THE EFFECTS OF OCEAN ACIDIFICATION ON WALLEYE POLLOCK

(*THERAGRA CHALCOGRAMMA*) EARLY LIFE HISTORY STAGES

By

Elena R. Fernandez

RECOMMENDED: _____

Dr. Michael Castellini

_____

Dr. Thomas Hurst
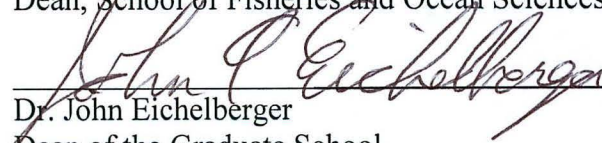
_____

Dr. Katrin Iken, Advisory Committee Co-Chair

_____
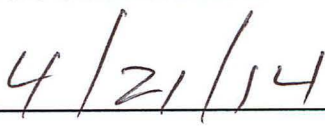
Dr. Larissa Horstmann, Advisory Committee Co-Chair

_____

Dr. Brenda Konar
Head, Graduate Program in Marine Sciences and Limnology

APPROVED: _____

Dr. Michael Castellini
Dean, School of Fisheries and Ocean Sciences

_____

Dr. John Eichelberger
Dean of the Graduate School

_____4/21/14_____

Date

THE EFFECTS OF OCEAN ACIDIFICATION ON WALLEYE POLLOCK

(*THERAGRA CHALCOGRAMMA*) EARLY LIFE HISTORY STAGES

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Elena R. Fernandez, B. A.

Fairbanks, Alaska

May 2014

Abstract

Since the Industrial Revolution of the late 1700's, atmospheric and marine carbon dioxide levels have drastically increased. Ocean acidification is the result of the shift in the marine carbon cycle caused by the increase in marine and atmospheric carbon dioxide. Changing environmental conditions caused by ocean acidification have been shown to have adverse effects on different marine species as well as life history stages. As a result, ecologically and economically important teleost fish species such as walleye pollock (*Theragra chalcogramma*) could be adversely affected by ocean acidification conditions. This study explores the responses of walleye pollock eggs and larvae incubated under different projected levels of ocean acidification, looking at hatch timing and growth parameters to examine potential adverse responses to more acidic conditions. Older walleye pollock juveniles (age 1+) were used to uncover potential physiological responses to ocean acidification pertaining specifically to stress, overall body condition indices, and blood chemistry. I found that while the two early life history stages of walleye pollock could survive under ambient, high, medium, and low pH conditions (pH 8.1, 7.9, 7.6, and 7.2, respectively), there were some physiological responses to projected levels of ocean acidification. Hatch timing was not delayed in the lowest pH treatment as expected. In addition, size at hatch, yolk area, and eye diameter did not differ among pH treatments. Walleye pollock juveniles reared under projected levels of ocean acidification demonstrated shifts in blood gas levels and blood pH. However, exposure to a lower pH environment of pH 7.9, 7.6, or 7.2 did not induce a response for either the stress indicators or body condition indices that were measured. To uncover the mechanism for their resilience, more testing is needed to gain further insight into underlying compensatory mechanisms of various life history stages and populations.

Dedication

To my family, for not letting me quit and having faith in me;
To my friends and co-workers, for keeping me sane and making me laugh;
And to everyone else, for your love and support when I needed it the most.

It's been an incredible journey. I don't know what I would've done without all of you.

"The oak fought the wind and was broken, the willow bent when it must and survived."

— Robert Jordan, *The Fires of Heaven*

Table of Contents

List of Figures

List of Tables

List of Appendices

Acknowledgements

Chapter 1. Ocean acidification and walleye pollock of the North Pacific

1.1. Introduction

Walleye pollock (*Theragra chalcogramma*) are an integral component of North Pacific and Bering Sea food webs and support major regional fisheries. With an annual harvest of about 1 million metric tons, they comprise the largest and one of the nation's most valuable fisheries (Ianelli et al. 2010). Successful recruitment of larval walleye pollock to the commercial fishery is highly dependent upon the development, growth, and survival of early life stages (i.e., eggs, larvae, and juveniles). Mortality rates of these stages are intrinsically high (e.g., through predation) and certain oceanographic conditions such as temperature variations, sea ice retreat, and stratification can create important bottlenecks in walleye pollock population size (Brodeur and Wilson 1996, Bailey et al. 1997, 1999, Hunt et al. 2011). Even slight reductions in growth and development from environmental stressors prolong the time fish stay in these vulnerable early life stages, thereby increasing mortality rates (Houde 1997). As a result, minor changes in larval or juvenile survival rates can result in order of magnitude responses in recruitment rates to the commercial fishery.

Since the Industrial Revolution of the mid-1700's, carbon dioxide (CO_2) levels have increased as a result of growing anthropogenic use of fossil fuels and shifts in land-use practices (e.g., deforestation) (Sabine et al. 2004). Prior to the Industrial Revolution, atmospheric concentrations of CO_2 were between 200 and 280 parts per million (ppm) per volume. Current concentrations are approaching 395 ppm (Tans and Keeling 2013), and it is projected that CO_2 concentrations could be over 800 ppm by 2100 (Prentice et al. 2001, Caldeira and Wickett 2003, Feely et al. 2004, Pelejero 2005, Feely et al. 2008). At the surface, the ocean interacts constantly with the atmosphere to absorb and release CO_2. The partial pressure gradient of CO_2 (pCO_2) between the ocean and the atmosphere causes more CO_2 to dissolve into the oceans. This is exacerbated in cold-temperate and polar waters, such as the North Pacific, because the cold waters of these regions allow for increased dissolution of CO_2. Once absorbed, a carbon atom remains in the ocean for hundreds of years, circulating from the ocean's surface to its depths and back to the

surface again (Sabine et al. 2004). On a global scale, the ocean currently acts as a carbon sink, with the amount of carbon stored in the ocean being roughly 50 times greater than in the atmosphere (IPCC 2007). With a net intake of approximately two billion metric tons of carbon per year, the global ocean absorbs the equivalent of about one-third of current annual anthropogenic emissions (Sabine et al. 2004, IPCC 2007). There are certain locations and times of the year where the ocean can act as a source of CO_2 to the atmosphere; however, on average, the global ocean acts as a net sink.

The CO_2 dissolved into the ocean spontaneously combines with water to form carbonic acid (H_2CO_3), which quickly releases two hydrogen ions (H^+) as it dissociates to bicarbonate (HCO_3^-) and ultimately carbonate (CO_3^{2-}) anions. As a result, the ocean surface water (depth of the mixed layer) becomes more acidic; however, the ocean has a high buffering capacity as a result of the carbonate equilibrium reaction, where HCO_3^- and CO_3^{2-} have the ability to take up H^+ and stabilize the pH. The current rate of pH decrease is an estimated 0.015 units per decade, or about 0.1 units per century (Haugan and Drange 1996, Brewer 1997, Petit et al. 1999, Feely et al. 2004, Sabine et al. 2004). By 2100, there will be a projected overall surface water pH decrease of 0.4 units since pre-industrial times (i.e., before 1800) (Orr et al. 2005, IPCC 2007, Feely et al. 2009). Forecasts utilizing several different models show that the marine pH could decrease by 0.3-0.5 pH units every 100 years, with a total decrease of nearly 0.8 pH by 2300 at the current rate of CO_2 increase in the atmosphere compared to pre-industrial conditions (Caldeira and Wickett 2005, Pelejero 2005, McNeil and Matear 2006). The ocean pH decrease caused by the increased CO_2 concentrations has the potential to affect plankton, invertebrates, and teleosts, ultimately with large impacts on marine food webs and higher trophic levels (Doney et al. 2009). Ocean acidification could impact the recruitment dynamics of early life stages and juvenile fish through several distinct pathways. First, there could be direct physiological stress associated with low pH environments, manifested as reduced rates of larval and juvenile growth and survival, increased disease susceptibility, and decreased fecundity (Pörtner et al. 2004). Second, changes in behavioral responses to stimuli (i.e., learning capabilities, predator-prey interactions,

responses to sensory cues) caused by ocean acidification can lead to increased mortality at early life history stages (Simpson et al. 2011, Dixson et al. 2010, Ferrari et al. 2012). Finally, ocean acidification could impact the production of lower trophic levels, hence altering the foraging environment of the early life stages as well as adult fish (Pörtner et al. 2004, Melzner et al. 2009a).

The present study aims to assess the effects of reduced pH conditions on early life stages of walleye pollock, specifically eggs (Chapter 2) and juveniles (Chapter 3). With the information gained through this study, we will have a better understanding of how this species will respond to changing ocean conditions associated with ocean acidification at different ontogenetic stages.

1.2. Importance of walleye pollock in the Gulf of Alaska and Bering Sea fisheries

Walleye pollock are semi-demersal fish that comprise over 40% of the global whitefish production (Ianelli et al. 2012). In U. S. waters, walleye pollock is the most abundant commercially exploited fish in the North Pacific and Bering Sea (Springer 1992, Brodeur and Wilson 1996). Products made from walleye pollock include fillets, whole fish, surimi, and roe (Ianelli et al. 2010). This species is widespread, ranging from the coast of Oregon and Washington, through the Gulf of Alaska into the Bering Sea, and west into Russian and Japanese waters (Figure 1.1, adapted from Bailey et al. 1999). To meet the market demand, fishermen target the large spawning aggregations and schools of adult walleye pollock during the commercial fishing seasons. Depending on the region (i.e., Bering Sea, Gulf of Alaska) and the time of year, there are generally two time periods open to commercial fishing: the "A" season from January to March, and the "B" season from June through October (Alaska Fisheries Science Center 2013).

A variety of state and federal fisheries management organizations monitor fluctuations in walleye pollock populations and use that information to regulate fishing activity in Alaskan waters. Acoustic surveys in 2006 suggest that populations decreased by 38%, indicating that some fish stocks were at the lowest levels in 30 years in the Bering Sea, Aleutian Islands, and Gulf of Alaska regions (Barbeaux et al. 2010, Dorn et

al. 2010, Ianelli et al. 2010). Overall, these three different stocks in Alaskan waters also decreased in the total commercial catch. Total allowable catch (TAC) in the Gulf of Alaska in 2009 was 44,003 metric tons as compared to the peak total catch of 307,401 metric tons in 1984 (Dorn et al. 2010). The eastern Bering Sea pollock stock averaged 1.17 million metric tons between 1977 and 2010; however, the 2009 and 2010 commercial catch dropped to 0.81 million metric tons as a result of stock declines and subsequent reductions in allowable harvest rates (Ianelli et al. 2010). The Aleutian Island stock also exhibited a marked decrease from their peak catch in 1991 when 98,604 metric tons were harvested, while in 2010, total harvest was recorded as only 1,238 metric tons (Barbeaux et al. 2010). Since 2010, surveys indicate that stocks recovered to their previous levels, with total harvest in both state and federally managed fisheries reaching 1.2 million metric tons in the Bering Sea and Aleutian Islands combined, and 813,000 metric tons in the Gulf of Alaska in 2011 (Fissel et al. 2012). This increase in harvest is the result of raising the 2011 TAC by 44.3% (Fissel et al. 2012).

Walleye pollock are an ecologically important species and function as an important food source for several species of marine organisms. Fish such as arrowtooth flounder (*Atheresthes stomias*), Pacific cod (*Gadus macrocephalus*), Pacific halibut (*Hippoglossus stenolepis*), and even adult walleye pollock rely on the large schools of juvenile walleye pollock as the primary component in their diet (Brodeur and Wilson 1996). Seabirds including puffins (*Fraterula* spp.) and the common murre (*Uria aalge*), as well as marine mammals, including harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*), also utilize walleye pollock as an important food source (Brodeur and Wilson 1996).

Table 1.1. Mean walleye pollock landings in Alaskan waters, 1997-2010. Mean walleye pollock landings (in metric tons) in Alaskan waters based on 2010 stock assessments and broken down by management stocks from 1997-2010.

Eastern Bering Sea (Ianelli et al. 2010)	Aleutian Islands (Barbeaux et al. 2010)	Gulf of Alaska (Dorn et al. 2010)	Total Catch (metric tons)
1,170,000	1,238	106,848	1,280,000
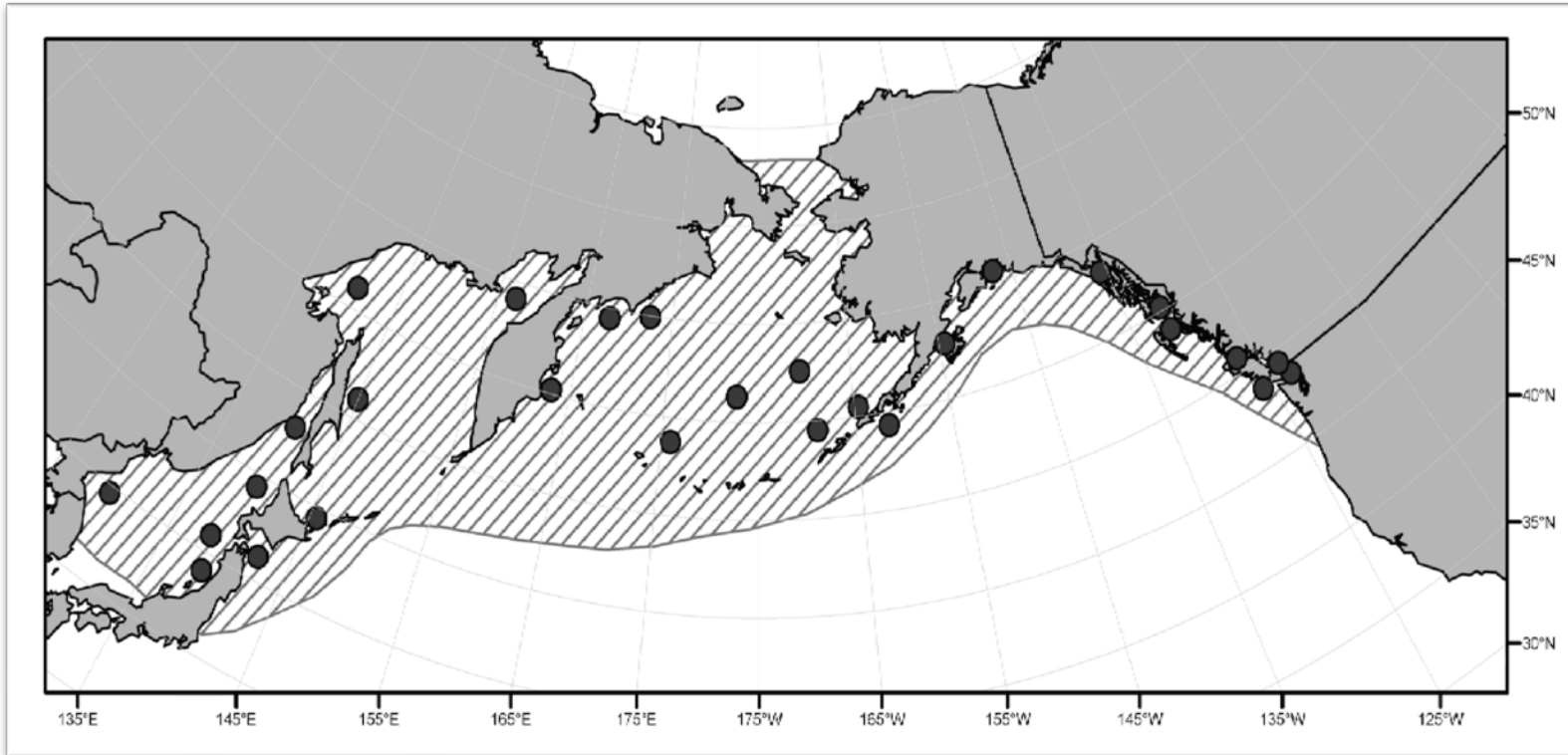
Figure 1.1. Map of walleye pollock distribution. The global distribution of walleye pollock (gray shaded area). Dark circles indicate known walleye pollock spawning grounds (adapted from Bailey et al. 1999).

