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The Effects of Ocean Acidification on the Gametogenesis of the Red Tree Coral, Primnoa pacifica

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THE EFFECTS OF OCEAN ACIDIFICATION ON THE GAMETOGENESIS OF THE
RED TREE CORAL, PRIMNOA PACIFICA

By
Ashley Madison Rossin
B.S. University of Maine, 2016

A THESIS
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
(in Marine Biology)

The Graduate School
The University of Maine
May 2018

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THE EFFECTS OF OCEAN ACIDIFICATION ON THE GAMETOGENESIS OF THE

RED TREE CORAL, PRIMNOA PACIFICA

By Ashley Madison Rossin

Thesis Advisor: Dr. Rhian Waller

An abstract of the Thesis Presented
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Primnoa pacifica, otherwise known as the Red Tree Coral, is one of the most ecologically important corals in the North Pacific. This species is an ecosystem engineer, providing essential habitat for commercially important fish and invertebrate species. Ocean acidification (OA) threatens corals and all calcifying organisms and is more prevalent in polar and sub-polar regions as the concentration of CO₂ is higher and there is a lowered buffering capacity due to low alkalinity in colder waters. The impact from a chemical shift in the oceans could alter the role of P. pacifica as an ecosystem engineer in predicted ocean conditions. An experiment was conducted to assess the impacts of OA on the gametogenesis of Red Tree Corals from Tracy Arm fjord in Alaska. Primnoa pacifica colonies were cultured for six to nine months in either projected 2100 pH (7.55 pH units) or Ambient pH (7.75 pH units), then were prepared for histology to investigate any changes in gametogenesis in acidified water. Oocyte diameters and fecundities were significantly lower in the 2100 samples. The females from the experiment also had a higher proportion of individuals experiencing reabsorption of vitellogenic oocytes, potentially to preserve the lipids for other ecological processes as a stress response. The highest percentage of oosorption was seen in the 2100 females, as well as the smallest oocytes and the
lowest fecundities. There was a “null tank effect” observed in all measurement types, however these only significantly affected the analyses of the exterior measurements and spermatogenesis. When compared to a published 2014 reproductive dataset, all the tank samples were statistically different from previous years and were significantly lower. These results indicate that reproduction in this species may not be possible in the current pH predictions for 2100, or that if spawning could still occur, any spawned oocyte may not be sufficiently equipped to support a larva. This study only investigated one life history stage of these long-lived organisms and more research spanning multiple life history stages needs to be done in their natural habitat as well as in homogeneous laboratory conditions. There is also a need to investigate the probability of these corals spawning under environmental stressors to identify if these important species will be present in the future.

**Keywords:** Gulf of Alaska, gametogenesis, Primnoa, octocoral, ocean acidification, histology, oogenesis, spermatogenesis
ACKNOWLEDGEMENTS

First and foremost, thank you to Dr. Rhian Waller for giving me the opportunity to work on the project of my dreams. Also, for the receipt of funds both from NOAA and the University of Maine to do this research. Thank you to my hardworking committee, Bob Steneck and Aaron Strong.

Thank you to Captain Dan and his crew for taking us to Tracy Arm, and allowing me to be a temporary captain during collections. Thank you to Bob and Michelle for your hospitality while we were in Alaska, the Three Little Pigs pizza, and the endless help following the collections. Thank you to Rhian Waller, Bob Stone, and Elise Hartill for your aid in the collection in Tracy Arm. Thank you to the staff at Kodiak Fisheries Research Center, especially Allie Bateman, for answering all my questions before I could access the files.

Tyler Fountain and Julia Johnstone, thank you for the sounding board, Hanley’s pizza, and answering the never-ending stats questions. My family, thank you for supporting me in my dreams, and understanding the shortened trips and repeated explanations of what OA stands for. For feeding me and encouraging me throughout my entire time at the DMC, thank you Paul, Gayle, and Yvette. Thank you to Randy Lackovic for finding all of the “lost” papers and helping me answer the big questions of this thesis. For the laboratory help and staining of approximately 40,000 slides: Elise Hartill, Augustus Pendleton, Genny Wilson, Jen Field, Lauren Rice, and Stephen Heald.
## TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................... ii

LIST OF TABLES ......................................................................................................................... v

LIST OF FIGURES ...................................................................................................................... vii

LIST OF THEOREMS AND EQUATIONS ................................................................................ viii

LIST OF ABBREVIATIONS ......................................................................................................... ix

LIST OF DEFINITIONS ............................................................................................................... xi

Chapter

1. INTRODUCTION ....................................................................................................................... 1

1.1 *Primnoa pacifica* (Kinoshita, 1907) ................................................................................ 1

1.2 Anthropogenic Impacts ......................................................................................................... 5

1.2.1 Physical Impacts ................................................................................................................. 5

1.2.2 Chemical Impacts ............................................................................................................... 6

2. MATERIALS AND METHODS ................................................................................................. 12

2.1 Study Area and Collection ................................................................................................. 12

2.2 Tank Experiment ................................................................................................................... 14

2.3 Randomization ...................................................................................................................... 17

2.4 Sprig Analysis ....................................................................................................................... 17

2.5 Histological Processing ....................................................................................................... 18

2.6 Histological Analysis .......................................................................................................... 19

3. RESULTS ............................................................................................................................... 21

3.1 Sprig Analysis ....................................................................................................................... 21

3.2 Gametogenesis ...................................................................................................................... 22

3.2.1 Spermatogenesis .............................................................................................................. 22
3.2.2 Oocytes ..................................................................................................................25
  3.2.2.1 Oocyte Size ........................................................................................................25
  3.2.2.2 Oosorption .........................................................................................................31
3.2.3 Fecundity ..................................................................................................................32

4. DISCUSSION ..................................................................................................................38
  4.1 Sprig Analysis ............................................................................................................38
  4.2 Gametogenesis ..........................................................................................................40
    4.2.1 Spermatogenesis ..................................................................................................40
  4.2.2 Oocytes ..................................................................................................................42
    4.2.2.1 Oocyte Size ....................................................................................................42
    4.2.2.2 Oosorption .....................................................................................................44
  4.2.3 Fecundity ................................................................................................................46
  4.3 Conclusions and Future Studies ..................................................................................47

REFERENCES .......................................................................................................................49

APPENDIX OF STATISTICS .............................................................................................55

BIOGRAPHY OF THE AUTHOR .........................................................................................61
<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Locations and months of specimen collection by sex</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.</td>
<td>Average results from exterior sprig measurements.</td>
<td>21</td>
</tr>
<tr>
<td>Table 3.</td>
<td>Number of individuals per sex per treatment from the tank experiment</td>
<td>22</td>
</tr>
<tr>
<td>Table 4.</td>
<td>End of experiment average oocyte diameter split by collection month</td>
<td>29</td>
</tr>
<tr>
<td>Table 5.</td>
<td>Average oocyte diameters for 2014 reproductive dataset</td>
<td>30</td>
</tr>
<tr>
<td>Table 6.</td>
<td>Average fecundity for 2014 dataset</td>
<td>36</td>
</tr>
<tr>
<td>Table 7.</td>
<td>All sprig measurement type datasets</td>
<td>55</td>
</tr>
<tr>
<td>Table 8.</td>
<td>OA dataset, Stage I spermatocysts</td>
<td>55</td>
</tr>
<tr>
<td>Table 9.</td>
<td>OA dataset, Stage II spermatocysts</td>
<td>55</td>
</tr>
<tr>
<td>Table 10.</td>
<td>OA dataset, Stage III spermatocysts</td>
<td>56</td>
</tr>
<tr>
<td>Table 11.</td>
<td>OA dataset, Stage IV spermatocysts</td>
<td>56</td>
</tr>
<tr>
<td>Table 12.</td>
<td>OA dataset with 2014 reproductive dataset, January 2013 spermatocyst stages</td>
<td>56</td>
</tr>
<tr>
<td>Table 13.</td>
<td>OA dataset with 2014 reproductive dataset, June Ambient to June 2011, Stage II spermatocysts</td>
<td>56</td>
</tr>
<tr>
<td>Table 14.</td>
<td>OA dataset with 2014 reproductive dataset, September OA to September 2011 Stage II spermatocysts</td>
<td>56</td>
</tr>
<tr>
<td>Table 15.</td>
<td>OA dataset with sprig length dataset and Stage I spermatocysts</td>
<td>56</td>
</tr>
<tr>
<td>Table 16.</td>
<td>OA dataset with sprig length dataset and Stage II spermatocysts</td>
<td>57</td>
</tr>
<tr>
<td>Table 17.</td>
<td>OA dataset, oocyte diameters</td>
<td>57</td>
</tr>
<tr>
<td>Table 18.</td>
<td>OA dataset, oocyte diameters: only Time 0 and September 2016</td>
<td>57</td>
</tr>
<tr>
<td>Table 19.</td>
<td>OA dataset, oocyte diameters: Time 0, Ambient, and 2100 split by month</td>
<td>57</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Table 20</td>
<td>OA dataset of oocyte diameters (split by month) with 2014 reproductive</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 21</td>
<td>OA dataset of oocyte diameters with sprig length dataset</td>
<td>58</td>
</tr>
<tr>
<td>Table 22</td>
<td>June Ambient dataset of oocyte diameters with June Ambient length</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 23</td>
<td>September Ambient dataset of oocyte diameters with September Ambient length</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 24</td>
<td>September 2100 dataset of oocyte diameters with September 2100 length</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 25</td>
<td>OA dataset, fecundity</td>
<td>59</td>
</tr>
<tr>
<td>Table 26</td>
<td>OA dataset of fecundity by month</td>
<td>59</td>
</tr>
<tr>
<td>Table 27</td>
<td>OA dataset with 2014 reproductive dataset of fecundity</td>
<td>59</td>
</tr>
<tr>
<td>Table 28</td>
<td>June OA dataset with June 2011 dataset of fecundity</td>
<td>60</td>
</tr>
<tr>
<td>Table 29</td>
<td>September OA dataset with September 2010 dataset of fecundity</td>
<td>60</td>
</tr>
<tr>
<td>Table 30</td>
<td>Ambient June dataset of fecundity with Ambient June sprig length</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 31</td>
<td>September Ambient dataset of fecundity with September Ambient sprig length</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
<tr>
<td>Table 32</td>
<td>September 2100 dataset of fecundity with September 2100 sprig length</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>dataset</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1. Study site in Southeastern Alaska .................................................................13
Figure 2. Tank setup at Kodiak Fisheries Research Center, AK .................................15
Figure 3. Histological section of a male and female .....................................................19
Figure 4. Boxplot distribution of percent change (%) versus treatment and measurement type ..............................................................22
Figure 5. Mean percent spermatocyst stage (%) versus treatment ...............................23
Figure 6. Mean percent spermatocyst stage (%) versus treatment, including the 2014 reproductive dataset ..........................................................24
Figure 7. Histogram of percent oocyte frequency (%) versus oocyte diameter (µm) ....27
Figure 8. Percent oocyte frequency (%) versus oocyte diameter (µm) by treatment and month .......................................................................................28
Figure 9. Boxplot distribution of oocyte diameter (µm) versus treatment and sprig condition ..............................................................31
Figure 10. Light microscopy photograph of female polyp containing a non-nucleated oocyte ..............................................................32
Figure 11. Maximum fecundity (oocytes per polyp) versus colony height ..................33
Figure 12. Average fecundity (oocytes per polyp) versus treatment ............................34
Figure 13. Boxplot distribution of fecundity (oocytes per polyp) versus treatment, separated by collection month .................................................35
Figure 14. Average fecundity (oocytes per polyp) versus treatment, including the 2014 reproductive dataset ..................................................35
Figure 15. Boxplot distribution of fecundity (oocytes per polyp) from June Ambient versus sprig condition .........................................................37
LIST OF THEOREMS AND EQUATIONS

