CONTEXTUALIZING THE IMPACT OF OCEAN ACIDIFICATION ON VELIGER GROWTH FOR THREE COMMERCIALLY IMPORTANT BIVALVE MOLLUSCS

by

François Edward Donald Bregha

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Dedication

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"The problem is known as ocean acidification
a phenomenon that may trigger a mass extinction.
It is attributed to the activity of man
And the destruction wrought by his hand.

5
This change reduces the presence of Carbonate,
Causing the conditions to build shells to dissipate.
Thus the communities of the many with a shell,
Have heard the first note of their death knell.

-František the half elf bard

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Abstract

The impact of ocean acidification on the growth rate of bivalve veligers for three commercially important species (*Crassostrea gigas, Crassostrea virginica* and *Mytilus edulis*) has been assessed experimentally for the last decade. Based on the results correlating an increase in pCO_2 with a reduction in growth rate, these studies have concluded that ocean acidification will have a strong impact on veligers. This study reassesses these results in the context of temperature dependant growth curves derived from historical benchmarks of veliger growth rates. Most of the ocean acidification data points fall within the confidence interval identified by the historical data. This study concludes that in terms of shell growth, ocean acidification exerts a minimal influence on maximum shell length. The differences in experimental methodologies are compared, and the different metrics used to relate ocean acidification to growth are discussed. Recommendations are included for future research and governmental action towards ocean acidification.

List of abbreviations used

ACC	Amorphous Calcium Carbonate
TA	Total Alkalinity
DIC	Dissolved Inorganic Carbon
IPCC	Intergovernmental Pannel on Climate Change
SRES	Special Report on Emissions Scenarios
DFO	Department of Fisheries and Oceans
USA	United States of America

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Chapter 1. Introduction

1.1 The Problem

The study of ocean acidification by increased atmospheric carbon dioxide concentration is a growing field of research. This phenomenon is expected to impact local marine ecosystems and the human communities that depend on the world's oceans. A growing body of experimental and observational research has produced a strong case for concern. Nevertheless, it is a challenge to piece together how these findings will affect real populations in the wild as well as dependent local coastal communities and economies. The research is currently in transition: many studies have characterized the chemical changes anticipated in the world's oceans, but work on their biological impact is mostly preliminary.

In the last two decades, ocean acidification research has focused on the potential impacts on animal growth, survival, and reproduction. This research blends regional variation, and has only begun to address interactive effects with other variables known to affect growth, survival and reproduction. Industries dependent on molluscan shellfish must take these predictions into account in their long-term development plans. Making sense of this information is a challenge without the context of all the variables that could affect a species life cycle.

Shellfish aquaculture is an important economic activity around the world. Atlantic Canada primarily cultivates the American oyster (*Crassostrea virginica*) and the Blue mussel (*Mytilus edulis*). These industries have benefitted the region immensely, producing 32 000 tonnes of shellfish, generating 71 million CAD in 2013 (DFO, 2013). Ocean acidification is thought as a threat to the long-term viability of the shellfish industry, particularly by reducing production capacity at existing sites as well as reducing the number of viable sites in the region (Cooley & Doney. 2009)

The problem stated simply: how does ocean acidification impact commercial bivalve veligers' shell growth in light of what is already known about their temperature-dependent growth rates? There is a corollary to this question: How does the affected

growth rate potentially impact the shellfish industry in a given location? This chapter provides an introduction to the problem and the background information required to understand the core components of related research. It starts with a broader view of bivalve biology, followed by reviews of oceanographic and biological research on ocean acidification. In the final section of this chapter, the elements of the rest of the study are presented.

1.2 A Primer on Bivalve Biology

Bivalves are filter-feeding animals of the phylum Mollusca (Stafford, 1913). Their adult forms are generally sessile or sedentary, the classic bivalve has a foot that can be used for slow locomotion and the highly-derived group of scallops has evolved the ability to swim. Bivalves have a very recognizable morphology. They are characterized by the two distinct shells, or valves, which grant them their name. These shells are actively maintained, and enclose a protein membrane known as the mantle that houses the vital organs (Stafford, 1913).

This study will focus on three species of bivalves, the Pacific oyster (*Crassostrea gigas*), the American oyster (*Crassostrea virginica*), and the blue mussel (*Mytilus edulis*). All three species tolerate a wide range of environmental conditions, though *M. edulis* and *C. gigas* have truly global distributions (DFO, 2003b). The adult forms of bivalves are very familiar to us; humans have fished for and eaten these benthic creatures for thousands of years. Despite our close relation with the adult stage, this study focuses on the earlier life stage known as the veliger, which is thought to be more susceptible to ocean acidification (Gazeau, 2013). The journey of this life stage is complicated, perilous and relatively short.

The life of bivalves such as the blue mussel, American oyster, or Pacific oyster begins in the water column. Adults release eggs and sperm once a temperature threshold is reached. Fertilization occurs in the water column (Stafford, 1913; DFO, 2003b). Of the many million eggs that can be released by a female, it is estimated that only a few will successfully grow into juveniles (Rumrill, 1990). The developmental timeline is very

similar between these species and other members of the class. Within twenty-four hours, the embryos will have metamorphosed into an initial larval stage known as a trochophore (Kurihara, Kato & Ishimatsu et al., 2007). By forty-eight hours after fertilization, they will develop into the veliger stage.

This transition is marked by the formation of the initial shell (termed prodissoconch 1) (Medaković, 2000). The mineral composition of this shell will vary by species but a typical construction is an initial amorphous calcium carbonate layer subsequently reinforced and covered by successive aragonitic layers during the larval lifespan (Medaković, 2000). The shell greatly increases the organism's weight and provides a protective barrier to the elements and predators found in the water column. The duration of the larval form can vary greatly and is heavily influenced by food availability, temperature, salinity, and genetics (Loosanoff & Davis, 1963; Dekshenieks et al., 1993). In optimal conditions this period can be as short as two to three weeks, but it can extend to a few months (Loosanoff & Davis, 1963). There are varying evolutionary strategies in the larval period resulting in an ontology that can favour varying periods of time. Shorter periods can reduce mortality caused by predation while longer periods can increase the probability of finding a suitable substrate for the sessile adult (Rumrill, 1990). Regardless of the duration, the planktonic phase is typically the main method of dispersal for bivalves, and individuals can potentially travel great distances in the ocean currents, sometimes more than twenty kilometers (Levin, 2006). Nevertheless, most survivors will settle closer to where they are spawned (Levin, 2006). During this time, the larvae are members of the oceans' zooplankton community and feed on phytoplankton.

Evaluating larval growth can be done using a few benchmarks. The most commonly used assessment is length, owing to its practicality and ease in understanding. Other benchmarks include developmental milestones (See figure 1), and cohort survival. These metrics are more complicated, but can capture the more subtle effects of external factors on the life of veligers.

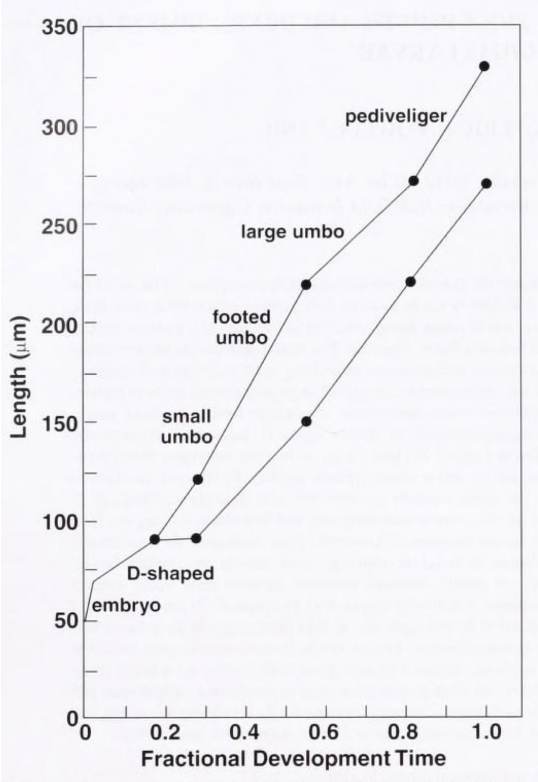

Figure 1: Typical growth of *C. gigas*, representing the main morphological benchmarks during the larval period. Fractional developmental time represents the proportion of time spent in each stage prior to metamorphosis. Reproduced from Bochenek et al., 2001

As stated previously, this study aims to incorporate the findings of ocean acidification within the context of a typical growth rate. Characterizing a typical pattern is no trivial matter, since larvae can be influenced by a myriad of factors (e.g. food, temperature, salinity, and genetics to name a few) many of which can dynamically fluctuate during the larval period (Pechenik, 1987). Two competing factors maintain the viability of this wide variation. On the one hand accelerating development minimizes the risk of death before reproducing, while on the other hand delaying development increases the chance of finding a suitable site for the adult stage. Based on growth curves measured during this lifespan, the trajectory of the veliger life stage is exponential (Bochenek et al., 2001).

Four primary factors influence the variation exhibited in this stage (Calabrese & Davis, 1970; Davis, 1953; Rico-Villa et al., 2009; Bochenek et al., 2001; Deksheniek et al., 1993). Temperature and salinity have an optimal condition that optimizes growth (Calabrese & Davis, 1970). Food influences growth based on quality and quantity, exhibiting a hyperbolic functional response (Rico-Villa et al., 2009; Davis, 1953). As the veliger develops, the ideal mixture of food changes. Initially they depends on internal energy reserves supplemented by feeding on small algae during the mixotrophic phase. Once internal reserves are exhausted, typically around the umbonate stage, they are said to enter the exotrophic period of growth (Rico-Villa et al., 2009). Genetic variation can exert an influence on observed growth (Newkirk, Haley, Waugh, & Doyle, 1977). Numerical models exist characterizing growth patterns in dynamic environments (Rico-

Villa et al., 2010; Bochenek et al., 2001; Deksheniek et al., 1993), however the paramterization doesn't fully capture the variation exhibited in laboratory conditions let alone those found in the wild.

Metamorphosis to the adult stage is triggered by a combination of cues that range from achieving a minimal length to detecting a suitable site. In some cases metamorphosis to the juvenile stage can be delayed for weeks until all requirements are met (Coon, Fitt & Bonar, 1990). Metamorphosis can be distinguished in two parts: metamorphosis proper, where the animal will cease feeding as it reorganizes its internal body structure to resemble a miniature adult, and setting, where the individual will actively sink to the benthos in search of a suitable site to remain as an adult (Coon et al., 1990). These two events tend to occur in synchrony.

In their adult form, *C. gigas* and *C. virginica* are physically very similar. These oysters and *M. edulis* will continuously grow during their lifespan and settle in mid- to lower-intertidal habitats, but have been reported as deep as 10m below sea level (DFO, 2003a; DFO, 2003b; Stanley & Sellers, 1986). Oysters and blue mussels have a few noteworthy peculiarities that distinguish them from other members of the class. Oysters have asymmetrical shells and cement themselves to hard surfaces using calcium carbonate (DFO, 2003a). Blue mussels attach themselves to hard surfaces using a proteinaceous polymer known as byssum woven into a byssal thread (O'Donnel et al., 2013; DFO, 2003b). These creatures are used to assess water quality, as their filtering ability captures toxins and heavy metals in the water column (Bricelj & Shumway, 1998; Chase et al., 2001). The adults of both species are highly gregarious and cluster in high densities; they can even produce large reefs by the agglomeration of the numerous individuals within a population. Their populations can have noticeable impacts on the surrounding environment, by altering local water quality or flow. As well, they provide other benthic creatures with new surfaces for attaching (Guitiérrez, Jones, Strayer & Iribarne, 2003). Large populations of cultured blue mussels also can alter the chemistry of local sediments by transferring suspended particles to the benthos by means of fecal and pseudo-fecal matter deposition (Grant et al., 1995).

1.3 Ocean Acidification

Since the industrial revolution, human societies around the world have dramatically increased their emissions of carbon dioxide (IPCC, 2013). For almost three decades, science has been identifying the impacts of this change on global climate, and more recently on change in ocean pH (IPCC, 2013). Ocean acidification is the phenomenon where the ocean's pH and carbonate pool are reduced by an increase in atmospheric carbon dioxide (The Royal Society, 2005). This change in ocean chemistry may have important consequences for life in the oceans and is the subject of a growing amount of research around the globe (The Royal Society, 2005; Washington State, 2012). The oceans' pH, defined as the negative logarithm of available hydrogen ions in the solution, is largely regulated by the carbonate buffer system. This buffer is maintained by the ratio of carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), and the baseline global surface water pH is currently ~8.1 (The Royal Society, 2005). The rise in atmospheric CO_2 triggers an increase in carbonic acid causing the buffer system to adjust to this influx. The new balance yields a decrease in carbonate in favour of bicarbonate and carbonic acid (The Royal Society, 2005). In its most basic form, this is the process that is occurring globally as more carbon dioxide accumulates into the atmosphere and is dissolved into the oceans.

1.3.1 Historical Context

This is not the first time that ocean acidification has manifested itself on the planet. The geological record for the last three hundred million years contains evidence of multiple time periods where atmospheric CO_2 rose to levels expected by the end of the 21st century and beyond (Caldeira & Wickett, 2003; Hönsich et al., 2012). The most notable ones are: the late Pleistocene 11.6 - 17.8 thousand years ago; the Pliocene warm period 2.97 - 3.29 million years ago; the Paleocene-Eocene Thermal Maximum 56 million years ago; the Cretaceous-Tertiary extinction event 65 million years ago, Multiple Anoxic episodes during Cretaceous and Jurassic periods 93, 120 and 183 million years ago; the Triassic-Jurassic extinction event 200 million years ago, and the Permian Triassic extinction event 252 million years ago (Hönsich et al., 2012). Many of these time

periods also coincide with mass extinction events in the fossil record (Hönsich et al., 2012). What makes the current situation unique is the elevated rate of CO_2 emissions derived from human activities (IPCC 2013) and the simultaneous decline of pH with this rise in atmospheric CO_2 (Hönsich et al., 2012). It is important to underscore that the rate of CO_2 emissions is key to understanding why the current ocean acidification event differs from previous ones. If the carbon release to the atmosphere is very slow, then the freshwater runoff from the land can balance the total alkalinity and prevent a substantial pH perturbation (Hönsich et al., 2012).

While each of these events has some analogue to the current situation, the conditions driving the current change are unique. Most alarmingly, the current rate of CO_2 emissions to the atmosphere greatly exceeds the estimates from any of the previous time periods associated with previous ocean acidification events (Hönsich et al., 2012). Consequently, the magnitude of the perturbation is therefore difficult to predict, as there are no true analogues known in the geological record. Given the current CO_2 emission rate and different seawater chemistry, biogeochemical models are the only place to turn for an indication of what may occur (Hönsich et al., 2012; Caldeira & Wickett, 2003). In the last three hundred years the oceans' pH level has not dropped by an amount comparable to current projections for the next 100 years, nor has any decline in the recent geological past been entirely caused by the absorption of excess CO_2 (Caldeira & Wickett, 2003). The projected effect of anthropogenic CO_2 emissions may therefore be significant (Caldeira & Wickett, 2003). These models predict a return to pre-industrial levels in a period of 10 000 years, a timeframe that is equivalent to the length of human history, based on the penetration of carbon dioxide into the depths of the world's oceans, where residence times can be very long (Hönsich et al., 2012).

The potential changes to the surface ocean chemistry are not likely to be uniform, and certain regions of the globe are more susceptible to this atmospheric influx than others (The Royal Society, 2005; Sabine et al., 2004; Wang et al., 2013). Many studies have established an ocean carbon inventory baseline, outlining the current state of carbon chemistry within the water column (see Sabine et al., 2004; Wang et al., 2013; Bates,

2007; Bates & Mathis, 2009; Feely, Sabine, Hernandez-Ayon, Ianson & Hales, 2008; Orr et al., 2005; Shadwick et al., 2010).

The study of ocean acidification is being conducted on multiple fronts. Using the anticipated increase in atmospheric CO_2 predicted by the Intergovernmental Panel on Climate Change (IPCC, 2013; IPCC, 2000), ocean acidification models are predicting a dramatic shift in carbonate chemistry (Hauri et al., 2013; Orr et al., 2005). The effect of this shift in chemistry is clear; however the broader impact that this will have on biology and human activities must still be determined (Orr et al., 2005). So far the hypothesis predicts that a reduction in carbonate must hinder the growth and development of creatures that use it. Evidence in many taxa is emerging from experiments that demonstrate a reduction in growth due to ocean acidification conditions similar to those projected for later in the century (Gazeau et al., 2013; Ross, Parker, O'Conner & Bailey, 2011). Constraints still exist when identifying the impacts of this phenomenon, particularly in the form of cumulative impacts of ocean acidification across the different life stages of a species. It is expected that research in the next decade will help to identify the nature of these cumulative impacts.

1.3.1 Carbonate Chemistry

The abundance of calcium and carbonate ions in the water column can be reported using the Ω factor, defined as:

$$\Omega = \frac{[Ca^{2+}]\,[CO_3^{2-}]}{K_{sp}}$$

$[Ca^{2+}]$ and $[CO_3^{2-}]$ refers to the concentration of calcium and carbonate ions in the water respectively, and the apparent solubility product (K_{sp}) to the solubility constant (Doney, Fabry, Feely & Kleypas, 2009; Mucci & Morse, 1984). Undersaturation and thus thermodynamic dissolution of calcium carbonate occurs when the value of Ω is less than one (The Royal Society, 2005).

The scientific and public concern about this chemical phenomenon centres on the impact that a shift in carbonate chemistry could have on calcium carbonate structures in the ocean. Many animals use this mineral as an essential structural support, building shells or coral reefs by depositing successive layers over time (Doney et al., 2009). The construction of such structures depends, in part, on the external concentration of the chemicals in the ocean, as well as the ability to concentrate the ions (Ca^{2+} and CO_3^{2-}) at the site of growth (McConnaughey & Gillikin, 2008). In bivalves this physiological process is still poorly understood. However, it is assumed that a reduction in dissolved carbonate due to more acidic conditions will force an increased allocation of energy towards shell construction and maintenance (Waldbusser et al., 2013). This shift in energy budget could come at the expense of other important processes including somatic growth and development (Waldbusser et al., 2013). In extreme conditions growth could cease all together, and the individual could die shortly thereafter.

To complicate matters further, multiple forms of calcium carbonate exist (e.g. aragonite, calcite), each with different levels of solubility, and a single organism may use several different forms throughout its lifespan or under different conditions (Weiss, Tuross, Addadi & Weiner, 2002). For example the Pacific oyster (*C. gigas*) will deposit an initial layer of amorphous calcium carbonate (ACC), followed by a layer of aragonite as a larva, and then multiple layers of calcite as an adult (Weiss, et al. 2002). Some shells will have traces of magnesium, increasing the shell's solubility relative to one with low or no magnesium present (Gazeau et al., 2013). Many carbon inventory studies track the more soluble aragonite, given that calcite tends to have an Ω factor that is roughly twice in value (Doney et al., 2009; Gruber, 2011). Furthermore many creatures such as corals and larval molluscs rely heavily on aragonite to form their complex biological structures (Doney, et al. 2009).

1.3.3 The Spatial Distribution

The carbonate found in seawater can arrive from five main sources: advective ocean currents, river discharge, biological activity, demineralization and atmospheric deposition (Wang et al., 2013; Thomas et al., 2012; Salisbury, Green, Hunt & Campbell,

2008; Feely et al., 2008). In the waters around Nova Scotia, it is known that the St. Lawrence River exerts a considerable influence on coastal waters, particularly on the northern coast. On the Atlantic side, the Labrador Current flows southward and mixes with water from the St. Lawrence along the coast (Shadwick et al., 2010). Further offshore the Gulf Stream can be found flowing towards the northeast. Nova Scotian inshore waters are known to be highly saline (30 psu) and cold (0 - 18ºC) for most of the year (Shadwick et al., 2010).

Quite a few studies that have assessed the carbon inventory in certain regions of the world and many supplement their inventories with modeling and projections. Carbon based molecules in various inorganic forms are present everywhere in the world's oceans and radioisotope markers (^{13}C vs ^{14}C) can help distinguish anthropogenic emissions found in the water column (Sabine et al., 2004). Over time, both models and in situ monitoring are indicating a change in carbonate chemistry related to climate change and ocean acidification (Bates, 2007; Hauri et al., 2013). Over the last three decades, a pattern has emerged where the oceans absorb slightly more CO_2 than prior to the industrial revolution, but, perhaps more importantly, they release less CO_2 to the atmosphere during the winter (Bates, 2007). This indicates that the system is retaining this additional CO_2.