1.3. Life history and diet of walleye pollock

Upon reaching sexual maturity at age 3+ or 4+ (approximately 40 to 45 cm total length), walleye pollock school and spawn annually between February and May, depending on the timing of adult migration to the populations' specific spawning grounds (Bailey et al. 1999, Dougherty et al. 2007). Distinct populations spawn at predictable times and in the same geographic location every year (Bailey et al. 1999). Individual females can produce up to 1.2 million eggs per season and release them during multiple spawning events occurring at depths between 100 and 400 m (Kendall et al. 1994). Eggs are about 1 mm in diameter and float in the water column at this depth for 7 to 30 days, depending on water temperature (Bailey et al. 1997). In the Shumagin Islands and Shelikof Strait, Alaska, peak hatch is generally around the last week in April or first week in May, with earlier hatch dates associated with warmer sea surface temperatures (Dougherty et al. 2007). At hatch, larvae are between 3.5 and 4.5 mm standard length and remain at depth until they start to feed, at about 5-7 days post hatch (Olla et al. 1996). Feeding larvae then migrate upward to the photic zone, to about 20 to 60 m depth, where they remain until metamorphosis (Olla et al. 1996, Bailey et al. 1997). Larvae metamorphose to juveniles at about 18 mm total length reaching 120 to 140 mm by the end of their first year (Bailey et al. 1997).

Walleye pollock spawning grounds are associated with oceanographic features (Figure 1.1), such as sea valleys, canyons, and indentations in the outer margin of continental shelves (Bailey et al. 1997). Some populations also favor deep-water regions and fjords as spawning habitats (Bailey et al. 1997). Eddies and island retention features associated with the spawning locations allow for the transport and retention of eggs and larval aggregations within surface currents (Stabeno et al. 1996). Coastal and shelf environments, such as seagrass beds, shelf habitats, and reefs are common, highly productive nursery grounds necessary for larval pollock survival (Ishimatsu and Dissanayake 2010). For other systems, such as the Bering Sea and Gulf of Alaska, larval and juvenile pollock rely on currents, eddies, and other oceanographic features that promote food production (Brodeur and Wilson 1996). In addition, vertical distribution

patterns of larval and juvenile walleye pollock allow for predator avoidance while maintaining foraging ability (Brodeur and Wilson 1996). In general, the timing and location of spawning events contribute to year-class variations in walleye pollock population structure (Stabeno et al. 1996).

Larval dispersal and survival are dependent mainly on major ocean currents, food availability, and predator protection in certain areas of the Gulf of Alaska and Bering Sea (Olla and Davis 1990, Brodeur et al. 1995, Stabeno et al. 1996). It is hypothesized that recruitment success in the Bering Sea is determined by a number of regional factors, such as timing of the sea ice formation and retreat, cold pool formation, summer stratification, localized upwelling, type of zooplankton prey and their energy content, and the presence of the "green belt" of primary production (Hartline 1980, Wespestad et al. 2000, Ciannelli et al. 2004, Mueter et al. 2006, Stabeno et al. 2008). All these processes are interconnected and can vary on annual, decadal and even interdecadal time scales (Sugimoto and Tadokoro 1998, Stabeno et al. 2001), thus potentially influencing walleye pollock larval recruitment on these similar time scales.

Vertical distribution of the various life history stages of walleye pollock depends on their body size and life history stage, tolerance for turbulence, temperature, light, predator avoidance strategies, and presence of desirable prey (Olla et al. 1996). Turbulence is necessary to induce mixing and bring nutrients to the surface to facilitate phytoplankton blooms as food for zooplankton. However, walleye pollock larvae also are known to sink if turbulence is too strong (Olla and Davis 1990, Olla et al. 1997). The movement of walleye pollock through the water column changes with ontogenetic stage. Juveniles <60 mm total length also tend to move offshore and into deeper water just above the thermocline to avoid predation by visual predators and to follow their preferred prey (Brodeur and Wilson, 1996). Feeding larval walleye pollock are found in surface waters above the thermocline and in the photic zone to efficiently make contact with their desired prey species (Olla et al. 1996). Age 0+ and 1+ fish are found in deeper waters (~20-50 m), and during the day migrate to even greater depths; however, the presence of a thermocline during the spring and summer will cause smaller juveniles to remain in the

upper portion of the water column to access desirable prey while avoiding cannibalistic adult walleye pollock found below the thermocline (Bailey 1989, Olla et al. 1996). As a result, vertical distribution of juvenile walleye pollock is driven by predator avoidance, food availability, and water temperature (Brodeur et al. 1995, Olla et al. 1996). Adults are considered semi-demersal, residing from 100 to 300 m depth.

Walleye pollock diet changes seasonally, spatially, and with developmental stage. This is related to prey abundance at different points during the year as well as walleye pollock size (Cooney 1981, Dwyer et al. 1987). Early juveniles in the Gulf of Alaska primarily consume copepodite and adult copepods (Grover 1991, Brodeur and Wilson 1996). As young of the year (YOY), their diet consists mostly of copepods (Dwyer et al. 1987, Brodeur and Wilson 1996). As fish get larger, euphausiids become an increasingly more important food source for juveniles to prepare for overwintering (Dwyer et al. 1987, Brodeur and Wilson 1996). The diet of YOY walleye pollock shifts to more epibenthic prey, such as mysids, shrimps, and cumaceans, due to scarcity of pelagic prey at the onset of winter (Brodeur and Wilson 1996). Both age 1+ and adult walleye pollock diets are dominated by euphausiids in the Gulf of Alaska (Brodeur and Wilson 1996, Adams et al. 2007), although cannibalism on younger age classes of pollock is also common (Dwyer et al. 1987, Brodeur and Wilson 1996). Similarly, in the Bering Sea, adult walleye pollock feed on euphausiids in the spring and summer, but primarily cannibalize younger age classes in the fall and winter months (Dwyer et al. 1987, Adams et al. 2007). Cannibalism is thought to be the main control of year-class strength rather than food availability for larvae and juvenile age classes in the Bering Sea (Walline 1985, Dwyer et al. 1987). Sea ice retreat and sea ice conditions are the primary drivers of cannibalism on younger age classes of walleye pollock (Hunt et al. 2011). Bottom-up controls in the Bering Sea caused by sea ice conditions result in shifts of phytoplankton blooms that will influence food availability for younger age classes (Mueter et al. 2006). With fewer food sources available when sea ice conditions are consistent with those of the beginning of a cold regime (e.g., La Niña conditions, cold phase of Pacific Decadal Oscillation), the need for

adult pollock to cannibalize on younger age classes increases, ultimately affecting year class strength (Hunt et al. 2011).

1.4. Ocean acidification and the marine carbon cycle

The marine carbon cycle is driven by both abiotic (i.e., CO_2 dissolution, acid/base chemistry) and biotic (i.e., photosynthesis, respiration, $CaCO_3$ formations) components. Two major parts of the carbon cycle and carbonate system are pH and the saturation state of carbonate minerals. Highest concentrations of CO_2 from the atmosphere are found in surface water (equal to the depth of the mixed layer) as a result of CO_2 diffusion across the sea surface interface (Sabine et al. 2004). Equilibrium of the surface water is achieved in a relatively short time frame. The marine carbon cycle begins with the dissolution of CO_2 in seawater and its subsequent hydration, which results in the formation of carbonic acid (Equation 1.1). Carbonic acid (H_2CO_3), a weak acid, instantly dissociates to the bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}), releasing two protons. The increase in hydrogen ion concentration in seawater from this equilibrium reaction is what lowers the pH of a solution and causes what is referred to as ocean acidification (Doney 2006).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+ \quad \text{(Equation 1.1)}$$
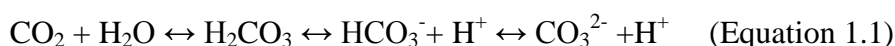
This equilibrium reaction is responsible for regulating the pH of the world's oceans. The introduction of anthropogenic CO_2 not only increases acidity, but also results in shifts in the concentrations of carbonate species within the seawater buffering system.

With the addition of CO_2, concentrations of HCO_3^-, H_2CO_3, and protons increase, while CO_3^{2-} ions decrease within seawater when their concentrations are considered as a function of pH (Sarmiento and Gruber 2006, Fabry et al. 2009). This pattern is caused by the nonlinearity of CO_2 solubility curves and the multiple products produced during dissolution (i.e., H_2CO_3, HCO_3^-, and CO_3^{2-}) (Sarmiento and Gruber 2006). Within the equilibrium equation (Equation 1.1), there are equilibrium rate constants (k) between each step in dissolution, with each k having its own unique value dependent on

temperature, salinity, and pressure (Sarmiento and Gruber 2006). Because of these different rates at each point in the equilibrium equation, the different dissolved inorganic carbon (DIC) species that exist in the equilibrium reaction (Equation 1.1) in seawater are 90% HCO_3^-, 9% CO_3^{2-}, and 1% CO_2 and H_2CO_3 (Feely et al. 2004, Sarmiento and Gruber 2006). As $[CO_3^{2-}]$ decreases, the concentrations of other solid carbonate-based compounds in seawater (i.e., $CaCO_3$) will also decrease (Fabry et al. 2009, Equation 1.2).

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \qquad \text{(Equation 1.2)}$$

The other component of the marine carbon cycle is the saturation state (Ω) of carbonate-based compounds, specifically calcite and aragonite. A saturation state of these minerals is calculated as the product of the ion component concentrations (generally in µmol/L) divided by the specific mineral's solubility constant (λ) (Equation 1.3).

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{\lambda} \qquad \text{(Equation 1.3)}$$

The $[Ca^{2+}]$ is measured from salinity, and the $[CO_3^{2-}]$ is calculated from DIC and total alkalinity (TA) measurements, where TA is the measure of excess cations that are balanced by anions from weak acids. For $CaCO_3$ precipitation, the saturation states have to be $\Omega > 1$. If $\Omega < 1$, dissolution will occur.

Where $\Omega = 1$, the rate of dissolution equals the rate of precipitation and occurs at the saturation horizon. A saturation horizon, or lysocline, is unique to every mineral and mineral structure, and is defined as the temperature and pressure conditions of the ocean where the rate of dissolution of a mineral (e.g., aragonite or calcite) is equal to the rate of precipitation. With the increased input of CO_2, saturation horizons are becoming shallower at all latitudes as a result of decreasing pH; however, there are regional differences that contribute to the overall depth of the saturation horizons of these compounds.

Aragonite and calcite saturation horizons are much shallower in the Indian Ocean and North Pacific compared to the North Atlantic because of upwelling caused by global thermohaline circulation, where the upwelling of old, cold water has high concentrations of DIC from accumulation through remineralization processes (Doney et al. 2009). Aragonite is more soluble than calcite at a given $[CO_2]_{aq}$, so its saturation horizon will be shallower. Oceans at higher latitudes, such as the Arctic and sub-Arctic seas, are more susceptible to increased acidification for a combination of several factors (Fabry et al. 2009). First, upwelling-favorable conditions from both wind-driven circulation and thermohaline circulation bring DIC-rich water from between 100-300 m depth or deeper in regions like the North Pacific Ocean or Bering Sea onto the continental shelf (Feely et al. 2008, Mathis et al. 2010). DIC created from decomposition and remineralization in midwater and benthic systems causes the pH of the deep water to decrease, in turn lowering the surface pH as this acidic deep water is pushed to the surface. These water masses are also extremely cold, causing the solubility of both aragonite and calcite to increase (Feely et al. 2004, Fabry et al. 2009). Second, upwelling (both wind-driven and from thermohaline circulation) carries biologically important nutrients to the surface, causing high primary productivity in these regions. Though the high primary production drives down pCO_2 at the surface, the subsequent remineralization processes increases [DIC] and causes pH to decrease in and below the photic zone (Feely et al. 2008, 2009). The water mass with higher [DIC] is then upwelled through wind-driven processes or mixing, resulting in a further pH decrease at the surface. Third, Arctic and sub-Arctic seas also are susceptible to ocean acidification because more gas dissolves at low water temperatures, allowing the water to reach supersaturation (where $pCO_{2\ atm} < pCO_{2\ ocean}$) with respect to CO_2 (Doney et al. 2009, Fabry et al. 2009).

On average, the North Pacific and the Bering Sea are both undersaturated (where $\Omega < 1$) with respect to calcite and aragonite as a result of low water temperatures and increased dissolution of CO_2 (Bates and Mathis 2009). However, there are regional and seasonal factors that affect the overall depth of $CaCO_3$ saturation horizons. The Bering Sea exhibits seasonal undersaturation of aragonite in deep water, because of the increased

uptake of CO_2 and subsurface remineralization of inorganic carbon (Fabry et al. 2009, Mathis et al. 2010). However, the surface waters are saturated in spring and summer as a result of high primary production depleting the CO_2. In the Gulf of Alaska, the saturation horizon is at ~150 m, and increases in depth toward the equator (Fabry et al. 2009). With the growing input of anthropogenic CO_2, the shoaling of undersaturated water and the saturation horizon are expected to continue to increase by 1-2 m per year until the entire water column becomes undersaturated (Feely et al. 2009). This happens at a faster rate at higher latitudes compared to waters near the equator, as the solubility of CO_2, calcite, and aragonite increases with decreasing temperature (Fabry et al. 2009). As the marine carbon system has a buffering system, aragonite, calcite, and other carbonate-based compounds are used to counteract the increased amounts of dissolved CO_2. This leads to an undersaturation of these minerals within the system, potentially causing problems for calcifying organisms in these regions (Fabry et al. 2008, 2009, Doney et al. 2009, Mathis et al. 2010).

Calcifying plankton and benthic organisms that comprise the diet of many commercially and ecologically important species secrete $CaCO_3$ to build shells and are thus integral to carbon cycling in the world's oceans (Fabry et al. 2008). Marine calcifiers can live below the saturation horizon of calcite or aragonite, provided that they have physiological mechanisms to build and maintain their $CaCO_3$-based structures. These mechanisms can include organic coatings or shifts in their energy budget (Fabry et al. 2009). Ultimately, changing the position of the saturation horizon will result in the reorganization of important species within marine food webs (Feely et al. 2004). Because teleosts are more difficult to study relative to plankton and other calcifiers, there is a lack of studies regarding the effects of ocean acidification on fishes.

1.5. Biological consequences of ocean acidification for teleosts

The chemistry of ocean acidification is well understood and not much debated, but the consequences of ocean acidification for marine organisms and ecosystems are only starting to be revealed. Most attention has been given to the effects of ocean

acidification on calcifying organisms (Kleypas et al. 2006, Fabry et al. 2008, Ishimatsu et al. 2008).

Of particular interest are acidification effects at the base of the food web that can indirectly affect productivity of higher trophic levels. The primary plankton species that most influence walleye pollock trophic interactions and production have chitin-based shells, containing calcium carbonate for added strength; however, there also are non-calcifying plankton that comprise a portion of their diet. These non-calcifying zooplankton and phytoplankton have the potential to experience adverse effects as a result of decreased pH, primarily affecting physiological processes (Hare et al. 2007, Kurihara and Ishimatsu 2008, Hopkinson et al. 2010, Dajuan et al. 2011). Phytoplankton in the Gulf of Alaska demonstrated increased primary production in response to elevated CO_2 conditions (Hopkinson et al. 2010). Similar results were found in the Bering Sea, where there was an increase in primary production (Hare et al. 2007). Under high temperature and high-CO_2 conditions, there was also a shift in the dominant phytoplankton species from large diatoms to nanophytoplankton species (Hare et al. 2007). The change in phytoplankton assemblages could have cascading effects on the zooplankton nutritional quality and survival of zooplankton species feeding on phytoplankton. Changes in environmental conditions can also directly affect zooplankton, as seen in subtropical copepods, where survival and egg production rates were adversely affected by high CO_2 conditions (Kurihara et al. 2004, Kurihara and Ishimatsu 2008, Dajuan et al. 2011). If such responses occur in sub-arctic zooplankton, they could create bottom-up effects for higher trophic levels, including walleye pollock.