Equation 1. Anthropogenic carbon dioxide and water..................................................8

Equation 2. Increases in bicarbonate in a system .........................................................8

Equation 3. Dissolution of calcium carbonate ...............................................................8

Equation 4. Calcium carbonate saturation state ...........................................................8

Equation 5. Average Fecundity......................................................................................20
LIST OF ABBREVIATIONS

GOA – Gulf of Alaska

POM – particulate organic matter

NOAA – National Oceanic and Atmospheric Agency

EFH – essential fish habitat

HAPC – habitat area of particular concern

ROV – remotely operated vehicle

SCUBA – Self-Contained Underwater Breathing Apparatus

pCO$_2$ – partial pressure of carbon dioxide

ppm – parts per million

ppt – parts per thousand

CO$_2$ – carbon dioxide

Ppm – parts per million

ASH – aragonite saturation horizon

OA – ocean acidification

CO$_3^{2-}$ – carbonate ion

H$^+$ – hydrogen ion

MgCO$_3$ – magnesium calcite

DIC – dissolved inorganic carbon
HCO₃⁻ - bicarbonate

H₂CO₃ – carbonic acid

Ca²⁺ - calcium ion

Kₛₚ – solubility product constant

CaCO₃ – calcium carbonate

RCP – Representative Concentration Pathways for greenhouse gas concentration

RCP8.5 – Representative Concentration Pathway for the highest greenhouse gas emissions

IPPC – Intergovernmental Panel on Climate Change

CESM4 – Community Earth System Model

PpOA – Primnoa pacifica ocean acidification project

DMC – Darling Marine Center

ANOVA – analysis of variance

RMANOVA – Repeated measures analysis of variance
LIST OF DEFINITIONS

Sprig – Branch from an individual, approximately 10 – 15 cm long, and containing a growing tip.

Time 0 – Indicates the subset of samples that were processed immediately for histology from the fjord in January 2016.

Ambient – The subset of samples from Kodiak Island that were in the ambient pH of 7.75 for the duration of the experiment.

2100 – The subset from Kodiak Island that were in the projected pH for 2100 (7.55 pH units) based on the RCP8.5 model from the IPCC.

Repeated Measures ANOVA – a test to detect any overall differences between related means.
CHAPTER 1
INTRODUCTION

1.1 *Primnoa pacifica* (Kinoshita, 1907)

Primnoidae is one of the most dominant gorgonian families in deep-sea and polar regions (Cairns and Bayer 2005; Taylor and Rogers, 2015) and the most abundant family of corals in Alaskan waters (Waller *et al.*, 2014). There are 266 species of the Primnoidae family known to date and they can occur from 8 – 5850 m worldwide (Taylor and Rogers, 2015). *Primnoa pacifica* has a wide geographic range in the north Pacific, including the Sea of Japan, the Sea of Okhotsk, the Aleutian Islands, the Gulf of Alaska, the Alexander Archipelago to the Dixon entrance, Glacier Bay, Alaska, and La Jolla, California (Cairns and Bayer, 2005). The red tree coral has a depth range of 9 - 1029 m (Cairns and Bayer, 2005; Matsumoto, 2007 and references therein), the shallowest of these depths are found in fjords in the Gulf of Alaska (GOA) due to a phenomenon called deep-water emergence (Waller *et al.*, 2014; Taylor and Rogers, 2015). The minimum annual survival temperature for *P. pacifica* is reported at 3.7℃ (Cairns and Bayer, 2005; Matsumoto, 2007). These corals prefer habitats dominated by bedrock with rough sea beds on a slope as well as in areas of high current speeds (Stone *et al.*, 2015).

Species that commonly inhabit depths of thousands of meters occur in the shallow waters of many sub-polar glacial fjords, however, the exact environmental processes that cause this phenomenon are unknown (Waller *et al.*, 2014; Taylor and Rogers, 2015). The cold, deep nutrient rich waters from beyond the shelf circulate into the fjord and remain for a long residence time beneath the freshwater runoff from the glacier (Freiwald *et al.*, 1997; Lindner *et al.*, 2008; Waller *et al.*, 2014). This freshwater layer creates a darkening effect, so that the saltwater beneath is as dark as it would be in the bathyal zone (Stone *et al.*, 2005; Lindner *et al.*, 2008;
Waller *et al.*, 2014). There are also strong currents within these fjords which mimic those in the deep sea (Stone *et al.*, 2005; Lindner *et al.*, 2008; Waller *et al.*, 2014). Fjords are important habitats at higher latitudes as they have high bathymetric relief, complex circulation patterns, geomorphic complexity, and have natural gradients in temperature, salinity, oxygenation, and water column properties (Syvitski *et al.*, 1987; Copeland *et al.*, 2013).

*Primnoa pacifica* is an ecosystem engineer, meaning colonies can modify their environment by directing small scale ocean currents to direct food and indirectly create living spaces for other organisms (Jones *et al.*, 1994; Waller *et al.*, 2014). They can also change the physical, chemical, and biological properties of sediments, along with alter siltation rates in the local environment (Jones *et al.*, 1994). Species that are considered to be ecosystem engineers do not create any of these resources, but rather provide external properties to other species (Jones *et al.*, 1994, Mortensen and Fosså, 2006; Waller *et al.*, 2014).

These corals are also known to form complex habitats which are then used by many species including invertebrates and commercially important fish species (Krieger and Wing, 2002; Roberts *et al.*, 2009 and references therein; Stone *et al.*, 2015 and references therein). *Primnoa pacifica* is an Essential Fish Habitat (EFH) where it forms thickets, as is common in the GOA (Stone, 2006). Due to the strong association between commercially important fish and invertebrate species at multiple life stages and *P. pacifica* thickets as a habitat structure, the corals are protected as an EFH for management purposes (Stone and Shotwell, 2007). This management practice acknowledges that the fish do not passively utilize the corals but rather actively use many aspects of the coral for multiple ecological purposes.

Within the GOA, Krieger (1993) observed small rockfish (*Sebastes* spp) associated with *Primnoa* spp and O’Connell and associates (1998) observed yelloweye rockfish (*Sebastes*...
ruberrimus) associated with red tree corals (Krieger and Wing, 2002). Krieger and Wing (2002) performed an additional observational study in the GOA to determine if megafaunal species were found within one meter of a Primnoa spp colony. They found 10 megafaunal groups associated with the corals; these species either feed on Primnoa spp polyps (sea stars, nudibranchs, and snails), use the branches to better their position for suspension feeding (crinoids, basket stars, anemones, and sponges), or use the colony for protection (crabs, shrimps, and rockfish). In the GOA, P. pacifica is a keystone species for 12 species of rockfish (Sebastes spp.) during multiple life history stages including juveniles and gravid females (Waller et al., 2014; Stone et al., 2015).

The exact association between commercial species and high-relief corals is unknown, however, their structure is known to provide refuge for species at various life stages as well as foraging ground (Auster, 2005; Stone, 2006; Stone et al., 2015). As such, certain areas of the GOA have been designated as Habitat Areas of Particular Concern (HAPC) (Stone et al., 2015). These areas were protected as a HAPC due to their associations with commercially important fish species in order to protect against bottom-contact fishing gear which damages and can kill the corals, thus eliminating their key role in habitat structure (Stone et al., 2015).

The red tree coral, Primnoa pacifica, is one of the most common habitat-forming cold-water soft corals in the Northeast Pacific Ocean (Matsumoto, 2007; Aranha et al., 2014; Morrison et al., 2015; Stone et al., 2015). Primnoa pacifica exhibits keystone species characteristics (King and Beazley, 2005; Waller et al., 2014; Stone et al., 2015), and is a foundation species (Gaylord et al., 2011), forming thickets (0.52 corals/m²) that provide essential habitat for ecologically important species (Krieger, 2001; Stone and Shotwell, 2007; Krieger and Wing, 2002; Waller et al., 2014; Stone et al., 2015). Keystone species play a critical role in community structure, and the removal of a keystone species would alter the composition of the
ecosystem (Jones et al., 1994). Keystone species all have a certain ecosystem trait which may be productivity, nutrient cycling, species richness or the abundance of a functional group (Power et al., 1996), and while they are typically top predators; they may occur at other trophic levels, examples include certain plant species, or ‘keystone modifiers’ and ecosystem engineers (Power et al., 1996). While corals would not have been defined as keystone species by Paine (1969), their unique importance to their ecosystem is not equivalent to their (low) abundance, placing them as keystone species.

*Primnoa pacifica* is a very long-lived species, living for decades to hundreds of years (Matsumoto, 2007 and references therein; Aranha et al., 2014; Waller et al., 2014; Stone et al., 2015). Due to their long life spans however, these corals are very slow growing, especially in colder waters (Stone et al., 2005; Stone, 2006; Matsumoto, 2007 and references therein; Roberts et al., 2009). These gorgonians have an axial skeleton composed of carbonate (CO$_3^{2-}$) and alternating layers of a horny protein called gorgonin (Matsumoto, 2007 and references therein). Sclerites in octocorals are composed of magnesium calcite (MgCO$_3$), whereas their axial skeletons are composed of a mixture of aragonite and calcite and the precise composition is dependent on water temperature (Matsumoto, 2007 and references therein).

Little is known about the reproductive processes and larval biology of most cold-water corals (CWCs) compared to their tropical water counterparts (Waller, 2005; Kahng et al., 2008; Simpson et al., 2007; Roberts et al., 2009; Waller et al., 2014). Patches of *P. pacifica* occur in distinct age cohorts in the GOA, indicating successful recruitment events are infrequent, occurring approximately once a decade (Stone et al., 2015).

The reproduction and seasonality of a shallow-emerged population of *P. pacifica* in Tracy Arm Fjord in southeastern Alaska has been studied by Waller and associates (2014). The
study found that an individual colony takes more than 16 months to form mature oocytes (female gametes), which then adhere to the outside of the polyps, where they are most likely fertilized.

Each female polyp produces approximately 17 mature oocytes in one cohort, while another cohort of previtellogenic oocytes develops within the mesentery concurrently (Waller et al., 2014). Spermatogenesis (production of sperm packets in males) was found to be shorter than oogenesis. Oocytes in octocorals tend to develop over a longer period of time than is required for spermatogenesis, as oocytes are energetically expensive to produce (Kahng et al., 2011 and references therein; Waller et al., 2014 and references therein).

