$_2$ consumed more oysters irrespective of whether oysters were acclimated at elevated or ambient $p$CO$_2$, suggesting that increased consumption was related to increased energy demand of the whelk to maintain shell strength, rather than thinner, weaker shells of the oyster prey.

1.3 Model and hypothesis 2

This study provides some support for the second hypothesis that growth and physiological defences of $C$. gigas will decrease as reduced seawater calcium carbonate saturation states make shell calcification and maintenance more difficult and greater energy is required for the maintenance of acid-base balance. Responses of oysters to elevated $p$CO$_2$ were variable and dependent on the species, family line and ploidy and size which in some cases interacted with the presence of the whelk.
**Responses of C. gigas**

In this study, there was a positive effect of elevated $p\text{CO}_2$ on shell growth of small sized *C. gigas* (mean mass ± S.E 0.45 ± 0.02 g; Table 6.2). This response was in contrast to all previous studies which have found the impacts of elevated $p\text{CO}_2$ on shell growth to be more profound for smaller, younger oysters as they attempt to deposit their calcium carbonate shell (Bamber 1990; Gazeau et al. 2010; Lannig et al. 2010; Parker et al. 2010). Small oysters may be able to invest energy into growth when exposed to elevated $p\text{CO}_2$ as a mechanism, to avoid negative impacts in oceans which are acidifying. Potentially faster growth at elevated $p\text{CO}_2$ may allow them to reach a less vulnerable life stage and perhaps even earlier reproductive maturity. Despite the positive shell growth of the smaller sized *C. gigas* under elevated $p\text{CO}_2$, there was a negative impact of elevated $p\text{CO}_2$ on their physiological defence. In the absence of the predator *T. marginalba* the SMR of *C. gigas* reared at elevated $p\text{CO}_2$ was similar to that of the ambient controls. In the presence of the predator, however, the SMR of *C. gigas* reared in the ambient controls was lower than that of the, the SMR of *C. gigas* reared at elevated $p\text{CO}_2$ (Table 6.2, Figure 3.4e). Although current evidence is limited, the ability to reduce SMR in the presence of a predatory cue may be a key defensive strategy of mollusc prey to reduce their metabolic output and avoid detection. This strategy is particularly important for sessile species such as oysters which lack the ability to escape. During exposure to elevated $p\text{CO}_2$, smaller sized *C. gigas* may not reduce their SMR in the presence of the predator as the energetic costs associated with the maintenance of acid-base balance and shell growth may be too great. Being unable to regulate metabolic output will make *C. gigas* more visible to *T. marginalba* and more susceptible to predation.

In contrast to the response of the smaller sized *C. gigas* (mean mass ± S.E 0.45 ± 0.02 g) in this study, the larger sized *C. gigas* (mean mass ± S.E 10.15 ± 0.01 g) showed a significant reduction in shell growth at elevated $p\text{CO}_2$ (Table 6.2). This result matched those of most previous studies which have shown that shell growth and calcification of oysters is reduced under elevated $p\text{CO}_2$ (eg.
Beniash et al. 2011; Parker et al. 2011; Barton et al. 2012; Coleman et al. 2014). Analysis of SMR in the larger C. gigas also showed that their metabolic response in the presence of the predator T. marginalba also differed compared with the smaller size oysters. In contrast to small sized oysters, the larger sized C. gigas did not display a reduction in SMR at ambient pCO$_2$ when in the presence of the predator. This lack of predatory defence in the larger sized oysters may occur because 1. the metabolic output of a larger sized oyster is higher and it will still be high enough for a predator to detect if it is reduced, and/ or 2. given that their shell is larger and that T. marginalba preferentially select smaller sized prey, larger C. gigas they may be less concerned about predation. In a future high-CO$_2$ world, as small sized oysters have weaker shells, they may be more prone to predation especially in the absence of predatory defensive mechanisms.