Ocean acidification, especially when combined with other environmental conditions (i.e., rising ocean temperatures, changing weather and oceanographic patterns) also affect many non-calcifying marine invertebrates, elasmobranchs, and teleosts through effects on physiological homeostasis (Melzner et al. 2009a, Chin et al. 2010). Chronic exposure to low pH can cause shifts in an organism's energy budget, forcing it to allocate more energy to maintaining homeostasis and less toward reproduction and growth (Pörtner 2008). Physiological functions, such as respiration rates, metabolism,

14

respiratory pigment O_2 saturation, and buffering capacity can also be impaired. As pH decreases, it becomes more difficult for marine organisms to maintain internal acid-base balance (Pane and Barry 2007, Pörtner 2008). To preserve homeostasis, organisms can actively buffer by either increasing bicarbonate concentrations through certain pathways (e.g., increased renal production of bicarbonate) or increase activity or presence of chloride cells, proton exchangers, and other similar transmembrane proteins to maintain physiological intracellular and extracellular pH (Robinson and Huxtable 1988, Pörtner 2008).

Because some species tolerate a broader range of environmental conditions than others, it is important to study their compensatory mechanisms to understand the organism's full capabilities to withstand environmental change. In particular, extracellular pH stabilization mechanisms are of importance in the context of low pH tolerance in fishes (Hayashi et al. 2004a, 2004b, Melzner et al. 2009a). The increase in environmental CO_2 induces a rise in ventilation rate to preserve internal homeostasis as a result of the Bohr and Root effects, describing the shifts in oxygen binding affinity and oxygen saturation maxima as a result of changes in blood pH, respectively (Perry and Gilmour 2002, Ishimatsu et al. 2004). The ability of fish to compensate and maintain extracellular homeostasis relies mainly on the efficacy, efficiency, and concentration of ion exchange proteins in the gill, and whether or not their concentration can be increased to promote osmoregulatory balance (Perry and Gilmour 2002). The tolerance mechanism requires that fish be able to accumulate and maintain high levels of bicarbonate in the blood to maintain a constant blood pH based on high buffering capacity (Hayashi et al. 2004a, 2004b, Melzner et al. 2009a). Marine teleosts exhibit decreased blood pH along with increased bicarbonate and blood pCO_2 upon exposure to low pH conditions (pH 6-7), as seen in the Japanese flounder (*Paralichthys olivaceus*), the yellowtail (*Seriola quinqueradiata*), and the starspotted dogfish (*Mustelus manazo*) (Hayashi et al. 2004a, 2004b). If animals cannot maintain cellular homeostasis, the resulting cascading effects may cause entire body systems to fail. Therefore, these blood gas parameters may be

useful indicators of the susceptibility of an organism to ocean acidification if they are variable under different environmental conditions.

Organisms with high metabolic rates and mechanisms to effectively expel CO_2 are likely best able to acclimate to high CO_2 environmental conditions (Hayashi et al. 2004a, 2004b). Presence of certain enzymes (e.g., carbonic anhydrase and lactate dehydrogenase) facilitates the removal of CO_2 and acid from blood and tissues, thus allowing the system to remain in homeostasis with respect to pH (Randall and Brauner 1998, Hayashi et al. 2004a, 2004b). Active swimmers like the yellowfin tuna (*Seriola quinqueradiata*) are very effective in reestablishing extracellular homeostatic conditions after exposure to high CO_2 conditions with the help of the above-mentioned enzyme systems (Hayashi et al. 2004a). In contrast, the more sedentary Japanese flounder experienced increased mortality after exposure to elevated levels of CO_2, indicating that its tolerance level was surpassed (Hayashi et al. 2004a).

Little is known about the different levels of sensitivity and which specific physiological traits are responsible for their sensitivity to ocean acidification among fish species or developmental stages over multiple generations (Pörtner et al. 2004, Fabry et al. 2008, Pörtner 2008). On this longer time scale, it is expected that short-lived species potentially have a better ability to adapt to ocean acidification than long-lived species, as they have faster generation times supporting higher levels of genetic modification and evolution (Melzner et al. 2009a). Offspring of adult fish that experienced large changes in water quality parameters (e.g., temperature, pH, salinity) during their early life history stages may have a greater overall tolerance than the offspring of adults reared under more stable conditions because genes of more tolerant adults are passed on to offspring, creating heritable adaptation to environmental stressors or pollutants (Nacci et al. 2010, Whitehead 2012). This can only occur if the rearing conditions are within the tolerance window of the species (Melzner et al. 2009a).

It also is often assumed that early life history stages are more sensitive to environmental stressors than older juvenile or adult stages, for example to ocean acidification, which may create population-level bottlenecks (Melzner et al. 2009a, Tseng

et al. 2013). As a result, there can be substantial population declines if mortality increases at any of these population bottlenecks. For example, eggs and larvae of the red sea bream (*Pagrus major*) experienced high mortality under low experimental pH conditions (pH 6.2; Kikkawa et al. 2004). However, mortality and development issues in the red sea bream and the Japanese sillago (*Sillago japonica*) were not affected by the increased acidity of the water caused by the addition of HCl. Rather, the increased amount of CO_2 caused changes in the chemistry of the perivitteline fluid (fluid within the egg surrounding the yolk and embryo) leading to either mortality or hindered cleavage and resulting in abnormal embryonic development (Kikkawa et al. 2003, 2004, Ishimatsu et al. 2004). In contrast, eggs and larvae of some fish species and populations can be naturally exposed to pH changes greater than those seen under projected ocean acidification levels, especially in urbanized areas, upwelling zones, and coastal areas (Melzner et al. 2009a, Ishimatsu and Dissanayake 2010). In some cases, CO_2 concentrations up to ~5800 ppm are encountered by some fish species (e.g., Atlantic cod, *Gadus morhua*) throughout development because of the local pCO_2 levels that occur seasonally at spawning or nursery grounds (Melzner et al. 2009a, 2009b). For this reason, the eggs of some broadcast spawners have mechanisms to compensate for large environmental pH changes (Melzner et al. 2009a), although these mechanisms may not completely prevent adverse effects of pH on their development.

1.6. Study objectives

The objectives of this study were to experimentally assess the response of walleye pollock eggs and juveniles to ocean acidification conditions projected for the next 300 years. Walleye pollock eggs were incubated under these pH levels to determine potential shifts in hatch timing and success that are a possible consequence of decreased environmental pH (Chapter 2). Morphometric parameters of larvae hatched from eggs incubated under these conditions were also assessed to determine if exposure to these conditions altered larval development (Chapter 2). In another experiment, an integrated bioassessment of several different physiological parameters was used to measure the

responses of age 1+ juvenile pollock to projected ocean acidification levels (Chapter 3).
The results from these experiments contribute to the growing body of knowledge
concerning marine teleost responses to ocean acidification.

Chapter 2. The response of eggs and first hatch walleye pollock (Theragra chalcogramma) to increased levels of ocean acidification

2.1. Abstract

With rising atmospheric CO_2, ocean pH is expected to decrease by 0.3-0.5 units by the year 2100. This study investigated the effects of low pH on the hatch and early larval development of the commercially important, subpolar walleye pollock (*Theragra chalcogramma*). Fertilized eggs from captive walleye pollock were incubated under current ocean pH conditions (pH 8.1) and at high, medium, and low pH treatment levels (pH 7.9, 7.6, and 7.2, respectively) reflecting projected levels of future ocean acidification. Upon hatch, several larval morphometric parameters were measured (i.e, standard length, myotome height, eye diameter, and yolk area) to assess overall condition and size of larvae. Overall, the eggs hatched between eight and ten days post-collection at 7.8ºC ± 0.5ºC, and this incubation time did not vary significantly among pH treatments. Morphometric parameters also did not vary significantly among pH treatments. These results indicate that walleye pollock eggs and post hatch larvae are resilient to the pH conditions tested in this experiment. However, the compensatory mechanisms and any physiological and energetic costs associated with reduced pH conditions are currently unknown.

2.2. Introduction

Sensitivity of teleost fish species to environmental stressors is dependent on life history stage (Kikkawa et al. 2003). Overall, the resilience of the early life history stages is vital for overall recruitment success. Early life stages represent population bottlenecks, where fluctuations in larval recruits, due to high mortality, can result in population regulation of older age classes (Houde 1997). The timing of hatch and proper larval development of marine teleosts is dependent on several abiotic (e.g., water temperature) and biotic (e.g., food availability, maternal investment) variables (Hjort 1914, Cushing 1990, Laurel and Blood 2011). The importance of these variables is species-specific, and may vary among populations (Melzner et al. 2009a). Therefore, it is essential to determine how changing environmental conditions will affect development and mortality of the early life history stages of marine teleosts. One such stressor of increasing importance in the marine system is ocean acidification.

Atmospheric CO_2 levels have increased from 281 ± 2 ppm in 1800 (Sabine et al. 2004) to current atmospheric CO_2 levels of 395 ppm (Tans and Keeling 2013). Based on current projections, atmospheric CO_2 levels are expected to reach 880 ppm by 2100 (Caldeira and Wickett 2003, Pelejero 2005, IPCC 2007, Feely et al. 2008). The ocean acts as a carbon sink, with net intake of carbon equal to about 2 billion metric tons per year, or one third of the current anthropogenic emissions (Sabine et al. 2004, IPCC 2007, Ballantyne et al. 2012). Because of the great absorptive capacity of the oceans for CO_2, the balance of the marine carbonate system equilibrium shifted, where the increased CO_2 dissolving into water as a result of the increased $pCO_{2(atm)}$ causes more H_2CO_3 to form and thus the pH to decrease. Under current CO_2 emission conditions, the marine pH is predicted to decrease globally by 0.3-0.5 units every 100 years, with a 0.8 pH unit decrease projected by 2300 (Caldiera and Wickett 2003). In high latitude oceans, it is expected that the pH will decline by about 0.45 pH units by 2100 (Steinacher et al. 2009). With the increased acidity from increased H_2CO_3, less CO_3^{2-} is available to precipitate as $CaCO_3$. Ultimately, this causes an undersaturation of aragonite and shallowing of the calcite saturation horizon, especially in high latitudes (Yamamoto-Kawai et al. 2009).

High latitude oceans, such as the Bering Sea and Gulf of Alaska, are especially at risk of decreasing pH, because the cold water temperature in these regions allows for more CO_2 gas to be dissolved (Doney et al. 2009, Fabry et al. 2009). These regions are extremely productive, because turbulent mixing provides nutrient- and dissolved inorganic carbon (DIC)-rich water, with high phytoplankton blooms developing in the seasonally stratified surface waters (Hunt et al. 2002, Bates and Mathis 2009, Fabry et al. 2009, Mathis et al. 2010). This high primary production supports prolific benthic and pelagic fisheries. However, with increasing ocean acidification, it remains largely unknown as to how the early life history stages of marine teleosts will respond to changing marine pH.

Research on the effects of ocean acidification on the early life history stages of marine organisms has so far focused mainly on calcifying organisms (e.g., Dupont et al. 2008, Foo et al. 2012, Landes and Zimmer 2012). The increase in marine pCO_2 results in the decreased availability of the carbonate ion, which is an integral component of the calcite and aragonite used in shell and skeletal formation of marine calcifiers (Fabry et al. 2008). Both calcite and aragonite saturation states (the thermodynamic potential for these minerals to precipitate) are declining in high latitude waters, partially due to the effects of ocean acidification (Yamamoto-Kawai et al. 2009, Mathis et al. 2010, Yamamoto et al. 2012). Less is known about the effects ocean acidification has on non-calcifying organisms such as marine teleosts, both on species-specific and population-specific levels. Changes in environmental pH result in shifts in acid-base metabolism in non-calcifiers, potentially leading to physiological consequences if the organisms are unable to compensate in some way (Pörtner 2008). There is a species-specific, life history dependence associated with effects of ocean acidification on fishes, where embryos and larvae of some species were observed to be more physiologically sensitive to changes in pH relative to adults (Brown and Sadler 1989, Pankhurst and Munday 2011). In addition, it has been observed that some early life stages of marine teleosts may also exhibit a variety of responses to low pH conditions (Melzner et al. 2009a). Among the few studies concerning the response of teleost eggs and larvae to ocean acidification (e.g., Kikkawa

et al. 2004, Munday et al. 2009a, 2011), minimal attention has been given to temperate, subpolar, and/or commercially important species.

Walleye pollock (*Theragra chalcogramma*) is a commercially important, semi-demersal fish species found throughout the Gulf of Alaska and Bering Sea, ranging into Russian and Japanese waters (Bailey et al. 1999). Worth about $1 billion annually, the walleye pollock fisheries in the Gulf of Alaska and Bering Sea are the largest single-species fishery by weight in the world, with about 1 million tons harvested per year (Barbeaux et al. 2010, Dorn et al. 2010, Ianelli et al. 2010). Walleye pollock reach sexual maturity and recruit to the commercial fishery at age 3+ or 4+ (Bailey et al. 1999, Dougherty et al. 2007). Females are oviparous and repeatedly spawn between the months of February and May (Bailey et al. 1999, Dougherty et al. 2007). Each female is capable of producing around 2.6 million eggs per year, which are released into the water column and externally fertilized (Kendall et al. 1994). Fertilized eggs are delivered from spawning grounds to the nursery grounds by ocean currents (Stabeno et al. 1996, Bailey et al. 1997). Eggs hatch after about 80 degree-days, with newly-hatched larvae being about 3.5-4.5 mm in total length (Bailey and Stehr 1986). Size-specific mortality is important in regulating overall populations of walleye pollock, with an estimated 87-90% mortality occurring in the yolk to first feeding stage, and up to 99% mortality in the late larval stages in northeast Pacific stocks (Houde 1997).

Based on the importance of walleye pollock in the Gulf of Alaska and the Bering Sea, and the fact that these regions could be strongly impacted by ocean acidification (Bates and Mathis 2009), this study focuses on the response of walleye pollock early life history stages to increased levels of ocean acidification. Eggs from wild-caught walleye pollock were used to determine potential shifts in both hatch timing and success in response to reduced pH conditions. Larvae were hatched under pH levels projected for the next 300 years to assess potential developmental consequences. Specifically, I hypothesize that there will be a delay in hatch timing associated with decreased marine pH because of the increased need to maintain osmoregulatory balance within the egg during development. I further hypothesize that there will be reductions in various

developmental parameters (i.e., standard length, eye diameter, myotome height, and yolk area) associated with exposure to lower pH levels. This is because the lower pH and higher CO_2 levels are potential stress sources for developing embryos and newly hatched larvae that could adversely affect developmental parameters.

2.3. Methods

2.3.1. Broodstock collection and maintenance

This study used an existing walleye pollock broodstock maintained at the Hatfield Marine Science Center (HMSC; Newport, OR). Briefly, juvenile (age 0) walleye pollock that served as the parent population for this experiment were collected in May 2006 from Port Townsend, WA in the nearshore waters of Puget Sound (48º 6' 59" N, 122º 46' 31" W) using a lighted lift net. Animals were held in ambient temperature and pH seawater for 24 h before transport to the Alaska Fisheries Science Center Laboratory at the HMSC. Animals were reared in 5,678 L round tanks with a 12 h light: 12 h dark photoperiod and temperature was maintained between 9-10 ºC. Throughout their holding period, fish were fed daily with live brine shrimp (*Artemia* spp.). Once fish reached age 1+, they were transferred to 15,142 L tanks maintained at 9-10 ºC and exposed to a 12 h light: 12 h dark photoperiod. Feeding was reduced to twice per week, and fish were fed a gelatinized combination of herring, amino acid supplements, commercial food, squid, krill, and vitamins (Appendix A). This routine was maintained until individuals reached sexual maturity, at 3+ or 4+ years of age.

Changes in photoperiod and water temperature mimicking natural conditions over the course of year were used to prepare the broodstock to spawn. For spawning, about 20 animals (an estimated 10 females and 10 males) were collected from the rearing group and placed in a separate 15,142 L tank at 12 h light:12 h dark photoperiod and 9-10ºC for three weeks. Sex of individuals was determined based on milt production. If milt was not produced, then individuals were assumed to be female and were given an intramuscular injection of 3.0 cc synthetic gonadotropin (luteinizing hormone releasing hormone (Salmon), Sigma-Aldrich, Inc.) to stimulate egg production.

2.3.2. Egg collection and setup

On two collection days (21 and 23 March 2010), fertilized walleye pollock eggs were collected using a 350 μm mesh basket hanging in the outflow of the broodstock rearing tanks for use in a series of hatch experiments under different pH treatments. Eggs were gently rinsed with ambient temperature and pH seawater and placed in 1 L seawater in a constant temperature room at 9ºC for 15-30 minutes, or until eggs stratified in the container. Walleye pollock have pelagic eggs (Bailey et al. 1997); therefore, only floating eggs were deemed viable and kept for experiments. Because eggs were of unknown individual parentage and all females were injected with synthetic gonadotropin, any possible effects of the hormone were equally distributed across all treatments.