Spawning was largely asynchronous with the majority of females maturing in January, whereas most males matured in three cycles throughout a year, from September to December, September to January, and March to June (Waller et al., 2014).

1.2 Anthropogenic Impacts

1.2.1 Physical Impacts

The natural predators of P. pacifica include species of sea stars, nudibranchs and snails (Krieger and Wing, 2002). Additionally, CWCs are often threatened by bottom trawling, long-line fishing, and being sold as display items such as jewelry (Häussermann and Försterra, 2007). The damage caused by fishing gear to these slow-growing corals is a concern due to their importance for structure in the ecosystem (Krieger and Wing, 2002). The amount of coral removed incidentally is unknown, however, coral removed during assessment surveys indicates it is frequently caught in bottom-fishing gear. A bottom trawl survey in the GOA and Aleutians removed Primnoa spp in 168 out of 3553 hauls (NMFS bottom-trawl data base, Alaska Fisheries Science Center, Seattle, Washington in Krieger and Wing, 2002).
Colonies are easily damaged or dislodged from the seabed by external forces; remotely operated vehicle (ROV) surveys from the GOA indicate that the thickets which form EFHs have been disturbed by fishing gear (Krieger, 2001; Stone et al., 2015). Most observed damage is caused by strain and entanglement which can lead to mortality or sublethal trauma (Stone et al., 2015). Removal of up to 50% of a colony is considered sublethal, however that amount of damage removes a considerable portion of the colony’s reproductive potential (Waller et al., 2014; Stone et al., 2015). A study by Aranha and colleagues in 2014 revealed that *P. pacifica* will recover slowly from disturbances such as trawling.

Larger colonies are more susceptible to trawling damage than smaller colonies (Waller et al., 2014; Stone et al., 2015). This is a concern for the habitat and population as those larger colonies are more fecund than smaller colonies (Hughes and Connel, 1999; Waller et al., 2014) thus contributing more gametes to the system. Those that are affected by external damage show a reduced fitness by a reallocation of energy and planulae expulsion (Waller et al., 2014 and references therein).

To further protect CWCs from these direct anthropogenic impacts, efforts need to be made to properly map their locations and ban longline fishing from areas of high concentrations (Häussermann and Försterra, 2007).

### 1.2.2 Chemical Impacts

In 1980, the annual atmospheric partial pressure of carbon dioxide (pCO$_2$) in the atmosphere was measured in parts per million (ppm) by the Global Monitoring Division of NOAA/Earth System Laboratory, the average was 338.8 ± 0.10 ppm. The average annual pCO$_2$ has continued to rise since then, in 2015 the average was 399.41 ± 0.10 ppm (Dlugokencky and Tans, 2018).
For the first time, the 2016 pCO$_2$ was over 400 ppm for the year and averaged at 402.81 ppm globally (Dlugokencky and Tans, 2018). 2017 raised the global average more than 2 ppm (Dlugokencky and Tans, 2018). In 2018, the average atmospheric pCO$_2$ has continued to rise, once again higher by an average of 2 ppm higher each week than the corresponding week from the 2017 dataset (Dlugokencky and Tans, 2018).

The ocean absorbs approximately one million metric tons of anthropogenic CO$_2$ per hour (Noone et al., 2013 and references therein); over the past 200 years, since the beginning of the Industrial Revolution, this translates to approximately 25% of the total emissions (Noone et al., 2013 and references therein). The ocean’s ability to naturally absorb this CO$_2$ creates a partial buffering system for anthropogenic climate change, however, it also leads to serious alterations of global ocean chemistry (Noone et al., 2013).

Recent increases in atmospheric CO$_2$ have lowered the average ocean pH by 0.1 pH units since the Industrial Revolution and is projected to lower by another 0.3 – 0.4 units by the end of the century (The Royal Society, 2005; IPCC, 2007; Albright, 2011; Form and Riebesell, 2012; Noone et al., 2013). Ocean acidification (OA) results from the mixing of atmospheric CO$_2$ in the ocean causing a decrease in oceanic pH. The average CO$_2$ emission rate is 8 Gt C/year which is 16 times the rate during the Paleocene – Eocene Thermal Maximum (PETM; Gibbs et al., 2006; Kurihara, 2008). OA was a primary driver of past mass extinctions and time periods of millions of years where reefs were recovering from those mass extinctions (Guinotte and Fabry, 2008 and references therein).

Future climate scenarios show that 15 – 37% of species and taxa will come extinct by 2050 (Thomas et al., 2004; Kurihara, 2008). The chemistry of the oceans is approaching conditions that haven’t been seen in millions of years and the rate of these changes is
unprecedented (Caldeira and Wickett, 2003; Guinotte and Fabry, 2008). This is then compounded as rapid evolution of calcifying organisms is unlikely since they are so long-lived (Berteaux et al., 2004; Kurihara, 2008).

The decrease in pH in the tropical waters of Hawaii since the Industrial Revolution is occurring at 0.019 pH units per decade (Doney et al., 2009; Noone et al., 2013), whereas the decrease in the Arctic Sea is declining at 0.024 pH units per decade. The dissolved inorganic carbon (DIC) in seawater at the average pH of 8.1 pH units and an average salinity of 35 parts per thousand (ppt) is comprised of <1% carbon dioxide (CO₂), <1% carbonic acid (H₂CO₃), 91% bicarbonate ions (HCO₃⁻), and 8% carbonate ions (CO₃²⁻). As CO₂ emissions increase, this increases the dissolved CO₂, H₂CO₃, and HCO₃⁻ as well as free H⁺ ions (Noone et al., 2013).

\[ CO₂ + H₂O ↔ H₂CO₃ ↔ H⁺ + HCO₃⁻ ↔ H⁺ + CO₃²⁻ \]

The decrease in the concentration of carbonate ions (CO₃²⁻) is caused by the reaction between carbon dioxide (CO₂) and carbonate (CO₃²⁻), which increases the number of bicarbonate ions (HCO₃⁻) in the system (Noone et al., 2013).

\[ CO₂ + CO₃²⁻ + H₂O → 2HCO₃⁻ \]

This decrease in carbonate availability increases the rate of dissolution of calcium carbonate (CaCO₃; Noone et al., 2013).

\[ CaCO₃ + CO₂ + H₂O → Ca^{2+} + 2HCO₃⁻ \]

OA results in a decrease of the concentration of carbonate ions ([CO₃²⁻]) in the system, thereby reducing the calcium carbonate saturation state which is determined by the formula:

\[ \Omega = [CO₃²⁻][Ca^{2+}]/K_{sp} \]

\[ \Omega < 1 = \text{undersaturation} \]

\[ \Omega > 1 = \text{super saturation} \]
$K_{sp}$ is the stoichiometric solubility of CaCO$_3$ (Kleypas et al., 2006; Kurihara, 2008) for aragonite at 25°C the $K_{sp}$ is $6.0 \times 10^{-9}$. $\Omega$ is the saturation state used to express of CaCO$_3$ saturation in seawater (Noone et al., 2013). The brackets ([[]]) indicate the concentration at equilibrium, neither dissolving or forming, which varies with temperature and pressure, and is more soluble at colder temperatures (Noone et al., 2013). Shells, skeletons and any other CaCO$_3$ structures start to dissolve in undersaturated conditions ($\Omega < 1$; Noone et al., 2013).

The aragonite saturation horizon (ASH) represents the depth at which aragonite shells can still be formed, below this depth aragonite dissolves. The aragonite and calcite saturation horizons in the North Pacific are currently very shallow (Feely et al., 2004) and are moving toward the surface at a rate of 1 – 2 m per year (Guinotte and Fabry, 2008).

The global ASH shoals an average of 4 m per year from the current depth of 1750 m (Noone et al., 2013). Deep-water coral ecosystems are often dominated by branching octocoral taxa, particularly in the North Pacific where the preexisting shallow calcium carbonate depth may limit the depth distribution of scleractinians (Kahng et al., 2011 and references therein).

This is likely to affect habitat-forming corals along with other calcifying species as the availability of carbonate ions (CO$_3^{2-}$) lowers due to more hydrogen ions (H$^+$) in the surrounding water column (Andersson et al., 2008).

Recent studies have suggested that calcifiers in high latitudes and in cold waters are at an immediate risk to OA due to the water being only slightly supersaturated with regard to carbonate, and the ASH will shoal as anthropogenic CO$_2$ emissions continue at their current rate (Andersson et al., 2008, Catarino et al., 2012). Gorgonians are composed of magnesium calcite (MgCO$_3$), which is the most soluble form of calcium carbonate (Noone et al., 2013).
Increased pCO$_2$ may have complex effects on the physiology, growth and reproductive success of marine calcifiers (Kurihara, 2008). A greater portion of an organism’s energy budget may be partitioned towards maintenance of the acid-base status and away from other fitness-sustaining processes such as shell and somatic growth, immune response, protein synthesis, behavior, and reproduction (Gazeau et al., 2013).

In order to better understand the effects of OA at the population level, the most sensitive life history stages, the early development and reproductive stages, need to be studied since the environmental requirements are more specific than at other stages (Thorson, 1950; Kurihara, 2008).

OA has the potential to negatively impact sexual reproduction, multiple early life stages, biological processes of many common coral species, and may contribute to substantial declines in recruitment that will be felt at the community or ecosystem scale (Albright, 2011; Nakamura et al., 2011; Noone et al., 2013). Calcifying organisms in an acidified ocean may shift from growth to dissolution due to the saturation depth shoaling and their global and local distribution may be restricted (Roberts et al., 2009).

*Primnoa pacifica*, other CWCs, and marine calcifying organisms, will be indicator species for climate change and OA (Anderson et al., 2008; Roberts et al., 2009). While studies continue to focus on calcification and growth, the interaction within these habitats in light of OA needs to be considered. OA could have significant indirect effects on fishes and other deep-sea organisms that rely on EFHs for protection and nutritional requirements (Guinotte and Fabry, 2008).

As information on the reproductive biology of CWCs increase and information on the importance of these habitats grows, the longevity of these habitats needs to be investigated. The
potential for organisms to upregulate certain processes in light of OA may indicate a potential for recovery, or possibly elucidate the loss of other expensive metabolic processes needed to continue a population.
CHAPTER 2

MATERIALS AND METHODS

2.1 Study Area and Collection

Tracy Arm Fjord in Southeastern Alaska is considered a “living laboratory” and was established as a study site for *P. pacifica* in 2006 (Waller *et al.*, 2014). The glacial fjord has a sill where it meets Holkham Bay in Southeast Alaska (Fig. 1) and is 49 km long, up to 378 m deep and ends in two tide-water glaciers, Sawyer and South Sawyer. The study site is 13.4 and 15.4 km from the Sawyer and South Sawyer glaciers respectively. Previous studies and surveys of this region show thickets of *P. pacifica* from as shallow as 10 m greater than 100 m (Waller *et al.*, 2014). The tides range more than seven meters and are diurnal. The vertical walls lining the fjord consist of greywacke and granodiorite that rise up to ~750 m above sea level.