The observed shift in pH is directly related to the buffering capacity of the body of water, and in the ocean this is determined by the ratio of total alkalinity to dissolved inorganic carbon (TA:DIC). A heavily buffered region like the Gulf of Mexico (~1.24 : 1), will have a smaller net change in pH than a less buffered region like the Gulf of Maine (~1.04 : 1) (Wang et al., 2013). The Gulf of Maine has experienced a steady decline in pH during the last few decades (Balch, 2014). The aragonite saturation state is also expected to decline in the region, as well as in Nova Scotia waters upstream of the Gulf of Maine.

A host of chemicals are capable of lowering pH and therefore meeting the definition of a cause of ocean acidification. After the experience with acid rain during the 1980s and 1990s, it is reasonable to assume that sulfurous and nitrous oxides will acidify

the ocean's surface and trigger ocean acidification. The public is already attuned to this environmental impact and assumes that ocean acidification and acid rain are intimately linked. However the influence of sulfur oxides and nitrous oxides on reductions of pH levels was minimal: they contributed only 2-5% of the total change in pH observed in the Bermuda Atlantic Time Series (Bates & Peters, 2007). These acids have a powerful local effect, but on the large scale do not impact the water column as much as the influx of CO_2 (Bates & Peters, 2007). The work of Bates & Peters (2007) also reported a mostly unchanged TA, but a rising level of DIC, further suggesting the important impact increased levels of CO_2 have on the water column.

Importantly, eutrophication can have a very noticeable impact on calcium carbonate saturation (Ω) as well as pH (Wallace, 2014). In close proximity to urban centres, changes in eutrophication account for most of the observed shift in pH and Ω (Borges & Gypens, 2010), obscuring the contribution from atmospheric deposition. Sewer discharge from New York City causes local water conditions to become inhospitable to marine life. Conditions during the summer can become anoxic at depth, poorly saturated with respect to aragonite, accompanied by a very high pCO_2 (>2000 µatm in surface waters and at depth in the immediate proximity of the source; Wallace, 2014). River discharge can also have a major impact because freshwater is naturally more acidic than ocean water. During a spring flood, major rivers will discharge a plume of water that acidifies ocean waters beyond the usual boundaries of the estuary (Salisbury et al., 2008). Frequent storms or more powerful spring floods can have a significant impact on local carbonate chemistry and thus mimic the effects of ocean acidification (Salisbury et al., 2008). In conjunction with eutrophication, freshwater input can mask the effects of ocean acidification by atmospheric deposition, but contribute to undersaturated Ω, high pCO_2 and harm shellfish in a similar manner.

1.4 The Biological Dimension of Ocean Acidification

Research about ocean acidification's impacts on the planktonic community focuses on a few key groups: coccolithophores, foraminifera and pteropods (Fabry, Seibel, Feely & Orr, 2008). These groups are responsible for the majority of $CaCO_3$ shell

construction among plankton and are represented around the world (Fabry et al., 2008). Pteropods build aragonitic shells, whereas the other two groups rely on calcite. Ocean acidification affects the construction of the test or shell built by these creatures, but it is still unclear how a reduction in calcification rates or deformities in the shell will affect fitness and population dynamics (Fabry et al., 2008). Given their size, generation time and limited individual ability to cope with changing conditions, the planktonic community is likely to respond faster than any other group. If the selection pressure from ocean acidification is as strong as predicted, these groups are likely to be less abundant by the end of the century and to force a change in ecosystem dynamics (Rost, Zondervan & Wolf-Gladrow, 2008).

Based on ocean climate models, zones undersaturated with respect to aragonite could occur very soon in the polar regions of the world (Comeau, Gorsky, Jeffree, Teyssié, & Gattuso, 2009). It is assumed that pteropod communities, which currently dominate these regions, will have a difficult time surviving in under-saturated waters and will move to the southern range of their distribution in order to find suitable growing conditions (Fabry et al., 2008; Comeau et al., 2009). *Limacina helicina* in particular is a major food source for small fish and other zooplankton in the Arctic Ocean, where ocean acidification could result in a smaller total biomass in the region (Comeau et al., 2009).

Macroinvertebrates and vertebrates are also affected, especially those dependent on support structures made of aragonitic $CaCO_3$. The most studied groups include corals, echinoderms and molluscs which will primarily be affected by impeded calcification rates. Arthropods and vertebrates seem to exhibit a larger tolerance though the arthropod phylum is so large that a wide range of potential responses is possible (Ries, Cohen & McCorkle, 2009). What is clear is that the responses of different phyla of the animal kingdom may be specific, and since many of these species form the core of marine ecosystems, the changes could have major consequences.

Corals are of particular concern, given their great importance in many benthic ecosystems. The observed impacts vary across the taxa. Corals are sensitive to the

aragonite saturation state; even small decreases can impede calcification rates. Tropical corals are projected to face poor growing conditions by the end of the century, but the waters should still be saturated with respect to aragonite (Ω_{ara}>1; Comeau, Edmunds, Spindel & Carpenter, 2013). Though growing conditions may decrease in quality, there are contrasting predictions of the impact, likely due to a combination of methodological differences in the studies and biological differences in the organisms (Comeau et al., 2013). Shallow corals with symbiotic algae may tolerate lower saturation conditions through an increase in photosynthetic ability (Comeau et al., 2013).

It is expected that large portions of the current range of cold water corals will become so depleted that aragonite will become thermodynamically unstable (Ω_{ara}<1), which could seriously jeopardize the survival of entire reefs (Turley, Roberts & Guinotte, 2007; Lunden, Georgian & Cordes, 2013). The shoaling of the saturation horizon is happening quickly, but this chemical factor doesn't seem to be the sole predictor of cold water coral presence (Lunden et al., 2013). The loss of these reefs is likely to have major ecological consequences, removing key habitat for fish and other marine life.

Echinoderms construct a calcium carbonate test (calcite) to protect their internal organs and are vulnerable to ocean acidification in a similar way to corals. Members of the group that rely more heavily on calcium carbonate, like the sea urchins, are most vulnerable to the potential effects of ocean acidification (Dupont, Ortega-Marínez & Thorndyke, 2010). Like molluscs, echinoderms have a pelagic larval phase that tends to rely on a very thin aragonitic shell for survival (Kurihara, 2008**)**. The adult form exhibits a greater tolerance for changes in carbonate chemistry, due to the more advantageous surface area to volume ratio, as well as a stronger metabolism to cope with the pressure (Kurihara, 2008). For instance ophiuroid brittlestar adults increased calcification rates when subjected to ocean acidification pressures anticipated during this century (Wood, Spicer & Widdicombe, 2008). However, the researchers noticed there was a tradeoff: the organism had atrophied muscle mass to compensate for this increased calcification (Wood et al., 2008). Ocean acidification can slow down growth at the larval stage as well as impede proper development (Kurihara, Asai, Kato & Ishimatsu, 2008; Dupont et al.,

2010). Adults seem more resistant to the acidification pressure, owing to their larger physiological capcity to resist the change, and their reliance on a calcite based shell. This trend is mirrored in other phyla (Dupont et al., 2010). This results in a hypothesis that changes in the pelagic environment will disrupt the population dynamics of the species, and based on the species-specific tolerance to the pressure, cause a shift in dominance of echinoderm species (Ross et al., 2011). This hypothesis can be extended to Arthropods and Molluscs. They also tend to have a pelagic larva reliant on an aragonite calcium carbonate structure and a benthic or epi-benthic (re Molluscs) adult tending to be very tolerant of ocean acidification.

While this study focuses on the impact of ocean acidification on the veliger larval stage of bivalve molluscs, other important findings are worthy of mention. The reviews by Gazeau et al. (2013) and Ross et al. (2011) provide detailed descriptions of all the documented impacts on each life stage for Mollusca. As with echinoderms, experiments have been conducted on each aspect of the lifecycle, highlighting the veliger stage as potentially the most vulnerable (Gazeau et al., 2013). There are varied impacts on gamete production, fertilization and trochophore development all based on the energy stores of the parent and not on the direct interference of shell construction (Kurihara et al., 2007; Gazeau et al., 2013; Ross et al., 2011). Ocean acidification is anticipated to increase density-dependent mortality during the transition from the pelagic veliger stage to the benthic juvenile (Green, Waldbusser, Reilly, Emerson & O'Donnell, 2009).

The transition during metamorphosis is already difficult and energy intensive; smaller individuals are typically less likely to survive this transition (Green et al., 2009). Under predicted conditions by the end of the century, adults and juveniles tend to have slower growth rates and minor impacts on survival, but this varies by species (Gazeau et al., 2013). Some adults maintain an aragonitic shell, which may explain why they are more susceptible than the calcite shells used in other species. Furthermore some species have an organic layer protecting the shell from the external environment, which enhances the resistance to ocean acidification (Gazeau et al., 2013). Impacts on behaviour have been noted, in particular, changes to byssal thread production, and phagocytosis by blue

mussels, and oxygen consumption in *L. littorea* (O'Donnel, George & Carrington, 2013; Ishimatsu & Dissanayake, 2010). Based on the meta analysis conducted by Gazeau et al. (2013), the two most studied molluscs are blue mussels and oysters (particularly *M. edulis* and *C. gigas,* respectively) and of the two, mussels are more resistant to ocean acidification.

A key problem with respect to echinoderms and molluscs is understanding how ocean acidification is altering organism function. There is the direct impact on calcification, but also an indirect impact on acid-base regulation, both of these being influenced by local environmental conditions and genetic variation. Teasing apart the potential sources of variation in order to connect ocean acidification to an impact on growth is difficult (Ross et al., 2011).

1.5 Study Objectives

The background above holds true from a global perspective. At the same time, it is important to provide context for each finding in order to meet the challenge of regional responses to ocean acidification. While shellfish aquaculture around the world will be affected, the regional industry in Atlantic Canada is about to face a specific set of conditions that will differ from those found along the Pacific coast of North America. The next chapter provides some context to the findings of ocean acidification experiments, by exploring the question: *Do the results of recent ocean acidification experiments on veliger larval growth of commercially important mollusc species fall outside the historically expected growth range of these species, based on previous laboratory experimentation?*

The scientific community attempted to characterize the impact of temperature, genetics, food and salinity on the early development of oysters and mussels. This study contrasts the known impacts of temperature with the experimental data from ocean acidification experiments, in order to demonstrate the impact of ocean acidification within the context of expected temperature based growth. This will add context to the scientific understanding of the net effect ocean acidification will have on the chosen

species. In addition, some management strategies are suggested that could make the aquaculture industry more robust in the face of changes expected from rising levels of ocean acidification. Finally, this study argues that the results of ocean acidification experiments on molluscs show less deviation from a standard growth rate than expected.

The final chapter expands on the conclusions found within Chapter 2. First, it discusses the different metrics that are used to measure ocean acidification as well as the biological response. Second, it will consider ways to compare methods and harness the information produced by studies on ocean acidification within the context of vulnerability.

Chapter 2. Contextualizing the Impact of Ocean Acidification Experiments on the Veliger Growth Rate for *C. gigas, C. virginica and M. edulis*

2.1 Introduction

The rise in atmospheric carbon dioxide is expected to alter the levels of carbonate and pH in the ocean (The Royal Society, 2005). Research and experimentation in the last decade have not only explored the mechanisms by which carbon dioxide can alter the ocean's pH, but also its far-reaching consequences on biology and human activity (The Royal Society, 2005). Lower metazoan trophic levels are assumed to be more susceptible, due primarily to the presence of calcium carbonate for physical structural support and/or defense (The Royal Society, 2005; Doney et al., 2009). By depleting the pool of available carbonate for construction, ocean acidification could impair the ability to build these structures and consequently compromise an organism's growth and/or survival.

Bivalves are potentially vulnerable to ocean acidification, particularly their veliger larval stage, which builds a thin shell using the more soluble aragonite crystal (Washington State, 2012; McConnaughey & Gillikin, 2008). Although ocean acidification will impact all aspects of the life cycle, this life stage could be particularly vulnerable to the phenomenon due to the size, thickness and chemistry of the shell (Gazeau et al., 2010; Ishimatsu & Dissanayake, 2013). An impact on shell construction would delay growth and/or increase the risk of death, which would impact later life stages, exerting a selective pressure on the population. Research so far has focused on ecologically and economically important species (Gazeau et al., 2013), such as the Pacific oyster (*Crassostrea gigas*), blue mussel (*Mytilus edulis*) and the American oyster (*Crassostrea virginica*).

Typically, research in this emerging field recreates the projected atmospheric concentration of CO_2 based on the IPCC Special Report Emissions Scenarios (SRES) A1F1 scenario (IPCC, 2000), and then observes the growth and development of bivalves in these anticipated conditions The benchmark of 700 ppm of atmospheric CO_2 by the year 2100 is typically used as the end-of-century projection for these experiments.

Experimental manipulation also often includes a mid point, as well as a future point at twice the anticipated concentration (Kurihara et al., 2007; Kurihara et al., 2008; Talmage & Gobler, 2012; Talmage & Gobler, 2011; Parker, Ross & O'Conner, 2010). Replicating this forecast is usually done using a bubbling technique, where air with a known concentration of CO_2 is forced into the experimental beaker, forcing the carbonate buffer system to adjust accordingly (Kurihara et al., 2007; Kurihara et al., 2008; Parker et al., 2010; Gazeau et al., 2010; Gazeau et al., 2011; Timmins-Schiffman, O'Donnell, Friedman & Roberts, 2012; Miller, Reynolds, Sobrino & Riedel, 2009; Talmage & Gobler, 2009). The net result allows for the detection of the isolated impact of elevated CO_2 conditions on the organism. It should be noted however that the coastal conditions where these organisms are typically found exhibit a large pCO_2 fluctuation (e.g. ranging from 350 - 700 μatm in the western Gulf of Maine)(Waldbusser & Salisbury, 2014).

The research effort on bivalve veligers has been ongoing for a decade, aiming to identify the extent of the impact of elevated pCO_2 on this life stage. Veligers of *C. virginica, C. gigas,* and *M. edulis* have been subjected to many variations of the basic ocean acidification experimental setup. Beyond the singular impact of elevated pCO_2 on the development of the organism to the veliger stage (Timmins-Schiffman, et al., 2012; Gazeau et al., 2010; Gazeau et al., 2011; Kurihara et al., 2007; Kurihara et al., 2008), and the development to metamorphosis (Talmage & Gobler 2010), other factors such as temperature (Talmage & Gobler, 2011; Parker, Ross & O'Conner, 2009; Parker et al., 2010) have been included to explore the more additive interaction with this phenomenon. In general, ocean acidification seems to interact with, or be influenced by, these factors to increase the magnitude of the observed reductions in growth or survival. A few studies have tracked the impact in terms of survival and morphological benchmarks (Talmage & Gobler, 2009; Talmage & Gobler, 2010; Talmage & Gobler, 2011; Ginger et al., 2013). This integrated approach of relating survival, development, and shell length has helped understand the multiple pathways that ocean acidification could affect the veliger stage.

In all experiments to date, local seawater and local animals were used. Each study had a different method of selecting the test organisms, ranging from acquiring seed from

local hatcheries to controlling maternal and paternal lines in house and producing test organisms. The experimental results are also a reflection of the regional population and species variation found around the globe, representing Japan, eastern Australia, the Netherlands as well as the north east and north west USA. Local seawater was used, and the observed salinity in each experiment reflects differences between the geographic locations where the studies were conducted. In most cases the salinities were high (28-34 psu), with two notable exceptions (18 and 26 psu) (Table 3). The food concentration was intended to be over abundant, though evaluated using different measurements (10^4 or 10^5 cells per mL using *Isocrysis galbana* mixed with other micro-algae) in order to prevent this factor from influencing the observed variation in growth (Gazeau et al., 2010; Talmage & Gobler, 2009; Waldbusser et al., 2013; See Table 3 for a comparison of the parameters in each experiment). The conclusions drawn from this set of experiments indicate that animals subjected to anticipated ocean pH conditions are, on average, smaller in length at the same age as their counterparts grown in current conditions.

2.1.1 Objectives of this Study

It is important to understand how ocean acidification influences growth, as well as its relative importance to other factors influencing growth, such as food, genetics, temperature, and salinity. There exists a considerable literature characterizing the environmental impacts on growth of *C. gigas, C. virginica* and *M. edulis* veligers, which can provide the necessary context to help understand the interaction. The primary question of this study is: do the results of recent ocean acidification experiments on veliger larval shell growth of commercially important mollusc species fall outside the historically expected growth range for these species?

This work, which is a re-analysis of publicly available data, will restrict its focus to two primary variables, temperature and pCO_2. The methods employed in precursor experiments had very similar variables for salinity, and food, but differing regimes for temperature as well as the ocean acidification pressure. Here, we seek to normalize the results from previous ocean acidification experimental work to historical data with respect to commercially important bivalve veligers. The aim is to understand the

magnitude of the influence exerted by ocean acidification on shell growth rates within the context of variable temperature regimes, saturated food conditions and high salinities.

2.2 Methods

Publications and data concerning ocean acidification research on veliger larvae of *M. edulis*, *C. gigas,* and *C. virginica* were gathered using the reviews provided by Gazeau et al. (2013) and Ross et al. (2011) through database searches in Google Scholar and Web of Science, as well as through the citations in the publications themselves. Relevant articles were retained if they used one of the three species of interest, documented the experimental conditions, and reported the impact of ocean acidification in terms of length of the larvae at a given point of time. The final list of studies retained for the re-analysis is highlighted in Table 1. This search began in February of 2013 and ended in June of 2014.

Table 1: List of ocean acidification studies assessing the impact on veliger growth rates for C. gigas, C. virginica, and M. edulis retained for the re-analysis. List of historical studies used to construct the growth curves.

Ocean Acidification studies	Historical Studies
C. gigas Kurihara et al., 2007 Parker et al., 2010 Gazeau et al., 2011 Timmins-Schiffman et al., 2012 Waldbusser et al., 2013 *C. virginica* Miller et al., 2009 Talmage & Gobler 2009 *M. edulis* Gazeau et al., 2010	*C. gigas* Helm & Millican, 1977 His & Maurer, 1988 His et al., 1989 Rico-Villa et al., 2009 Kheder et al., 2010 *C. virginica* Medcof, 1939 Davis, 1953 Davis & Calabrese, 1964 Calabrese & Davis, 1970 Rhodes & Landers, 1973 *M. edulis* Brenko & Calabrese, 1969 Hayhurst, 2001 Pechenik et al., 1990

In conjunction with the literature search on ocean acidification experiments relating to veliger growth, a separate search was conducted to identify reference length

based growth curves expected for each species under various temperature regimes. A similar strategy was employed using the citations found in the publications retained in Table 1, as well as the reference lists used by numerical modeling studies (Bochenek, Klinck, Powell & Hofmann, 2001; Dekshenieks et al., 1993; Rico-Villa, Bernard, Robert & Pouvreau, 2010) on veliger larvae as a starting point for the search. Publications were kept if they documented growth under conditions of high salinities and saturated food conditions. The final list of articles used to construct baseline growth curves is also contained in Table 1.

Reference curves were reconstructed for each species using data from the historical publications noted in Table 1, separated into temperature ranges reflecting the intervals used in the ocean acidification studies (Appendix 1). When length at age coordinates were only reported graphically, they were transcribed using Image J (Abramoff, Magalhaes & Ram, 2014). All of the data points were organized in an Excel spreadsheet and paired with the corresponding experimental conditions, authorship and species (Microsoft, 2008). The data set was divided into temperature ranges of 3 degrees Celsius, reflecting intervals found in the ocean acidification data set. Due to the lack of historical data for the temperature range of 24-26ºC for *C. virginica,* two regressions were conducted. The first regression using a wider temperature interval (22-28ºC), while the second with data generated from a numerical model (Deksheniek et al., 1993). An exponential curve was fitted to each temperature range and species combination using Matlab's curve fitting tool pack, yielding 14 growth curves (MathWorks, 2012). These curves were bound by a starting condition of 50 µm reflecting an assumption in the literature that oyster eggs have an average size of 50 µm (Dekshenieks et al., 1993; Bochenek et al., 2001; Loosanoff & Davis, 1963; Table 2). The validity of the relationship was assessed using a linear regression model on the logarithmically transformed data.

Larval length at age coordinates from the ocean acidification experiments were then transcribed, and stored in an Excel spreadsheet alongside the experimental conditions employed (Appendix 2). Where these data points were only available

graphically they were transcribed using Image J. These points were superimposed onto the corresponding species temperature baseline growth rate graphs using Matlab. The ocean acidification data clusters were analyzed using a coefficient of variation to compare the variability between the ocean acidification data to the variation predicted by the exponential regressions.