Based on egg availability in the collection basket, the collected eggs were randomly divided up into groups of 130 to 730 eggs by volume. These eggs were subsequently transferred to eight 1000 mL plastic beakers with 220 μm mesh bottoms on each of the collection days. Beakers were distributed randomly into 12 tanks, with three tanks set to either low, medium, or high pH treatments (pH 7.2, 7.6, or 7.9) and three control tanks remained at ambient conditions for the Oregon coast (pH 8.1 ± 0.1). Treatment pH conditions were created using a gravity feed system with a conditioning tank set at the lowest pH treatment, several header tanks to achieve experimental pH conditions, and the treatment tanks. In the conditioning tank, a pH computer (Aquamedic) and pH probe (Aquamedic) controlled a solenoid valve to inject CO_2 gas through a gas membrane exchanger so that a pH 7.2 was achieved (detailed description in Chapter 3). Water from the conditioning tank was pumped to a series of header tanks (n=3, 100 L each), where it was mixed with ambient pH water (pH 8.1) to obtain the desired experimental pH levels: low (pH 7.2), medium (pH 7.6), and high (pH 7.9). The header tanks gravity-fed water to the treatment tanks (3 per treatment, 144 L each). Ambient pH water (pH 8.1) was also pumped to a 100 L header tank, where it was gravity fed to control treatment tanks (n=3, 144 L each). Each set of treatment tanks was monitored using a pH data logger (SympHony, VWR) equipped with a pH probe (glass combination pH probe, VWR) and a combination temperature/dissolved oxygen (DO) probe (VWR) to monitor pH, DO, and

temperature throughout the experimental period. All tanks were set to a flow rate of 0.5 ± 0.1 L/min and temperatures were maintained at 7.8 ± 0.5ºC, and all pH probes were calibrated with three pH standards (pH 4.0, 7.0, and 10.0) three days per week. The pH probes collected pH data every 15 min for the duration of the experiment.

2.3.3. Larval collection

Each beaker containing fertilized eggs was checked for hatching once per day and all hatched larvae were counted and removed from the beakers for morphometric measurements. If there was a large hatch event of more than 15 individuals, 15 of those larvae were randomly used as a subsample for measurements and the remainder was discarded. If less than 15 individuals hatched, then all hatched larvae were collected for measurements. Larvae were stored in chilled seawater of the treatment pH until measurements of developmental parameters were completed. The experimental trials were terminated when eggs were no longer hatching.

Larvae were individually photographed in a small dish of seawater of the same pH as their rearing tanks under 20x magnification using a dissecting microscope (Zeiss Stemi
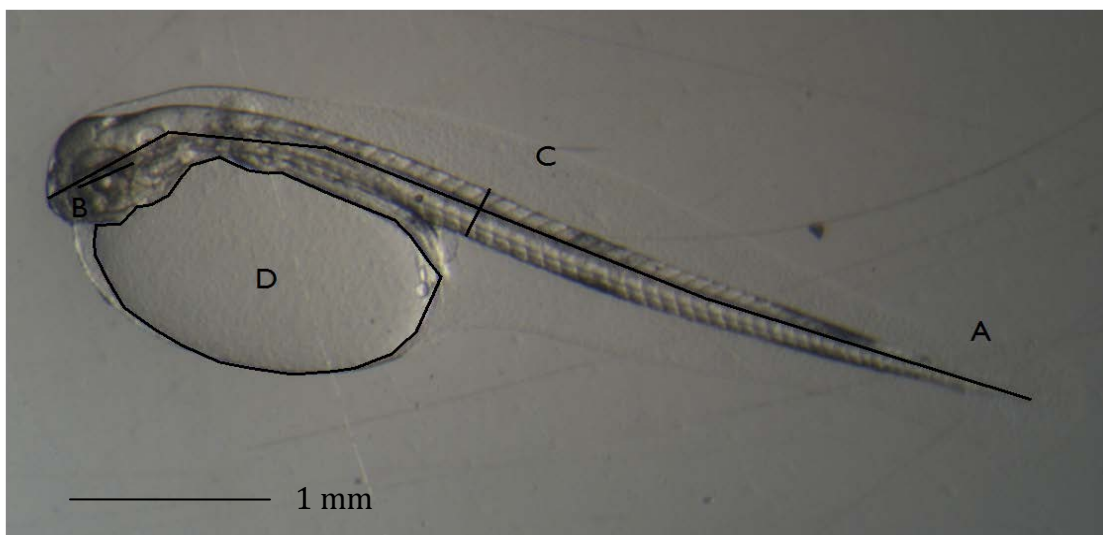
Figure 2.1. Larval walleye pollock developmental parameters. Developmental parameters used in determining size at hatch of walleye pollock larvae. Parameters of interest include (A) standard length, (B) eye diameter, (C) myotome height, and (D) yolk area. Magnification: 20x.

SR, Bartels and Stout, Inc.) with digital camera (Go-3, Q-Imaging, Inc.). After photographing, all larvae were euthanized using a lethal dose of MS-222 (tricane methanesulfonate, ~250 mg/L) until the larvae stop moving for several minutes. Larval photographs were measured using the ImagePro Plus Express 6.0 software package (Media Cybernetics) for standard length, eye diameter, yolk area, and myotome height (Figure 2.1). Myotome height is defined as the widest part of the muscle behind the anus. Standard length, yolk area, and myotome height are development parameters were used to determine size, condition, and overall variation of the hatching larvae. Rearing conditions, sampling protocols, and euthanasia were conducted in accordance with University of Alaska Fairbanks Institute of Animal Care and Use Committee assurance #09-25 (Appendices B and C).

2.3.4. Data analysis

A repeated measures ANOVA was conducted for each treatment (pH) to determine whether or not the incubation conditions were consistent over time and close to their target pH levels throughout the course of the experiment. Significance was set to $\alpha = 0.05$ for all analyses.

Because the egg collection dates were close together (2 days apart) and eggs were from the same group of spawning adults for both release dates, data from the two collection dates were combined for analysis. However, it is unknown which individual fish within the spawning tanks contributed to the fertilization of the eggs used in this experiment. A hierarchical linear model (HLM) was used for subsequent data analyses, with the beaker and tank numbers included in the model as random effects, which were analyzed using a reduced maximum likelihood test (REML). Pooling all individuals from all three tanks within a treatment, the HLM measured treatment effects on the hatched date. To assess developmental parameters, specifically total length, myotome height, eye diameter, and yolk area, data for all individuals from each of the three tanks per treatment were pooled. An HLM was conducted for each parameter to measure the effect of the pH conditions. All data were analyzed using JMP® 9 statistical software (SAS, Inc.).

2.4. Results

2.4.1. pH treatment condition

Throughout the course of the incubation experiments, there were fluctuations in the treatment pH, possibly as a result of natural pH variations in the ambient seawater or some change in the CO_2 injection system. Even though there was significant variation of the treatment pH conditions from the target pH (repeated measures ANOVA, p<0.0001 for each treatment, Table 2.1), this variation was no greater than the variation deemed acceptable (±0.05 units) for each treatment to account for any natural variation in pH that the ambient seawater exhibits during the course of the experiment. For the duration of incubation and hatch, the pH readings in the four treatments were significantly different from each other (p≤ 0.0001).

Table 2.1. Water conditions for larval walleye pollock experiments. Mean values for each of the treatments for the duration of the incubation experiments as determined by the VWR SympHony loggers (one-way ANOVA, p<0.0001). Different letters indicate significant differences among treatment groups. Experimental duration was ~12 days.

Target Treatment	Mean pH (±SD)
Ambient control (8.1)	8.143 (±0.017) [a]
High (7.9)	7.905 (±0.028) [b]
Medium (7.6)	7.627 (±0.029) [c]
Low (7.2)	7.185 (±0.053) [d]

2.4.2. Hatch timing and larval developmental parameters

Walleye pollock eggs started to hatch 7 days post fertilization (55 degree-days), with peak hatching occurring 8-10 days post fertilization (63-78 degree-days) in all experimental treatments. There was no effect of pH treatments on the hatch timing of the larvae (p=0.99) (Table 2.2). None of the

Table 2.2. Walleye pollock hatch timing. The effects of pH treatments on the hatch timing of walleye pollock eggs incubated under projected levels of ocean acidification (no significant differences with hierarchical linear model with reduced maximum likelihood (REML) test, p=0.99).

Target Treatment	n	Days to Peak Hatch (±SE)
Ambient control (8.1)	464	9.628 (±0.066)
High (7.9)	408	9.542 (±0.069)
Medium (7.6)	406	9.677 (±0.069)
Low (7.2)	445	9.894 (±0.066)

developmental parameters were affected by the pH treatment: standard length (p=0.86), eye diameter (p=0.42), yolk area (p=0.79), and myotome height (p=0.70) were all not significantly different among pH treatments (Table 2.3). Beaker or tank effects were random effects and were not a significant source of error within the experimental treatments.

Table 2.3. Larval walleye pollock developmental parameters. Developmental parameters of walleye pollock eggs incubated under different pH treatments. None of the parameters were significantly different among treatments (p>0.05, hierarchical linear model (HLM) with the reduced maximum likelihood (REML), with both beaker and tank listed as random effects).

Target Treatment	n	Standard Length (mm) ($\pm$SE)	Eye Diameter (mm) ($\pm$SE)	Yolk Area (mm^2) ($\pm$SE)	Myotome Height (mm) ($\pm$SE)
Ambient control (8.1)	464	4.158 ($\pm$0.067)	0.2679 ($\pm$0.0026)	0.7635 ($\pm$0.0905)	0.2458 ($\pm$0.0046)
High (7.9)	408	4.231 ($\pm$0.068)	0.2755 ($\pm$0.0027)	0.8004 ($\pm$0.0904)	0.2495 ($\pm$0.0046)
Medium (7.6)	406	4.194 ($\pm$0.067)	0.2734 ($\pm$0.0026)	0.8358 ($\pm$0.0907)	0.2459 ($\pm$0.0046)
Low (7.2)	445	4.199 ($\pm$0.067)	0.2747 ($\pm$0.0027)	0.8609 ($\pm$0.0903)	0.2427 ($\pm$0.0046)

2.5. Discussion

Hatch timing of walleye pollock was not affected by changes in environmental pH. The hatch timing of walleye pollock in this study was consistent with the findings of Bailey and Stehr (1986), where they determined that walleye pollock hatch in 9-10 days at 8.4°C (or 75-84 degree-days). Similarly, developmental parameters of standard length, eye diameter, yolk area, and myotome height were also not affected by the decreased environmental pH. These developmental parameter measurements in all treatments were also consistent relative to those of other walleye pollock populations (Bailey and Stehr 1986) and other related species reared under ambient conditions or found in the wild (i.e., Pacific cod *Gadus macrocephalus*, Laurel et al. 2010). These results indicate some resiliency of early life stages of this particular broodstock of walleye pollock to ocean acidification.

The results of this study agree with some previously published data indicating that early life stages of most marine teleost species may be more resilient to low pH

conditions than initially thought (Melzner et al. 2009a). Similar to walleye pollock in this study, spiny damselfish (*Acanthochromis polyacanthus*) did not show consistent response in size at hatch to pH levels that could be reached within the next 100 years (Munday et al. 2011). Atlantic herring (*Clupea harengus*) post hatch larvae did not exhibit differences among treatments with regards to total length, yolk area, dry weight, and otolith area at hatch when incubated under different pH levels (Franke and Clemmesen 2011). Clownfish (*Amphiprion pericula*) egg survivorship and size at hatch did not change under ocean acidification levels projected for the next 100 years (Munday et al. 2009a). Instead, decreased environmental pH resulted in greater weight and length gain in clownfish larvae in the same set of experiments, showing that some species can exhibit positive reactions to changing environmental conditions as a result of ocean acidification (Munday et al. 2009a). Conversely, some studies suggest the early life history stages are more sensitive to changes in environmental factors than adults, if the environmental conditions are outside the adult's physiological window of tolerance. Some fish species exhibit a delay in hatch with decreasing pH, such as Atlantic salmon (*Salmo salar*) and European perch (*Perca fluviatilis*) (Peterson et al. 1980, Rask 1983). However, the majority of these studies indicating delayed hatch focus on the effects of acid rain in freshwater ecosystems (Kamler 2002) and not CO_2-based acidification of the marine system. The medaka (*Oryzias latipes*) was also found to have a developmental delay of 1.7 days under elevated CO_2 conditions and upregualtion of important genes responsible for acid-base regulation and metabolism (Tseng et al. 2013).

Early life stages of fishes are often considered highly vulnerable to changing or extreme environmental conditions or pollutants (Brooks et al. 1997). However, it is possible for some species to naturally experience high-CO_2 conditions during their early development, depending on their habitat (Melzner et al. 2009a). For example, species with pelagic eggs may be more sensitive than those with demersal eggs, because the pH levels in the pelagic realm do not fluctuate as dramatically as in nearshore benthic areas. However, adequate tests have not been conducted to compare the different sensitivity levels of demersal and pelagic eggs (Pankhurst and Munday 2011). The sensitivity of

both demersal and pelagic eggs may also depend on other aspects of spawning habitat, as some species spawn in relatively shallow areas. For example, Pacific herring (*Clupea pallasii*) spawn less than 10 m below low water (Rooper et al. 1999). Thus, species with demersal eggs that spawn nearshore may have developed ways to combat some lower pH conditions to facilitate normal development of the early life history stages.

Adaptations to low or variable pH in marine teleosts could include an increased number of chloride cells, which are present on the yolk membrane and the integument of larval fishes and the gills of adult fishes (Shirashi et al. 1997, Katoh et al. 2000). Chloride cells are integral for osmoregulatory balance as well as acid-base regulation in fresh and marine fish species (Perry 1997). The increased number of chloride cells or their increased activity results in lower blood chloride (Cl^-) concentrations by exchanging internal Cl^- for bicarbonate ions (HCO_3^-) from the environment, thus moderating internal pH for the fish (Shirashi et al. 1997, Katoh et al. 2000). Though Cl^- is not directly responsible for the increase and decrease of pH, the exchange of Cl^- and HCO_3^- across the lamellar membrane via chloride cells allows for pH to increase. However, this is an active transport mechanism requiring the use of adenosine tripohosphate (ATP), making this an energy demanding mechanism. Chloride cells increase in number between the cleavage and embryo stages, and species with greater numbers of these cells are more likely to adapt to more acidic conditions (Ishimatsu et al. 2004). However, there is high energy expenditure as yolk sac larvae shift from having their main osmoregulatory mechanism associated with the yolk membrane to osmoregulation occurring at the gills (Melzner et al. 2009a), as seen in Atlantic cod (Frommel et al. 2011) and in the four teleost species studied by Kikkawa et al. (2003). This shift occurs as larvae become more dependent on external food sources rather than relying on their yolk stores for energy (Melzner et al. 2009a, Franke and Clemmesen 2011). Maintaining osmoregulatory balance throughout metamorphosis is integral for larvae in this transition state (Pankhurst and Munday 2011). Ultimately, the formation and transition of chloride cells from the body and yolk membrane to gills occurs throughout larval development (Varsamos et al. 2002). Insufficient yolk size and poor yolk quality can cause increased mortality, decreased size,

and smaller energy stores for other physiological functions in teleost larvae. In several marine species, such as Atlantic cod (*Gadus morhua*), red sea bream (*Pagrus major*), and Japanese sillago (*Sillago japonica*), there was an observable drop in CO_2 tolerance as larvae aged, likely due to the reduced energy availability in the yolk (Kikkawa et al. 2003) or due to energy allocation to growth over organ development causing osmoregulatory and metabolic imbalances (Frommel et al. 2011). It is possible that the pH resiliency of the walleye pollock eggs and larvae observed in the present study was due to high chloride cell numbers and activity as a result of being incubated under high-CO_2 conditions. Chloride cell density or activity was not measured in this experiment, but could be included in future studies through histology or measurement of cell activity to assess the degree of response to increased CO_2 concentrations and lower pH due to ocean acidification. Future studies should assess overall energetics and metabolic rates of newly hatched larvae during development and metamorphosis to assess yolk and food consumption due to this compensation, especially since I did not observe effects on yolk sac size.