In January of 2016, three branches, approximately 10 – 15 cm in length, each with a growing tip (from here on referred to as “sprigs”) from 54 individual colonies were collected from 9.8 – 18.6 m deep by SCUBA. Of the three sprigs from each colony, one was immediately fixed in a 4% formalin solution for 24 hours, then moved to 70% ethanol for the remainder of the cruise. They were shipped to the Darling Marine Center (DMC), in Walpole, ME for histological processing. The other two sprigs were kept alive in circulating ambient seawater on the cruise until they were transported to Kodiak Fisheries Research Center in Kodiak Island, Alaska for the acidification experiment.
Figure 1. Study site in Southeastern Alaska. The study area in Tracy Arm is denoted by the star. In the inlet map, the solid box indicates the zoomed in region and the dotted box marks Kodiak Island, where the tank experiment took place.
Sex was initially determined by Dr. Rhian Waller on the cruise by splitting open polyps under the dissecting microscope to see if oocytes or spermatocysts were in the polyp as there are no external structures indicating sex in octocorals (Watling et al., 2011). Based on the sexes determined on the cruise, 54 colonies were collected to maintain close to a 1:1 ratio. This initial analysis was later reconfirmed by histology, this experiment has 30 females, 20 males, and 4 non-reproductive colonies (Table 1).

**Table 1.** Locations and month of specimen collection by sex. Treatment is not included; all specimens were collected in 2016.

<table>
<thead>
<tr>
<th>Collection</th>
<th>Location</th>
<th>Female</th>
<th>Male</th>
<th>Non-reproductive</th>
<th>Specimens collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 8 - 11</td>
<td>Tracy Ann</td>
<td>30</td>
<td>20</td>
<td>4</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Kodiak Island</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>June 21 - 29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 22</td>
<td>Kodiak Island</td>
<td>40</td>
<td>24</td>
<td>8</td>
<td>72</td>
</tr>
</tbody>
</table>

**2.2 Tank Experiment**

All information regarding the tank experiment was provided by Allison Batemen, Fisheries Biologist and Seawater Laboratory Coordinator at the Kodiak Fisheries Research Center.

Two live sprigs from the 54 individuals were sent to Kodiak Fisheries Research Center where one was put in a flowing seawater tank with the expected pH concentration for 2100 (7.55 pH units) and the other was in another tank with ambient pH (7.75 pH units) for 6 – 9 months. The ambient pH of 7.75 pH units is the current pH at 175 m in the Eastern Gulf of Alaska. 7.55 pH units is the projected pH following RCP8.5 (high scenario) from the Intergovernmental Panel on Climate Change (IPPC) as used in the Community Earth System Model 4 (CESM4; Jessica Cross, Oceanographer at NOAA Pacific Marine Environmental Laboratory, pers. comm.).
In order to identify the treatment for each of the sprigs, samples from Tracy Arm were ordered PpOA01 – 54 (Time 0), and the corresponding sprigs from Kodiak Island were either labeled with that original number and an A or B, denoting the tank treatment. Treatment “A” marked the ambient treatment of 7.75 pH units (Ambient), and treatment “B” marked the acidity treatment of 7.55 pH units (2100). Sprigs of both treatments were randomized among the three tanks per treatment to avoid a “tank bias” (pers. comm. R. Stone, 2017; Fig. 2).

![Tank setup diagram](image)

**Figure 2.** Tank setup at Kodiak Fisheries Research Center, AK. Tanks 1, 5, and 6 were treatment A and tanks 2, 3, and 4 were treatment B. Each tank held 18 sprigs split between four rows. Highlighted cells represent individuals that were “spares” as their sex could not be determined to absolute certainty on the cruise.

Sprigs were suspended in the water column, tip facing downwards and tied with a Spectrafiber microfilament braided line (10-pound test). They were fed 25 ml of a unique mixture of six marine microalgae (Reed Mariculture Inc., Shellfish Diet 1800) that was diluted in
450 ml of unfiltered seawater once a week. The mixture was slowly and evenly poured throughout each tank, and the main water line was removed to ensure the food was not flushed out. The food would take approximately an hour and a half to completely drain from the tank once the water line was put back in. Any remaining food bits or sediment were siphoned away every 10 days to avoid disturbing the corals but also maintain a clean flow-through.

Water temperature was maintained at 4.5 - 5°C in continuously circulating seawater (2 l/min) following the same exposures used for recent acidification experiments including Alaskan crabs and fishes (e.g. Long et al., 2013). Each day, the pH and temperature were measured in each of the tanks. If a tank’s pH differed by .02 pH units, the set temperature was readjusted. If the temperature of a tank was out of range, the flowrate within that tank was readjusted, if any of the tanks were out of range by 0.2°C, the flowrates for each tank was rechecked and adjusted. Once a week, water samples were taken for alkalinity and dissolved inorganic carbon (DIC) analysis.

The tank experiment ran from January 14 to June 21, 23, 29, or September 22 of 2016 (Table 1). In June, the circulating system of Tank 3 (2100 treatment; Fig. 2) failed, causing all but three of the sprigs to shed their tissues which were then collected from the bottom of the tank. Those sprigs were then removed and processed for histology and sent to the DMC. The health of the remaining three sprigs in the tank began to deteriorate within the week and were removed and processed as well. The corresponding sprigs from the Ambient treatment were also removed within the week so that the reproductive data from the failed tank could potentially still be compared to the corresponding sprigs as originally intended.

After nine months, the remaining sprigs began to shed their tissues and were prepared for histological processing and shipped to the Darling Marine Center in Walpole, Maine.
2.3 Randomization

In order to further avoid bias when analyzing gametes between the two experiments, samples were randomized prior to histological processing. 109 samples from Kodiak Fisheries Research Center were arranged in five trays, and were mixed among themselves, then renumbered to follow the untreated corals (PpOA - 55 to PpOA - 163). Each tray held 25 individuals, and each sample was selected from each tray at random, and from each position at random. Samples were mixed among the trays approximately every 15 individuals to ensure they remained completely unordered. The initial 54 samples maintained their original numbering to verify sex for the corresponding sprigs from the same individual in later analysis. The label denoting the original number and tank identification code were covered by tape to ensure it was no longer visible to avoid potentially biasing the data. After the 163 samples were processed completely and all data were collected, the treatments and the tank set up were revealed.

2.4 Sprig Analysis

Volumetric measurements were done at the beginning and end of the experiment at Kodiak Fisheries Research Center. No volumetric data were collected on June 21, 2016 on the failed tank since the tissue was fragile and preserved immediately so as to not cause further desiccation or damage. Pictures were also taken of each individual sprig before and after the tank experiment to determine any difference in the tissue in response to the tank conditions. Comparisons were made between the Time 0 and September images, excluding the sprigs from the failed tank. Length and surface areas of live tissue were measured three times per sprig using ImageJ software. Volumetrics, lengths and surface areas were compared between the Ambient and 2100 treatments to determine if there was a significant difference between treatments. Analysis was done in R Studio, using the “car” package (Fox and Weisberg, 2011).
2.5 Histological Processing

Three to nine polyps were dissected from each sprig for histological processing following previous protocols from Waller and associates (2014). The polyps were decalcified with Rapid Bone Decalcifier for approximately 20 minutes, then dehydrated in serial ethanol dilutions from 30% to 100% over two and a half hours and left overnight in 100% ethanol. The following day, samples were cleared in Toluene solution for 20 minutes and then immersed in paraffin wax (Leica ParaPlast Plus) for approximately 48 hours at 56°C.

Tissue was then embedded in paraffin wax blocks and left to cool for at least 24 hours, then placed in a freezer at least one hour prior to sectioning with the microtome (Microm HM 325). All wax blocks were serially sliced through all material. Specimens were sliced 6 µm thick to maintain tissue quality; the distance between serial sections is 90 µm between slides, which is the average diameter of the oocyte nucleus in *P. pacifica* (Waller et al., 2014). Sectioned tissue was mounted on glass slides, dried on slide warmers, and stained (Fig. 3). Samples from Time 0 (PpOA01 – 54) were stained with Hematoxylin and Eosin and samples from the end of the experiment (PpOA 55 – 163) were stained with Masson’s Trichrome (Humason, 1962).
2.6 Histological Analysis

Slides were examined using an Olympus (CX31) compound microscope with a Motic video camera attachment. Images were captured using Motic Image Plus and analyzed with ImageJ (NIH) software to calculate oocyte and nucleus diameter.

Unless tissue limited, 100 spermatocysts were staged from I-IV, indicating increasing maturity, following the classification by Waller et al. (2014). Spermatocysts are surrounded by a thin layer of follicle cells which form a sack (Fig. 3). Stage I spermatocysts are smaller, loosely packed, and no sperm tails are visible. Stage II spermatocysts are slightly more densely packed, and show a congregation of sperm heads toward the center, where the lumen will be formed. In Stage III, the lumen is apparent, the sperm heads are organized and congregating on the outer edge near the follicle layer, and sperm tails can sometimes be seen. Stage IV spermatocysts have a large, irregularly shaped lumen full of sperm tails.
Fecundity was measured by counting all oocytes (both previtellogenic and vitellogenic; Fig. 3) in three polyps per individual and then averaged following Waller et al. (2014) to determine the average fecundity of the colony. Oocytes with a visible nucleus were the only oocytes counted to ensure there was no double counting. The same methodology was used for measuring the oocytes, as the nucleus occurs roughly central in the oocyte, meaning it is a true maximum diameter. 100 oocytes were measured for each female sprig, unless there was not enough tissue available to do so.

Histological analyses were initially performed using Microsoft Excel, and comparison between treatments and individuals were performed using R Studio Version 3.4.1. To determine normality prior to running all tests, a histogram, boxplot, and scatterplot were made. Since the data are dependent upon one another, a standard ANOVA or t-test could not be performed. A Repeated Measures ANOVA (RMANOVA) was performed for all data comparisons to satisfy the “within subjects” assumption from the “car” package (Fox and Weisberg, 2011). To determine if the difference in variance was significant, a paired t-test was performed within the same package.
CHAPTER 3

RESULTS

3.1 Sprig Analysis

Table 2 shows the average results from the volumetric, length and surface area measurements. The RMANOVA found that regardless of external measurement type, treatment had no effect on percent change in tissue (p > 0.05, Fig. 4, Table 7).

Table 2. Average results from exterior sprig measurements. For all measurement types and sampling dates, the number of sprigs was 35. SE indicates standard error.

<table>
<thead>
<tr>
<th></th>
<th>Volume (ml)</th>
<th>SE</th>
<th>Length (cm)</th>
<th>SE</th>
<th>Surface Area (cm²)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>January</td>
<td>7.26</td>
<td>0.43</td>
<td>14.25</td>
<td>0.62</td>
<td>13.46</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>1.39</td>
<td>0.12</td>
<td>12.6</td>
<td>0.68</td>
<td>4.45</td>
</tr>
<tr>
<td></td>
<td>Percent Change (%)</td>
<td>-80.89</td>
<td>7.18</td>
<td>-11.69</td>
<td>3.22</td>
<td>-67.2</td>
</tr>
<tr>
<td>2100</td>
<td>January</td>
<td>7.69</td>
<td>0.51</td>
<td>14.19</td>
<td>0.63</td>
<td>14.06</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>1.39</td>
<td>0.12</td>
<td>12.24</td>
<td>0.73</td>
<td>4.56</td>
</tr>
<tr>
<td></td>
<td>Percent Change (%)</td>
<td>-80.66</td>
<td>10.09</td>
<td>-13.69</td>
<td>3.57</td>
<td>-68.09</td>
</tr>
</tbody>
</table>
Figure 4. Boxplot distribution of percent change (%) versus treatment and measurement type. N represents number of individuals. For each treatment and methodology, N = 35.