Table 2: Regression equations (±95% confidence interval and r^2) derived from data contained in baseline studies for *C. virginica*, *C. gigas* and *M. edulis* for each temperature interval in terms of larval length and days post fertilization. Summary of regression analysis performed on $\log_e$ transformed data. In addition the coefficient of variation (C.V.) for the variation in length of the superimposed ocean acidification data sets is included for the appropriate temperature intervals.

Species and Temp range	Regression equation	n	Relationship		Slope		r^2	C.V.
			F test	p value	T test	p value		
(1) *C. gigas* 15-17 ºC	Length = (53.19±4.01) x $e^{((0.06282±0.005) \times Days)}$	20	214	p<<0.05	102	p<<0.05	0.966	n/a
(2) *C. gigas* 18-20 ºC	Length = (55±10.12) x $e^{((0.06603±0.03295) \times Days)}$	12	12.4	0.005	66	p<<0.05	0.378	0.046
(3) *C. gigas* 21-23 ºC	Length = (55±13.83) x $e^{((0.08107±0.01613) \times Days)}$	17	349.6	p<<0.05	111.4	p<<0.05	0.824	0.266
(4) *C. gigas* 24-26 ºC	Length = (55±5.56) x $e^{((0.1116±0.0088) \times Days)}$	30	736	p<<0.05	125	p<<0.05	0.959	0.025
(5) *C. gigas* 27-29 ºC	Length = (55±82.1) x $e^{((0.09666±0.09904) \times Days)}$	6	17.67	0.013	22.6	p<<0.05	0.363	n/a
(6) *C. gigas* 30-32 ºC	Length = (55±13.81) x $e^{((0.1007±0.0201) \times Days)}$	28	187	p<<0.05	79	p<<0.05	0.659	0.025
(7) *C. virginica* 18-20 ºC	Length = (60±13.13) x $e^{((0.0408±0.0081) \times Days)}$	97	182.6	p<<0.05	82.27	p<<0.05	0.322	n/a
(8) *C. virginica* 21-23 ºC	Length = (60±10.64) x $e^{((0.0699±0.0092) \times Days)}$	87	327.7	p<<0.05	84.99	p<<0.05	0.692	n/a
(9) *C. virginica* 24-26 ºC	Length = (60±6.97) x $e^{((0.07838±0.00655) \times Days)}$	65	1429	p<<0.05	123.5	p<<0.05	0.921	0.126
(10) *C. virginica* 22-28 ºC	Length = (60±30.42) x $e^{((0.08667±0.04223) \times Days)}$	37	24	p<<0.05	31.4	p<<0.05	0.192	0.126
(11) *M. edulis* 5 ºC	Length = (55±24.32) x $e^{((0.03204±0.02204) \times Days)}$	17	1.57	0.22	25	p<<0.05	0	n/a
(12) *M. edulis* 10 ºC	Length = (80±16.45) x $e^{((0.02749±0.01049) \times Days)}$	17	23	p<0.05	69	p<<0.05	0.220	n/a
(13) *M. edulis* 15-16 ºC	Length = (80±20.6) x $e^{((0.04321±0.01057) \times Days)}$	43	53	p<<0.05	66.3	p<<0.05	0.118	0.003
(14) *M. edulis* 20 ºC	Length = (80±51.8) x $e^{((0.05273±0.03824) \times Days)}$	14	264.1	p<<0.05	52	p<<0.05	0.633	0.045

Table 3: A comparison of experimental conditions contained in the selected ocean acidification studies about bivalve veliger larvae for *C. gigas, C. virginica*, and *M. edulis*, sorted by species

Author (date)	Species	Temperature (°C)	Food	Salinity (psu)	Ocean acidification parameters				
					Duration (days)	pH	pCO_2 (ppm)	Ω_{ara}	Carbonate ($\mu mol\ kg^{-1}$)
Gazeau (2011)	*C. gigas*	18.9	none	34	3	8.03 - 7.41	448.7 - 2170.5	2.8 - .8	181.9 - 50.4
Parker (2010)	*C. gigas*	18 - 30	Saturated[1]	35	23	8.19 - 7.85	375 - 1000	2.78 - 1.34 3.57 - 1.81	181 - 87.93 223 - 113
Timmins-Schiffman (2012)	*C. gigas*	20.31	none	28.29	3	7.99 - 7.66	468 - 1065	2 - .95	120 - 61
Kurihara (2007)	*C. gigas*	23±.5	none	33.7	2	8.21 - 7.42	348 -2268	3 - 0.68	161.4 - 36.4
Waldbusser (2013)	*C. gigas*	25	Saturated[2]	26.5	19	8.15	396	2.38	
Miller (2009)	*C. virginica*	25	Saturated[3]	18	30	8.16 - 7.91	284-840	1.2-0.6	72.4 - 32.7
Talmage (2009)	*C. virginica*	24.5	Saturated[4]	28	20	8.07 - 7.5	355-1480	2.91-.92	180 - 58
Gazeau (2010)	*M. edulis*	19.5	Saturated[5]	31.4	15	8.03 - 7.78	642 - 1213	2.30 - 1.37	135 - 80
Gazeau (2010)	*M. edulis*	16.5	none	32	2	8.15 - 7.58	468 - 1929	2.75 - .81	166 - 49

[1] defined as 15-40 x 10^5 algae
[2] defined as 5-7 x 10^{10} cells m^{-3}
[3] defined as 1.5x 10^4 cells mL^{-1}
[4] defined as 2 x 10^4 cells mL^{-1}
[5] defined as 29.12 µmol ChlA

2.3 Results

2.3.1 Comparing Methods

The experimental conditions varied moderately between authors (Table 3) (Appendix 2), reflecting local seawater conditions. The methodologies employed to simulate anticipated pCO_2 conditions as well as test organism selection differed, but are comparable between studies. Some of the investigators reared adults in-house and then cross-fertilized them to produce the larvae used in the experiment (Gazeau et al., 2010; Gazeau et al., 2011). Others imported eggs or even young larvae from a local hatchery (Timmins-Schiffman, et al., 2012; Miller et al., 2009; Waldbusser et al., 2013). Thus out of the eight experiments analyzed here, only Gazeau et al. (2010) and Gazeau et al. (2011) controlled the genetic variation between individuals by sourcing them from the same parents. Assessing the hidden impact of genetics is difficult, since it will influence the optimal environmental conditions for growth and is beyond the scope of this study.

The benchmark pCO_2 conditions used to simulate future conditions are very similar between studies, generally using contemporary (~400 ppm) pCO_2 conditions as a control, and then establishing treatments to represent mid-century, end-of-century (~750 ppm), and beyond end-of-century conditions. Because each experiment relied on seawater local to where the experiments were conducted, the infusion of extra CO_2 resulted in different ranges of pH (7.41 - 8.21), Ω_{ara} (0.6 - 4.18), and carbonate (72.4 - 256 μmol kg^{-1}) between treatments (Table 3). This variation is due to the relative variation between dissolved inorganic carbon (DIC), and total alkalinity (TA). As the TA:DIC ratio increases, the flux of CO_2 has a weaker impact on the depletion of carbonate in the water (Wang et al., 2013).

Broadly, two methods were used to achieve the desired state for each treatment, either by continuous bubbling of CO_2, or by intermittent addition. In a continuous injection system, gas with a known concentration of CO_2 is bubbled into the test conditions and by varying the flow (Kurihara et al., 2007; Parker et al., 2010) or the concentration (Gazeau et al., 2010; Gazeau et al., 2011), the desired conditions are achieved. In the intermittent design, the recirculating water is analyzed and CO_2 is added

to maintain the desired pH (Timmins-Schiffman, et al., 2012; Miller et al., 2009; Talmage & Gobler, 2009). All studies predicted the required amount of CO_2 by analyzing the total alkalinity of the filtered seawater and maintained conditions by measuring the pH of each treatment instead of Ω_{ara} or carbonate. Waldbusser et al. (2013) did not manipulate the water conditions to mimic future conditions, but measured the ocean acidification parameters (pCO_2, pH, Carbonate) throughout the development of the *C. gigas* cohort.

From the above analysis, the effect of the different methodologies employed to construct the test conditions seems minor. Moreover the mechanism that delivers the CO_2 to these beakers is different from what is expected to occur in nature. The forced injection differs considerably from the slow dissolution expected to occur naturally, though the end product is the same. The other environmental parameters are mostly consistent between treatments (Table 3). The experimental design for all of the studies emphasized local salinities and saturated food conditions. Miller et al. (2009) explicitly tested for the impact of ocean acidification in estuarine conditions (18 psu), while the rest had higher salinities (26 - 35 psu) (Table 3). The temperatures selected for study span a wide range (12ºC for *C. gigas*, .5ºC for *C. virginica*, and 3ºC for *M. edulis*)(Table 3). The experimental duration focused either on early shell formation within the first three days (Gazeau, 2011; Timmins - Schiffman, 2012; Gazeau 2010; Kurihara, 2007), or on the entire veliger larval span (Waldbusser, 2013; Gazeau 2010; Talmage, 2009; Parker, 2010).

2.3.2 Regression Analysis

Using the data contained in the "historical" publications (Table 1)(Appendix 1), fourteen growth curves were constructed to represent veliger growth in saturated food conditions, high salinity, across three species in a range of temperatures (Table 2, Figures 1 - 9). Equations describing the growth curves include the 95% confidence interval for the growth rate as well as the initial egg size. An exponential regression was chosen based on the shape of the historical data set, as well as the behaviour of numerical models describing veliger growth (Bochenek et al., 2001; Dekshenieks et al., 1993)

Growth rate has a thermal optimum, and in *C. virginica* this is believed to be approximately 28ºC (Davis 1966; Davis & Calabrese, 1964). The reconstructed baseline curves do not fully support this thermal optimum (Table 3). In the *C. gigas* regressions, the range from 27-29ºC had a slightly lower growth rate than the cooler 24-26ºC and the warmer 30-32ºC curves (Table 3). According to Parker et al. (2010), 26ºC is the optimal temperature for survival for *C. gigas*. In the *C. virginica* regressions, the lack of data prevented a regression at or beyond the point of 28ºC, however as the temperature rises, the growth rate increases. In the *M. edulis* regressions the growth rate increased with temperature though the range of temperatures for which growth data were available.

In all cases, the exponential regressions suffered from limited datasets, though it is noted that each point included in the regression is an experimental mean. The boundary on initial egg size influenced the slope and intercept of the regressions; however, its use is justified by the commonly held assumption that oyster eggs begin at 50 µm in size (Bochenek et al., 2001; Rico-Villa et al., 2010; Loosanoff & Davis, 1963), and reach 75 µm by day 2 (Davis, 1953). Identifying the appropriate starting size for blue mussels is still difficult, and it is either the same as oysters or as large as 70 µm (Moran, 1989).

Based on the size of the confidence interval (Table 2), certain temperature intervals are lacking data for the regression (such as *C. gigas* 27-29 ºC or *C. virginica* 22-28ºC), however only one regression (*M. edulis* 5 degrees) did not have a significant relationship (Table 2). The paucity of data reduces the confidence of identifying a typical growth curve for each species in each temperature range.

2.3.3 Analyzing the Ocean Acidification Data

2.3.3.1 *C.gigas*

Figure 1 describes the regression analysis of length and time conducted for *C. gigas* for the temperature interval of 18-20 ºC. This temperature interval had sparse baseline data, and the resulting confidence interval is large, but significant ($r^2 = .378$; $p = 0.005$)(Table 2). Three ocean acidification studies are captured by this range (Gazeau et al., 2011; Parker et al. 2010 and Timmins-Schiffman et al. 2012), and

cluster primarily in the first three days (coefficient of variation = 0.046). The data points representing the different levels of pCO_2 from Parker (2010) fall very close to each other at all time points, while the Timmins (2012) data points are further apart. All fall within the confidence interval predicted by the regression. The inset in Figure 1 shows the clustering of data points in the first three days. It highlights the overlap between the historical data, the predicted mean, and the values obtained by the three studies (Figure 1).

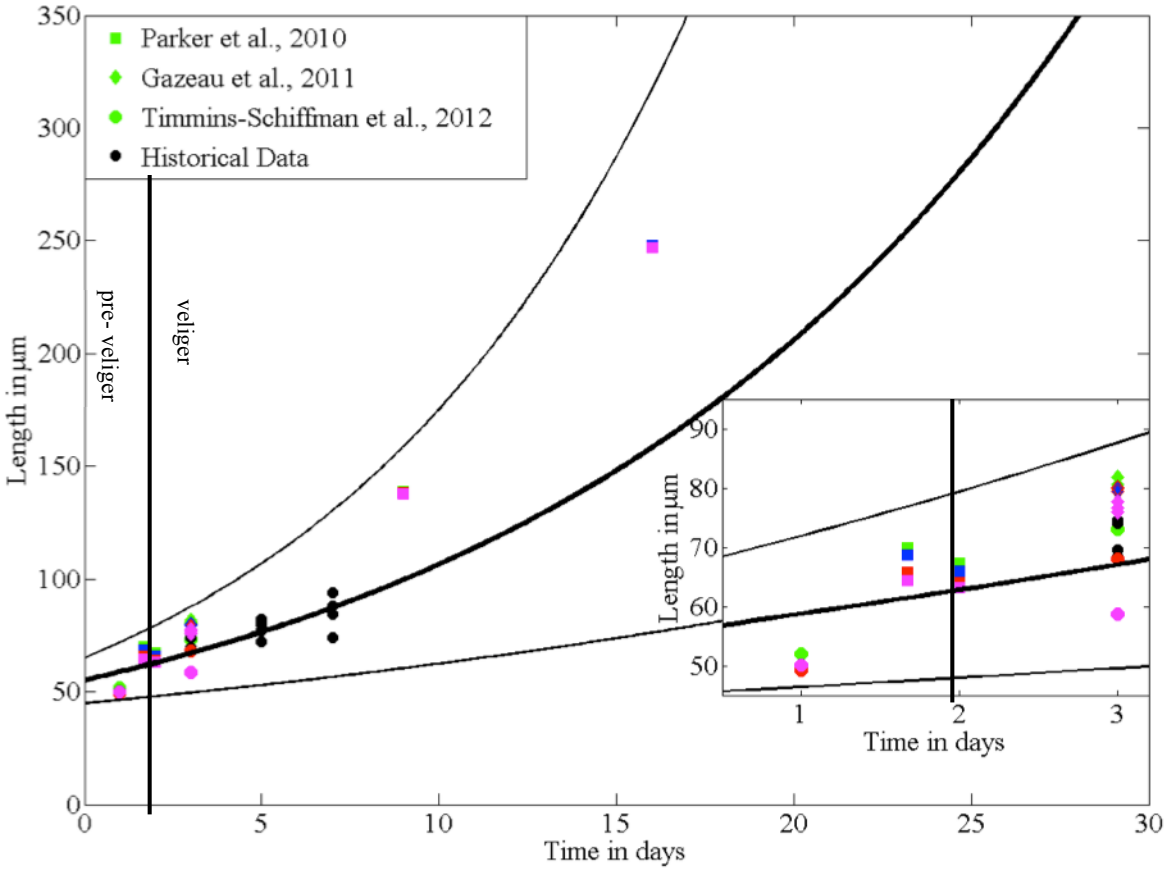

Figure 2: Exponential regression determining the growth rate of *C gigas* in the 18-20ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression (Table 1). Square points correspond to the experimental values obtained by Parker (2010). Diamonds correspond to the experimental values obtained by Gazeau (2011). Coloured circles correspond to the experimental values obtained by Timmins (2012). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

Figure 2 describes the temperature interval between 21 and 23 ºC for *C. gigas*. Data used for this regression covers the entire span of the veliger stage and is heavily influenced by two studies, (His & Seaman, 1992, and Rico-Villa et al., 2009). The confidence interval is relatively narrow, and the correlation is stronger

(r^2 = .824; p <<0.05) (Table 2). Two ocean acidification experiments are represented in this interval, Parker (2010) and Kurihara (2007) (Coefficient of variation = 0.266). The cluster of data in the first three days is highlighted in the inset of Figure 2, exposing the close grouping of the treatments used in the Parker (2010) study. Kurihara (2007) focused on the impacts on the pre-veliger stage, under current and extreme conditions. These data points fall below the regression line, but remain within the confidence limits.

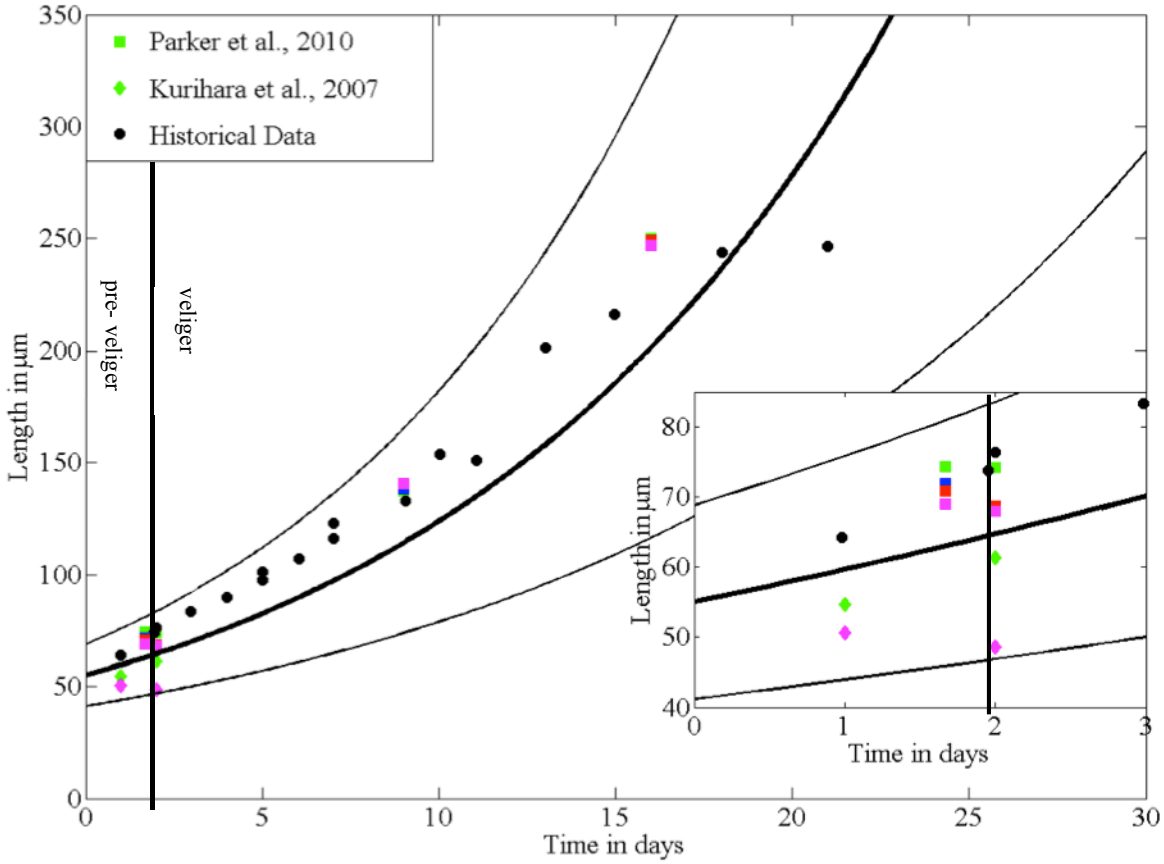

Figure 3: Exponential regression determining the growth rate of *C. gigas* in the 21-23ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression (table 1). Square points correspond to the experimental values obtained by Parker (2010). Diamonds correspond to the experimental values obtained by Kurihara (2007). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

Figure 3 describes the temperature interval between 24 and 26ºC for *C. gigas*. This regression has the narrowest confidence interval (r^2 = .959; p<<0.05; Table 2), though the historical data comes from two publications (His et al., 1989; Rico-Villa et al., 2009). This temperature interval captures two ocean acidification studies, Parker (2010)

and Waldbusser (2013) (Coefficient of variation = 0.025). As in Figures 1 and 2, the data derived from Parker (2010) cluster tightly together, however the effect of the treatment levels is more noticeable at each time point. The Parker (2010) fall closely to the regression line, with the exception of the cluster at 16 days (Figure 3).