The results of this study suggest that the pH levels within the walleye pollock experiments were not outside their physiological tolerance window, either because of the potential for increased activity of chloride cells or other compensatory mechanisms. The amount of yolk and type of yolk fatty acids are important for larval fish, as they provide nutrients to the developing embryo and while the larva is transitioning to exogenous feeding (Rønnestad et al. 1998, Laurel et al. 2010). Although there was no difference among treatments in the yolk area in walleye pollock in this study, it remains unknown whether or not environmental pH as a result of ocean acidification could adversely affect the yolk quality after hatch as a result of increased stress response and potential increased energy uptake during development, which could be a direction of future studies. Most studies related to the negative effects of pH on yolk quality are within the context of freshwater systems and acid rain (e.g., northern pike *Esox lucius*; Johansson and Kihlström 1975), providing context for comparative marine studies. It would be valuable to explore how quickly yolk stores are used up during development and how yolk

composition changes over time in response to ocean acidification, e.g., using analytical methods such as fatty acid analysis.

Reduced pH had little effect on developing walleye pollock eggs and hatching larvae in this study, but physiological tolerance of the species could be exceeded if more than one stressor were at work synergistically. Food limitation, disease, and increasing ocean temperatures are several potential stressors that eggs and/or larvae could encounter during development that may interact with the currently non-detectable effects of reduced pH. For example, as water temperature influences the incubation time, growth rates, and metabolic rate of larval fish, understanding how both increased temperature and decreased pH will influence larval growth rates and energy demands will yield valuable information regarding the resilience of fish species to several stressors. Synergistic effects of pH and increased temperature have been studied in an adult clownfish (Munday et al. 2009b), showing an overall decrease in aerobic capacity as a result of decreased pH as well as the combined effect of increased temperature and decreased pH. However, little is known about the combined effects of ocean acidification and other stressors that both fish eggs and larvae could encounter during growth and development. Hence, despite the apparent resilience of developing walleye pollock eggs to ocean acidification effects, there is a need for further studies to assess how this valuable fisheries species might be affected by changing environmental conditions.

Chapter 3. Responses of juvenile walleye pollock (Theragra chalcogramma) to projected increases in ocean acidification

3.1. Abstract

With rising atmospheric CO_2, ocean pH is predicted to decrease 0.3 to 0.5 units by 2100. Biological consequences of ocean acidification have been studied in marine organisms; however, comparatively few studies focused on the response of marine teleosts to decreasing environmental pH. Walleye pollock (*Theragra chalcogramma*) are an important marine resource to commercial fisheries and ecosystem dynamics in the North Pacific, and it is crucial to understand how this species will respond to changing ocean conditions. Age-1 juvenile walleye pollock were reared at current ocean pH for the North Pacific (8.1) and at high, medium, and low pH treatment levels (pH 7.9, 7.6, and 7.2) to simulate projected levels of acidification. After a six-week exposure period, their physiological response was assessed using a suite of bioindicators: blood gas, plasma cortisol, cortisol secretion, body condition, hepatosomatic index, and growth rate. Marked differences were observed in several blood parameters in response to decreasing pH. Mean bicarbonate concentrations significantly increased from 9.72 mmol/L at ambient pH treatment to 15.07 mmol/L at the low pH level (p< 0.0001), and mean blood pCO_2 also significantly increased from 5.12 kPa at ambient pH level to 6.18 kPa in the low pH (7.2) treatment (p< 0.0001). Blood pH significantly increased as treatment pH decreased, from 7.02 at ambient pH treatment to 7.13 at the low pH level (p< 0.0001). However, these blood gas results were likely affected by the anesthesia solution that was not adjusted for treatment pH. Cortisol parameters showed that juvenile walleye pollock did not exhibit a stress response under lowered pH treatments. There was a trend toward elevated plasma cortisol levels at lower pH, although this trend was not statistically significant (p=0.87). In addition, there was a significant effect of sampling order on blood cortisol levels, suggesting a possible source of stress at the termination of the experiment (p< 0.0001). Basal and maximum interrenal secretion rates were not affected by decreased environmental pH (p= 0.26 and p=0.51, respectively), pointing to the resiliency of walleye pollock to the experimental conditions. Total length and weight changes did

not differ among pH treatments (p=0.88 and p=0.34, respectively). Both the body condition index and hepatosomatic index of juvenile walleye pollock did not vary significantly among treatments (p=0.66 and p=0.21, respectively). Overall, these results indicate that walleye pollock are largely able to withstand the pH conditions tested over the time frame of this experiment. However, it remains unknown whether chronic exposure to these conditions may have long-term effects on walleye pollock in the wild.

3.2. Introduction

Anthropogenic CO_2 levels have been rising in the atmosphere since the Industrial Revolution due to the increased use of fossil fuels and changes in land use practices. Since the late 18th century, atmospheric CO_2 concentrations have increased from ~280 parts per million (ppm) to more than 392 ppm, and CO_2 levels could reach 880 ppm by 2100 (Caldeira and Wickett 2003, Pelejero 2005, IPCC 2007, Feely et al. 2008, Tans and Keeling 2013). Model results show that the average ocean pH could decrease by another 0.3-0.5 pH units every 100 years if CO_2 emissions continue unchecked, with a decrease of nearly 0.8 pH units by 2300 (Pelejero 2005, McNeil and Matear 2006). Even though ocean acidification affects all marine biomes, oceans at higher latitudes are more susceptible to increased acidification (e.g., Fabry et al. 2009). This is especially true for cold water masses found in areas such as the North Pacific and the Bering Sea, where the combination of upwelling from global thermohaline circulation and cold water temperatures allow for more CO_2 gas to remain dissolved in solution. These high latitude regions are also extremely productive, resulting in seasonal shifts in pH and carbonate parameters (Bates et al. 2009, Fabry et al. 2009, Mathis et al. 2010). Large commercially important benthic and pelagic fisheries are supported by this high primary production. Changing ocean conditions can have implications for species playing key roles in these fisheries and their supporting food web. It is also possible that pH influences could affect the distribution and survival of important commercially harvested species such as walleye pollock (*Theragra chalcogramma*) through either direct or indirect bottom-up control effects (Fabry et al. 2009).

The physiological tolerance window for pH is likely species-specific and can be dependent on the overall life history and life stage of an organism (Hayashi et al. 2004a, Pörtner and Farrell 2008, Munday et al. 2009a, 2009b). Under reduced pH conditions associated with ocean acidification, organisms could exhibit compromised extracellular acid-base regulation (Hayashi et al. 2004a, Hayashi et al. 2004b) and osmoregulation (Miles et al. 2007, Pane and Barry 2007, Pörtner 2008); lower growth rates or smaller overall size (Kurihara 2008, Melzner et al. 2009a); reduced metabolic scope (Seibel and Walsh 2003, Munday et al. 2009b); and increased mortality rates associated with early life stages (Ishimatsu et al. 2008). Currently, there is a paucity of data on the specific effects of ocean acidification on fish species (Ishimatsu et al. 2008). The few studies available indicate that any effects resulting from ocean acidification are species-specific; one species could be extremely sensitive, whereas the same environmental conditions may not elicit a response in another species. Some adult marine fishes (i.e., Japanese flounder *Paralichthys olivaceus*, yellowtail *Seriola quinqueradiata*, starspotted dogfish *Mustelus manazo*, and the gulf toadfish *Opsanus beta*) exhibit changes in blood chemistry during laboratory ocean acidification studies (Hayashi et al. 2004a, Hayashi et al. 2004b, Kikkawa et al. 2004, Esbaugh et al. 2012). Such physiological responses could potentially result in whole body effects, such as decreased survival or body condition, but these responses are species dependent. Environmental stressors may shift an organism's energy budget, requiring greater allocation of energy to maintaining homeostasis. This allows for less investment in reproduction and growth (Ishimatsu et al. 2004, Pörtner 2008, Melzner et al. 2009a) if organisms cannot employ other compensatory mechanisms (e.g., increase food intake). Understanding potential effects on physiological indicators of stress, body condition, and acid-base balance can lead to a greater understanding of how an organism like walleye pollock can respond to changing environmental pH. Overall, the responses to changing ocean conditions could impact not only the individual, but also have population and ecosystem-wide implications with potential community shifts (Doney et al. 2009).

The purpose of this study was to evaluate how juvenile walleye pollock respond to ocean acidification, employing several physiological mechanisms as bioindicators. To assess potential responses to altered environmental pH as a result of ocean acidification, I measured whole body indices (i.e., hepatosomatic index, body condition index, and growth rates), blood gas parameters (i.e., blood pH, bicarbonate, and pCO_2), and stress parameters (i.e., plasma cortisol levels and cortisol secretion) in juvenile walleye pollock. With decreasing pH, I expected that juvenile walleye pollock would exhibit decreased growth rate and lower condition indices because more energy could be allocated to the increased activity of compensatory mechanisms than somatic growth. Blood pCO_2 and blood bicarbonate were expected to increase, and blood pH to decrease, with lower pH exposure. Increased blood cortisol and cortisol secretion were expected as a stress response to lowered pH.

3.3. Methods

3.3.1. The pH dosing system

Experiments were conducted at the Hatfield Marine Science Center (HMSC) in Newport, OR, in 2009. To simulate the potential pH conditions that walleye pollock could be exposed to in the next several decades, an injection system was developed (Figure 3.1) allowing for the controlled addition of carbon dioxide (CO_2) into the laboratory's flowing seawater. Flowing seawater was conditioned to the lowest set point (pH = 7.2) at 9°C using an automated CO_2 injection system, which was monitored using a pH probe attached to a computer-controlled solenoid valve to regulate the CO_2 gas flow into a membrane contactor. A small pump provided circulation to prevent stratification in the conditioning tank and ensure uniform pH. Conditioned water (pH 7.2) flowed into three elevated header tanks, where it was mixed with untreated flowing seawater (pH = 8.1±0.07) in fixed volume ratios to create the high, medium, and low treatment pH levels (pH 7.9, 7.6, and 7.2, where the latter received no addition of natural seawater). One header tank received only untreated seawater to represent the ambient environmental pH treatment. Water from the elevated header tanks was then gravity fed to 16 144 L

experimental tanks (four treatment tanks for each pH level) where the fish were held during the experiment. To continually monitor pH in the experimental tanks, each tank group was outfitted with a pH meter (SympHony, VWR, ±0.001 pH units) equipped with both a glass combination pH probe and a dissolved oxygen/temperature probe. pH, dissolved oxygen (DO, ±0.01 mg/L), and temperature (±0.1 °C) were monitored every 15 minutes throughout the experiment. Flow rates were set at 1 L/min, tank temperatures were maintained at 9.0°C ± 0.6°C (Table 3.1) based on the probe measurements, and DO was ensured to remain above 70 mg/L by adding an air stone bubbler to each tank. pH meters were calibrated once per week with three standards (pH= 4.0, 7.0, and 10.0).

Carbonate parameters of treatment water were determined from samples collected twice per week from each set of treatment tanks and 200 μL saturated mercuric chloride solution was added to halt any biological activity. These water samples were analyzed for dissolved inorganic carbon (DIC) and total alkalinity (TA) using a VINDTA 3C (Versatile INstrument for the Determination of dissolved inorganic carbon and Total Alkalinity) coupled to a UIC 5014 coulometer (±1μmol/kg). These data were used to calculate actual pH, pCO_2, and carbonate mineral saturation states (Ω) of the experimental waters using the program developed by Lewis and Wallace (1995) (Table 3.1).

Table 3.1. Water conditions for juvenile walleye pollock experiments. Tank carbonate conditions for the different pH treatment groups during the 6-week experiment. pH was calculated from carbonate parameters gathered by the VINDTA. Data are presented as mean ± standard deviation.

Target Treatment	Temperature (°C)	pH	pCO_2 (μatm)	Calcite Saturation State (Ω_{calc})	Aragonite Saturation State (Ω_{arag})
Ambient Control (8.1)	9.33 ± 1.83	8.02 ± 0.05	377 ± 6	4.41 ± 0.13	2.85 ± 0.13
High (7.9)	8.48 ± 0.36	7.91 ± 0.04	526 ± 5	3.49 ± 0.12	2.25 ± 0.12
Medium (7.6)	8.82 ± 0.27	7.64 ± 0.08	772 ± 6	2.61 ± 0.13	1.68 ± 0.13
Low (7.2)	8.57 ± 0.24	7.28 ± 0.16	1069 ± 6	2.00 ± 0.13	1.29 ± 0.13
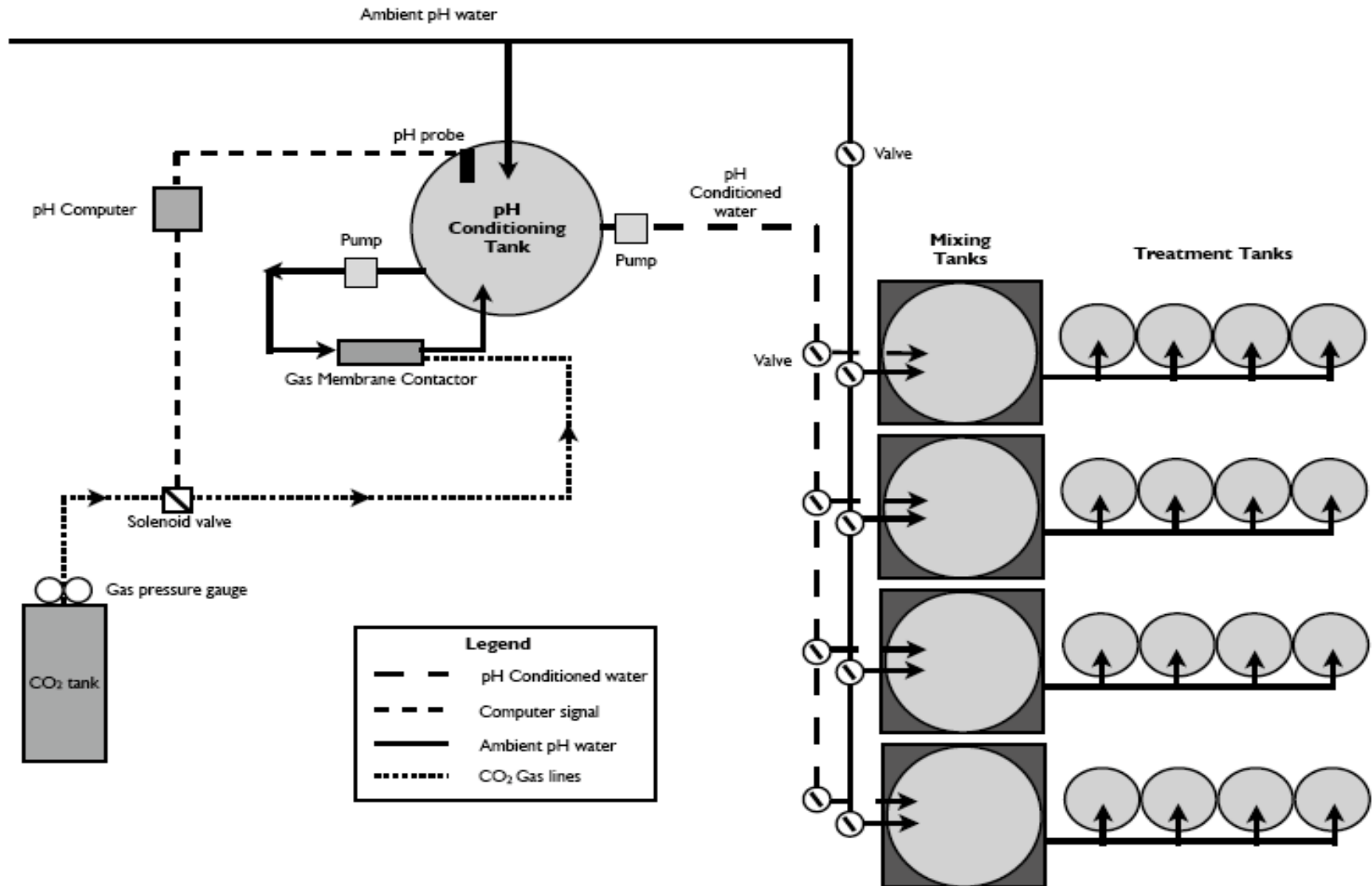
Figure 3.1. CO$_2$ injection system schematic. Schematic overview of CO$_2$ system used to simulate ocean acidification conditions for juvenile walleye pollock experiments.