3.2 Gametogenesis

3.2.1 Spermatogenesis

Tissue from 20 male individuals were analyzed in this study. There were 20 males from Time 0, 18 males from the Ambient treatment, and 12 males from the 2100 treatment (Table 3).

Table 3. Number of individuals per sex per treatment from the tank experiment. Change in number of reproductive individuals results from either the failed tank where no viable sample was retrieved, or no gametes were found in the tissue available.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Time 0</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Males from the Time 0 subsample show a mixed composition of all four spermatocyst stages within an individual. Stage II was the mode stage (Figs. 5 and 6).
Figure 5. Mean percent spermatocyst stage (%) versus treatment. Sperm stages increase with maturity. N indicates number of individuals and n indicates number of spermatocysts (Time 0: N = 20, n = 2000; Ambient: N = 18, n = 1710; 2100: N = 12, n = 1070).

Males from the Ambient treatment show a homogenous composition with a majority of the spermatocyst stages seen were from Stage I. Where Stage I was not the only spermatocyst stage, 83 – 99% of the spermatocysts were Stage I, and the corresponding 17 – 1% were from either Stage II or III (Figs. 5 and 6).

Males from the 2100 treatment also show a slightly varied composition with a majority of the spermatocyst stages were Stage I. Where Stage I was not the only stage present in an individual, 66 – 99% were Stage I, and the other 34 – 1% were from Stages II or III, except for two of the individuals which had all four stages (Figs. 5 and 6).
Figure 6. Mean percent spermatocyst stage (%) versus treatment including the 2014 reproductive dataset. Sperm stages increase with maturity. N indicates number of individuals and n indicates number of spermatocysts (September 2010: N = 15, n = 1621; June 2011: N = 15, n = 1600; September 2011: N = 8, n = 766; January 2012: N = 14, n = 895; January 2013: N = 15, n = 1149; Time 0: N = 20, n = 2000; Ambient: N = 18, n = 1710; 2100: N = 12, n = 1070).

The RMANOVA was used to determine if there was a difference in composition of each spermatocyst stage between treatments (Time 0, Ambient, and 2100). Tests were run on both the raw and percent data, to ensure both yielded the same result. Each test also assumed an unbalanced design, given that many individuals did not have usable tissue for histological analysis. Regardless of the spermatocyst stage or data type, the statistical result was the same; treatment had an effect on mean percent of each stage (p < 0.05; Tables 8-11). Time 0 was statistically different from both Ambient and 2100 (p < 0.05; Tables 8-11), but Ambient and 2100 were not statistically different from one another (p > 0.05; Tables 8-11).
In order to compare the OA dataset to the 2014 reproductive dataset (Fig. 6), a RMANOVA was used to compare the spermatocyst stages from January of 2013 to Time 0. Stages I, III, and IV were statistically different from January of 2013 to Time 0. Stage II from both time points had a true difference of zero. Since Stage II was the only stage where the sprigs from 2016 were statistically the same as those from the 2014 reproductive study (Table 12), the Stage II spermatocysts from both treatments were compared to the previous data set.

The RMANOVA showed that between June Ambient and June 2011, the Stage II percentages were statistically the same (p > 0.05; Table 13). For the 2100 treatment, however, individuals had no usable histological tissue and cannot be compared.

For September 2016, eight males were compared between the old reproductive data, seven from the Ambient treatment, and eight from the 2100 treatment. The RMANOVA showed that treatment once again had an effect (p < 0.05; Table 14). The old reproductive data was significantly different from Ambient and 2100 treatments (p < 0.05; Table 14), however, Ambient and 2100 were not significantly different from one another (p > 0.05; Table 14).

A RMANOVA was run to determine if tissue loss throughout the experiment had an effect on spermatocyst stages. Regardless of the stage investigated, external indicators had no effect on spermatocyst percent composition (p > 0.05; Tables 15, 16).

### 3.2.2 Oocytes

Tissue from 30 female individuals was examined in this study. There were sprigs of these 30 females from Time 0 and from the Ambient treatment, and 20 female sprigs (due to losses) from the 2100 treatment (Table 3).

#### 3.2.2.1 Oocyte size

The mean oocyte diameter for Time 0 was 106.8 ± 1.09 µm, Ambient was 77.79 ± 1.12 µm and 2100 was 67.18 ± 1.09 µm (Fig. 7). The RMANOVA for oocyte diameter
comparing the three treatments found that treatment had an effect on oocyte diameter (p < 0.05; Table 17) and that each treatment was statistically different from one another (p < 0.05; Table 17).
Figure 7. Histogram of percent oocyte frequency (%) versus oocyte diameter (µm). From top to bottom: Time 0, Ambient, and 2100. N indicates number of individuals, n indicates number of oocytes measured, and \( \mu_x \) is the mean, which is shown by the arrows.
Figure 8. Percent oocyte frequency (%) versus oocyte diameter (µm) by treatment and month. From top to bottom and left to right: September 2010, June 2011, September 2011, January 2012, January 2013, Time 0, Ambient, and 2100. N indicates number of individuals, n indicates number of oocytes measured, and $\mu_x$ is the mean, shown by the arrows.
The oocyte diameters were compared between Time 0, June Ambient and 2100, and September Ambient and 2100 to determine if the June oocytes were statistically different from the September oocytes (Fig. 8). The mean oocyte diameters split between collection months are shown in table 4. The RMANOVA showed that treatment and month had an effect on oocyte diameter ($p < 0.05$; Tables 18, 19) and that each month and treatment was significantly different from one another ($p < 0.05$; Tables 18, 19).

**Table 4.** End of experiment average oocyte diameter split by collection month. SE indicates standard error.

<table>
<thead>
<tr>
<th>Collection Month</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oocyte diameter (µm)</strong></td>
<td><strong>SE</strong></td>
<td><strong>Oocyte diameter (µm)</strong></td>
</tr>
<tr>
<td><strong>June</strong></td>
<td>95.47</td>
<td>1.52</td>
</tr>
<tr>
<td><strong>September</strong></td>
<td>70.12</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Oocyte diameters were then compared between the OA dataset, separated by month and treatment, and the 2014 reproductive dataset (Fig. 8). The mean oocyte diameters from the 2014 reproductive dataset are listed in table 5. The RMANOVA found that treatment and month had an effect on oocyte diameter ($p < 0.05$; Table 20), and that regardless of month, study, or treatment, all were statistically different from one another ($p < 0.05$; Table 20).
Table 5. Average oocyte diameters for 2014 reproductive dataset. SE indicates standard error.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Oocyte diameter (µm)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>2010</td>
<td>115.49</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>122.77</td>
<td>4.07</td>
</tr>
<tr>
<td>June</td>
<td>2011</td>
<td>112.3</td>
<td>1.68</td>
</tr>
<tr>
<td>January</td>
<td>2012</td>
<td>125.23</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>157.9</td>
<td>2.76</td>
</tr>
</tbody>
</table>

A RMANOVA was run to determine if there was a relationship between oocyte diameter and external tissue loss or gain (Fig. 9). While treatment did have an effect ($p < 0.05$; Tables 21-24) there was no conclusive significance between treatments regarding tissue loss or gain and oocyte diameter.
Figure 9. Boxplot distribution of oocyte diameter (µm) versus treatment and sprig condition. “Loss” refers to the overall length of the sprig being reduced during the experiment, and “gain” refers to the overall length of the sprig growing during the experiment.

3.2.2.2 Oosorption. 38 of the 80 females (48%) had structures near oocytes which did not have a nucleus, however had concentrations of lipids (Fig. 10). These would have been the approximate size of vitellogenic oocytes (~220 – 802 µm; Waller et al., 2014) if a nucleus had been present, and were observed alongside both previtellogenic and vitellogenic oocytes. Due to the small sample size of individuals from all treatment types with these structures, a RMANOVA would not be statistically robust. 20% of the Time 0 females, 63% of the Ambient females, and 65% of the 2100 females had these structures. There was no correlation between presence and treatment. Time 0 presence could not predict presence in Ambient or 2100 sprigs from the same individual. There also was no relationship between whether 100 oocytes were measured per sprig and presence of non-nucleated oocytes.
Figure 10. Light microscopy photograph of female polyp containing a non-nucleated oocyte at 100 times magnification on the left and 400 times magnification on the right. Scale bars are 100 µm and 500 µm respectively. PV = previtellogenic oocyte, V = vitellogenic oocyte, OO = oocyte undergoing oosorption.

3.2.3 Fecundity

All reproductive females have a colony height larger than 42 cm, colony height also has a positive relationship with maximum fecundity (Fig. 11).
Figure 11. Maximum fecundity (oocytes per polyp) versus colony height. Height measurements taken in situ. Trendline is logarithmic.

The average fecundity for Time 0 was 56.43 ± 3.13 oocytes per polyp, Ambient was 25.1 ± 3.19 oocytes per polyp, and 2100 was 17.32 ± 2.24 oocytes per polyp (Fig. 12). The RMANOVA determined that treatment had an effect on average fecundity (p < 0.05; Table 25), and that each treatment was statistically different from the others (p < 0.05; Table 25).

The OA dataset was then split between end month and treatment (Fig. 13). The mean fecundities were as follows: June Ambient was 45.07 ± 6.7 oocytes per polyp, June 2100 was 22.67 ± 2.46 oocytes per polyp, September Ambient was 14 ± 2.15, and September 2100 was 16.36 ± 2.59 oocytes per polyp (Fig. 14). The RMANOVA found that treatment and month did have an effect on fecundity (p < 0.05; Table 26). June Ambient with June 2100, and Time 0, June 2100 with all other months and treatments, September Ambient with September 2100, all ambient samples with all 2100, and September 2100 with all ambient and all 2100 fecundities were statistically the same (p > 0.05; Fig. 13; Table 26). June Ambient fecundity was statistically
different from both September Ambient and 2100, and the inclusive Ambient and 2100 fecundities (p < 0.05). September Ambient and 2100 were statistically different from Time 0 (p < 0.05; Fig. 13; Table 26).

**Figure 12.** Average fecundity (oocytes per polyp) versus treatment. Error bars indicate standard error. N represents number of individuals (Time 0: N = 30; Ambient: N = 30; 2100: N = 20).
Figure 13. Boxplot distribution of fecundity (oocytes per polyp) versus treatment, separated by collection month. N indicates number of individuals (Time 0: N = 29; June Ambient: N = 10; June 2100: N = 3; September Ambient: N = 20; September 2100: N = 17).