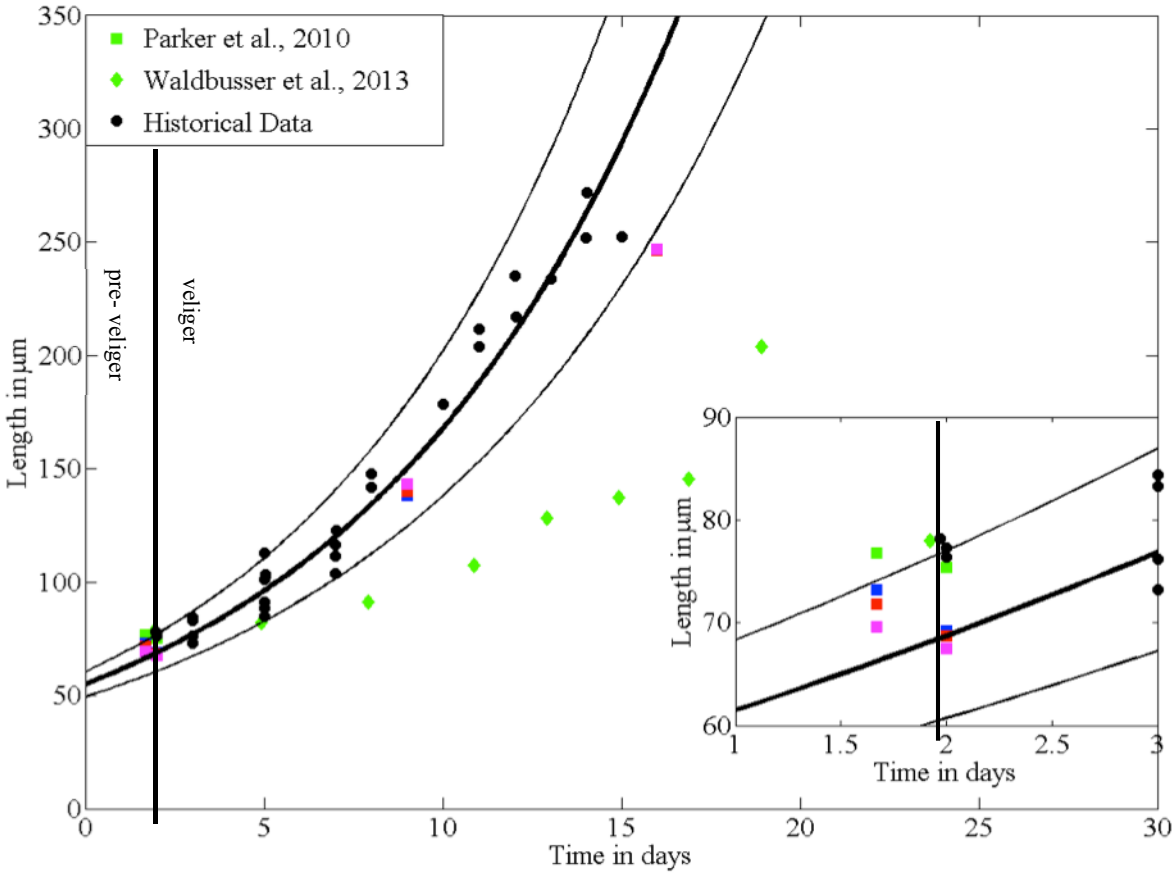

Figure 4: Exponential regression determining the growth rate of *C. gigas* in the 24-26ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression. Square points correspond to the experimental values obtained by Parker (2010). Diamonds correspond to the experimental values obtained by Waldbusser (2013). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

The Waldbusser et al. (2013) data series departs considerably from the regression line (Figure 3). There is nothing from the methodological description that can account for such a strong deviation from the regression; the temperature and food were comparable to other studies, but the salinity was not at full seawater strength. Furthermore the regression line is derived from experiments conducted in a similar time period, and therefore the change in baseline pCO_2 cannot be the cause for this deviation.

Figure 4 describes the confidence interval between 30ºC and 32ºC. One ocean acidification experiment is represented in this temperature range (Parker et al., 2010) (coefficient of variation = 0.025). The exponential regression is strong, and contains data points across the time range (r^2 = .659; p <<0.05). All ocean acidification points fall within the confidence limits of the regression analysis, and are grouped closely to each other. This cluster can be seen in the inset of Figure 4, where the treatment levels in Parker et al. 2010 exhibit a wider variation than similar conditions in cooler temperatures (Figures 1, 2 and 3).

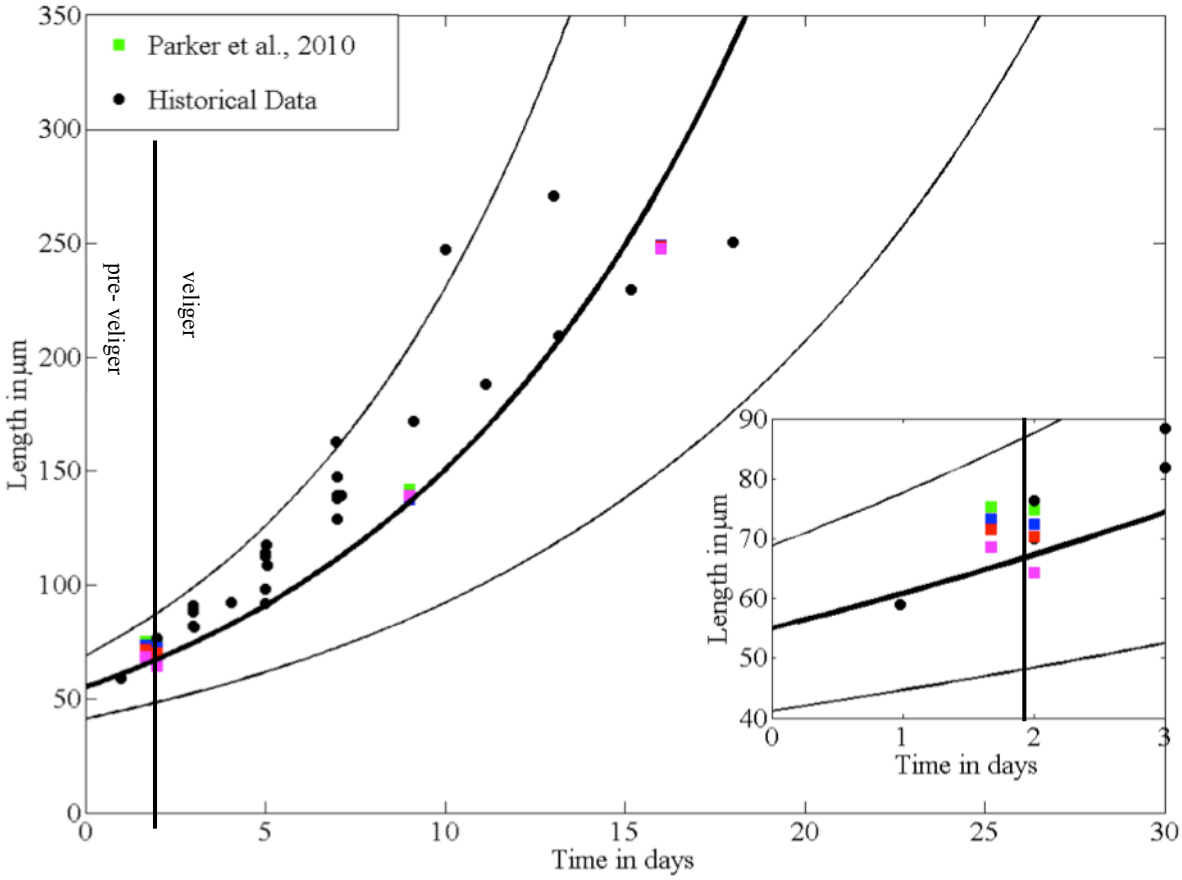

Figure 5: Exponential regression determining the growth rate of *C gigas* in the 30-32ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression. Square points correspond to the experimental values obtained by Parker (2010). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

2.3.3.2 *C.virginica*

The regression analysis for *C. virginica* spans a similar temperature range to *C. gigas* (18-28ºC), however the data were not evenly distributed across this range (Table 2). Data from ocean acidification studies were only found in the 24-26ºC, which had no corresponding historical data. Thus two regressions were constructed, one with a wider range of temperature (22-28ºC, Table 2, Figure 5), and one using data from a numerical model (Table 2, Figure 6). The narrower range yielded a stronger correlation (r^2 = .921, Figure 6), with a tight confidence interval, while the wider range of 22-28ºC has a wide confidence interval and a poorer correlation coefficient (r^2 = 0.192). Both describe a significant relationship ($p<<0.05$; Table 2)

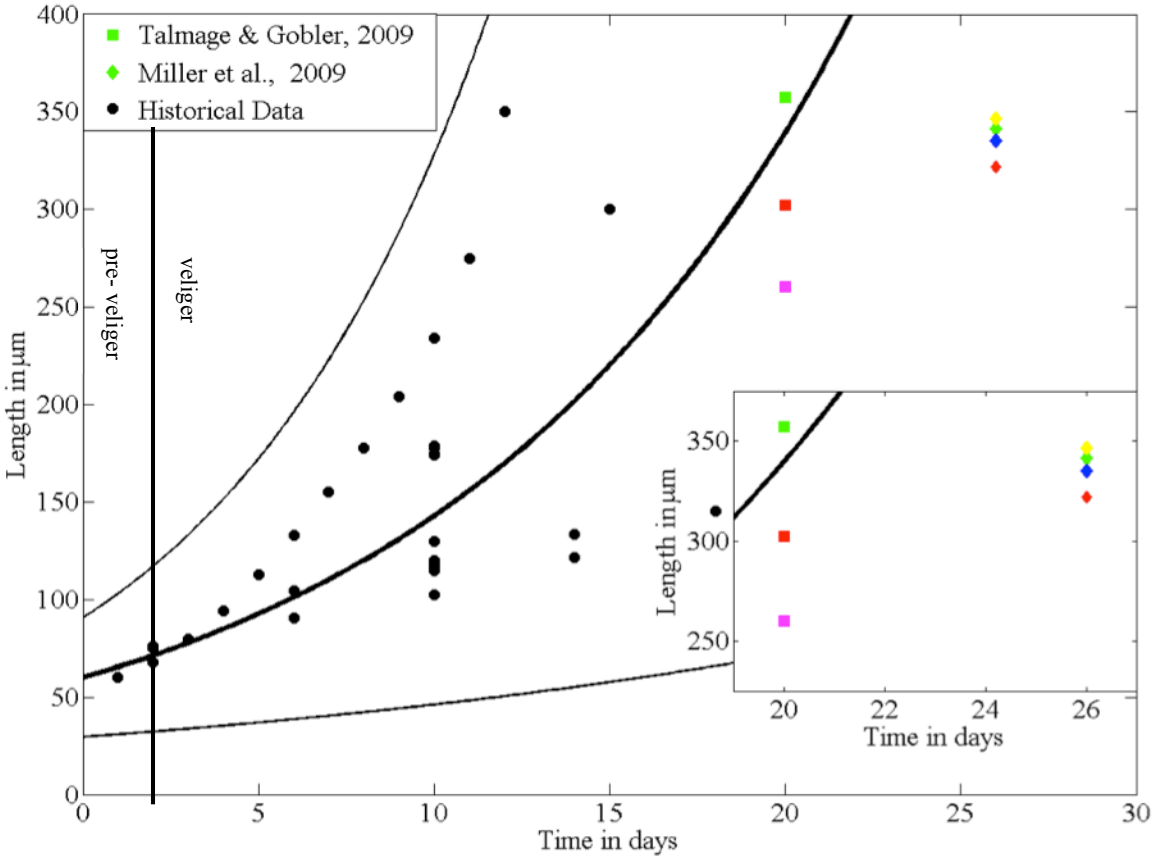

Figure 6: Exponential regression determining the growth rate of *C. virginica* in the 22-28ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression. Square points correspond to. the experimental values obtained by Talmage & Gobler (2009). Diamonds correspond to the experimental values obtained by Miller et al. (2009). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

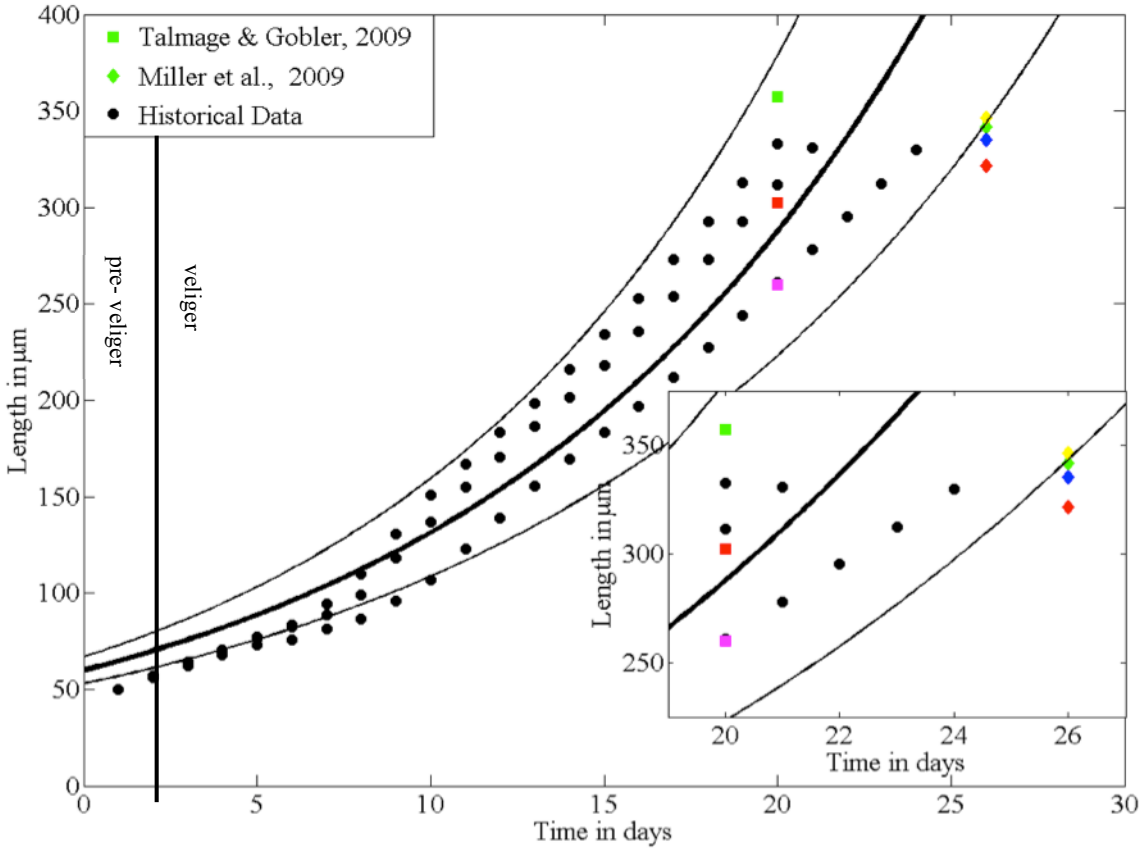

Figure 7: Exponential regression determining the growth rate of *C. virginica* in the 24-26ºC temperature interval (±95% confidence interval). Black data points represent historical data, and modeled data from Deksheniek et al. 1993 (Table 1). Square points correspond to the experimental values obtained by Talmage (2009). Diamonds correspond to the experimental values obtained by Miller (2009). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

The ocean acidification data superimposed on these regressions fall closely to the predicted mean (Figures 5 and 6) (coefficient of variation (0.126). The Talmage & Gobler (2009) data series falls within the confidence limits in both regressions. In Figure 5 the average length in current conditions lies next to the anticipated mean, however in Figure 6 it is the end-century prediction that intersects with the anticipated length. The Miller et al. (2009) data series falls partially below the lower confidence boundary in Figure 5. This reduction in growth may be attributed to the lower salinity levels used in the study (18 psu), which also reduce growth rates (Calabrese & Davis, 1970).

2.3.3.3 *M. edulis*

Figure 7 describes the regression analysis for *M.edulis* at 15-16ºC. The regression analysis is strong, and a large amount of historical data are available (r^2= .118; p<<0.05; Table 2). One ocean acidification study is represented in this temperature interval, and all data points are represented on the second day (coefficient of variation = 0.003) (Figure 8) (Gazeau et al., 2010). The ocean acidification points are under the regression mean, and the impact of the treatment levels is noticeable, though all are contained within the margins of the confidence interval.

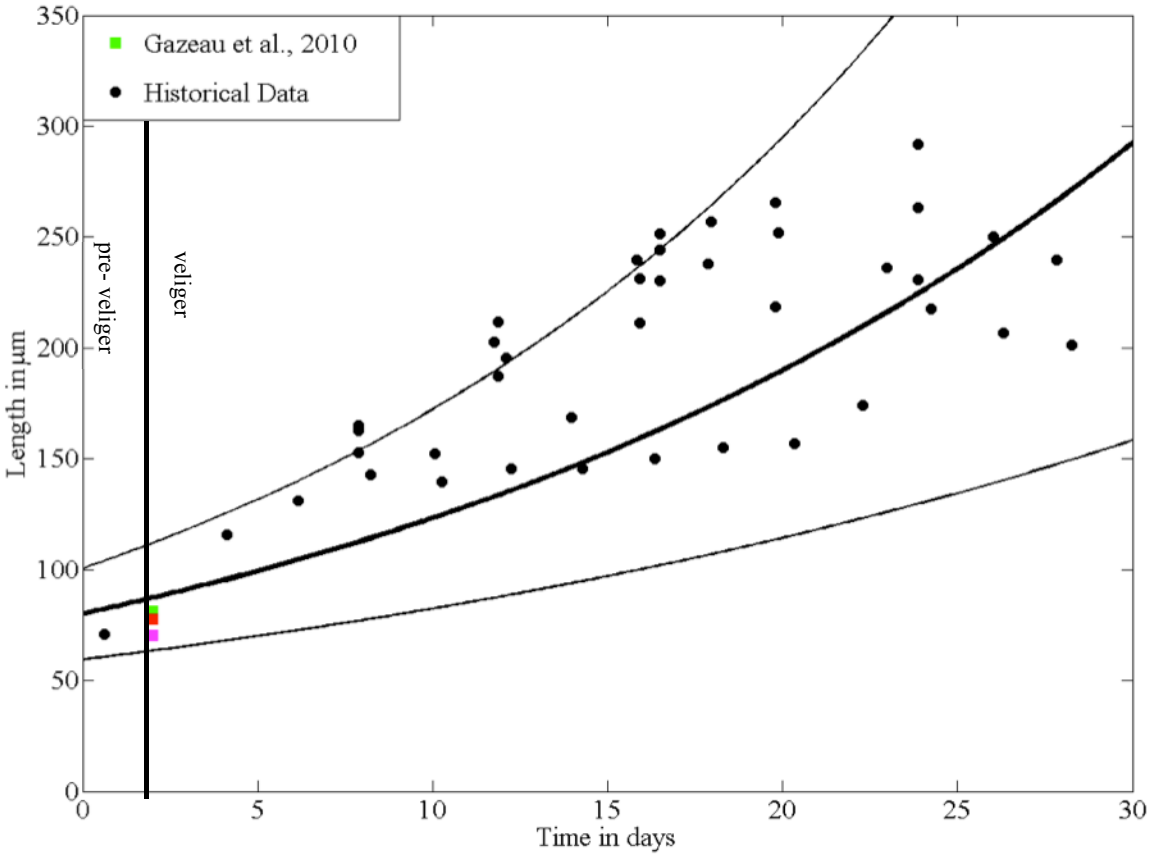

Figure 8: Exponential regression determining the growth rate of *M. edulis* in the 15-16ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression (Table 1). Square points correspond to the experimental values obtained by Gazeau (2010). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

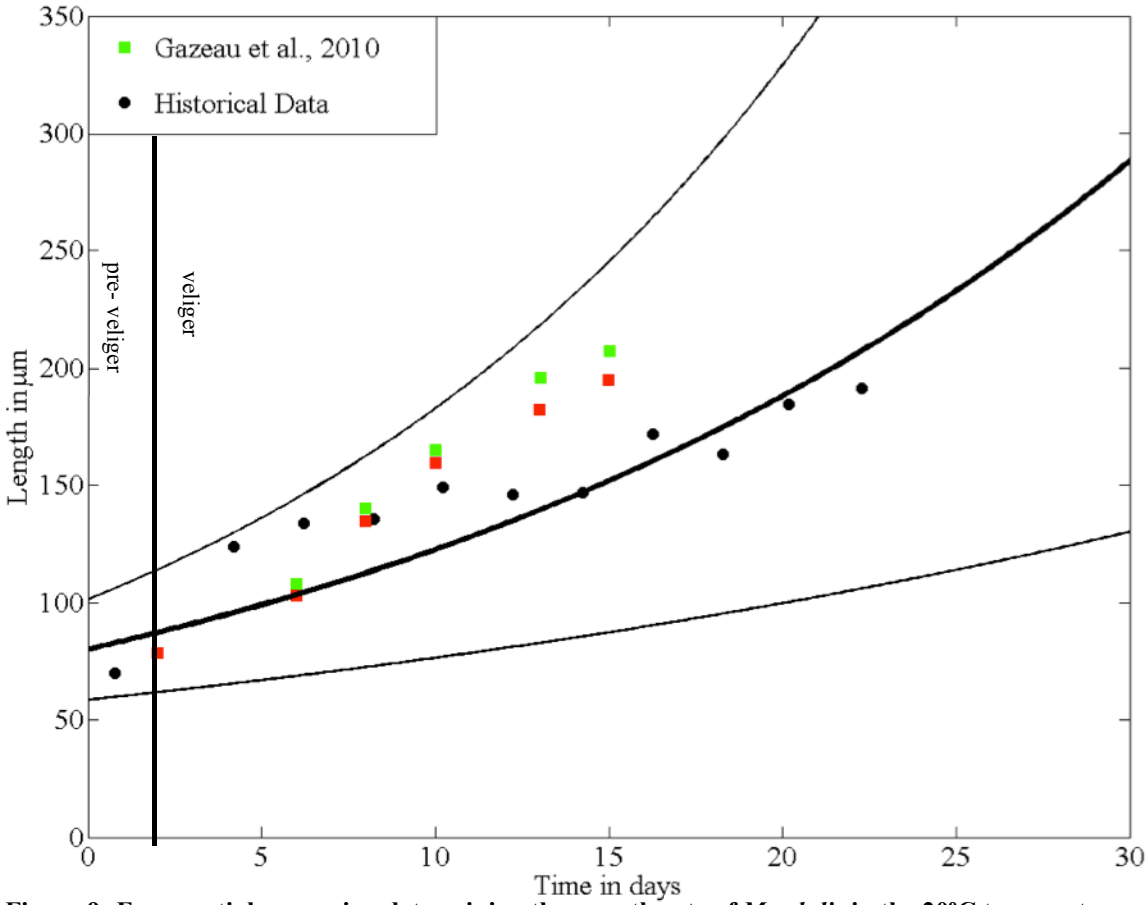

Figure 9: Exponential regression determining the growth rate of *M. edulis* in the 20ºC temperature interval (±95% confidence interval). Black data points represent historical data used in the regression (Table 1). Square points correspond to the experimental values obtained by Gazeau (2010). pCO_2 treatment level is differentiated by colour, where green corresponds to current pCO_2, blue to mid-century, red to end-of-century, and magenta to beyond end-of-century predictions.