**Responses of S. glomerata**

There was no effect of elevated pCO$_2$ on the size of S. glomerata with or without the presence of the predator T. marginalba (Table 6.2). In addition, SMR of S. glomerata was decreased under elevated pCO$_2$ (Table 6.2). A reduction in SMR during exposure to elevated pCO$_2$ has been shown for a number of bivalve species and has been suggested to be a short-term acclimation mechanism to withstand the negative impacts of elevated pCO$_2$ (Michaelidis et al. 2005; Liu and He 2012; Sokolova et al. 2012). It is likely, however, that over a more chronic exposure period S. glomerata would no longer be able to maintain fitness under such reduced SMR.

Unlike C. gigas, S. glomerata did not reduce their SMR in the presence of the predator T. marginalba. This may suggest that S. glomerata do not utilise reduced metabolic output as a defensive strategy to avoid detection by T. marginalba, perhaps because they have thicker, stronger shells compared to C. gigas. Caution in interpretation must be used here, however, as S. glomerata used in this study were medium sized (6.34 ± 0.10 g). Smaller sized S. glomerata may have displayed similar defensive mechanisms as the smaller sized C. gigas.
Despite a reduced SMR, *S. glomerata* did not experience a decrease in shell growth under elevated $pCO_2$. This suggests a greater partitioning of energy to shell growth and less to other fitness sustaining processes such as early reproductive conditioning. Impacts on other fitness sustaining processes of *S. glomerata* were not measured in this study. Even with no reduction in shell growth under elevated $pCO_2$, the consumption of *S. glomerata* by *T. marginalba* was still significantly increased (Tables 6.1 and 6.2). Maintaining calcification can come at a cost. For example, Wood et al. (2008) found that ophiuroid brittle stars, *Amphiura filiformis*, can increase the rates of many of their biological processes (i.e. metabolism and the ability to calcify) to compensate for increased seawater acidity. However, this upregulation of metabolism and calcification, although it potentially ameliorates some of the effects of increased acidity comes at a substantial cost (i.e. muscle wastage) and is therefore unlikely to be sustainable in the long term. Oysters are known to partition energy when under stress (Lannig et al. 2010; Stapp et al. 2015; Sussarellu et al. 2016).

*Among family lines*

This thesis demonstrates that there are significantly different sensitivities to ocean acidification even within populations of *C. gigas*. Overall there was an impact of elevated $pCO_2$ on shell growth and an impact of family lines on shell growth, but there were a limited number of interactions between elevated $pCO_2$ and family line. After 10 days, *T. marginalba* that were exposed to elevated $pCO_2$ consumed significantly more oysters regardless of whether *C. gigas* had been acclimated for 6 weeks to ambient or elevated $pCO_2$, but this was not dependent on the family line and the effect was not significant after 17 days. Differences within populations of oysters in response to elevated $pCO_2$ are becoming increasingly well known. For example, Parker et al. (2011) showed differences in the growth of family lines of *S. glomerata* under elevated $pCO_2$, with two out of ten family lines not affected. Further, spat of *S. glomerata* oyster populations selected for fast growth or disease resistance had greater growth than wild populations in response to elevated $pCO_2$ (Parker et al. 2011). Similarly Waldbusser et al. (2011) found significant variability in the dissolution of shells
among populations of the Eastern oyster *Crassostrea virginica* at elevated pCO₂. Such variability in response by oysters and other molluscs may be the key to species survival in a climate changed ocean (Sunday et al. 2011; Sunday et al. 2014; Reusch 2014), however, in the family lines tested in this study there were mainly no significant differences in response to both elevated pCO₂ and predation were detected. Only one or two family lines demonstrated some resilience to elevated pCO₂ and predation. Future studies need to look at a larger number of family lines from geographically distinct locations to determine whether sufficient within-species (intra-specific) differences exist within populations of *C. gigas* to allow the persistence of these ecologically and economically important species over this century.