3.3.2. Fish Capture and Husbandry

Juvenile walleye pollock (age 0+) were captured in May 2008 in nearshore waters of Puget Sound off of Port Townsend, WA (48° 6′ 59″ N, 122° 46′ 31″ W) and maintained in ambient seawater at the HMSC. Prior to use in the experiments, fish were reared in 144 L round tanks in ambient pH treatments with a 12 h light: 12 h dark photoperiod and temperatures maintained at 9-10℃. Once the fish reached the age 1+ age class, they were transferred and reared in 5,678 L tanks with a density of 70 individuals per tank at 9℃ with a 12 h light: 12 h dark photoperiod. Throughout their holding period and before use in the experiments, fish were fed a mixture of commercially available food (Otohime EP) and thawed krill (*Euphausia pacifica*) daily until apparent satiation (i.e., when fish stopped eating). As fish grew, feeding to apparent satiation was eventually reduced to two days per week using a blended and gelatinized combination of herring, amino acid supplements, commercial food, squid, krill, and vitamins (Appendix A). All age 1+ walleye pollock were reared and handled under the University of Alaska Fairbanks Institutional Animal Care and Use Committee assurance #09-25 (Appendices B and C).

3.3.3. Experimental Setup

In late October 2009, 48 age 1+ fish were transferred to the experimental tanks and randomly distributed between treatment tanks at a density of three fish per tank (12 fish total for each pH treatment). Experimental fish averaged 21.0 ± 1.1 cm (mean $\pm$ SE) total length (L_T) and 72.0 ± 12.2 g wet mass (M_w), and there were no significant differences in either size measurement among the treatment groups (one-way ANOVA, M_w p= 0.39; L_T p= 0.13). The fish were kept at a 12 h light: 12 h dark photoperiod and were allowed to acclimate to these experimental conditions for one week at ambient pH conditions prior to initiating the pH treatments. pH was adjusted in treatment tanks over a 24 h period for the 7.9 treatment, and over a 48 h period for the 7.6 and 7.2 treatment tanks to not introduce sudden stress on the fish. Fish were reared for six weeks under these

experimental conditions. During the experimental period, fish were fed the gelatinized food (Appendix A) to apparent satiation once per day to maximize growth potential.

Following the six week experimental exposure period, fish were anesthetized in ambient pCO_2 seawater using 100 mg/L buffered tricane methanosulfonate (MS-222) and morphometric parameters and blood samples were collected. After whole body parameters were collected, fish were euthanized using a lethal dose of MS-222 (~250 mg/L), and a complete necropsy was conducted to isolate tissues of interest and determine the presence of ovaries.

3.3.4. Whole Body Parameters

Growth was calculated as the difference between final and initial total length (L_T) and wet mass (M_W), averaged within a treatment group, and are presented as mean ± SE. The body condition (I_C) was calculated using the residual weight method, where the individual variation from the relationship between $\log_{10} M_W$ and $\log_{10} L_T$ is used (Blackwell et al. 2000, Hurst 2004). The liver is a primary organ for energy storage in many fishes, including gadids (Jobling 1988, Grant et al. 1998, Gildberg 2004, Lekva et al. 2010). Therefore, the hepatosomatic index (I_H) was used to assess the variation in energetic status of walleye pollock under experimental conditions. I_H was calculated as the individual deviation from the relationship between $\log_{10}$ liver wet mass (weighed to the nearest 0.01 g) and $\log_{10} L_T$ x 100 as described in Hurst (2004).

3.3.5. Blood Gas Parameters

Whole blood was drawn from anesthetized fish from the caudal vein using a 3cc heparinized syringe and a 25 gauge needle. Syringes were capped using syringe plugs designed for blood chemistry analysis, and samples were transported on wet ice to the Samaritan Pacific Community Hospital's analytical laboratory in Newport, OR within 3h after collection. The samples were analyzed at room temperature on an Omni-6 Blood

Gas Analyzer (#GD0485, Roche Diagnostics) within 3 h of collection for pH (calculated), total bicarbonate, and pCO_2 (measured).

3.3.6. Cortisol Measurements

As much whole blood as possible was collected from each fish using a sodium heparin Vacutainer[TM]. Vacutainers were spun in a centrifuge for three min at 3000 x gravity (*g*) to separate blood components. Plasma was isolated and stored in microcentrifuge tubes at -80ºC until analysis (see below).

In addition to whole blood, interrenal tissues were cultured to measure basal and maximum interrenal cortisol secretion after six weeks of exposure to pH conditions, following protocols outlined in Patiño et al. (1986) and Patiño and Schreck (1988). Following fish sacrifice (within five min), interrenal tissue was isolated, weighed, masticated, and placed in a static system consisting of a 24 well plate with 2 mL cell culture made of 5 M NaCl, 0.5 M KCl, 0.3 M $CaCl_2$, 0.5 M $MgSO_4$, 0.5 M, KH_2PO_4, 0.5 M $NaHCO_3$, 0.28 M glucose, 0.20 M glutamine, and Eagles-MEM (Minimum Essential Medium) solution for two, two h washes. Tissues were incubated in the cell culture medium for 3 h with either 25 mU/L adrenocorticotropic hormone (ACTH) or untreated to obtain maximum and basal secretion rates, respectively (25 mU ACTH= 133.3 µg ACTH in 400 mL MEM/Ringers Solution). ACTH is a hormone that is integral to the hypothalamus-pituitary-interrenal (HPI) axis. ACTH is produced by the pituitary gland and stimulates the secretion of cortisol by the interrenal tissue upon detection of a stressor (Schreck et al. 1989). Cultures were incubated in a 9ºC cold room on a slow plate shaker. After three h, the tissue incubation solution was placed in microcentrifuge tubes and stored at -80ºC.

Both plasma and interrenal cell culture media were assayed in duplicate using the protocol outlined in the Cortisol EIA (Enzyme Immunoassay) kit (Assay Designs, Inc.). Cortisol EIA plates were read using a SpectraMax microplate spectrophotometer with the wavelength set at 340 nm. According to the kit protocol, the following hormones could potentially cross-react with this particular EIA and their percent reactivity if they come

into contact with the assay components: prednisolone (122%), corticosterone (27.7%), 11-deoxycortisol (4%), progesterone (3.64%), prednisone (0.85%), testosterone (0.12%), androstenedione (<0.1%), cortisone (<0.1%), and estradiol (<0.1%). Cortisol concentrations were calculated using a standard curve, with an acceptable deviation of 5% between duplicates. Because all duplicate samples were within the acceptable deviation, no samples were re-run. This particular kit and protocol has previously been validated for four different fish species (channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, red pacu *Piaractus prachypomus*, and golden shiner *Notemigonous crusoleucas*; Sink et al. 2008).

3.3.7. Data Analysis

Because of the natural fluctuation in pH of the ambient seawater source, a repeated measures ANOVA tested if treatment conditions were constant throughout the duration of the experiment for data collected from both the VINDTA and pH meters. The accuracy of the data collected from the pH meter was compared with the calculated values from the VINDTA using a two-way ANOVA with repeated measures.

A nested ANOVA was conducted for all measured biological parameters to consider tank effects, sampling order effects, and effects of the individual's sex for the various physiological biomarkers. If a component of the nested ANOVA (e.g., tank within a treatment) was not statistically significant, a one-way ANOVA with subsequent Tukey's post-hoc test was conducted. All statistical analyses and graphics were done using the statistical program JMP 8 (SAS Institute), with a significance level set at $\alpha = 0.05$.

3.4. Results

3.4.1. Tank pH Conditions

There was significant variation in all treatment pH levels as measured by the pH meters (repeated measures ANOVA, $p > 0.0001$; Figure 3.2). All four pH conditions also remained significantly different from each other throughout the six week experimental

period (repeated measures ANOVA, p< 0.0001). The cause of this variation is likely the changes in the injection system because of the gas injection setpoint on the pH computer and gas dissolution process rather than natural variation; the ambient control did not exhibit a high degree of pH variability. Deviations between the pH meter readings taken at the time of water sample collection and
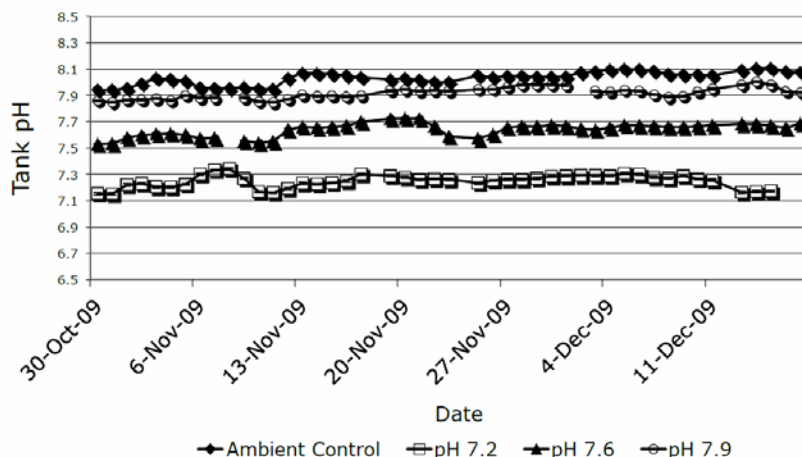
Figure 3.2. Daily mean pH for juvenile walleye pollock experiments.
Daily mean pH for juvenile walleye pollock ocean acidification experiments from 30 October 2009 to 17 December 2009 for four experimental pH levels. Error= ±SD small and not visible in figure.

VINDTA measurements were statistically different (two-way repeated measures ANOVA, p= 0.02). However, deviations from the mean of each treatment condition were overall small, with a maximum of 0.009 pH units. Despite the statistically significant variation in pH within the experimental tanks, the treatments themselves remained different through the duration of the experiment. The variation within a treatment was within the acceptable limit of ±0.05 units for the 6-week experimental period. As a result, I determined that the experimental pH conditions were stable enough within each of the four treatments to effectively conduct the experiments.

3.4.2. Whole Body Parameters

There were no mortalities associated with the experimental pH conditions. However, one fish jumped out of the high pH treatment tank and died (n= 11 fish for this treatment). All fish used in the experiment had an average L_T increase of 30.11 ± 9.95 mm (mean ± SE), and M_W gain of 24.83 ± 10.47 g over the course of the experiment.

Changes in L_T (p= 0.43) and M_W (p= 0.58) were not statistically significant among pH treatment groups (Table 3.2).

Table 3.2. Juvenile walleye pollock somatic indices. Somatic parameters of juvenile walleye pollock exposed to different pH conditions for a 6-week period. The somatic parameters of interest were changes in wet weight (M_w), changes in total length (L_t), condition factor (I_c), and hepatosomatic index (I_H). Data are presented as mean ± standard error.

Treatment	n	M_W (g)	L_T (cm)	I_C	I_H
Ambient Control (8.0)	12	25.28 ± 3.06	2.96 ± 0.29	-0.0041 ± 0.1188	1.302 ± 3.62
High (7.9)	11	26.27 ±3.06	2.79 ± 0.29	0.0121 ± 0.1188	-0.086 ± 3.78
Medium (7.6)	12	20.98 ± 3.19	2.86 ± 0.30	-0.0003 ± 0.1241	1.499 ± 3.62
Low (7.2)	12	26.47 ± 3.06	3.42 ± 0.29	-0.0084 ± 0.1241	-1.543 ± 3.62

The I_C was not significantly different among treatment groups (Table 3.2, p= 0.66). Overall, I_H also was not significantly different among experimental pH treatments (Table 3.2, p= 0.84). Gender was not a contributing factor for any differences among treatments with regards to L_T (p= 0.88), M_W (p= 0.82), I_C (p= 0.34), or I_H (p= 0.83). The combined factors of the individual rearing tank within a treatment and sampling order did not significantly affect mean L_T (p= 0.19), M_W (p= 0.64), I_C (p= 0.62), or I_H (p= 0.21) in any of the four pH treatment groups.

3.4.3. Blood Gas Parameters

Blood gas parameters exhibited marked differences among pH treatment groups after the 6-week incubation period. Blood pCO_2 increased significantly as treatment pH decreased (Figure 3.3a, p< 0.0001). There also was a significant increase in blood bicarbonate concentrations associated with the exposure to reduced treatment pH (Figure 3.3b, p< 0.0001). Lastly, an increase in blood pH was observed in the lower pH treatments (Figure 3.3c, p< 0.0001). However, this increase in blood pH is believed to be an artifact caused by CO_2 off-gassing in the ambient pH water used in the anesthesia

process. As a result, the blood pH findings are likely not reflective of how the fish are physiologically responding to decreased environmental pH.

3.4.4. Cortisol Parameters

There was no overall difference in the plasma cortisol levels among experimental treatments (Table 3.3, p= 0.87). However, sampling order of individuals proved to be a significant source of variation, with the last fish sampled from a given tank regardless of pH treatment having the highest plasma cortisol levels (p< 0.0001, Table 3.4). The plasma cortisol levels of fish sampled first (one-way ANOVA, p= 0.33), second (one-way ANOVA, p= 0.68), or third (one-way ANOVA, p= 0.75) showed no difference among treatment groups. Plasma cortisol levels ranged from 0.68 to 20.59 ng/mL in all fish sampled first in this experiment, showing the high variability of this parameter among individuals.

Table 3.3. Juvenile walleye pollock cortisol parameters. Cortisol parameters measured in walleye pollock exposed to different pH levels for a 6 week time period. Statistical differences as a result of only pH treatment conditions were determined using a Tukey's HSD and are denoted by different letters (p≤0.05). Data are presented as mean ± standard error.