Figure 8 describes the regression analysis for *M. edulis* at 20ºC. The regression is heavily influenced by a single data set (Hayhurst, 2001), and the confidence limits are wide with a poor correlation ($r^2 = .633$; $p << 0.05$; Table 2). A single ocean acidification study is represented in this temperature interval, and the data points fall close to the regression line with little separation between the treatment levels (coefficient of variation = 0.045) (Figure 9) (Gazeau et al., 2010).

2.3.3.4 Remaining Regressions

Figure 9 illustrates the regression analyses that did not have ocean acidification data superimposed. The *C. virginica* regression at 18-20ºC has two competing historical growth trends. The lower series is derived from a regression conducted on the Bideford

river oysters in PEI; this trajectory seems abnormally slow (Figure 9d, $r^2 = 0.322$). The other temperature intervals across the range of three species illustrate the gaps in historical data. Figure 9e describe a non-significant relationship ($r^2 = 0$; $p = 0.22$; Table 2). Some regressions are heavily influenced by a single author (Figure 9a, e, f), while others have a wealth of data characterizing average length within the respective temperature interval (Figure 9b, c, d).

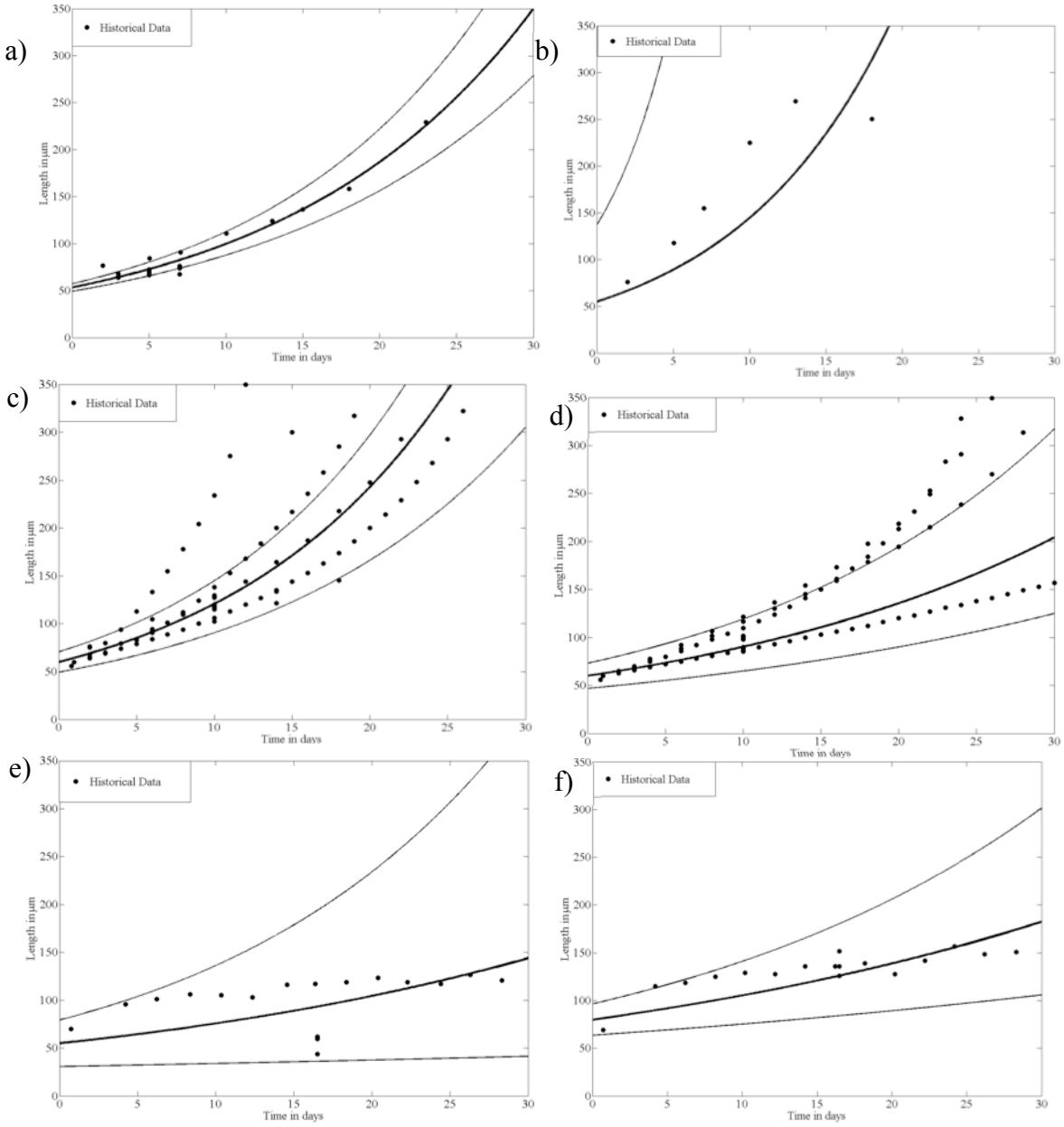

Figure 10: Growth rate regressions for *C. gigas*, *C. virginica*, and *M. edulis* with their respective historical data (Table 1). *C. gigas* 15-17ºC (a), *C. gigas* 27-29ºC (b), *C. virginica* 21-23ºC (c), *C. virginica* 18-20ºC (d), *M. edulis* 5ºC (e), and *M. edulis* 10ºC (f).

Generally, the superimposed ocean acidification experimental data points did not deviate substantially from the regression line or fall outside the boundaries of the confidence interval (Figures 1 - 8). Increasing pCO_2 did consistently yield smaller larvae at a similar age. However even the most extreme treatment levels do not deviate substantially from the average.

2.4 Discussion

Research has tested the impact of ocean acidification on bivalve veliger growth in isolation (Kurihara et al., 2007; Kurihara et al., 2008), as well as in the context of other environmental factors (Talmage & Gobler, 2012; Talmage & Gobler, 2011; Parker et al., 2010). This re-analysis of data from experiments with bivalve larvae helps provide context to the findings by including a reference growth curve based on historical data. Most of the authors concluded that the application of the acidification pressure, by simulating anticipated pCO_2 conditions, triggered an observable reduction in length for organisms of a similar age. There are a few exceptions to this general trend. Gazeau et al. (2010) indicated that there was no significant difference between treatments that were undertaken to simulate future pCO_2 conditions. *C. virginica* seemed more susceptible to ocean acidification than the other tested animals (Talmage & Gobler, 2009). This conclusion is also supported by (Miller et al., 2009), which further states that both tested species (*C. virginica and C. ariakensis*) can build shells in low carbonate conditions. Talmage & Gobler (2009) also concludes that current and mid pCO_2 treatment levels produced similar results, and only the end-of-century conditions produced a significant difference.

Based on the results from this study however, the observed influence exerted by ocean acidification is captured by the variation predicted by the temperature dependant growth regressions. Based on inferences from the historical literature (Table 1; Pechenik, 1987) the influence exerted by food, salinity, and temperature can predict a general growth trajectory for the veliger larval stage. The influence exerted by ocean acidification may explain variation within this trajectory, especially the occurrence of smaller individuals of the same age (Talmage & Gobler, 2009). This does not rule out ocean

acidification as a phenomenon of concern, but rather that shell length, i.e. growth rate, alone is an inappropriate metric for evaluating the effect of ocean acidification on bivalve veligers.

Shifting the mean larval size towards the lower boundaries would increase the frequency of smaller individuals that would perish in size dependent mortality during metamorphosis (Green et al., 2009). Mortality during metamorphosis is influenced by other factors, notably energy reserves, location, and environmental conditions (Rico-Villa, Le Coz, Mingant & Robert, 2006; Bochenek et al., 2001; Coon et al., 1990); however, length does play a role in achieving metamorphic competence (Coon et al., 1990). The reduced survival during the veliger life stage in combination with a higher frequency of small individuals within the cohort could accentuate mortality during metamorphosis to the juvenile stage (Green et al., 2009).

The results from this re-analysis also demonstrate that characterizing a typical temperature-dependant, food-saturated growth pattern for bivalve veligers is difficult. Given the potential interactive effects between environmental parameters, genetics, and hidden variation, teasing apart the influence of a single factor is difficult (Pechenik, 1987). The reference data are not evenly distributed between the temperature regimes, resulting in wide confidence intervals. Furthermore the superimposed data from the ocean acidification studies are inconsistent; the data clusters fall on either side of the regression line or straddled it. This study is aggregating data from around the world, and the genetic variation between regions is only one of many underlying factors that can influence the observed difference between results (Pechenik, 1987). In particular, *C. gigas* stands out from the three species examined. This study includes representative ocean acidification experiments from Japan, Spain, the USA and Australia, yet the source material used to generate the reference curves comes primarily from France. The geographic overlap for *C. virginica,* and *M. edulis* is stronger, but still point to a difficulty in predicting a typical temperature based growth pattern in saturated food conditions.

2.4.2 Explaining the Inconsistencies

This study relies on publicly available data to construct baseline growth curves. The oldest study included dates back to 1939 (Medcof, 1939), while the most recent study is from 2009 (Rico-Villa, Pouvreau & Robert, 2009). Aggregating these studies highlights the variability in growth of the veliger larval stage for the three species. Stafford (1913) describes American oyster larvae appearing over a period of two months in Atlantic Canadian waters, and estimates an average of a six-week growing period from smallest larvae to setting size. In 1939, Medcof (1939) re-examined this benchmark in the context of temperature. Based on observations over a two-month period, he determined the larvae were reaching a setting size (>300 μm) in 28 days at 19ºC, and 23 days at 21ºC. Davis & Calabrese (1964) describes a much slower benchmark of at least 36 days at 20ºC in experiments conducted in Milford, Connecticut, USA. The fastest setting time was achieved at 30 - 32.5ºC, within an astounding 10 days post fertilization (Davis & Calabrese, 1964). This difference could be attributed to genetic variation between populations native to these regions, but it is nevertheless a big difference. Interestingly the three week timeframe from spawn to set is at 25ºC, suggesting that perhaps the selection for the growth rate may be in relation to a 3-4 week time period in the water column and is therefore not about maximizing speed. For *C. gigas,* Rico-Villa et al. (2009) describes the effects of temperature on the larval development of a population in Brittany, France. In this case, 27ºC and 32ºC yield the fastest rate, with a larval lifespan of 13 days (Rico-Villa et al., 2009). For *M. edulis*, the benchmark was harder to find, although Hayhurst (2001) does provide a few growth curves from 5ºC to 20ºC, and settled in 22 - 30 days for the 15ºC and 20ºC trials.

This empirical benchmark from spawn to set in three to four weeks may hold in local conditions, but there is considerable variation within a population. Even in the controlled conditions used by Davis & Calabrese (1964), oyster larvae still exhibited great variation in growth rates, where some larvae were growing twice as fast as other members of the same cohort. This variation likely explains the difficulty in obtaining a consistent standard. The regression analyses performed in this study highlight the wide range of data available to inform this baseline growth rate (see Table 2).

Many ocean acidification experiments on veligers have focused on the impacts in the first three days; in this study, data from four such studies are included. This time period is certainly critical to veliger development, however, its brevity limits the conclusions drawn from these studies. During this time frame, embryos will develop, complete the trochophore stage and initiate the shell formation associated with the onset of the veliger (Stafford, 1913; Loosanoff & Davis, 1963). These four studies (Table 1) focused on this initial shell genesis, and reduced the complexity of the environmental setup. The species used do not feed most this time, eliminating a potentially confounding variable (Waldbusser et al., 2013). The embryo and trochophore stages are not affected by extreme pCO_2 conditions, however the successful transition to the veliger stage is affected (Kurihara et al., 2007; Kurihara et al., 2008). Amongst all the data concerning the impact of ocean acidification in the first three days of development, the variation in observed length is very small. Based on the historical data, the scope for variation in the first two days is minimal, and most of the observed variation caused by different feeding, temperature, or salinity regimes occurs after the organisms begin to feed (Davis, 1953; Rico-Villa et al., 2009).

Results obtained in experiments beyond the first few days may hide the potential range of sizes at each treatment level. Only Talmage et al. (2009) track survival continuously during the experiment in addition to length and developmental stage. The projected conditions may be selecting out the individuals with slower growth rates, thus reducing the observed effect. Indeed most of the studies had tight clusters of data at each time point (Figures 1 - 8), and without information on mortality at each stage it is impossible to assess this claim of directional selection. Based on the results of the current study, the net effect of ocean acidification appears to be minor within the broader context of temperature based growth.

Making sense of this result requires an examination of the mechanism that could affect the growth of these organisms. Gazeau et al. (2011) and Waldbusser et al. (2013) identify two elements of this mechanism, thereby improving our understanding of the link between ocean acidification and the growth of veliger larvae. Gazeau et al. (2011)

tested impacts of ocean acidification in terms of shell length on the Pacific oyster; in addition to the routine current, mid-century and end-century predictions with all other elements of seawater chemistry held constant, the research team includes two additional trials that modify total alkalinity in addition to the net pH decrease. Their results demonstrate that the concentration of carbonate is a much stronger predictor of growth, a factor that is incorporated into the calculation of Ω, and is influenced by the pCO_2 (Gazeau et al., 2011). Because carbonate is a primary building block in shell construction, limiting its concentration would directly impede growth. It is therefore not surprising that the ambient concentration of carbonate yields the strongest relationship with observed growth (Gazeau et al., 2011). Unlike temperature, this relationship seems to lack an optimal concentration for growth, and has a lower threshold to achieve the maximum growth rate.

Waldbusser et al. (2013) provides a complementary conclusion by tracking the energy demand for shell building during the veliger lifespan. Calcification rates are not continuous, but exhibit two major surges corresponding to the formation of the early shell layer at the D-hinge stage, and again prior to metamorphosis (Waldbusser et al., 2013). Their study traces the energy source used to build the shell during the first two weeks by tracking carbon isotopes. It concludes that the isotopic composition of the shell in the first six days is dominated by the carbon signature found within the energy reserves of the egg and are not derived from the ingested food (Waldbusser et al., 2013). This implies that the ingested food during this timeframe is fuelling other developmental needs, and the energy needed to build this initial shell must be primarily sourced from the maternal investment in the egg. If the maternal investment doesn't provide enough energy, and the organism prioritizes shell construction, then depleting the carbonate pool could cause the energetic needs to exceed the budget provided, and the individual's survival to be compromised (Waldbusser et al., 2013).

2.4.3 Implications for Future Experimental Work on Ocean Acidification

One of the critical hurdles for future research is to capture properly the impact of ocean acidification over the entire lifespan of an organism (Ginger et al., 2013). The

results of this study temper the conclusions drawn from experiments on veliger larvae, and underscore two main conclusions. First, that veliger larvae have a wide variation in observed growth rate, making it difficult to establish a typical rate from which future conditions will deviate. Second, that in light of this variable growth, the magnitude of the impact of ocean acidification on the size (i.e. length) of veliger larvae is minimal and will probably not become a determining factor. Impacts on survival and cascading impacts on other life stages of the three bivalves were not examined.

The tone and content of the ocean acidification discussion has dampened since the earliest publication in 2006 (Gazeau et al., 2013). Older experiments demonstrated a significant effect in conditions that would only be reached in two to three centuries time under a business as usual emissions scenario. Such a long timeframe makes it difficult to grasp the impact of ocean acidification, given that other prime determinates of growth, such as temperature, food, and genetics, may also change considerably. More recent publications have focused on implications of emission projections within the next century, and their conclusions are tempered with greater uncertainty. Mid-century projections do not produce a distinctive pattern, and even the blue mussel experiments conducted by Gazeau et al. (2010) did not produce significant differences across the entire range of pCO_2 for the century (Miller et al., 2009; Gazeau et al., 2010; Parker et al., 2010). This could be attributed to the natural variability already occurring in the near shore habitats where these creatures are found (Waldbusser & Salisbury, 2014). In the pelagic system, a similar variation in pCO_2 will occur over much wider spaces and depths (Shadwick et al., 2010).

In the aftermath of the first studies demonstrating a severe impact on growth and fitness on marine larvae under extreme conditions (two the three times beyond end of century projections; Kurihara et al., 2007; Kurihara et al., 2008), more recent work has incorporated other environmental conditions (Talmage & Gobler, 2012; Talmage & Gobler, 2011; Parker et al., 2010). The focus has shifted to observing the impacts of ocean acidification in the context of other growth factors such as temperature, salinity, or food (Talmage & Gobler, 2012; Talmage & Gobler, 2011; Parker et al., 2010).

Investigating the joint or combined effects of ocean acidification and other variables, as discussed above, is of prime importance. Parker et al. (2010) noted that *C. gigas* had the strongest response when combining excessively warm conditions with elevated pCO_2. Based on this re-analysis ocean acidification acting on its own will only produce a negligible impact on length, and yield observations that would still be considered normal based on the temperature in which these individuals are grown. Testing for interactive effects would be the logical next step in research, to push the conclusions found within the ocean acidification literature. In particular more experiments like Parker et al. (2010) could help identify an interaction between ocean acidification and environmental factors.

2.5 Conclusions

The wider literature on the topic of ocean acidification highlights the variable biological response to this phenomenon across different taxa and lifestages (Ross et al., 2011). Based on the results from this study, reducing the impact of ocean acidification to a single metric will likely obscure the true effect of this phenomenon. Echinoderms, crustaceans, corals and molluscs are all displaying different responses to the same pressure (Doney et al., 2009), and the survival rate variable will play a determining role in the dominance of certain species within our future oceans. Making a sound prediction is still difficult because of the complexity of combining the different biological responses. Moreover this exercise is compounded by uncertainty in predicting how chemical and physical factors including temperature, aragonite saturation horizons, and atmospheric pCO_2 will change (Ross et al., 2011; Ries et al., 2009). Presently, oceanographic work on ocean acidification emphasizes the spatial distribution of carbonate/aragonite/Ω/pCO_2/pH in the ocean (Wang et al., 2013; Sabine et al., 2004; Wallace, 2014; Hauri et al., 2013). Biological research on the other hand emphasizes the temporal nature of the phenomenon, including impacts on the different stages of the early life cycle, and long term impacts on populations and ecosystems (Kurihara et al., 2007; Talmage & Gobler, 2009; Parker et al., 2010; Gazeau et al., 2013; Waldbusser et al., 2013). In the coming decade, clarifying these uncertainties and aligning the perspectives of oceanography, biology and management will be critical to enabling society to adapt to the changes caused by ocean acidification.