### Table 6.1 Summary of the effect of elevated pCO₂ on the growth and SMR of *T. marginalba*.

<table>
<thead>
<tr>
<th>Whole mass of the whelk at the beginning of the experiment</th>
<th>Duration of exposure</th>
<th>Direction of response of growth</th>
<th>Direction of response of SMR</th>
<th>Predation</th>
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<td>1.75 ± 0.04 g (Chapter 3)</td>
<td>8 weeks + 17 days</td>
<td>=</td>
<td>↑</td>
<td>↑*</td>
</tr>
<tr>
<td>2.85 ± S.E 0.38 g (Chapter 4)</td>
<td>4 and 8 weeks</td>
<td>↓ &amp; = size**</td>
<td>↓ &amp; =</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑shell strength</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.62 ± S.E 0.52 g (Chapter 5)</td>
<td>6 weeks + 15 days</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
</table>

* (both species) = *C. gigas* (17 days)

**4 and 8 weeks results
Table 6.2 Summary of the effect of elevated $p$CO$_2$ and *T. marginalba* on the growth and SMR of diploid *S. glomerata* and *C. gigas*. *whole mass recorded at the beginning of the experiment*

<table>
<thead>
<tr>
<th>Species</th>
<th>Whole mass*</th>
<th>Duration of exposure</th>
<th>Direction of response of growth</th>
<th>Direction of response of SMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Without <em>T. marginalba</em></td>
<td>With <em>T. marginalba</em></td>
</tr>
<tr>
<td><em>Saccostrea glomerata</em></td>
<td>6.34 ± 0.10g (chapter 3)</td>
<td>8 weeks + 17 days</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td><em>Crassostrea gigas – 2 n</em></td>
<td>0.45 ± 0.02g (chapter 3)</td>
<td>8 weeks + 17 days</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>1.20 ± 0.24g (chapter 4)</td>
<td>4 and 8 weeks</td>
<td>n/a</td>
<td>↑↓</td>
</tr>
<tr>
<td></td>
<td>10.15 ± 0.24g (chapter 5)</td>
<td>6 weeks + 15 days</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td><em>Crassostrea gigas – 3 n</em></td>
<td>6.12±0.45g (chapter 5)</td>
<td>6 weeks + 15 days</td>
<td>↑shell height = shell length = shell depth</td>
<td>↑shell height = shell length ↓ shell depth</td>
</tr>
</tbody>
</table>

*whole mass recorded at the beginning of the experiment

Diploid versus triploid *C. gigas*

Triploid oysters were more resilient than diploids oysters to elevated $p$CO$_2$. Triploid *C. gigas* grew faster than diploid *C. gigas*. Further, diploid oysters had a significant reduction in growth at elevated $p$CO$_2$ while the growth of triploid oysters was not reduced by elevated $p$CO$_2$, except for shell depth. Triploid oysters had greater growth in shell height at elevated $p$CO$_2$. Investment in height (outward growth) is a more common response to stress than shell thickening because it is believed to be less energetically costly. However when a predator is present they do the opposite and invest in
thickening and change their shape rather than height (Trussell and Nicklin 2002; Bourdeau 2012; Melatunann et al. 2013), but this depends on whether the strategy of the predator is through crushing or drilling. A crushing predator will be less able to be successful to handle a long thin prey (Bibby et al. 2007), and a drilling predator will be less able to be successful on increased thickness (Sanford et al. 2014). This result has significant economic implications as the vast majority of farmed C. gigas in New South Wales are triploids. Triploids are, however, usually specifically reared for commercial exploitation and unlikely to appear naturally in the wild. As a result, triploid oysters may be used to help sustain important aquaculture industries in Australia and around the world but will not improve the ecological resilience and sustainability in the next century of C. gigas. In contrast to diploid oysters, triploid oysters allocate less energy to reproduction and more to somatic and shell growth (Maguire et al. 1995; McCarthy et al. 2015). As calcification and acid-base balance becomes more energetically costly under exposure to elevated $pCO_2$ the limitations of both the amount of available energy and the rates of its acquisition and metabolic conversions will result in trade-offs between the energy diverted to basal maintenance costs and other fitness-related functions (Sokolova et al. 2012) such as reproduction, development and growth (Shirayama and Thornton 2005; Berge et al. 2006; Green et al. 2009; Lischka et al. 2010; Range et al. 2011) . Since triploid oysters divert significantly less energy into reproduction compared to diploid oysters, they have a greater pool of energy available for acid-base balance and shell growth under elevated $pCO_2$ stress, likely contributing to their increased resilience. Many more studies are finding that pre-exposure to elevated $pCO_2$ has a positive carry-over effect on offspring, but how long this lasts in generational times is still unknown (Parker et al. 2012; Ross et al. 2016; Zhao et al. 2017). What is known, suggests that transgenerational plasticity (TPP) may help alleviate the impacts of OA on predator defences (Parker et al. 2017).
6.4 Summary