The average fecundity for the 2014 reproductive dataset is provided in table 6. The RMANOVA found that treatment and month had an effect on fecundity (p < 0.05; Tables 27-29). Apart from September 2010 and January 2013, all the old dataset months and Time 0 are statistically the same (p < 0.05; Tables 27-29). All the old dataset fecundities were statistically different from Ambient and 2100 (p < 0.05; Fig. 14; Tables 27-29).
Table 6. Average fecundity (oocytes per polyp) for 2014 dataset. SE indicates standard error.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Fecundity (oocytes/polyp)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>2010</td>
<td>73.39</td>
<td>8.56</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>63</td>
<td>9.4</td>
</tr>
<tr>
<td>June</td>
<td>2011</td>
<td>50.62</td>
<td>5.68</td>
</tr>
<tr>
<td>January</td>
<td>2012</td>
<td>59.07</td>
<td>5.49</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>40.9</td>
<td>4.31</td>
</tr>
</tbody>
</table>

Figure 14. Average fecundity (oocytes per polyp) versus treatment, including the 2014 reproductive dataset. Error bars represent standard error. N indicates number of individuals (September 2010: N = 19; June 2011: N = 19; September 2011: N = 7; January 2012: N = 19; January 2013: N = 13; Time 0: N = 29; Ambient: N = 30; 2100: N = 20; June Ambient: N = 10; June 2100: N = 3; September Ambient: N = 20; September 2100: N = 17).
Fecundity was also compared between end month of the OA experiment and the 2014 reproductive dataset. For June, treatment had an effect (p < 0.05; Table 28), Ambient was statistically the same as June 2011 (p > 0.05; Table 28), but 2100 was not (p < 0.05, Table 28). For September, treatment had an effect (p < 0.05; Table 29), and both Ambient and 2100 were statistically different from the old dataset (p < 0.05; Table 29).

A RMANOVA was used to determine a relationship between tissue change over the course of the experiment and fecundity, and tissue loss or gain had no effect on fecundity (p > 0.05; Fig. 15; Tables 30-32).

![Figure 15](image_url)  
**Figure 15.** Boxplot distribution of fecundity (oocytes per polyp) from June Ambient, versus sprig condition. Loss indicates a decrease in length during the study and gain indicates an increase in length during the study. N represents the number of individuals (Ambient Loss: N = 7; Ambient Gain: N = 3; Ambient: N = 10).
CHAPTER 4

DISCUSSION

The Time 0 samples were originally collected to be compared to the Ambient condition on the premise that the tank water conditions would be the same and the Ambient corals would show a similar reproduction results. The sprigs from the Ambient treatment, however, were significantly different than the Time 0 samples. The Ambient and 2100 results were statistically the same for both the exterior measurements and the spermatocyst stages, while the oocyte diameters and fecundities were statistically different between treatments. This negative trend in reproduction and exterior condition can be attributed to the animals being stressed by being in the tanks, regardless of treatment. This “null tank effect” likely reflects the absence of a homogeneity between the tank environment and the corals’ natural habitat (Anthony, 1999). This “null tank effect” is too large to determine any significance between treatments for the exterior measurements and spermatogenesis.

The null tank effect did cause a significant effect for the oocyte diameters and fecundities; however, treatment effects can still be discerned. Females in the 2100 treatment had a smaller average oocyte size and lower fecundity than those in the Ambient treatment. The 2100 females also had the highest proportion of resorbing oocytes. We hypothesize it would be unlikely that these females could have ever spawned naturally in the projected pH for 2100.

4.1 Sprig Analysis

The sprigs were measured before and after the experiments to determine any growth or decay while in the tanks and to identify a difference between treatments. Regardless of treatment, an overall loss of tissue was observed throughout the experiment. The largest percent change observed was from the volumetric measurements (Figs. 5 and 6), however, this may be a
limitation from the measurement methodology. The sprigs were suspended by line which would sometimes pull the last centimeter of tissue off the axis, causing the sprig to fall to the bottom of the tank. The lost tissue did not have a standard volume as there is no standard volume for a coral polyp and was not accounted for in the end of experiment measurements. This was, however, accounted for in the length measurements, but not the surface area for the same reason as the volumetric measurements.

The volumetric measurements had the largest percent loss, regardless of treatment type (listed Ambient then 2100), at -80.89 and -80.66%, while for length they were -11.69 and -13.69%, and surface area was -67.2 and -68.09% (Fig. 4). This difference in percent loss between measurement types may also indicate that while skeletal growth was occurring, the density was decreasing due to stress and a less robust and more soluble skeleton was being produced. Beniash and associates (2010) found that the juvenile Eastern oysters (*Crassostrea virginica*) shell mass changed but the shell area did not, indicating that the juveniles were depositing thinner shells (Gazeau *et al.*., 2013).

Corals can actively increase the pH in the organic matrix where calcification occurs so that calcification can occur even in undersaturated conditions (Hennige *et al.*, 2014 and references therein). The low abundance (~5%) of CWCs below the ASH suggest that the increased energetic demands cannot be met by most corals, and that dissolution of exposed skeletal framework may be greater than net calcification of tissue-covered skeleton (Hennige *et al.*, 2014).

The extent to which shell growth is dependent on aragonite saturation or related change in metabolism under low pH conditions is not well understood (Thomsen and Melzner, 2010; Gazeau *et al.*, 2013). The impacts on calcification could result in an altered energy allocation
(Wood et al., 2008), lower growth rates, reduced reproductive output, and decreased survival amongst calcifying organisms under conditions of OA (Kroeker et al., 2010).

A similar long-term experiment on the cold-water scleractinian, Lophelia pertusa, from the North Atlantic which compared the short- and long-term effects of increased pCO$_2$ on the growth of the coral (Form and Riebesell, 2012). In the short-term, a drop of even 0.1 pH units decreased the growth rate by 26 – 29%. In the long-term, there was no correlation between the increased pCO$_2$ and growth rate (Form and Riebesell, 2012). They suggested this increase could be a result of the slow growth rate of the species (Rodolfo-Metalpa et al., 2010; Form and Riebesell, 2012), which also applies to the slow growth rate of P. pacifica. This trend could also indicate the fluctuating environment that CWCs are exposed to, and the species’ necessity to cope with natural nutrition changes leading to shift this adaptability for corrosive conditions (Form and Riebesell, 2012).

Given the assumption of a reallocation of energy reserves from reproduction to calcification or growth, these exterior measurements were compared with the gametogenic data to determine if there was a correlation with any of the aspects of gametogenesis. This could indicate that the exterior condition could be a proxy for energy allocation as reproductive output. Long and associates (2008) noted that condition index and fecundity were correlated in a clam species in the Chesapeake Bay, however, this study did not determine a correlation between tissue loss or gain and gametogenic data.

**4.2 Gametogenesis**

**4.2.1 Spermatogenesis**

The Time 0 samples can be added to the existing reproductive dataset from Waller and associates (2014). While only Stage II was seen to be statistically equivalent to the previous
dataset, the varied composition was expected given the noted sporadic composition of the stages (Waller et al., 2014). The exact composition shifts each year and month, however in all January time points, all four spermatocyst stages were present (Fig. 6). This long-term reproductive series can aid future research to better understand environmental and seasonal cues for gametogenesis, spawning, and larval development.

The Time 0 samples had a heterogeneous composition with a mode of Stage II, followed by a slightly varied composition in 2100 with the mode being Stage I, and the Ambient condition which was composed almost entirely of Stage I, with an average of 2.28% from Stage II, 0.47% from Stage III, and no Stage IV spermatocysts were found. The results from the Ambient and 2100 spermatocyst data were not significantly different, however, the absence of Stage IV sperm in Ambient and presence from 2100 could be noteworthy.

While this study did not determine a significant difference between treatments for spermatocyst development, some studies have observed a decrease in sperm motility following spawning under acidified conditions. For the sea urchin *Strongylocentrotus purpuratus*, sperm motility is suppressed at some pH value less than 7.0 pH units (Christen et al., 1983; Kurihara, 2008); while decreases in sperm swimming speed and percent motility have been seen for sperm from the sea urchin, *Heliocidaris erythrogramma* exposed to a pH as high as 7.7 pH units (Havenhand et al., 2008; Kurihara, 2008).

Disregarding the negative null tank effect, there is a potential for the visually apparent but not statistically significant presence of Stage IV in the 2100 treatment. Caldwell and associates (2011) performed a study looking at sperm swimming speeds in sea urchins at different pH ranges and temperatures. Subsamples from each treatment were taken at 10, 20, and 30 minutes following an induced spawning. For each subsample, measurements of pH,
temperature, mean percent motility and swimming velocity were taken. The lower pH treatments found a higher mean percent motility and swimming velocity. It was also noted, however, that while those increased, it would come at a metabolic cost, indicating a decrease in sperm longevity (Caldwell et al., 2011). This study did not look at fertilization success within these pH regimes, but concluded that ocean acidification has a potential to disrupt the reproductive and development processes and should still be considered a primary concern (Caldwell et al., 2011).

While there was no significantly negative difference between Ambient and 2100 treatments with regards to spermatocyst composition, there is still more to be understood regarding sperm and OA, as well as the effects of combining eggs and sperm under acidified conditions. Any improved potential in sperm swimming could potentially be cancelled out by reduced oocyte fitness, fertilization success, or embryonic condition (Reuter et al., 2011; Caldwell et al., 2011).

4.2.2 Oocytes

While a 2100 tank failed in June, there were three females which did not initially show signs of stress (i.e. shedding tissues). The average oocyte diameter for 2100 includes those smaller oocytes, as it is difficult to determine if the average oocyte diameter was smaller from June 2100 (Figs. 7 and 8) due to the stress of the projected pH of the tank, or because of the tank failure itself. The presence of these oocytes did not alter the statistics, so they are included in the overall 2100 oocyte frequency and average calculations.

4.2.2.1 Oocyte Size. The average oocyte diameter for Time 0 was 106.8 µm, Ambient was 77.8 µm, and 2100 was 67.2 µm (Fig. 7), which were all significantly distinct from one another. While this difference between Time 0 and Ambient oocyte diameters indicates the null tank effect, the difference between the Ambient and 2100 may indicate a more notable trend. The
2100 oocytes are smaller than those from Ambient, indicating less lipid reserves available for gametogenesis and reproduction which can impact many life history stages. Smaller eggs can have reduced lipid content (Emlet and Hoegh-Guldberg, 1997; Dupont et al., 2013), and these can have negative consequences for larval fitness such as increasing time in the pelagic before metamorphosis and reducing juvenile quality (Dupont et al., 2013 and references therein). Larval longevity is dependent on their high lipid content (Tay et al., 2011 and references therein) which is provided from the oocyte.

These long-term tank experiments are important to identify the effects of environmental conditions on a more realistic timescale. Short-term studies may conclude that OA has no effect on an observed biological process because the organism is able to upregulate that process for that time, however over six to nine months, there may be a different result. This was shown between those individuals removed in June 2016 versus September 2016 (Fig. 8). The average oocyte diameter for June Ambient is significantly higher than in September (95.5 μm and 70.12 μm, respectively). The June 2100 and September 2100 comparison is not robust unfortunately as there were only three females from June 2100, therefore just shows a trend.

The difference in the Ambient oocytes between months shows the overall stress from the tank experiment shown in a significant decrease in size. It also shows an energy allocation shift between six and nine months of the experiment.