Chapter 3. Further Analysis

3.1 The Appropriate Indicator

Four metrics can be used to characterize ocean acidification (pH, pCO_2, Ω_{ara}, and carbonate). Each has value in representing the effect of ocean acidification, but together they can confuse the discussion about the impacts of the phenomenon. During the process of conducting the primary research outlined in Chapter 2, a lot of effort was put in to understanding the relationship of these four indicators. Although they are all valid in terms of describing ocean acidification, they do not necessarily pair well with the biological processes that are or may be affected by the phenomenon. Thus, identifying an appropriate and accepted indicator is key to connecting the findings in oceanography to the ones in biology. In addition, the biological metric of growth (i.e. length) is also imperfect, some of the flaws in its use are shown below.

3.1.1 pH

Amongst the four metrics of ocean acidification, pH is surely the most universal. Its value and scale are already widely used in society, and it is a very simple and effective means to measure ocean acidification. Ocean acidification studies examining biological impacts tend to report the water chemistry in different forms, but pH is the most frequently included and the simplest to compare between studies (Gazeau et al., 2013). However there is more to ocean acidification than a simple increase in hydrogen ions within the water column.

Work on the pH tolerance of *C. virginica* is over 40 years old and identifies a very strong resistance to a singular decrease in pH (Calabrese & Davis, 1970). In the ocean acidification scenario, the same animals are suddenly more sensitive to minor declines in pH. The perturbation to the carbonate buffer system through ocean acidification magnifies the impact of a decline in pH, as the addition of CO_2 depletes the carbonate pool to a much greater extent than the addition of a comparable amount of another acid (Hönsich et al., 2012; The Royal Society, 2005)**.** Therefore pH is a useful metric of the context for these conditions and is the most comparable between experiments, but it

doesn't properly reflect the biological responses that may be triggered by ocean acidification.

3.1.2 pCO_2

pCO_2 is a metric used to evaluate the partial pressure of carbon dioxide in the atmosphere. Given Henry's Law, this value also expresses the proportion of CO_2 dissolved in the water column to that in air. Since predictions about climate change involve an estimate of the amount of atmospheric CO_2 expected in coming centuries, this measure is very useful for relating ocean acidification to climate change and underscores the importance of this change. It brings ocean acidification into the dimension of climate science and facilitates the comparison of impacts around the world (IPCC, 2000, IPCC, 2013).

Paradoxically, pCO_2 doesn't correlate well with biological response, and makes comparisons between regions difficult. Because of the variation in water chemistry, a similar pressure translates into different magnitudes of change of Ω, pH and carbonate; thus different biological responses. Based on Table 3, the benchmark pCO_2 was very similar, employing a current condition (~400 ppm), mid-century (~500-600ppm) and an end-of-century (~700 ppm). Yet because of the difference in species, local alkalinity, and local temperature, the observed response was different. Like pH, pCO_2 is only indirectly tied to the biological consequences of ocean acidification and similar pressures can yield markedly different results, given subtle differences in the experimental or ambient water chemistry.

3.1.3 Ω_{ara} or Ω_{cal}

Much of the literature concerning ocean acidification tries to explain the calcium carbonate saturation state known as Ω. It is an index, influenced by the amount of calcium and carbonate, but also local temperature, salinity and atmospheric pressure (Doney et al. 2009). To complicate matters there are many forms of calcium carbonate: the two most common being aragonite and calcite (using Ω_{ara} and Ω_{cal} as their symbols in the literature). The value of Ω is that it reflects local conditions, and has been used to help

connect physical conditions to biological potential in the form of shell or coral building (Gruber, 2011; CBD, 2009). It is important to highlight that Ω only indicates thermodynamic stability. From a purely chemical standpoint, in waters with a value greater than one, an aragonite shell will remain stable, but in waters with a value less than one, it will begin to dissolve.

There is some value in using Ω to translate the chemical change to a biological response. Both growth and weight are dependent on shell formation which can be limited when conditions are relatively under-saturated. Unlike temperature or salinity, there does not appear to be an optimal Ω level, but rather a minimal threshold that must be reached in order to achieve normal development (Ries et al., 2009). Even in super-saturated conditions (Ω > 3 or 4) calcium carbonate precipitates very slowly, and it is a biological intermediary that greatly accelerates mineral formation (McConnaughey & Gillikin, 2008). For this reason Ω is not an ideal prediction of the biological response because species will respond differently to a similar value of Ω. Some creatures can successfully build and maintain a shell in unstable conditions (Ω < 1), while others exhibit a stress response in supersaturated conditions (Ω = 3) (Wanninkhof, 2013; Miller et al., 2009). Given the current difficulty in understanding how shellfish build their shells, Ω_{ara} has a mixed ability to predict shellfish growth. This concept is grounded in chemistry, and though it is a better indicator of biological performance than pH or pCO_2, it still doesn't correlate as well as carbonate.

3.1.4 Carbonate

Carbonate is one of the three primary constituents of the carbonate buffer system in the ocean. However it (along with calcium) is the main building block in calcite and aragonite. Conceptually this makes the measure of carbonate a very valuable metric; ultimately this is the raw material that is being used by organisms to construct these structures. The conclusion from Gazeau (2010) exposes how the abundance of carbonate seems to drive veliger performance and not Ω or alkalinity. Low carbonate levels could impact the veliger in two ways. First, the under-saturated conditions (Ω <1) would cause the shell to actively precipitate. In this scenario, the organism cannot compensate for the

thermodynamic instability associated with low carbonate in the external environment, and cannot deposit shell fast enough to compensate for the erosion caused by the external environment. In the second scenario, the biological mechanism for shell deposition is handicapped, where carbonate is the limiting factor. The reduction in ambient carbonate would require the organism to increase the concentrating mechanisms to maintain a normal shell construction speed. It is not clear which mechanism is acting on or within molluscs, but at this point both are possible results of low carbonate during the development of these bivalves. From a biological standpoint, carbonate is the best indicator of ocean acidification. Converting Ω, pCO_2 or pH values to their corresponding carbonate values helps explain the inconsistencies in biological response. The downside is that carbonate is inconvenient to measure directly and requires the calculation of the other parameters.

3.1.5 The Appropriate Response Metric for Biology

Despite the debate on how best to represent the effect of ocean acidification, it is clear that various processes are occurring in the water. In order to link an oceanographic change with a biological one, a consensus must be reached not only on what metric is used to evaluate the change in chemistry, but also a metric used to evaluate the biological response. So far, many quantitative and qualitative observations have been made about the consequence of ocean acidification. With respect to veliger larvae, the most commonly reported quantitative metrics are changes in survival and changes in mean length after a specific time (Talmage & Gobler, 2009).

There is a serious problem with evaluating the impact of ocean acidification in terms of reduced length. The conceptual effect of dissolution affects the surface area on a three-dimensional shape, which is poorly represented by length. Growth in the larval stages of bivalves is fairly isometric. Therefore a proportional reduction in surface area will translate to a reduction in length, although this equivalence may be uneven. Given the plasticity of growth in some adults, this metric may be even harder to account for in later life stages. Especially since somatic and shell growth are influenced by many environmental factors in addition to ocean acidification (Calabrese & Davis, 1970).

Based on this study, and the literature, ocean acidification should have secondary influence to the other factors on observed growth (Davis & Calabrese, 1964). Length is a simple and convenient measurement, but can be inconsistent. Certain experiments inferred length based on the retention of individuals across a sifting mesh instead of directly measuring it (Talmage & Gobler, 2011).

Ocean acidification has many documented qualitative effects including reduced shell thickness and morphological defects during the larval stage (Talmage & Gobler, 2010; Green et al., 2009; Kurihara et al., 2008; Ginger et al., 2013). The effects must have consequence on the mortality at the veliger stage, as well as the long-term fitness of the entire life history. However, determining how a change in carbonate chemistry may cause death or increase the risk of death of an individual larva is still unclear. There are many potential reasons but, until the mechanisms behind shell formation in veliger larvae are better understood, this will remain a mystery. Thinner shells have great consequence during settling and can mean the difference between life and death. The results of the present study suggests that such secondary effects may be where the true impact of ocean acidification lies. For instance, the findings from Waldbusser et al. (2013) imply that low levels of carbonate could compromise the energy budget of the individual. Although the organism will maintain growth at a normal pace, it will come at the cost of depleted reserves and bring the risk of being unable to metamorphose successfully to the adult form.

3.2 Addressing Vulnerability and Ocean Acidification

Ocean acidification is being noticed around the world, with international bodies such as the Intergovernmental Panel on Climate Change (IPCC) and the Convention on Biological Diversity (CBD), and others publishing important summaries on the topic (IPCC, 2007; CBD, 2009). In the process, these organizations have condensed the wide array of information on the topic, while presenting the problem in a more generalized and accessible manner. The motivation is to present the information in a manner that can facilitate a response by individuals and organizations at any scale; so far it has been effective in raising awareness on the issue. The tone of these publications is often grave;

based on the description of the phenomenon in Chapter 1, the tone appears warranted. There are many recent references to ocean acidification in the public media; each is accompanied by alarming headlines (Abbot, 2011; Hume, 2014; Acidic ocean, 2014). However, these stories tend to identify singular instances in the broader narrative of ocean acidification. Identifying how this phenomenon will manifest itself in the future, while also assessing the vulnerability of local industries is still a challenge; especially at a local and regional level.

The concept of vulnerability is useful to enable the dissemination of information and insight regarding ocean acidification to the wider public. Vulnerability is a perception of how resistant the current state of being is to change, either generally or in response to a defined threat (Adger, 2006). It can be quantified, or remain as a quality attributed to the being in question. Although it is conceptualized differently to address each specific scenario, the fundamental approach is still universal (Adger, 2006). Aligning the local conditions with the general trends to understand specific vulnerabilities enables, an appropriate response to ocean acidification.

3.2.1 Sources of Vulnerability

The ocean acidification literature contains contributions from many disciplines of which three in particular are of importance to this study. The findings from oceanography, biology and resource management are critical to understand the vulnerability of an industry like aquaculture in Atlantic Canada. Unfortunately aligning the perspectives and approaches of these three disciplines (and others) is a challenge for effective policy. The core of this study is devoted to an analysis aimed at comparing different experiments within one subfield of biology, and a similar exercise would need to be employed to align these different perspectives.

Each discipline provides an important perspective to the study of ocean acidification. For instance, the carbon inventory maps produced by oceanographers underscore the heterogeneous nature of carbonate chemistry and they are crucial to explaining the local differences in aragonite saturation and pH (Wang et al., 2013; Feely

et al., 2008; Sabine et al., 2004; 322). In contrast, work on the biological dimension has a strong temporal emphasis. The studies considered in Chapter 2 investigate the impact of projected atmospheric CO_2, honing in on how organisms will react at different moments in their larval development based on manipulating current local conditions (See Tables 1 and 3)(Kurihara et al., 2007; Gazeau et al, 2010; Talmage & Gobler 2009).

There are two major stumbling blocks that can bias the dissemination of information about ocean acidification. First is underplaying the local oceanographic context when reporting the anticipated changes, while the second is failing to distinguish influencing factors such as food and genetics, which are often controlled in the experiments. Based on how this information is typically integrated in the literature, the spatially resolved oceanographic conditions are used as a background to the biological projections on ocean acidification. This is the starting point for decision makers to anticipate the consequence of ocean acidification.

The first major convergence between biology, oceanography and industry occurred with the release of the Washington State Blue Ribbon Panel on Ocean Acidification (Washington State, 2012). The story from the Pacific Coast of North America was shared using the term Canary in Coal Mine (Abbot, 2011) especially when referring to shellfish based industries. Within Canadian waters, shellfish industries on Vancouver Island have also attributed losses to ocean acidification (Hume, 2014; Acidic ocean, 2014). This link isn't entirely false, but the oceanographic context of the Pacific coast of North America is quite different from the Atlantic coast or other regions. The Pacific coast is home to a large upwelling system, where water depleted of its carbonate is brought to the surface along the coast from California to British Columbia (Feely et al., 2008; Hauri et al., 2013). This system contrasts with the Atlantic coast where the influence of deep water rising to the surface isn't as strong (Shadwick et al., 2010). Waters in this region will experience stronger impacts from river runoff and atmospheric deposition (Salisbury et al., 2008; Shadwick et al., 2010). The sudden spikes in under-saturated water occur through different mechanisms; the timing and the response may differ between the regions. Furthermore, a permanent under-saturation caused by

atmospheric deposition may take a century to manifest itself and these stochastic impediments are only a precursor to what could be a permanent change.

The experiments on the biological response to ocean acidification have kept a few key variables under control, most notably genetic makeup. Although it is clear that the response to ocean acidification will be species-specific, the role of genetics within sub populations still has not been properly addressed. There are two important considerations to make on the long-term implications of genetics on ocean acidification. First, selecting for fast growth rates in an aquaculture setting also improves the resistance of those animals to ocean acidification, purely in terms of impaired growth rates (Ross et al., 2011). Second, certain populations or individuals within populations are likely to possess a set of traits that will be positively selected for over time, ultimately mitigating the magnitude of the impact (Ross et al., 2011). Ocean acidification may be a strong selection pressure. Nevertheless, the impact on the aquaculture and shellfish harvesting industries may go unnoticed, given that they may already have resistant sub-populations.

The research conducted so far has not controlled for regional variation. Although the same species was used in many of the experiments reported in Chapter 2, the regional variation can potentially explain some of the differences in the observed results. There is a possibility that microevolution can keep pace with the projected changes. Phenotypic plasticity, genetic variation, combined with generation time will play a major role in determining the adaptive capacity of these species facing the effects of climate change (Berteaux, Réale, McAdam & Boutin, 2004). The three species of concern have advantageous characteristics, being broadly distributed, exhibiting a wide tolerance for different environmental conditions across all populations, and a fast generation time. Given that the pressure of ocean acidification will unfold slowly over the course of a few centuries, the net effect of ocean acidification may be mitigated.

3.2.2 Efforts to Counter Vulnerability.

Preparing a science-based response can require multiple approaches. In the case of ocean acidification, government reports are an important tool, acknowledging the need to

identify and assess the vulnerability of local ecosystems and human activity. Along with non-government reports they further understanding of how the complex spectrum of observations and theories on ocean acidification may be distilled into a coherent response by society.

The most impressive government report to date on ocean acidification comes from the Washington State Blue ribbon panel (Washington State, 2012). In the aftermath of catastrophic upwelling events killing entire cohorts of veliger larvae that were ready to settle, the local shellfish industry in Washington State was seriously harmed. This report provides a succinct summary of ocean acidification, explores how the phenomenon has harmed, and suggests how the government and industry should respond (Washington State, 2012). While the content is specific to the region, the process used by Washington State to determine this content should be noted as a model for other jurisdictions. By bringing together interested parties and experts, Washington State managed to match its local industry, biology and oceanographic conditions to the broader understanding of ocean acidification. The response was manifested in a commission studying the phenomenon, as well as a directed response from the state government (Washington State, 2012). This included direct investment into ocean acidification monitoring, partnering science with the aquaculture industry, and coordinating the different agencies to address this problem in unison (Washington State, 2012). Nothing similar has been accomplished in other similar jurisdictions.

Another example of a strong policy document that provides clarity on the topic of ocean acidification comes from the IPCC (IPCC, 2007). The language employed echoes the variability and uncertainty outlined earlier. However, it cuts through this uncertainty to provide recommendations to support a policy response. Critically, it underscores the potential interactive effects of ocean acidification with other processes caused by human activity (like pollution and resource extraction) or by climate change (such as rising water temperatures; IPCC, 2007). This report cites a high confidence level that molluscs are one of the most sensitive group of species to ocean acidification due to the emerging evidence about ocean acidification on all aspects of their life cycle (IPCC, 2007). This

conclusion is not necessarily contrary to the findings in Chapter 2, which examined the relative impact of a single life stage of three widely distributed species within the much larger phylum. It must, however, be stressed that commercially important species may already be robust in response to changes caused by ocean acidification if this study's results are verified.

Another important point is the possible synergy between ocean warming and ocean acidification (Parker, 2010; Gruber, 2011). The analysis in Chapter 2 did not greatly exceed the thermal optimum, and is therefore is unable to identify potential combined effects between rising temperatures and ocean acidification. It is expected that environmental conditions that greatly surpass this thermal optimum, while also having low carbonate, will exacerbate the impact on growth rate and survival to a much greater extent than either factor operating on their own (IPCC, 2007).

In summary, it is possible to customize information about ocean acidification to meet a local region's need for useful data. It must be remembered, however, that the specific conditions in which the information was produced is critically important to any appreciation of how ocean acidification may affect another region.

Chapter 4. Conclusion

Two major conclusions stem from the core content of this study. First, identifying a typical or baseline growth rate for bivalve veligers is difficult. The range of studies is limited, and the confidence limits are wide. Moreover, the variability is enhanced by the number of factors known to control growth, which in turn increases the confidence limits. Second, when superimposing the projected growth of bivalve veligers in acidified conditions onto these confidence intervals, few of the points breach the confidence limits. Although the pattern between the data points is consistent (an increase in pressure yields a decrease in length), the broader alignment falls well within the confidence zone. This suggests that, even in the worst-case scenario, ocean acidification may not yield growth rates that are unusual by today's standards.

Based on the evidence reviewed in this thesis, ocean acidification will either have a minimal or negligible impact on veliger growth rates. Based on the current literature the adults should also be resistant to the conditions expected during this century. Attention, however, must be paid to the qualitative impacts, including changes to shell quality, which will affect survival. More work must be done to clarify how impacts on the quality of shell construction will affect individual survival, and populations as a whole. Given the importance and variability of temperature, genetics, food, and salinity, the industry may not observe a uniform reduction in average length or mortality rate in an acidified future. However, this phenomenon may accentuate stochastic patterns within the population. For instance, development may be delayed because of insufficient carbonate during the planktonic phase, or because of an increased energetic requirement for shell construction and maintenance. This effect could combine with reduced food abundance or with poor conditions for metamorphosis, to negatively impact population recruitment.

Finally, growing conditions in our coastal oceans will likely become more unpredictable in light of climate change, ocean acidification, coastal pollution, boat traffic, and invasive species. A tempering of the gravity of ocean acidification is warranted given the conclusions of this thesis, but nevertheless the phenomenon exists

and has the potential to impact other aspects of the molluscan life cycle. Given that the change caused by elevated atmospheric CO_2 will be long lasting in the world's ocean, understanding what the future holds remains paramount. Work like this that bridges information between disciplines will be an important tool to help apply the knowledge about ocean acidification to adaptive measures in coastal communities.