In this study *T. marginalba* were relatively resilient to ocean acidification. Unlike other molluscan predators, there was no change in foraging of *T. marginalba*. Although SMR of *T. marginalba* was increased, they were able to prevent any potential negative long term impacts of elevated CO$_2$ on their shell by increasing their rates of consumption of prey. As prey are increasingly negatively impacted by ocean acidification, reduced prey availability may have negative consequences for *T. marginalba* if they are unable to find and consume prey at an adequate rate to meet their increased energy demands. Further, more research is needed to determine whether other fitness sustaining processes in *T. marginalba* such as reproduction, acid-base balance, somatic growth and immune response are negatively affected.

There were mixed effects of elevated pCO$_2$ on the growth and physiological defences of oysters. For *C. gigas*, smaller sized juveniles (0.45 g, approximately 6 months old) had greater shell growth under elevated pCO$_2$ but this positive response was negated by a loss of ability to reduce metabolic output to avoid predatory detection. In larger *C. gigas* (10.15 g, approximately 12 months old), shell growth was reduced under elevated pCO$_2$. The larger *C. gigas* did not reduce their metabolic output under ambient or elevated pCO$_2$ in response to *T. marginalba*. For *S. glomerata* (6.34 ± 0.10 g), medium sized juveniles showed a significant reduction in SMR under elevated pCO$_2$ but there was no impact on shell growth. While the response of oysters differed with species and size, it is likely that over an extended period of exposure (months to years) juveniles of both species will become more sensitive to predation under elevated pCO$_2$ across all size ranges (Figure 6.1).

Given the large global consumption of oysters as the most abundant harvested shellfish (He et al. 2002), near-future ocean acidification is predicted to have serious consequences for the sustainability of this vital aquaculture industry. It is often difficult to predict change as multiple factors (including ocean acidification, warming and oxygen reduction) will interact to influence species adaptive potential. Further studies should assess the adaptive potential of oysters and their
predator and prey interactions with whelks to synergistic changes in the physical environment. Physiological responses of marine organisms in response to stress have traditionally been assessed in laboratory experiments as they provide controlled environments for the manipulation of environmental stressors (Kleypas et al 2006). Extrapolation of results from laboratory based studies is often challenging as the results may not be replicable in the field. Further, the responses of molluscs to climate stressors are often assessed in experiments where the duration of exposure is acute. Acute exposure experiments do not mimic well the non-acute, longer time frame expected for oceans to acidify. Results from acute experiments make it difficult to extrapolate the longer term impacts of ocean acidification.

Improvements in research predictions would result if experiments are done on synergistic impacts of elevated $pCO_2$ and in the field and locations where natural variation in $pCO_2$ occurs. This thesis provided evidence that utilising triploid breeding programs to produce oysters that can divert a greater proportion of their energy budget into growth and acid-base balance may be a viable option to reduce the predicted impacts of elevated $pCO_2$ on oyster aquaculture over this century.

Preliminary evidence for selecting oyster family lines that are resilient to both elevated $pCO_2$ and predation suggests that this may be a challenge. Further research is required to determine whether selective breeding is a feasible option to help ‘climate-proof’ aquaculture industries and oyster populations in Australia and around the world.
Figure 6.1 Conceptual framework of the influence of ocean acidification on the pathways that determine predator-prey interactions of *T. marginalba* and its oyster prey *S. glomerata* and *C. gigas*.

Modified after (Kroeker et al. 2014), original diagram is in the section 1.6 of the introduction.
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