The oocyte diameters from the 2014 reproductive dataset were significantly different between all months (Fig. 8). There were natural fluctuations ranging from 112.3 μm to 157.9 μm (Fig. 8). Time 0 was smaller than this range, still within 10 microns, whereas the average diameters from the OA experiment were much smaller. The large, yolky, vitellogenic oocytes (> 400 μm) are mostly absent from the OA experiment (Fig. 8), not only lowering the average
oocyte diameter, but potentially having negative impacts for the population as those larger oocytes are considered to be developed and become ready to spawn as they grow to as large as 802 µm (Waller et al., 2014).

4.2.2.2 Oosorption. The combined results of a smaller average oocyte diameter between the 2100 and Ambient treatments (67.2 µm and 79.1 µm, respectively), along with an increased presence of these large, non-nucleated lipid structures (65% and 63%) suggest that lipid reserves may have been redirected from gametogenesis under experimental conditions.

The presence of multiple cohorts of oocytes within one polyp is not uncommon for octocorals, where a portion will mature during one season to be followed by another, without depleting the entire stock of oocytes (Kahng et al., 2011 and references therein). This continual pool of oocytes may be a mechanism for delayed egg maturation within a season, rather than at onset of development (Brazeau and Lasker, 1989).

The lipid-dense structures were seen within reproductive polyps, alongside both previtellogenic and vitellogenic eggs, and generally near the gastrovascular wall. None of these structures were the size of previtellogenic eggs, as those would lack sufficient lipid reserves to be worth resorption (Eckelbarger et al., 1984).

Initially, we considered them to be unidentified extracellular material, however due to the consistency with other oosorption observations, they align as oosorbed oocytes. These structures had a membrane surrounding the mass of cells, similar to the vitelline envelope in oocytes (Fig. 10). Surrounding the membrane were small cells which seemed to have suspended nuclei which were connected by a less rigid conglomerate, similar to observations from Eckelbarger and associates (1984) of intracellular gaps leading to an unorganized association of cells on the exterior of the oocyte. Within the membrane were small, round concentrations of cells which
stained as lipids, as oocytes do, however, were more densely packed and unorganized than those in vitellogenic oocytes. In *Capitella* (sp. I) oosorption, it was observed that within the oocyte, the yolk body and lipid droplets became closely packed, and were up to 6 \( \mu \text{m} \) in diameter (Eckelbarger *et al.*, 1984). No nuclear membrane was seen, however the interiors of these masses had structures which stained as nucleic acid, though they lacked a nuclear membrane.

For this study, there was no relationship between fecundity or oocyte size with the presence of oosorption. Oosorption does not prevent other oocytes from developing within the same individual, and smaller eggs are likely held in a reserve to develop from the resorbed lipids (Eckelbarger *et al.*, 1984).

Since these structures were seen in Time 0 females as well as Ambient and 2100, it seems the process occurs naturally, which is similar for oosorption in other long-lived species. Oosorption for polychaete species usually occurs in long-lived species which reproduce more than once in a lifetime as a mechanism for converting that energy back into stored energy for somatic growth (Eckelbarger *et al.*, 1984).

The increased proportion of oosorption from the tanks indicates the stress response of these corals to the acidity and difference from natural environmental conditions. Oosorption has been seen as a stress response for individuals that were kept with minimum food, stagnant water, and varying water temperatures (Eckelbarger *et al.*, 1984). Species without significant nutrient reserves who devote a large portion of their energy budget to gametogenesis may resorb gametes in response to starvation or stressors that induce a rapid energy deficit (Eckelbarger *et al.*, 1984).

Kaniewska and associates (2012) indicated a possible breakdown of lipid reserves, which could provide the energy needed to maintain net calcification rates with the suppressed metabolism observed in several OA studies. This increase in lipid breakdown would not be
apparent through changes in tissue mass over that relatively short (21 days) period (Hennige et al., 2014), however, may be demonstrated by the oosorption observed in this long-term study.

The increased occurrence of oosorption coinciding with smaller oocytes could indicate that spawning of fully developed oocytes, or natural spawning may not be possible under acidified conditions.

4.2.3 Fecundity

The fecundity and height relationship from this study corroborates Waller and associates’ (2014) conclusion that colonies larger than 42 cm are reproductive, and there is a slightly positive relationship with size and fecundity as shown by the logarithmic line (Fig. 11).

The average fecundity from Time 0 was 56.4 oocytes per polyp, Ambient was 25.2 oocytes per polyp, and 2100 was 17.3 oocytes per polyp (Fig. 12). While all three fecundities are statistically different from one another, the 2100 average fecundity is significantly lower than the Ambient treatment. As fecundity can be used as a proxy for reproductive effort of a colony (Leuzinger et al., 2003; Kahng et al., 2011), this marked decrease in fecundity with an increase in acidity elucidates a decrease in reproductive effort under stress.

When compared to the 2014 reproductive dataset, the OA experiment fecundities were significantly different and smaller (Fig. 14), apart from June Ambient and June 2011, which were statistically equivalent. While the 2014 dataset showed natural heterogeneity in fecundities over various months and years, there is a significant drop in polyp fecundity after exposure to increased pCO₂ conditions. Decreased fecundity is likely to reflect the increased energy costs needed for survival in an acidified environment (Dupont et al., 2013).
4.3 Conclusions and Future Studies

The results from this study are just the beginning for studying the effects of OA on the allocation of energy using the processes of gametogenesis and reproduction as proxies for CWCs. While the spermatogenesis results cannot be fully compared due to the observed null tank effect, the oogenesis results are concerning. The smaller and more infrequent occurrence of oocytes from the 2100 females indicate their inability to allocate energy to oogenesis in acidified conditions. These results combined with the increased presence of oosorption could have potentially deleterious effects for the population in future oceans if CO₂ emission rates do not change.

Future studies need to investigate both gametogenesis and reproduction under OA. Better understanding of spawning cues for cold-water species as well as larval development and behavior is needed to accurately cue reproduction under predictable conditions.

Recent studies which allude to upregulation or homeostasis for growth and calcification need to also compare which biological processes are being down-regulated to accommodate the shift (Gazeau et al., 2013; Hennige et al., 2014). Merely observing the exterior is no longer sufficient for truly understanding the condition of organisms living in an acidified ocean and other environmental shifts.

The apparent null tank effect from this study calls for a need to better understand the natural environment of cold-water species to replicate the in-situ conditions in tank experiments. This will aid in truly understanding the effects of OA without compounding factors.

Biological and ecological baselines need to be studied and understood both in situ and in laboratory conditions to research essential species and life history stages in a changing
environment. Studies need to include multiple life history stages in order to understand potential carry-over effects from generation to generation under stress.

While commercially important species have been the primary focus of OA studies in previous studies, there are ecosystem effects which are unknown due to the lack of research in those areas. There needs to be a renewed focus on the species which create habitat and structure for those other species, particularly keystone species.
REFERENCES


Dlugokencky, E., Tans, P. NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/)


APPENDIX OF STATISTICS

Results of the Repeated Measures ANOVAs, all results are p-values from a paired t-test within the R Studio package “car” (Fox and Weisberg 2011). Significant p-values (p < 0.05) are indicated by italics.

A1. Sprig Analysis

Length dataset with Ambient and 2100
H₀: The percent change of length does not depend on treatment
ANOVA: p-value = 5.75e-1

Surface area dataset with Ambient and 2100
H₀: The percent change of surface area does not depend on treatment
ANOVA: p-value = 5.65e-1

Volumetric dataset with Ambient and 2100
H₀: The percent change of volume does not depend on treatment
ANOVA: p-value = 8.99e-1

Table 7 All sprig measurement type datasets
H₀: The percent change does not depend on measurement type
ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th>Measurement Type</th>
<th>Surface Area</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
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<td>&lt;6.6e-16</td>
<td>&lt;6.6e-16</td>
</tr>
<tr>
<td>Surface Area</td>
<td></td>
<td>5.21e-12</td>
</tr>
</tbody>
</table>

A2. Males

Table 8 OA dataset, Stage I spermatocysts.
H₀: The percent of stage I spermatocysts does not depend on treatment
ANOVA: p-value = 9.485e-07

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>5.118e-09</td>
<td>4.321e-05</td>
</tr>
<tr>
<td>Ambient</td>
<td></td>
<td>4.28e-1</td>
</tr>
</tbody>
</table>

Table 9 OA dataset, Stage II spermatocysts.
H₀: The percent of stage II spermatocysts does not depend on treatment
ANOVA: p-value = 6.102e-11

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>4.728e-10</td>
<td>1.068e-10</td>
</tr>
<tr>
<td>Ambient</td>
<td></td>
<td>4.34e-1</td>
</tr>
</tbody>
</table>
Table 10 OA dataset, Stage III spermatocysts

H₀: The percent of stage III spermatocysts does not depend on treatment
ANOVA: p-value = 2.718e-6

<table>
<thead>
<tr>
<th>Treatment</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>7.569e-07</td>
<td>2.968e-04</td>
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<tr>
<td>Ambient</td>
<td>2.92e-1</td>
<td></td>
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</table>

Table 11 OA dataset, Stage IV spermatocysts

H₀: The percent of stage IV spermatocysts does not depend on treatment
ANOVA: p-value = 2.38e-3

<table>
<thead>
<tr>
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<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>7.03e-3</td>
<td>2.48e-2</td>
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<tr>
<td>Ambient</td>
<td>3.43e-1</td>
<td></td>
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</tbody>
</table>

Table 12 OA dataset with 2014 reproductive dataset, January 2013 spermatocyst stages

H₀: The percent of each stage of spermatocyst does not depend on treatment

<table>
<thead>
<tr>
<th>Stage</th>
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<tr>
<td>Stage III</td>
<td>1.17e-3</td>
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<tr>
<td>Stage IV</td>
<td>1.35e-2</td>
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</table>

Table 13 OA dataset with 2014 Reproductive Dataset, June Ambient to June 2011 Stage II Spermatocysts

H₀: The percent of stage II spermatocyst does not depend on treatment

<table>
<thead>
<tr>
<th>Stage II</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.05e-1</td>
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</table>

Table 14 OA dataset with 2014 reproductive dataset, September OA to September 2011 Stage II spermatocysts

H₀: The percent of stage II spermatocysts does not depend on treatment
ANOVA: p-value = 1.0e-3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
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</thead>
<tbody>
<tr>
<td>September 2011</td>
<td>2.7e-2</td>
<td>2.7e-2</td>
</tr>
<tr>
<td>Ambient</td>
<td>3.86e-1</td>
<td></td>
</tr>
</tbody>
</table>

Table 15 OA dataset with sprig length dataset and Stage I spermatocysts

H₀: The percent of stage I spermatocysts does not depend on treatment
ANOVA: p-value = 2.27e-1

<table>
<thead>
<tr>
<th></th>
<th>2100 Loss</th>
<th>Ambient Gain</th>
<th>2100 Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient Loss</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2100 Loss</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ambient Gain</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Table 16 OA dataset with sprig length dataset and Stage II spermatocysts
H₀: The percent of stage II spermatocysts does not depend on treatment
ANOVA: p-value = 0.2948