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Appendix 1: Historical Data

Species	Author	time	length	±	Temp	Sal	Food
C. gigas	His 1989	3.0	64.7	0.8	15.0	20.0	50 cell/µl
C. gigas	His 1989	3.0	63.6	0.8	15.0	25.0	50 cell/µl
C. gigas	His 1989	3.0	67.8	0.8	15.0	30.0	50 cell/µl
C. gigas	His 1989	3.0	67.9	0.8	15.0	35.0	50 cell/µl
C. gigas	His 1989	5.0	66.6	0.6	15.0	20.0	50 cell/µl
C. gigas	His 1989	5.0	69.2	0.8	15.0	25.0	50 cell/µl
C. gigas	His 1989	5.0	69.7	1.3	15.0	30.0	50 cell/µl
C. gigas	His 1989	5.0	72.2	1.1	15.0	35.0	50 cell/µl
C. gigas	His 1989	7.0	67.5	0.8	15.0	20.0	50 cell/µl
C. gigas	His 1989	7.0	76.0	1.6	15.0	25.0	50 cell/µl
C. gigas	His 1989	7.0	75.6	1.6	15.0	30.0	50 cell/µl
C. gigas	His 1989	7.0	74.0	1.5	15.0	35.0	50 cell/µl
C. gigas	Rico-Villa 2009	2.0	76.4		17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	5.0	84.3		17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	7.1	90.5	5.3	17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	10.0	110.7	4.4	17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	13.0	123.9	6.2	17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	15.0	136.3	6.2	17.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	18.0	158.3	6.2	17.0	34.5	30 per µl
C. gigas	Kheder 2010	23.0	229.1	38.5	17.0		
C. gigas	His 1989	3.0	69.6	0.8	20.0	20.0	50 cell/µl
C. gigas	His 1989	3.0	68.3	1.1	20.0	25.0	50 cell/µl
C. gigas	His 1989	3.0	74.1	1.3	20.0	30.0	50 cell/µl
C. gigas	His 1989	3.0	74.5	1.1	20.0	35.0	50 cell/µl
C. gigas	His 1989	5.0	72.2	2.0	20.0	20.0	50 cell/µl
C. gigas	His 1989	5.0	77.4	0.8	20.0	25.0	50 cell/µl
C. gigas	His 1989	5.0	79.7	1.7	20.0	30.0	50 cell/µl
C. gigas	His 1989	5.0	82.0	1.8	20.0	35.0	50 cell/µl
C. gigas	His 1989	7.0	73.9	1.3	20.0	20.0	50 cell/µl
C. gigas	His 1989	7.0	88.0	1.2	20.0	25.0	50 cell/µl
C. gigas	His 1989	7.0	84.3	2.2	20.0	30.0	50 cell/µl
C. gigas	His 1989	7.0	93.8	2.8	20.0	35.0	50 cell/µl
C. gigas	His 1992	1.0	64.3		22.0	25.0	
C. gigas	His 1992	2.0	73.8		22.0	25.0	
C. gigas	Rico-Villa 2009	2.0	76.4		22.0	34.5	30 per µl
C. gigas	His 1992	3.0	83.3		22.0	25.0	
C. gigas	His 1992	4.0	90.0		22.0	25.0	
C. gigas	Rico-Villa 2009	5.0	101.1		22.0	34.5	30 per µl
C. gigas	His 1992	5.0	97.6		22.0	25.0	
C. gigas	His 1992	6.0	107.1		22.0	25.0	
C. gigas	Rico-Villa 2009	7.0	123.1	6.2	22.0	34.5	30 per µl
C. gigas	His 1992	7.0	116.2		22.0	25.0	
C. gigas	His 1992	9.1	132.9		22.0	25.0	
C. gigas	Rico-Villa 2009	10.0	153.9	7.0	22.0	34.5	30 per µl
C. gigas	His 1992	11.1	151.0	8.0	22.0	25.0	

Species	Author	time	length	±	Temp	Sal	Food
C. gigas	Rico-Villa 2009	13.0	201.4	9.7	22.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	15.0	216.4	10.6	22.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	18.0	243.7	12.3	22.0	34.5	30 per µl
C. gigas	Kheder 2010	21.0	246.6	33.4	22.0		
C. gigas	Rico-Villa 2009	2.0	78.2		25.0	34.5	40 per µl
C. gigas	Rico-Villa 2009	2.0	77.3		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	2.0	76.4		25.0	34.5	30 per µl
C. gigas	His 1989	3.0	76.2	1.1	25.0	20.0	50 cell/µl
C. gigas	His 1989	3.0	73.2	1.9	25.0	25.0	50 cell/µl
C. gigas	His 1989	3.0	84.3	2.1	25.0	30.0	50 cell/µl
C. gigas	His 1989	3.0	83.3	1.0	25.0	35.0	50 cell/µl
C. gigas	His 1989	5.0	84.8	2.1	25.0	20.0	50 cell/µl
C. gigas	His 1989	5.0	88.5	3.1	25.0	25.0	50 cell/µl
C. gigas	His 1989	5.0	112.8	2.9	25.0	30.0	50 cell/µl
C. gigas	His 1989	5.0	91.3	2.7	25.0	35.0	50 cell/µl
C. gigas	Rico-Villa 2009	5.0	101.1		25.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	5.0	102.6		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	5.0	103.6		25.0	34.5	40 per µl
C. gigas	His 1989	7.0	104.0	2.7	25.0	20.0	50 cell/µl
C. gigas	His 1989	7.0	116.5	5.5	25.0	25.0	50 cell/µl
C. gigas	His 1989	7.0	116.6	4.6	25.0	30.0	50 cell/µl
C. gigas	His 1989	7.0	111.5	3.3	25.0	35.0	50 cell/µl
C. gigas	Rico-Villa 2009	7.0	123.1	6.2	25.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	8.0	142.1		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	8.0	147.7		25.0	34.5	40 per µl
C. gigas	Rico-Villa 2009	10.0	178.5	7.9	25.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	11.0	204.1		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	11.0	211.7		25.0	34.5	40 per µl
C. gigas	Rico-Villa 2009	12.0	235.2		25.0	34.5	40 per µl
C. gigas	Rico-Villa 2009	12.0	217.3		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	13.0	234.0	12.3	25.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	14.0	252.1		25.0	34.5	20 per µl
C. gigas	Rico-Villa 2009	14.0	271.8		25.0	34.5	40 per µl
C. gigas	Rico-Villa 2009	15.0	252.5	14.1	25.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	2.0	76.4		27.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	5.0	117.8		27.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	7.0	154.8	7.0	27.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	10.0	225.2	10.6	27.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	13.0	269.2	14.1	27.0	34.5	30 per µl
C. gigas	Kheder 2010	18.0	250.1	27.3	27.0		
C. gigas	His 1992	1.0	59.0		30.0	30.0	
C. gigas	His 1992	2.0	70.0		30.0	30.0	
C. gigas	His 1989	3.0	81.8	1.4	30.0	20.0	50 cell/µl
C. gigas	His 1989	3.0	88.4	1.8	30.0	25.0	50 cell/µl
C. gigas	His 1989	3.0	90.6	1.6	30.0	30.0	50 cell/µl

Species	Author	time	length	±	Temp	Sal	Food
C. gigas	His 1989	3.0	90.9	1.9	30.0	35.0	50 cell/µl
C. gigas	His 1992	3.0	81.5		30.0	30.0	
C. gigas	His 1992	4.0	92.5		30.0	30.0	
C. gigas	His 1989	5.0	92.0	3.1	30.0	20.0	50 cell/µl
C. gigas	His 1989	5.0	112.5	2.6	30.0	25.0	50 cell/µl
C. gigas	His 1989	5.0	98.3	2.7	30.0	30.0	50 cell/µl
C. gigas	His 1989	5.0	114.1	2.5	30.0	35.0	50 cell/µl
C. gigas	His 1992	5.1	108.5	6.0	30.0	30.0	
C. gigas	His 1989	7.0	128.8	5.6	30.0	20.0	50 cell/µl
C. gigas	His 1989	7.0	147.3	7.0	30.0	25.0	50 cell/µl
C. gigas	His 1989	7.0	139.1	5.1	30.0	30.0	50 cell/µl
C. gigas	His 1989	7.0	137.9	6.4	30.0	35.0	50 cell/µl
C. gigas	His 1992	7.1	139.5	8.0	30.0	30.0	
C. gigas	His 1992	9.1	172.0	13.0	30.0	30.0	
C. gigas	His 1992	11.1	188.0	16.0	30.0	30.0	
C. gigas	His 1992	13.2	209.5	20.0	30.0	30.0	
C. gigas	His 1992	15.2	229.5	19.0	30.0	30.0	
C. gigas	Rico-Villa 2009	2.0	76.4		32.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	5.0	117.8	6.2	32.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	7.0	162.7	7.9	32.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	10.0	247.2	12.3	32.0	34.5	30 per µl
C. gigas	Rico-Villa 2009	13.0	271.0	13.2	32.0	34.5	30 per µl
C. gigas	Kheder 2010	18.0	250.6	29.4	32.0		
C. gigas	Bochenek 2001	0.0	48.9		n/a	n/a	n/a
C. gigas	Bochenek 2001	0.0	68.9		n/a	n/a	n/a
C. gigas	Bochenek 2001	0.2	90.4		n/a	n/a	n/a
C. gigas	Bochenek 2001	0.3	120.6		n/a	n/a	n/a
C. gigas	Bochenek 2001	0.6	219.4		n/a	n/a	n/a
C. gigas	Bochenek 2001	0.8	271.9		n/a	n/a	n/a
C. gigas	Bochenek 2001	1.0	331.3		n/a	n/a	n/a
C. gigas	His 1988	1.0	67.0	1.0			
C. gigas	His 1988	2.0	71.0	1.0			
C. gigas	His 1988	3.0	76.0	1.0			
C. gigas	His 1988	6.0	95.0	2.0			
C. gigas	His 1988	7.0	102.0	3.0			
C. gigas	His 1988	9.0	124.0	3.0			
C. gigas	His 1988	14.0	183.0	6.0			
C. gigas	His 1988	16.0	200.0	10.0			
C. gigas	His 1988	21.0	269.0	10.0			
C. gigas	His 1988	23.0	274.0	9.0			
C. gigas	His 1988	25.0	320.0	5.0			
C. gigas	His 1988	27.0	324.0	6.0			
M. edulis	Brenko 1969	16.5	61.8		5.0	25.0	saturated
M. edulis	Brenko 1969	16.5	59.4		5.0	30.0	saturated
M. edulis	Hayhurst 2001	0.7	70.1		5.0	30.0	100 cell/ml

Species	Author	time	length	±	Temp	Sal	Food
M. edulis	Hayhurst 2001	4.2	95.8		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	6.2	101.1		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	8.3	106.4		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	10.4	105.5		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	12.4	102.8		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	14.6	116.1		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	16.4	117.0		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	18.4	118.8		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	20.4	123.2		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	22.3	118.8		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	24.4	117.0		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	26.3	126.7		5.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	28.3	120.5		5.0	30.0	100 cell/ml
M. edulis	Brenko 1969	16.5	43.5		5.0	35.0	saturated
M. edulis	Brenko 1969	16.5	135.9		10.0	25.0	saturated
M. edulis	Brenko 1969	16.5	151.5		10.0	30.0	saturated
M. edulis	Hayhurst 2001	0.7	69.2		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	4.2	115.0		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	6.2	118.3		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	8.2	125.0		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	10.2	129.2		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	12.2	127.5		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	14.2	135.8		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	16.2	135.8		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	18.2	139.2		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	20.2	127.5		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	22.2	141.7		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	24.2	156.7		10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	26.2	148.3	5.8	10.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	28.3	150.8	6.7	10.0	30.0	100 cell/ml
M. edulis	Brenko 1969	16.5	125.7		10.0	35.0	saturated
M. edulis	Brenko 1969	16.5	244.5		15.0	25.0	saturated
M. edulis	Brenko 1969	16.5	251.7		15.0	30.0	saturated
M. edulis	Hayhurst 2001	0.6	70.9		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	4.1	115.5		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	6.2	131.0		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	8.2	143.0		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	10.3	139.6		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	12.2	145.6		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	14.3	145.6	6.0	15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	16.3	149.9	6.9	15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	18.3	155.0	12.9	15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	20.3	156.7	15.5	15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	22.3	173.9		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	24.2	217.7		15.0	30.0	100 cell/ml

Species	Author	time	length	±	Temp	Sal	Food
M. edulis	Hayhurst 2001	26.3	206.5		15.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	28.3	201.4		15.0	30.0	100 cell/ml
M. edulis	Brenko 1969	16.5	230.4		15.0	35.0	saturated
M. edulis	Pechenik 1990	7.9	165.2	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	11.9	211.9	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	15.8	239.9	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	18.0	256.8	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	19.8	265.6	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	23.9	291.8	25.0	16.0	31.0	$30x10^4$
M. edulis	Pechenik 1990	7.9	162.8	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	11.8	202.5	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	15.9	231.1	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	17.9	238.1	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	19.9	252.1	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	23.9	263.2	25.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	7.9	152.9	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	11.9	187.4	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	15.9	211.3	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	19.8	218.3	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	23.9	230.5	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	27.8	239.9	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	31.8	256.8	25.0	16.0	31.0	$9x10^4$
M. edulis	Pechenik 1990	10.1	152.6	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	14.0	168.5	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	12.1	195.3	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	23.0	236.2	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	26.0	250.2	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	30.9	283.4	12.0	16.0	31.0	$15x10^4$
M. edulis	Pechenik 1990	37.0	298.1	12.0	16.0	31.0	$15x10^4$
M. edulis	Brenko 1969	16.5	288.3		20.0	25.0	saturated
M. edulis	Brenko 1969	16.5	296.4		20.0	30.0	saturated
M. edulis	Hayhurst 2001	0.8	69.9		20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	4.2	123.8		20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	6.2	134.0		20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	8.2	135.7		20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	10.2	149.4	5.1	20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	12.2	146.0	6.0	20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	14.2	146.8	8.5	20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	16.3	171.6	6.8	20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	18.3	163.1	7.7	20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	20.2	184.4		20.0	30.0	100 cell/ml
M. edulis	Hayhurst 2001	22.3	191.3		20.0	30.0	100 cell/ml
M. edulis	Brenko 1969	16.5	260.4		20.0	35.0	saturated
M. edulis	Brenko 1969	16.5	193.8		25.0	25.0	saturated
M. edulis	Brenko 1969	16.5	194.4		25.0	30.0	saturated

Species	Author	time	length	±	Temp	Sal	Food
M. edulis	Brenko 1969	16.5	147.6		25.0	35.0	saturated
M. edulis	Brenko 1969	16.5	0.0		30.0	25.0	saturated
M. edulis	Brenko 1969	16.5	0.0		30.0	30.0	saturated
M. edulis	Brenko 1969	16.5	0.0		30.0	35.0	saturated
M. edulis	Brenko 1969	16.5	24.0		5.0	40.0	saturated
M. edulis	Brenko 1969	16.5	88.5		10.0	40.0	saturated
M. edulis	Brenko 1969	16.5	150.3		15.0	40.0	saturated
M. edulis	Brenko 1969	16.5	160.8		20.0	40.0	saturated
M. edulis	Brenko 1969	16.5	71.4		25.0	40.0	saturated
M. edulis	Brenko 1969	16.5	0.0		30.0	40.0	saturated
C. virginica	Davis 1964	10.0	85.4		17.5	27.5	
C. virginica	Davis 1964	10.0	87.9		17.5	25.0	
C. virginica	Davis 1964	10.0	90.0		17.5	22.5	
C. virginica	Davis 1964	10.0	88.8		17.5	20.0	
C. virginica	Bideford	1.0	60.0		19.0		
C. virginica	Bideford	2.0	63.0		19.0		
C. virginica	Bideford	3.0	66.0		19.0		
C. virginica	Bideford	4.0	69.0		19.0		
C. virginica	Bideford	5.0	72.0		19.0		
C. virginica	Bideford	6.0	75.0		19.0		
C. virginica	Bideford	7.0	78.0		19.0		
C. virginica	Bideford	8.0	81.0		19.0		
C. virginica	Bideford	9.0	84.0		19.0		
C. virginica	Bideford	10.0	87.0		19.0		
C. virginica	Bideford	11.0	90.0		19.0		
C. virginica	Bideford	12.0	93.0		19.0		
C. virginica	Bideford	13.0	96.0		19.0		2.5 cell/µl
C. virginica	Bideford	14.0	100.0		19.0		10 cell/µl
C. virginica	Bideford	15.0	103.0		19.0		15 cell/µl
C. virginica	Bideford	16.0	106.0		19.0		17.5 cell/µl
C. virginica	Bideford	17.0	109.0		19.0		25 cell/µl
C. virginica	Bideford	18.0	112.0		19.0		35 cell/µl
C. virginica	Bideford	19.0	116.0		19.0		35 cell/µl
C. virginica	Bideford	20.0	120.0		19.0		
C. virginica	Bideford	21.0	123.0		19.0		
C. virginica	Bideford	24.0	134.0		19.0		
C. virginica	Bideford	25.0	138.0		19.0		
C. virginica	Bideford	26.0	141.0		19.0		
C. virginica	Bideford	27.0	145.0		19.0		
C. virginica	Bideford	28.0	149.0		19.0		
C. virginica	Bideford	29.0	153.0		19.0		
C. virginica	Bideford	30.0	157.0		19.0		
C. virginica	Bideford	31.0	161.0		19.0		
C. virginica	Bideford	32.0	165.0		19.0		
C. virginica	Bideford	33.0	170.0		19.0		

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Bideford	34.0	174.0		19.0		
C. virginica	Bideford	35.0	179.0		19.0		
C. virginica	Bideford	36.0	183.0		19.0		
C. virginica	Bideford	37.0	188.0		19.0		
C. virginica	Bideford	38.0	192.0		19.0		
C. virginica	Bideford	39.0	197.0		19.0		
C. virginica	Bideford	40.0	202.0		19.0		
C. virginica	Medcof 1939	0.8	55.8		19.0	?	
C. virginica	Medcof 1939	2.0	62.6		19.0	?	
C. virginica	Medcof 1939	4.0	74.8		19.0	?	
C. virginica	Medcof 1939	6.0	88.5		19.0	?	
C. virginica	Medcof 1939	8.0	101.7		19.0	?	
C. virginica	Medcof 1939	10.0	116.5		19.0	?	
C. virginica	Medcof 1939	12.0	130.2		19.0	?	
C. virginica	Medcof 1939	14.0	145.0		19.0	?	
C. virginica	Medcof 1939	16.0	159.3		19.0	?	
C. virginica	Medcof 1939	18.0	178.8		19.0	?	
C. virginica	Medcof 1939	20.0	194.7		19.0	?	
C. virginica	Medcof 1939	22.0	214.8		19.0	?	
C. virginica	Medcof 1939	24.0	238.5		19.0	?	
C. virginica	Medcof 1939	26.0	270.2		19.0	?	
C. virginica	Medcof 1939	28.0	313.5		19.0	?	
C. virginica	Medcof 1939	29.8	366.3		19.0	?	
C. virginica	Bideford	1.0	60.0		20.0		?
C. virginica	Bideford	2.0	65.0		20.0		
C. virginica	Bideford	3.0	70.0		20.0		
C. virginica	Bideford	4.0	75.0		20.0		
C. virginica	Bideford	5.0	80.0		20.0		
C. virginica	Bideford	6.0	86.0		20.0		
C. virginica	Bideford	7.0	92.0		20.0		
C. virginica	Bideford	8.0	98.0		20.0		?
C. virginica	Bideford	9.0	104.0		20.0		?
C. virginica	Bideford	10.0	110.0		20.0		?
C. virginica	Bideford	11.0	117.0		20.0		?
C. virginica	Bideford	12.0	124.0		20.0		?
C. virginica	Bideford	13.0	132.0		20.0		?
C. virginica	Bideford	14.0	141.0		20.0		?
C. virginica	Bideford	15.0	150.0		20.0		?
C. virginica	Bideford	16.0	161.0		20.0		?
C. virginica	Bideford	17.0	172.0		20.0		?
C. virginica	Bideford	18.0	184.0		20.0		?
C. virginica	Bideford	19.0	198.0		20.0		?
C. virginica	Bideford	20.0	213.0		20.0		?
C. virginica	Bideford	21.0	231.0		20.0		?
C. virginica	Bideford	22.0	253.0		20.0		?

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Bideford	23.0	283.0		20.0		?
C. virginica	Bideford	24.0	328.0		20.0		?
C. virginica	Davis 1964	10.0	97.9		20.0	27.5	
C. virginica	Davis 1964	10.0	99.0		20.0	25.0	
C. virginica	Davis 1964	10.0	100.4		20.0	22.5	
C. virginica	Davis 1964	10.0	101.6		20.0	20.0	
C. virginica	Medcof 1939	0.8	55.8		20.0	?	
C. virginica	Medcof 1939	2.0	63.7		20.0	?	
C. virginica	Medcof 1939	4.0	77.4		20.0	?	
C. virginica	Medcof 1939	6.0	92.2		20.0	?	
C. virginica	Medcof 1939	8.0	106.5		20.0	?	
C. virginica	Medcof 1939	10.0	121.8		20.0	?	
C. virginica	Medcof 1939	12.0	136.6		20.0	?	
C. virginica	Medcof 1939	14.0	154.0		20.0	?	
C. virginica	Medcof 1939	16.0	173.0		20.0	?	
C. virginica	Medcof 1939	18.0	197.9		20.0	?	
C. virginica	Medcof 1939	20.0	218.5		20.0	?	
C. virginica	Medcof 1939	22.0	249.1		20.0	?	
C. virginica	Medcof 1939	24.0	290.8		20.0	?	
C. virginica	Medcof 1939	26.0	349.4		20.0	?	
C. virginica	Medcof 1939	26.3	365.8		20.0	?	
C. virginica	Bideford	1.0	60.0		21.0		
C. virginica	Bideford	2.0	64.0		21.0		
C. virginica	Bideford	3.0	70.0		21.0		
C. virginica	Bideford	4.0	74.0		21.0		
C. virginica	Bideford	5.0	83.0		21.0		
C. virginica	Bideford	6.0	92.0		21.0		?
C. virginica	Bideford	7.0	101.0		21.0		?
C. virginica	Bideford	8.0	112.0		21.0		?
C. virginica	Bideford	9.0	124.0		21.0		?
C. virginica	Bideford	10.0	138.0		21.0		?
C. virginica	Bideford	11.0	153.0		21.0		?
C. virginica	Bideford	12.0	168.0		21.0		?
C. virginica	Bideford	13.0	184.0		21.0		?
C. virginica	Bideford	14.0	200.0		21.0		?
C. virginica	Bideford	15.0	217.0		21.0		?
C. virginica	Bideford	16.0	236.0		21.0		?
C. virginica	Bideford	17.0	258.0		21.0		?
C. virginica	Bideford	18.0	285.0		21.0		?
C. virginica	Bideford	19.0	317.0		21.0		?
C. virginica	Bideford	20.0	359.0		21.0		?
C. virginica	Bideford	1.0	60.0		21.0		?
C. virginica	Bideford	2.0	64.0		21.0		?
C. virginica	Bideford	3.0	69.0		21.0		?
C. virginica	Bideford	4.0	74.0		21.0		?