Treatment	n	Plasma cortisol [ng/mL]	Basal Interrenal Secretion [ng/mL]	Maximum Interrenal Secretion [ng/mL]
Ambient Control (8.0)	12	22.12 ± 6.40	15.78 ± 3.41	28.42 ± 4.75 [ab]
High (7.9)	11	23.02 ± 6.69	13.56 ± 3.56	19.69 ± 4.96 [b]
Medium (7.6)	12	26.57 ± 6.40	15.77 ± 3.41	24.97 ± 4.75 [ab]
Low (7.2)	12	28.78 ± 6.40	9.62 ± 3.41	33.45 ± 4.75 [a]

Basal cortisol secretion from the interrenal tissue exhibited no differences as a result of pH treatments (Table 3.3, nested ANOVA p= 0.26), and were comparable to cortisol levels in blood plasma (two-way ANOVA, p= 0.51). Maximum secretion was significantly affected by treatment conditions (p= 0.046) in the nested model, with significantly higher maximum cortisol secretion rates observed in low pH (7.2) treatment. Sampling order was not a significant contributor to the variation in either basal (p= 0.54) or maximum cortisol interrenal secretion (p= 0.31) within these individuals. However,

A

B

C
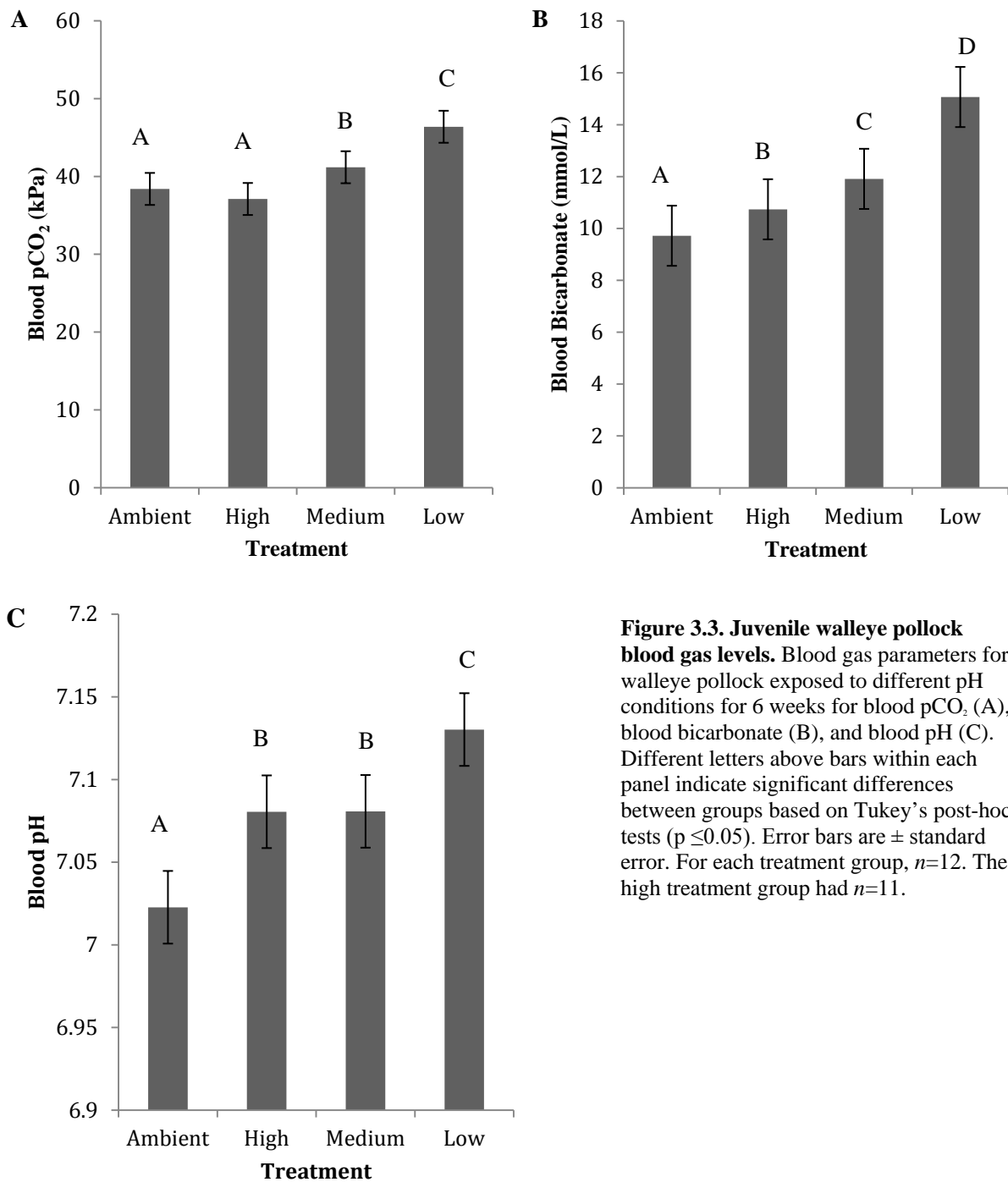
Figure 3.3. Juvenile walleye pollock blood gas levels. Blood gas parameters for walleye pollock exposed to different pH conditions for 6 weeks for blood pCO_2 (A), blood bicarbonate (B), and blood pH (C). Different letters above bars within each panel indicate significant differences between groups based on Tukey's post-hoc tests ($p \leq 0.05$). Error bars are ± standard error. For each treatment group, $n=12$. The high treatment group had $n=11$.

Table 3.4. Walleye pollock sampling order plasma cortisol concentrations. Plasma cortisol as a result of sampling order (capture stress) after individuals were reared under different pH levels for 6 weeks. Data are presented as mean ± standard error. Different letters indicate significant differences among sampling order and among treatments (p≤ 0.05).

Treatment	n	First sampled blood cortisol [ng/mL]	Second sampled blood cortisol [ng/mL]	Third sampled blood cortisol [ng/mL]
Ambient Control (8.0)	12	2.09 ± 5.70 [a]	18.77 ± 5.70 [b]	45.51 ± 5.70 [c]
High (7.9)	11	2.96 ± 8.06 [a]	21.28 ± 8.06 [b]	44.83 ± 8.06 [c]
Medium (7.6)	12	4.06 ± 6.62 [a]	26.08 ± 6.62 [b]	57.23 ± 7.65 [c]
Low (7.2)	12	7.98 ± 4.86 [a]	30.59 ± 4.86 [b]	47.78 ± 4.86 [c]

there were significant differences among replicate tanks within pH treatment in both basal (p= 0.001) and maximum cortisol secretion (p= 0.02).

3.5. Discussion

Despite the potential implications that ocean acidification may have on commercially important fish species, this is the first study where a multifactorial evaluation was utilized to measure the response of juvenile walleye pollock to lowered pH conditions. With a longer exposure period to decreased environmental pH levels relative to previous studies, it was anticipated that there would be detectable differences between treatments with regards to the tested parameters. There was no significant response detected with regards to whole body indices, suggesting that either fish were able to compensate for any additional energetic costs through increased food intake or that conditions were within the levels of tolerance for this population. The same patterns for whole body indices were observed in subsequent experiments with larval walleye pollock, where environmental CO_2 levels had a minor effect on overall body sizes (Hurst et al. 2013). Stress parameters were also not affected by the decrease in environmental pH. Conversely, there was a physiological compensation with regards to blood gas levels in response to hypercapnia from reduced environmental pH: elevated bicarbonate, blood pCO_2 levels, and blood pH correlated with decreasing environmental pH. Overall, these results indicate that either, (a) the treatment conditions did not affect or were not severe

enough to illicit a response of these particular physiological mechanisms in walleye pollock; or (b) the walleye pollock compensated for the energetic cost of acclimating to these circumstances during satiation feeding conditions. However, these experiments with juveniles and others with larvae (Hurst et al. 2013) indicate that the early life history stages exhibit a high degree of resiliency to ocean acidification conditions. Interestingly, slightly different results were found in subsequent experiments, likely caused by a suite of other, unknown factors that influenced growth rates and body size (Chapter 2, Hurst et al. 2012, 2013).

3.5.1. Whole Body Parameters

Whole body parameters in juvenile walleye pollock in this study were little affected by lowered experimental pH treatments, indicating that experimental conditions did not exceed their physiological window. This could be a species-specific trait, or it could be particular to the tested population. The walleye pollock used in these experiments were from a population at the southern edge of the species' range in Puget Sound, near the Strait of Juan de Fuca. This region has a high degree of eutrophication caused by high freshwater runoff from both urbanized locales and areas rich in organic material (Feely et al. 2010). The resulting dissolved inorganic carbon (DIC)-rich water is mixed in the water column by both wind and tidal forcing. There also is influence of seasonal upwelling that draws DIC-rich water from deeper regions (Feely et al. 2010). Annually, pH in Puget Sound ranges between 7.6-7.8 (Feely et al. 2010), covering much of the pH exposure range applied in the experiment. However, conditions found in Puget Sound are much lower in pH than those found in at the Hatfield Marine Science Center located on Yaquina Bay, OR, where the ambient conditions were 8.1 during the course of the experiment. Organisms found in habitats with a naturally variable pH are thought to have wider physiological tolerance windows than those living in more stable environments (Ishimatsu et al. 2004; Ishimatsu and Dissanayake 2010). It is therefore possible that the walleye pollock broodstock in this study had, a) previous exposure to variable pH conditions as juveniles before their capture; and/or, b) the tolerance levels of

the parents was passed on to the juveniles. It is currently unknown how other walleye pollock populations would respond to the pH levels as applied in this study. Conducting a similar set of experiments on individuals from different populations could offer some insight into the natural physiological variability occurring within this species, and to how the species as a whole may respond to ocean acidification.

Alternatively or in addition to high tolerance windows, maintenance of whole body parameters could also be related to high-energy (food) supply during the experiments. All animals in this study were fed daily to apparent satiation, so they were potentially able to sufficiently utilize food energy to compensate for any energy diverted to maintain homeostasis under decreased pH conditions. For example, some invertebrates were able to re-allocate metabolic costs under lowered pH conditions, especially if energy supply was maintained (Melzner et al. 2009a). A similar process may have allowed the walleye pollock in this study to maintain similar growth (both length and mass) under all experimental pH conditions for the duration of this experiment, and I_C and I_H also did not differ with pH treatment. In general, I_C values indicate the resiliency of the species to a broad range of pH. I_H exhibited no trend in relation to exposure to different pH levels, again pointing to the resiliency of the species to these conditions. Both I_c and I_H are commonly used by fisheries biologists as indices of health and condition of many teleost species as they are easy to measure. According to Lambert and Dutil (1997), different forms of both I_c and I_H have been used as indicators of the general nutritional status of gadids, specifically Atlantic cod. However, there are species, such as the three-spined stickleback (*Gasterosteus aculeatus*), that demonstrate the inaccuracy and unreliability of these and other (e.g., Fulton's condition index) indices (Chellappa et al. 1995). The amount of fat, protein, and water in the liver can affect these indices, as well as the overall energy available to the individual. Coupling these condition indices with biochemical analysis of tissue would allow for a more precise evaluation of the overall energetic status of individuals. Future experiments with limited food access could test the hypothesis that food energy can deflect adverse low pH effects on whole body parameters in walleye pollock.

The duration of the experiment could have implications for the observed results. Long-term responses, such as whole body parameters, may only be measurably affected over time periods longer than the 6-week period applied here. This may be especially true if experimental time were prolonged until walleye pollock sexually matured to assess if there are potential effects on fecundity and egg quality, similar to a previous study with salmonids and exposure to chronic stress (Schreck 2000).

3.5.2. Response in Blood Gas Parameters

Though it was possible to detect a physiological response to the different treatment conditions by using blood gas measurements, it is probable that the specific results were affected by aspects of the sampling procedures. The anesthesia protocol, which utilized ambient pH water, likely caused significant offgassing of CO_2 by the fish, altering the internal blood gas composition. This CO_2 offgassing happens almost instantly. As a result, the offgassing should not impact the stress parameters and whole body indices that were measured because of their longer response time to change. In the experiments with juvenile walleye pollock, blood pCO_2 significantly increased at the lowest two pH treatments. Generally, blood pCO_2 levels exceeding 4.6-5.2 kPa indicate hypercapnia within an organism (Robinson and Huxtable 1988). Based on this criterion, walleye pollock in the two lowest pH treatment groups were experiencing hypercapnia. Fishes can adjust to this with physiological shifts in the acid-base balance (Larsen and Pörtner 1997). To maintain a concentration gradient favorable for the expulsion of CO_2 from the body, some teleost fish have slightly higher (0.3-0.5 kPa) blood pCO_2 concentrations compared to their environment (Melzner et al. 2009a). This allows for more efficient diffusion of CO_2 at the gills from the fish into the environment, but plays an overall small part in maintaining internal pH (Melzner et al. 2009a). The difference in blood pCO_2 between the ambient and the medium pH (7.6) treatment in the present walleye pollock experiments was about 0.3 kPa, well in accordance with the adjustment levels previously reported (Melzner et al. 2009a). However, the 1.1 kPa difference in

blood pCO_2 between the ambient and the lowest pH (7.2) treatment is nearly double the maximum compensation levels previously reported (Melzner et al. 2009a).

The blood stores of CO_2 within teleosts are large relative to its production, and even minor changes to the CO_2 stores can have profound effects on overall acidosis or alkalosis of the blood (Randall and Daxboeck 1984). Ultimately, the degree of CO_2 tolerance in marine teleosts is directly dependent on both the ability of the organism to accumulate bicarbonate from seawater and the non-bicarbonate buffering mechanisms within the individual (Melzner et al. 2009a, Esbaugh et al. 2012). In this experiment, the increase in blood pCO_2 caused increased blood bicarbonate concentrations with decreasing treatment pH, indicating the buffering response by walleye pollock. The trends observed in the bicarbonate levels in this study match those found in several published accounts observing the response of fish to elevated CO_2 conditions (Hayashi et al. 2004a, 2004b), where the trends of high blood CO_2 and high blood bicarbonate concentrations also coincided in several marine fishes exposed to low environmental pH (Esbaugh et al. 2012). The elevated bicarbonate levels should subsequently induce a decrease in blood pH according to the carbonate equilibrium reaction (Equation 1.1). As a result, fish undergo a metabolic compensation by changing ventilation rates to excrete more CO_2 in response to raised plasma bicarbonate levels to reestablish homeostasis (Lloyd and White 1967). However, the expected inverse correlation of blood pH and bicarbonate levels was not observed in this study.

The water used to anesthetize the fish was at ambient pH (8.1) and not at the pH of the treatment water used in the experimental tanks. As a result, the anesthesia water contained a lower pCO_2 than the water in which fish were reared, causing the fish to offgas CO_2 during handling. As a result of this offgassing, there probably was an over-buffering response in the bloodstream, causing the highest blood pH levels to be observed in the lowest pH treatment. For complete compensation after exposure to the treatment conditions, it could take about 2-4 h for the blood pH, bicarbonate, and blood pCO_2 at exposure levels of pCO_2= 1900 µatm (192.5 kPa), as seen in the gulf toadfish (Esbaugh et al. 2012).

3.5.3. Stress Hormone (Cortisol) Responses

Exposure to a stressor causes the secretion of cortisol from interrenal tissue as a result of stimulation by ACTH (Mazeaud et al. 1977). The amount of cortisol found in the blood stream post stress is dependent on the intensity and duration of the stressor as well as the cortisol clearance rate from the bloodstream (Mazeaud et al. 1977, Barton et al. 1987, Barton 2000). Cortisol secretion by the interrenal kidney is almost immediate once a stressor is perceived (Mazeaud et al. 1977). No plasma cortisol response to the experimentally reduced pH conditions was observed in the walleye pollock, with plasma levels being between 20-30 ng/mL and no distinctive trend observed across all treatments. Previously published cortisol data on walleye pollock under ambient pH conditions had basal blood cortisol levels of about 7 ng/mL when subjected to a stress associated with capture in a trawl (Olla et al. 1997). Increasing the towing duration at high speeds resulted in acute stress plasma cortisol levels as high as 860 ng/mL (Olla et al. 1997). According to Pickering et al. (1991), plasma cortisol levels in rainbow trout (*Oncorhynchus mykiss*) of 10 ng/mL are considered chronic stress. However, such stress could be caused by confinement or overcrowding within the treatment tanks during the course of the experiment, and not from the pH treatment conditions themselves. It is possible that the consistently lower blood cortisol levels across all treatments relative to those found in Pickering et al. (1991) are an indicator of hardening or acclimation to the stressor. It has been previously shown that some fish, like rainbow trout *Onchorhynchus mykiss*, become desensitized to prolonged stressors (e.g., daily handling) over a long period of time (Barton et al. 1987). Either through pre-adaptation of the population with wide physiological tolerance windows from which experimental fish were obtained (see above), or through acclimation to chronic (6-week experimental period) exposure, the walleye pollock in this experiment seemed resilient to the stress of decreased pH conditions.

Plasma cortisol level is an effective bioindicator of acute stress (Barton 2000), which was likely seen in the influence of sampling order on cortisol levels in experimental walleye pollock in this study. Plasma cortisol levels increased significantly

with sampling order within each treatment (Table 3.4), indicating that the stress of the tank disturbance induced an acute stress response in the individuals independent of pH treatment. Future experiments should take this into account, and rapidly sample all individuals within a tank to reduce this sampling artifact as much as possible. Alternatively, cortisol could be extracted from biological materials (e.g., feces, tissue) that are indicative of long-term cortisol storage and relatively unaffected by acute stress (Lupica and Turner 2009, Peterson and Booth 2010).

Cortisol secretion from interrenal tissue is increased by exposure to ACTH, which is secreted by the pituitary gland when the organism comes into contact with a stressor (Schreck et al. 1989). Exposure to an acute stressor would cause a spike in cortisol secretion because of activation of the hypothalamus-pituitary-interrenal (HPI) axis and ACTH secretion (Pickering et al. 1991). In this experiment, the basal secretion from interrenal tissue from the lowest pH treatment did not differ from the ambient control. It was expected that the addition of ACTH did not change the possible stress response when chronically exposed to a stressor.