<table>
<thead>
<tr>
<th></th>
<th>2100 Loss</th>
<th>Ambient Gain</th>
<th>2100 Gain</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1</td>
</tr>
<tr>
<td>2100 Loss</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ambient Gain</td>
<td></td>
<td></td>
<td>1</td>
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</tbody>
</table>

A3. Females

Table 17 OA dataset, oocyte diameters
H₀: The oocyte diameter does not depend on treatment
ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>&lt;6.6e-16</td>
<td>&lt;6.6e-16</td>
</tr>
<tr>
<td>Ambient</td>
<td></td>
<td>6.831e-4</td>
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</tbody>
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Table 18 OA dataset, oocyte diameters: only Time 0 and September 2016
H₀: The oocyte diameter does not depend on treatment
ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>&lt;6.6e-16</td>
<td>&lt;6.6e-16</td>
</tr>
<tr>
<td>Ambient</td>
<td></td>
<td>3.257e-05</td>
</tr>
</tbody>
</table>

Table 19 OA dataset, oocyte diameters: Time 0, Ambient, and 2100 split by month
H₀: The oocyte diameter does not depend on treatment
ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th></th>
<th>June 2000</th>
<th>June 2100</th>
<th>September 2010</th>
<th>September 2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>7.71e-05</td>
<td>3.74e-15</td>
<td>3.74e-15</td>
<td>3.74e-15</td>
</tr>
<tr>
<td>June Ambient</td>
<td></td>
<td>4.485e-13</td>
<td>3.74e-15</td>
<td>3.74e-15</td>
</tr>
<tr>
<td>June 2100</td>
<td></td>
<td></td>
<td>3.05e-2</td>
<td>3.05e-2</td>
</tr>
<tr>
<td>September Ambient</td>
<td></td>
<td></td>
<td></td>
<td>7.864e-04</td>
</tr>
</tbody>
</table>
Table 20 OA dataset of oocyte diameters (split by month) with 2014 reproductive dataset

H₀: The oocyte diameter does not depend on treatment

ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th></th>
<th>September 2010</th>
<th>June 2011</th>
<th>January 2013</th>
<th>Time 0</th>
<th>June Ambient</th>
<th>June 2100</th>
<th>September Ambient</th>
<th>September 2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2010</td>
<td>8.12e-2</td>
<td>4.73e-1</td>
<td>1.7e-1</td>
<td></td>
<td>3.74e-15</td>
<td>3.74e-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 2011</td>
<td>5.98e-1</td>
<td>4.83e-6</td>
<td></td>
<td></td>
<td>3.74e-15</td>
<td>3.74e-15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2011</td>
<td>5.98e-1</td>
<td></td>
<td>3.74e-15</td>
<td>3.74e-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2012</td>
<td>1.43e-2</td>
<td>2.33e-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2013</td>
<td></td>
<td></td>
<td>3.3e-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 21 OA dataset of oocyte diameters with sprig length dataset

H₀: The oocyte diameter does not depend on treatment

ANOVA: p-value = <2.2e-16

<table>
<thead>
<tr>
<th></th>
<th>June Ambient Loss</th>
<th>June Ambient Gain</th>
<th>June 2100 Loss</th>
<th>September Ambient Loss</th>
<th>September Ambient Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>June Ambient Loss</td>
<td>6.61e-3</td>
<td>7.98e-11</td>
<td>4.62e-15</td>
<td>5.91e-10</td>
<td>7.02e-6</td>
</tr>
<tr>
<td>June 2100 Loss</td>
<td>5.24e-1</td>
<td>1.94e-1</td>
<td>9.18e-11</td>
<td>9.04e-1</td>
<td></td>
</tr>
<tr>
<td>September Ambient Loss</td>
<td>4.18e-2</td>
<td>4.62e-15</td>
<td>2.13e-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>September Ambient Gain</td>
<td>5.34e-4</td>
<td>5.88e-4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 2100 Loss</td>
<td></td>
<td></td>
<td>3.88e-12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22 June Ambient dataset of oocyte diameters with June Ambient length dataset

H₀: The oocyte diameter does not depend on treatment

ANOVA: p-value = 7.52e-4

<table>
<thead>
<tr>
<th></th>
<th>June Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>June Ambient Loss</td>
<td>6.12e-1</td>
</tr>
<tr>
<td>June Ambient Gain</td>
<td>6.16e-3</td>
</tr>
</tbody>
</table>

June 2100 dataset oocyte diameter with June 2100 Length dataset

H₀: The oocyte diameter does not depend on treatment

ANOVA: p-value = 1
Table 23 September Ambient dataset of oocyte diameters with September Ambient length dataset
H0: The oocyte diameter does not depend on treatment
ANOVA: p-value = 4.73e-4

<table>
<thead>
<tr>
<th>September Ambient Loss</th>
<th>September Ambient Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.202e-7</td>
<td>4.67e-1</td>
</tr>
</tbody>
</table>

Table 24 September 2100 dataset of oocyte diameters with September 2100 length dataset
H0: The oocyte diameter does not depend on treatment
ANOVA: p-value = 3.22e-16

<table>
<thead>
<tr>
<th>September 2100 Loss</th>
<th>September 2100 Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.51e-3</td>
<td>5.61e-6</td>
</tr>
</tbody>
</table>

Table 25 OA dataset, fecundity.
H0: The fecundity does not depend on treatment
ANOVA: p-value = 7.57e-13

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0</td>
<td>2.8e-9</td>
<td>3.99e-2</td>
</tr>
<tr>
<td>Ambient</td>
<td></td>
<td>3.312e-12</td>
</tr>
</tbody>
</table>

Table 26 OA dataset of fecundity by month.
H0: The fecundity does not depend on treatment
ANOVA: p-value = 9.357e-5

<table>
<thead>
<tr>
<th></th>
<th>Time 0</th>
<th>June 2100</th>
<th>September Ambient</th>
<th>September 2100</th>
<th>Ambient</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>June Ambient</td>
<td>1</td>
<td>1</td>
<td>2.2e-3</td>
<td>5.08e-3</td>
<td>2.48e-3</td>
<td>1.45e-12</td>
</tr>
<tr>
<td>June 2100</td>
<td>8.61e-2</td>
<td>3.24e-1</td>
<td>8.61e-2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>September Ambient</td>
<td>1.91e-11</td>
<td>1</td>
<td>2.53e-1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 2100</td>
<td>9.9e-9</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 27 OA dataset with 2014 reproductive dataset of fecundity
H0: The fecundity does not depend on treatment
ANOVA: p-value = 1.07e-9

<table>
<thead>
<tr>
<th></th>
<th>June 2011</th>
<th>September 2011</th>
<th>January 2012</th>
<th>January 2013</th>
<th>Time 0</th>
<th>Ambient</th>
<th>2100</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2010</td>
<td>5.82e-1</td>
<td>5.0e-1</td>
<td>9.11e-1</td>
<td>2.02e-2</td>
<td>8.77e-1</td>
<td>2.26e-6</td>
<td>3.67e-7</td>
</tr>
<tr>
<td>June 2011</td>
<td>1</td>
<td>9.11e-1</td>
<td>5.82e-1</td>
<td>1</td>
<td>4.06e-5</td>
<td>3.13e-6</td>
<td></td>
</tr>
<tr>
<td>January 2012</td>
<td></td>
<td></td>
<td>9.35e-2</td>
<td>1</td>
<td>2.65e-7</td>
<td>2.09e-7</td>
<td></td>
</tr>
<tr>
<td>January 2013</td>
<td></td>
<td></td>
<td></td>
<td>2.83e-1</td>
<td>3.16e-5</td>
<td>4.17e-4</td>
<td></td>
</tr>
</tbody>
</table>

Table 28 June OA dataset with June 2011 dataset of fecundity
**H₀**: The fecundity does not depend on treatment
ANOVA: p-value = \(4.73 \times 10^{-3}\)

<table>
<thead>
<tr>
<th></th>
<th>June 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>June Ambient</td>
<td>2.34e-1</td>
</tr>
<tr>
<td>June 2100</td>
<td>4.9e-2</td>
</tr>
</tbody>
</table>

**Table 29** September OA dataset with September 2010 dataset of fecundity

**H₀**: The fecundity does not depend on treatment
ANOVA: p-value = \(1.32 \times 10^{-12}\)

<table>
<thead>
<tr>
<th></th>
<th>September 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>September Ambient</td>
<td>6.17e-8</td>
</tr>
<tr>
<td>September 2100</td>
<td>1.7e-7</td>
</tr>
</tbody>
</table>

**Table 30** Ambient June dataset of fecundity with Ambient June sprig length dataset

**H₀**: The fecundity does not depend on sprig quality
ANOVA: p-value = 8.9e-1

<table>
<thead>
<tr>
<th></th>
<th>June Ambient Gain</th>
<th>June Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>June Ambient Loss</td>
<td>1</td>
<td>8.37e-1</td>
</tr>
<tr>
<td>June Ambient Gain</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 31** September Ambient dataset of fecundity with September Ambient sprig length dataset

**H₀**: The fecundity does not depend on sprig quality
ANOVA: p-value = 1

<table>
<thead>
<tr>
<th></th>
<th>September Ambient Gain</th>
<th>September Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>September Ambient Loss</td>
<td>2.03e-1</td>
<td>7.7e-1</td>
</tr>
<tr>
<td>September Ambient Gain</td>
<td></td>
<td>3.39e-1</td>
</tr>
</tbody>
</table>

**Table 32** September 2100 dataset of fecundity with September 2100 Sprig length dataset

**H₀**: The fecundity does not depend on sprig quality
ANOVA: p-value = 5.07e-2

<table>
<thead>
<tr>
<th></th>
<th>September 2100 Loss</th>
<th>September 2100 Gain</th>
<th>September Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2100 Loss</td>
<td>1</td>
<td></td>
<td>8.45e-1</td>
</tr>
<tr>
<td>September 2100 Gain</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>September 2100 No Change</td>
<td>8.45e-1</td>
<td>8.45e-1</td>
<td>8.45e-1</td>
</tr>
</tbody>
</table>
BIOGRAPHY OF THE AUTHOR

Ashley Madison Rossin was born in Lakeville, Minnesota. She graduated from Blue Valley Northwest High School in Overland Park, Kansas in May 2013. She attended the University of Maine and graduated after three years in May 2016 with a Bachelor of Science in Marine Science with a concentration in Marine Biology. She worked at the Darling Marine Center in Dr. Rhian Waller’s Deep-Sea Coral Histology Laboratory as a laboratory intern, and then did her senior research project in the lab. Her capstone studied the reproduction of the deep-sea sea whip, Primnoella chilensis, from Patagonian Chile, and this research project became her first published paper in the summer of 2017, titled, “Reproduction of the cold-water coral Primnoella chilensis (Philippi 1984).” During her undergraduate career, she was a member of the Pi Beta Phi sorority and the Greek life honor society, Order of Omega, where she was Vice President during her junior year. She was also president of the Darling Marine Center SCUBA Club for a year as a masters student. She is a candidate for the Master of Science degree in Marine Biology from the University of Maine in May 2018.