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Bideford	5.0	79.0		21.0		?
C. virginica	Bideford	6.0	84.0		21.0		?
C. virginica	Bideford	7.0	89.0		21.0		?
C. virginica	Bideford	8.0	94.0		21.0		?
C. virginica	Bideford	9.0	100.0		21.0		?
C. virginica	Bideford	10.0	106.0		21.0		?
C. virginica	Bideford	11.0	113.0		21.0		?
C. virginica	Bideford	12.0	120.0		21.0		?
C. virginica	Bideford	13.0	127.0		21.0		
C. virginica	Bideford	14.0	135.0		21.0		
C. virginica	Bideford	15.0	144.0		21.0		
C. virginica	Bideford	16.0	153.0		21.0		
C. virginica	Bideford	17.0	163.0		21.0		
C. virginica	Bideford	18.0	174.0		21.0		
C. virginica	Bideford	19.0	186.0		21.0		
C. virginica	Bideford	20.0	200.0		21.0		
C. virginica	Bideford	21.0	214.0		21.0		
C. virginica	Bideford	22.0	229.0		21.0		
C. virginica	Bideford	23.0	248.0		21.0		
C. virginica	Bideford	24.0	268.0		21.0		
C. virginica	Bideford	25.0	293.0		21.0		
C. virginica	Bideford	26.0	322.0		21.0		
C. virginica	Bideford	27.0	360.0		21.0		
C. virginica	Medcof 1939	0.8	55.8		21.0	?	
C. virginica	Medcof 1939	2.0	64.8		21.0	?	
C. virginica	Medcof 1939	4.0	79.5		21.0	?	
C. virginica	Medcof 1939	6.0	94.3		21.0	?	
C. virginica	Medcof 1939	8.0	109.6		21.0	?	
C. virginica	Medcof 1939	10.0	127.1		21.0	?	
C. virginica	Medcof 1939	12.0	144.0		21.0	?	
C. virginica	Medcof 1939	14.0	164.6		21.0	?	
C. virginica	Medcof 1939	16.0	186.8		21.0	?	
C. virginica	Medcof 1939	18.0	217.9		21.0	?	
C. virginica	Medcof 1939	20.0	247.5		21.0	?	
C. virginica	Medcof 1939	22.0	292.9		21.0	?	
C. virginica	Medcof 1939	24.0	358.4		21.0	?	
C. virginica	Medcof 1939	24.1	364.8		21.0	?	
C. virginica	Bideford	1.0	60.0		22.0		
C. virginica	Bideford	2.0	68.0		22.0		
C. virginica	Bideford	3.0	80.0		22.0		
C. virginica	Bideford	4.0	94.0		22.0		
C. virginica	Bideford	5.0	113.0		22.0		
C. virginica	Bideford	6.0	133.0		22.0		
C. virginica	Bideford	7.0	155.0		22.0		
C. virginica	Bideford	8.0	178.0		22.0		

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Bideford	9.0	204.0		22.0		
C. virginica	Bideford	10.0	234.0		22.0		
C. virginica	Bideford	11.0	275.0		22.0		
C. virginica	Bideford	12.0	350.0		22.0		
C. virginica	Davis 1953	2.0	76.2	0.0	22.0	30.0	
C. virginica	Davis 1953	6.0	90.8	1.9	22.0	30.0	
C. virginica	Davis 1953	10.0	102.3	2.0	22.0	30.0	
C. virginica	Davis 1953	14.0	121.5	4.9	22.0	30.0	
C. virginica	Davis 1953	18.0	145.5	6.4	22.0	30.0	
C. virginica	Davis 1953	2.0	75.1	0.0	22.0	30.0	
C. virginica	Davis 1953	6.0	104.6	1.5	22.0	30.0	
C. virginica	Davis 1953	10.0	129.6	2.6	22.0	30.0	
C. virginica	Davis 1953	14.0	133.6	3.7	22.0	30.0	
C. virginica	Davis 1964	10.0	120.2		22.5	27.5	
C. virginica	Davis 1964	10.0	117.9		22.5	25.0	
C. virginica	Davis 1964	10.0	116.0		22.5	22.5	
C. virginica	Davis 1964	10.0	114.9		22.5	20.0	
c. virginica	B. Mook	15.0	300.0		23.0		
C. virginica	Davis 1964	10.0	178.6		27.5	27.5	
C. virginica	Davis 1964	10.0	174.0		27.5	25.0	
C. virginica	Davis 1964	10.0	174.6		27.5	22.5	
C. virginica	Davis 1964	10.0	178.0		27.5	20.0	
C. virginica	rhodes 1973		74.7		28.0	31.0	
C. virginica	rhodes 1973		80.1		28.0	31.0	
C. virginica	rhodes 1973		109.4		28.0	31.0	
C. virginica	rhodes 1973		142.4		28.0	31.0	
C. virginica	rhodes 1973		172.0		28.0	31.0	
C. virginica	rhodes 1973		200.4		28.0	31.0	
C. virginica	rhodes 1973		237.7		28.0	31.0	
C. virginica	Davis 1964	10.0	200.4		30.0	27.5	
C. virginica	Davis 1964	10.0	192.8		30.0	25.0	
C. virginica	Davis 1964	10.0	194.6		30.0	22.5	
C. virginica	Davis 1964	10.0	202.1		30.0	20.0	
C. virginica	Davis 1964	10.0	196.3		32.5	27.5	
C. virginica	Davis 1964	10.0	191.9		32.5	25.0	
C. virginica	Davis 1964	10.0	193.5		32.5	22.5	
C. virginica	Davis 1964	10.0	193.7		32.5	20.0	
C. virginica	Deksheniek	1.0	50.0		24.0		
C. virginica	Deksheniek	2.0	56.3		24.0		
C. virginica	Deksheniek	3.0	62.5		24.0		
C. virginica	Deksheniek	4.0	68.0		24.0		
C. virginica	Deksheniek	5.0	73.4		24.0		
C. virginica	Deksheniek	6.0	75.8		24.0		
C. virginica	Deksheniek	7.0	81.3		24.0		
C. virginica	Deksheniek	8.0	86.7		24.0		

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Deksheniek	9.0	96.1		24.0		
C. virginica	Deksheniek	10.0	107.0		24.0		
C. virginica	Deksheniek	11.0	122.7		24.0		
C. virginica	Deksheniek	12.0	139.1		24.0		
C. virginica	Deksheniek	13.0	155.5		24.0		
C. virginica	Deksheniek	14.0	169.5		24.0		
C. virginica	Deksheniek	15.0	183.6		24.0		
C. virginica	Deksheniek	16.0	196.9		24.0		
C. virginica	Deksheniek	17.0	211.7		24.0		
C. virginica	Deksheniek	18.0	227.3		24.0		
C. virginica	Deksheniek	19.0	243.8		24.0		
C. virginica	Deksheniek	20.0	260.9		24.0		
C. virginica	Deksheniek	21.0	278.1		24.0		
C. virginica	Deksheniek	22.0	295.3		24.0		
C. virginica	Deksheniek	23.0	312.5		24.0		
C. virginica	Deksheniek	24.0	329.7		24.0		
C. virginica	Deksheniek	1.0	50.0		25.0		
C. virginica	Deksheniek	2.0	57.0		25.0		
C. virginica	Deksheniek	3.0	64.0		25.0		
C. virginica	Deksheniek	4.0	70.1		25.0		
C. virginica	Deksheniek	5.0	76.3		25.0		
C. virginica	Deksheniek	6.0	82.4		25.0		
C. virginica	Deksheniek	7.0	88.5		25.0		
C. virginica	Deksheniek	8.0	99.0		25.0		
C. virginica	Deksheniek	9.0	118.3		25.0		
C. virginica	Deksheniek	10.0	136.6		25.0		
C. virginica	Deksheniek	11.0	155.0		25.0		
C. virginica	Deksheniek	12.0	170.8		25.0		
C. virginica	Deksheniek	13.0	186.5		25.0		
C. virginica	Deksheniek	14.0	201.4		25.0		
C. virginica	Deksheniek	15.0	218.0		25.0		
C. virginica	Deksheniek	16.0	235.5		25.0		
C. virginica	Deksheniek	17.0	253.9		25.0		
C. virginica	Deksheniek	18.0	273.1		25.0		
C. virginica	Deksheniek	19.0	292.4		25.0		
C. virginica	Deksheniek	20.0	311.6		25.0		
C. virginica	Deksheniek	21.0	330.9		25.0		
C. virginica	Deksheniek	1.0	50.0		26.0		
C. virginica	Deksheniek	2.0	57.3		26.0		
C. virginica	Deksheniek	3.0	64.5		26.0		
C. virginica	Deksheniek	4.0	70.8		26.0		
C. virginica	Deksheniek	5.0	77.2		26.0		
C. virginica	Deksheniek	6.0	83.5		26.0		
C. virginica	Deksheniek	7.0	94.4		26.0		
C. virginica	Deksheniek	8.0	109.8		26.0		

Species	Author	time	length	±	Temp	Sal	Food
C. virginica	Deksheniek	9.0	130.7		26.0		
C. virginica	Deksheniek	10.0	150.6		26.0		
C. virginica	Deksheniek	11.0	166.9		26.0		
C. virginica	Deksheniek	12.0	183.2		26.0		
C. virginica	Deksheniek	13.0	198.6		26.0		
C. virginica	Deksheniek	14.0	215.8		26.0		
C. virginica	Deksheniek	15.0	234.0		26.0		
C. virginica	Deksheniek	16.0	253.0		26.0		
C. virginica	Deksheniek	17.0	272.9		26.0		
C. virginica	Deksheniek	18.0	292.9		26.0		
C. virginica	Deksheniek	19.0	312.8		26.0		
C. virginica	Deksheniek	20.0	332.8		26.0		

Appendix 2: Ocean Acidification Data

author	time	length	±	Ω	carbonate	pCO_2	temperature
Parker 2010	2.0	67.3	0.6	2.8	181.2	375.0	18.0
Parker 2010	2.0	65.9	1.0	2.0	126.6	600.0	18.0
Parker 2010	2.0	64.0	2.0	1.7	109.4	750.0	18.0
Parker 2010	2.0	63.1	0.1	1.3	87.9	1000.0	18.0
Parker 2010	1.7	69.9	0.3	2.8	181.2	375.0	18.0
Parker 2010	9.0	138.6	1.9	2.8	181.2	375.0	18.0
Parker 2010	16.0	247.7	1.6	2.8	181.2	375.0	18.0
Parker 2010	1.7	68.7	0.4	2.0	126.6	600.0	18.0
Parker 2010	9.0	137.7	1.0	2.0	126.6	600.0	18.0
Parker 2010	16.0	247.9	1.7	2.0	126.6	600.0	18.0
Parker 2010	1.7	65.8	0.6	1.7	109.4	750.0	18.0
Parker 2010	9.0	138.4	1.7	1.7	109.4	750.0	18.0
Parker 2010	16.0	247.0	0.9	1.7	109.4	750.0	18.0
Parker 2010	1.7	64.4	1.3	1.3	87.9	1000.0	18.0
Parker 2010	9.0	137.7	1.0	1.3	87.9	1000.0	18.0
Parker 2010	16.0	246.9	0.7	1.3	87.9	1000.0	18.0
Gazeau 2011	3.0	81.9	3.6	2.8	181.9	448.7	18.9
Gazeau 2011	3.0	80.7	3.7	2.8	181.9	448.7	18.9
Gazeau 2011	3.0	80.5	2.5	2.8	181.9	448.7	18.9
Gazeau 2011	3.0	79.3	3.6	1.5	97.3	1019.9	18.9
Gazeau 2011	3.0	80.0	3.7	1.5	97.3	1019.9	18.9
Gazeau 2011	3.0	80.0	3.4	1.5	97.3	1019.9	18.9
Gazeau 2011	3.0	77.7	3.8	0.8	50.4	2170.5	18.9
Gazeau 2011	3.0	76.6	3.4	0.8	50.4	2170.5	18.9
Gazeau 2011	3.0	76.0	4.6	0.8	50.4	2170.5	18.9
Gazeau 2011	3.0	71.6	2.8	1.6	40.0	493.5	18.9
Gazeau 2011	3.0	72.8	3.9	1.6	40.0	493.5	18.9
Gazeau 2011	3.0	72.1	3.2	1.6	40.0	493.5	18.9
Gazeau 2011	3.0	81.9	3.6	4.5	226.5	3730.4	18.9
Gazeau 2011	3.0	80.2	4.1	2.6	226.5	3730.4	18.9
Gazeau 2011	3.0	80.2	3.4	2.6	226.5	3730.4	18.9
Timmins 2012	3.0	68.0		1.3	81.7	781.0	20.1
Timmins 2012	1.0	49.3		1.2	75.5	812.4	20.3
Timmins 2012	3.0	58.8		1.1	65.5	1030.7	20.4
Timmins 2012	3.0	73.0		2.1	131.0	428.4	20.5
Timmins 2012	1.0	50.1		0.9	56.5	1150.0	20.5
Timmins 2012	1.0	52.0		1.4	120.3	466.3	20.5
Parker 2010	2.0	74.2	0.1	3.6	223.1	375.0	22.0
Parker 2010	2.0	68.6	1.1	2.6	163.1	600.0	22.0
Parker 2010	2.0	68.8	0.4	2.2	141.1	750.0	22.0
Parker 2010	2.0	68.0	0.6	1.8	113.3	1000.0	22.0
Parker 2010	1.7	74.4	0.4	3.6	223.1	375.0	22.0
Parker 2010	9.0	137.4	0.7	3.6	223.1	375.0	22.0
Parker 2010	16.0	250.0	3.8	3.6	223.1	375.0	22.0
Parker 2010	1.7	72.0	0.3	2.6	163.1	600.0	22.0
Parker 2010	9.0	138.3	1.6	2.6	163.1	600.0	22.0
Parker 2010	16.0	247.6	1.4	2.6	163.1	600.0	22.0
Parker 2010	1.7	70.8	0.6	2.2	141.1	750.0	22.0
Parker 2010	9.0	140.7	4.0	2.2	141.1	750.0	22.0
Parker 2010	16.0	249.5	3.3	2.2	141.1	750.0	22.0
Parker 2010	1.7	69.0	0.4	1.8	113.3	1000.0	22.0
Parker 2010	9.0	140.7	4.0	1.8	113.3	1000.0	22.0

author	time	length	±	Ω	carbonate	pCO_2	temperature
Parker 2010	16.0	246.9	0.7	1.8	113.3	1000.0	22.0
Kurihara 2007	1.0	54.7		3.0	161.4	348.0	23.0
Kurihara 2007	2.0	61.4		3.0	161.4	348.0	23.0
Kurihara 2007	1.0	50.6		0.7	36.4	2268.0	23.0
Kurihara 2007	2.0	48.6		0.7	36.4	2268.0	23.0
Waldbusser 201	1.9	77.9		2.4		396.0	25.0
Waldbusser 201	4.9	82.1		2.4		396.0	25.0
Waldbusser 201	7.9	91.3	6.2	2.4		396.0	25.0
Waldbusser 201	10.9	107.7	9.2	2.4		396.0	25.0
Waldbusser 201	12.9	128.2	13.3	2.4		396.0	25.0
Waldbusser 201	14.9	137.4	16.4	2.4		396.0	25.0
Waldbusser 201	16.9	145.6	22.6	2.4		396.0	25.0
Waldbusser 201	18.9	204.1	40.0	2.4		396.0	25.0
Parker 2010	2.0	75.4	0.3	2.8	181.2	375.0	26.0
Parker 2010	2.0	69.2	0.3	2.0	126.6	600.0	26.0
Parker 2010	2.0	68.8	0.7	1.7	109.4	750.0	26.0
Parker 2010	2.0	67.5	1.5	1.3	87.9	1000.0	26.0
Parker 2010	1.7	76.8	0.3	2.8	181.2	375.0	26.0
Parker 2010	9.0	139.1	2.4	2.8	181.2	375.0	26.0
Parker 2010	16.0	246.5	0.3	2.8	181.2	375.0	26.0
Parker 2010	1.7	73.2	0.4	2.0	126.6	600.0	26.0
Parker 2010	9.0	138.3	1.6	2.0	126.6	600.0	26.0
Parker 2010	16.0	246.5	0.3	2.0	126.6	600.0	26.0
Parker 2010	1.7	71.8	0.3	1.7	109.4	750.0	26.0
Parker 2010	9.0	140.0	3.3	1.7	109.4	750.0	26.0
Parker 2010	16.0	246.5	0.3	1.7	109.4	750.0	26.0
Parker 2010	1.7	69.6	1.1	1.3	87.9	1000.0	26.0
Parker 2010	9.0	143.3	6.6	1.3	87.9	1000.0	26.0
Parker 2010	16.0	247.2	1.0	1.3	87.9	1000.0	26.0
Parker 2010	2.0	74.8	0.3	3.6	223.1	375.0	30.0
Parker 2010	2.0	72.4	1.3	2.6	163.1	600.0	30.0
Parker 2010	2.0	70.3	1.3	2.2	141.1	750.0	30.0
Parker 2010	2.0	64.3	1.7	1.8	113.3	1000.0	30.0
Parker 2010	1.7	75.4	0.1	3.6	223.1	375.0	30.0
Parker 2010	9.0	141.9	5.2	3.6	223.1	375.0	30.0
Parker 2010	16.0	249.1	3.0	3.6	223.1	375.0	30.0
Parker 2010	1.7	73.2	1.0	2.6	163.1	600.0	30.0
Parker 2010	9.0	137.7	1.0	2.6	163.1	600.0	30.0
Parker 2010	16.0	249.3	3.1	2.6	163.1	600.0	30.0
Parker 2010	1.7	71.5	0.4	2.2	141.1	750.0	30.0
Parker 2010	9.0	139.1	2.4	2.2	141.1	750.0	30.0
Parker 2010	16.0	248.8	2.6	2.2	141.1	750.0	30.0
Parker 2010	1.7	68.6	1.5	1.8	113.3	1000.0	30.0
Parker 2010	9.0	139.5	2.8	1.8	113.3	1000.0	30.0
Parker 2010	16.0	247.9	1.7	1.8	113.3	1000.0	30.0
Talmage 2009	20.0	357.2	13.7	2.9	180.0	390.0	24.0
Talmage 2009	20.0	302.2	17.0	1.9	121.0	750.0	24.0
Talmage 2009	20.0	260.2	12.9	0.9	38.0	1500.0	24.0
Miller 2009	26.0	346.5	2.4	1.2	72.4	284.0	25.0
Miller 2009	26.0	341.6	2.2	1.0	60.4	380.0	25.0
Miller 2009	26.0	335.1	4.1	0.8	45.1	560.0	25.0
Miller 2009	26.0	321.8	1.7	0.6	32.7	840.0	25.0

author	time	length	±	Ω	carbonate	pCO_2	temperature
Gazeau 2010	2.0	81.5	0.8	2.8	166.0	468.0	16.6
Gazeau 2010	2.0	77.7	0.5	1.4	84.0	1124.0	16.7
Gazeau 2010	2.0	81.0	0.3	2.4	144.0	537.0	16.5
Gazeau 2010	2.0	70.6	0.6	0.8	49.0	1929.0	16.2
Gazeau 2010	2.0	78.6	4.3	2.3	135.0	642.0	19.4
Gazeau 2010	6.0	108.1		2.3	135.0	642.0	19.4
Gazeau 2010	8.0	140.0		2.3	135.0	642.0	19.4
Gazeau 2010	10.0	165.2		2.3	135.0	642.0	19.4
Gazeau 2010	13.0	195.9	5.5	2.3	135.0	642.0	19.4
Gazeau 2010	15.0	207.0	4.3	2.3	135.0	642.0	19.4
Gazeau 2010	2.0	78.6		1.4	80.0	1213.0	19.2
Gazeau 2010	6.0	103.2		1.4	80.0	1213.0	19.2
Gazeau 2010	8.0	134.5		1.4	80.0	1213.0	19.2
Gazeau 2010	10.0	159.7		1.4	80.0	1213.0	19.2
Gazeau 2010	13.0	182.4	3.7	1.4	80.0	1213.0	19.2
Gazeau 2010	15.0	194.7		1.4	80.0	1213.0	19.2