Prior exposure to ACTH can also enhance interrenal tissue sensitivity to increased ACTH levels, as is the case of repeated stressors or concurrent stressors (Schreck 1981). This can lead to a hyper-response (increased secretion rate) by the tissue when high ACTH levels are delivered to the tissue under stressful conditions (Schreck et al. 1989). However, consistent exposure to one particular level of stress (i.e., chronic stress), like the extended exposure to decreased environmental pH, can inhibit the responsiveness of interrenal tissue to ACTH (Patiño and Schreck 1988). Ultimately, this results in hardening or desensitization of the tissue to that level of ACTH exposure (Patiño and Schreck 1988), resulting in lower stress response because of decreased sensitivity or dulling seen in previous studies (Schreck 1981, Schreck et al. 1989). Because a decreased secretion rate was not found, the walleye pollock in this set of experiments were not stressed by the projected levels of ocean acidification. The results found in this experiment also indicate that chronic stress or desensitizing of interrenal tissue to cortisol did not occur, nor was a hyper-response of interrenal tissue to cortisol detected.

Even though basal and maximum secretion rates are species-specific, the values seen in this experiment are similar to a study that also measured basal and maximum cortisol secretion rates in coho salmon, *Oncorhynchus kisutch* (Schreck et al. 1989). Changes in plasma cortisol levels and cortisol secretion rates can affect a number of different physiological mechanisms, such as whole body indices measured in this experiment (Schreck 1981, Barton 2000). However, such deleterious effects were not observed in this experiment, indicating that the conditions were within the tolerance window of walleye pollock.

3.6. Conclusions

Ocean acidification is a stressor that has the potential to elicit a wide array of responses from many different species and life history stages. For walleye pollock, the rearing conditions presented in this experiment did not cause overall deleterious effects based on the parameters observed in this set of experiments. However, it remains to be seen as to how other ecosystem-wide effects as a result of ocean acidification (e.g., change or loss of a food source) could potentially alter the overall condition, abundance, and distribution of this commercially important species.

Chapter 4. General Conclusions

Since the Industrial Revolution in the late 1700's, there has been a substantial increase in atmospheric CO_2 levels as a result of increasing fossil fuel use and changes in land use practices (Sabine et al. 2004). As a result, it is expected that the pH of high-latitude oceans will decrease by 0.45 units within the next century (Steinacher et al. 2009; Yamamoto-Kawai et al. 2009). Ultimately, ocean acidification could cause detrimental effects on important marine species, such as the dissolution of carbonate skeletons in calcifiers and physiological effects in teleosts. Despite the increasing awareness and urgency, ocean acidification and its effects on marine life remains poorly understood.

This project used experimental manipulation of two life history stages, egg and age 1+ juveniles, of walleye pollock (*Theragra chalcogramma*) to evaluate the responses of these stages under levels of ocean acidification predicted over the next 300 years. Eggs and post-hatch larvae were used to assess possible adverse effects of ocean acidification on early development and growth (Chapter 2). Age 1+ walleye pollock were studied using a multifaceted assessment of responses including blood gas levels, stress response, and whole body condition indices to ocean acidification (Chapter 3). Combining the results of the two experiments, I found that early life history stages of walleye pollock demonstrated resiliency to projected levels of ocean acidification; however, I was able to detect a physiological response to these ocean pH levels. Therefore, the experiments outlined in this thesis contribute to the limited knowledge of responses and adaptive potential to projected levels of ocean acidification in subpolar fishes, specifically gadids.

There are several developmental bottlenecks present at distinct points in the life history of walleye pollock with large mortality events occurring between the post-hatch larvae and age-0+ stages (Houde 1997). As a result, it is necessary to study the physiological response to ocean acidification during several critical developmental stages of walleye pollock to gain a comprehensive view of potential impacts on this species under changing ocean pH conditions. Different life history stages may have varying levels of sensitivity to ocean acidification and may face several other challenges (e.g., predator avoidance behavior, ion regulation) caused by lower environmental pH during

development into reproductive adults (Hayashi et al. 2004, Dixson et al. 2010). The effects that changing pH conditions may have, especially on these early life stages, will have implications for the overall population structure of the species. Understanding and predicting changes in survival of certain age classes is critical to management of important commercial fishes (Melzner et al. 2009a).

Marine teleost fishes, such as walleye pollock, inhabit dynamic environments, causing these fish to have compensatory mechanisms to acclimatize to changing environmental conditions if needed. Throughout their life history, the walleye pollock's environment changes as a function of upwelling, storm mixing, and seasonal variability of terrestrial nutrient input (Bailey et al. 1997, 1999). This natural variability is likely to also include fluctuations in ocean pH. However, under the projected conditions of ocean acidification, the reaction and tolerance levels of a commercially important species, like walleye pollock, to decreasing environmental pH is important to explore. To investigate this question, adult and juvenile walleye pollock were collected from Port Townsend, WA for use in a series of experiments to assess the developmental and physiological responses to projected ocean conditions in early life stages. The lack of developmental and most physiological responses suggests that the conditions experienced by juvenile or larval walleye pollock in this study were probably within the range of pH levels of tolerance for this population, as pH levels as low as 7.4 occur seasonally in regions of the highly dynamic Puget Sound area (Feely et al. 2010).

Investigations on egg and larval stages provide necessary details on how the earliest and least developed, rapidly growing life stages will respond to an environmental stressor, such as ocean acidification. In the experiments presented in this thesis, walleye pollock eggs were exposed to three pH levels, from 7.2 (predicted in ~300 years) to 8.0 (currently ambient). Eggs hatched in all pH treatments (at 8°C) 8-10 days after fertilization, and none of the measured developmental and growth parameters were affected by low pH conditions (Chapter 2). Hatch timing of experimental fish was similar to that of walleye pollock in the wild, where hatch timing can vary from 7 to 30 days depending on water temperature (Bailey et al. 1997). The tolerance to low pH of this life stage is possibly the

result of several compensatory mechanisms (e.g., chloride cells) within the egg, allowing them to develop in areas with large daily and seasonal pH shifts (Melzner et al. 2009a). Larval walleye pollock also likely have several compensatory mechanisms (e.g., chloride cells on yolk membrane and gills) to tolerate low pH mechanisms similar to the egg stage. The location of these mechanisms shifts as larvae grow and become juveniles (Melzner et al. 2009a). It is also possible that juvenile walleye pollock exposed to low pH levels in the experiments were able to increase their food intake as a compensatory mechanism to offset energetic deficits encountered during ATP-dependent regulation of homeostasis. However, energy intake was not quantified in this study, as fish were fed to satiation, and future research should include this important parameter to assess overall energetic cost of adaptive responses. Another consideration is how larval energetics change once the yolk is completely used up, and if lower pH levels cause either slower growth or smaller overall size in exogenously feeding walleye pollock larvae. If the yolk is utilized faster at lower pH levels to facilitate compensation to different environmental conditions, there could be an overall disadvantage for larvae because of potentially smaller body size relative to those reared under ambient conditions. To assess yolk utilization and larval growth rates, future studies need to rear walleye pollock larvae through the exogenous feeding stage. Though the exact mechanism for compensatory responses was not part of this set of experiments, the results suggest that the overall physiological window of the egg and larval life stage of walleye pollock includes pH ranges projected in climate change scenarios over the next 100 years.

Juvenile (age 1+) walleye pollock had a physiological response to changing pH conditions between pH 7.2 to 8.0 as demonstrated by changing blood gas levels (Chapter 3). However, these results have to be interpreted with caution and future studies should carefully monitor pH conditions of the anesthesia solution to avoid confounding any physiological responses due to offgassing in rapidly changing pH conditions. Despite the possible physiological response in observed blood gas concentrations, indicators of stress (i.e., plasma cortisol and interrenal secretion) and whole body condition (as determined by several morphometric parameters) did not vary with treatment pH conditions.

Consequently, juvenile walleye pollock appear to be resilient to the pH ranges they were exposed to in this study and thus, the experimental pH conditions were probably not outside their physiological window of tolerance. However, future studies should include proximate composition parameters as an additional indicator of fish body condition as well as monitor parameters such as bone density and food intake (in particular intake of calcium from prey) of fishes. Nevertheless, the approach outlined in Chapter 3 of this thesis yields not only baselines for select physiological parameters for juvenile walleye pollock, but also provides a starting point for future work with other marine species and populations.

In future studies, it will be important to assess other populations of walleye pollock, e.g., the Bering Sea population, to ensure that the patterns and resilience observed in walleye pollock from Puget Sound analyzed in this study apply to the species as a whole. Several studies have now measured various physiological and behavioral responses of marine teleosts to ocean acidification (e.g., Hayashi et al. 2004, Dixson et al. 2010, Munday et al. 2009b), but few researchers have assessed similar parameters of condition and blood gas indices in addition to the response of temperate or subpolar species to ocean acidification during different developmental stages. Using an experimental CO_2 injection and rearing system, like the one outlined in this thesis to simulate projected pH changes as a result of ocean acidification, provides the means to measure the response of commercially important fish species, such as walleye pollock, to this particular ecosystem change. The long-term effects of ocean acidification on adult walleye pollock were beyond the scope of this study and it remains unknown if they will be able to spawn successfully or if effects from this pH stressor will carry over to the next generation. Future studies should address these important questions to fully understand population-level effects of ocean acidification on ecologically and commercially important species.

Chapter 5. References

Adams, C. F., A. I. Pinchuk, K. O. Coyle (2007) Seasonal changes in the diet composition and prey selection of walleye pollock (*Theragra chalcogramma*) in the northern Gulf of Alaska. *Fish. Res.* 84(3): 378-389.

Alaska Fisheries Science Center (2013). Bering Sea and Aleutian Islands management area pollock seasons, 1991-2011. NOAA Fisheries, National Marine Fisheries Service Alaska Regional Office publication. Accessed 31 December 2013. http://alaskafisheries.noaa.gov/sustainablefisheries/plckseas.pdf.

Bailey, K. M. (1989) Interaction between the vertical distribution of juvenile walleye pollock *Theragra chalcogramma* in the eastern Bering Sea, and cannibalism. *Mar. Ecol. Prog. Ser.* 53: 205-213.

Bailey, K. M., T. I. Quinn, P. Betzen, W. S. Grant (1999) Population structure and dynamics of walleye pollock, *Theragra chalcogramma*. *Adv. Mar. Biol.* 37: 179-255.

Bailey, K. M. and C. L. Stehr (1986) Laboratory studies on the early life history of the walleye pollock, *Theragra chalcogramma* (Pallas). *J. Exp. Mar. Biol. Ecol.* 99: 233-246.

Bailey, K. M., P. J. Stabeno, D. A. Powers (1997) The role of larval retention and transport features in mortality and potential gene flow of walleye pollock. *J. Fish. Biol.* 51(Supplement A): 135-154.

Ballantyne A. P., C. B. Alden, J. B. Miller, P. P. Trans, J. W. C. White (2012) Increase in observed net carbon dioxide uptake by land and oceans during the past 50 years. *Nature* 488: 70-72.

Barbeaux, S., J. Ianelli, S. Gaichas, M. Wilkins (2010) Assessment of the pollock stock in the Aleutian Islands. Aleutian Island Pollock Stock Assessment 2010. National Marine Fisheries Service Alaska Fisheries Science Center. pp. 157-234.

Barton, B. A. (2000) Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *Nor. Amer. J. Aquat.* 62(1): 12-18.

Barton, B. A., C. B. Schreck, L. D. Barton (1987) Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Dis. Aquat. Org.* 2: 173-185.

Bates, N. R. and J. T. Mathis (2009) The Arctic Ocean marine carbon cycle: evaluation of air-sea CO_2 exchanges, ocean acidification impacts and potential feedbacks. *Biogeosci.* 6: 2433-2459.

Bates, N. R., J. T. Mathis, L. Cooper (2009) Ocean acidification and biologically induced seasonality of carbonate mineral saturation states in the western Arctic Ocean. *J. Geophys. Res.* 114: C11007.

Blackwell, B. G., M. L. Brown, D. W. Willis (2000) Relative weight (Wr) status and current use in fisheries assessment and management. *Rev. Fish. Sci.* 8(1): 1-44.

Brewer, P. G. (1997) Ocean chemistry of the fossil fuel CO_2 signal: the haline signal of "business as usual". *Geophys. Res. Lett.* 24(11): 1367-1369.

Brodeur, R. D., M. S. Busby, M. T. Wilson (1995) Summer distribution of early life stages of walleye pollock, *Theragra chalcogramma*, and associated species in the western Gulf of Alaska. *Fish. Bull.* 93: 603-618.

Brodeur, R. D. and M. Wilson (1996) A review of the distribution, ecology and population dynamics of age-0 walleye pollock in the Gulf of Alaska. *Fish. Oceanogr.* 5(Suppl. 1): 148-166.

Brooks, S., C. R. Tyler, J. P. Sumpter (1997) Egg quality in fish: what makes a good egg? *Rev. Fish Biol. Fish.* 7: 387-416.

Brown, D. J. A. and K. Sadler (1989) Fish survival in acid waters. In: *Acid Toxicity and Aquatic Animals.* R. Morris, E. W. Taylor, D. J. A. Brown, and J. A. Brown, eds. Cambridge University Press: Cambridge, UK. pp. 31-44.

Caldeira K. and M. E. Wickett (2003) Anthropogenic carbon and ocean pH. *Nature* 425: 365.

Caldeira K. and M. E. Wickett (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J. Geophys. Res.* 110: C09S04.

Chellappa, S., F. A. Huntingford, R. H. C. Strang, R. Y. Thomson (1995) Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *J. Fish Biol.* 47: 775-787.

Chin, A., P. M. Kyne, T. I Walker, R. B McAuley (2010) An integrated risk assessment for climate change: analysing the vulnerability of sharks and rays on Australia's Great Barrier Reef. *Glob. Change Biol.* 16: 1936-1953.

Ciannelli, L., R. D. Brodeur, J. M. Napp (2004) Foraging impact on zooplankton by age-0 walleye pollock (*Theragra chalcogramma*) around a front in the southeast Bering Sea. *Mar. Biol.* 144: 515-526.

Cooney, R. T. (1981) Bering Sea zooplankton and micronekton communities with emphasis on annual production. In: *The Eastern Bering Sea Shelf: Oceanography and Resources.* D. W. Hood and J. A. Calder, eds. Vol. 2. (pp. 947-974). Seattle, WA: Office of Marine Pollution Assessment, NOAA University of Washington Press. 1339 pp..

Cushing, D. H. (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv. Mar. Biol.* 26: 249-293.

Dajuan, Z., L. Shaojing, W. Guizhong, G. Donghui (2011) Impacts of CO_2-driven seawater acidification on survival, egg production rate and hatching success of four copepods. *Acta Oceanol. Sin.* 30(6): 86-94.

Dixson, D. L., P. L. Munday, G. P. Jones (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* 13: 68-75.

Doney, S. C. (2006) The dangers of ocean acidification. *Sci. Am.* 294: 58-65.

Doney, S. C., V. J. Fabry, R. A. Feely, J. A. Kleypas (2009) Ocean acidification: the other CO_2 problem. *Annu. Rev. Mar. Sci.* 1(1): 169-192.

Dorn, M., K. Aydin, S. Barbeaux, M. Guttormsen, K. Spalinger, M. Wilkins (2010) Assessment of the walleye pollock stock in the Gulf of Alaska. Gulf of Alaska Walleye Pollock. National Marine Fisheries Service Alaska Fisheries Science Center. 104 pp.

Dougherty, A. B., K. M. Bailey, K. L. Mier (2007) Interannual differences in growth and hatch date distributions of age-0 year walleye pollock *Theragra chalcogramma* (Pallas) sampled from the Shumagin Islands region of the Gulf of Alaska, 1985-2001. *J. Fish Biol.* 71(3): 763-780.

Dupont, S., J. Havenhand, W. Thorndyke, L. Peck, M. Thorndyke (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser.* 373: 285-294.

Dwyer, D. A., K. M. Bailey, P. A. Livingston (1987) Feeding habits and daily ration of walleye pollock (*Theragra chalcogramma*) in the Eastern Bering Sea, with special reference to cannibalism. *Can. J. Fish. Aquat. Sci.* 44(11): 1972-1984.

Esbaugh, A. J., R. Heuer, M. Grosel (2012) Impacts of ocean acidification on respiratory gas exchange and acid-base balance in a marine teleost, *Opsanus beta*. *J. Comp. Physiol. B* 182: 921-934.

Fabry, V. J., J. B. McClintock, J. T. Mathis, J. M. Grebmeier (2009) Ocean acidification at high latitudes: the bellwether. *Oceanogr.* 22(4): 160-171.

Fabry, V. J., B. A. Siebel, R. A. Feely, J. C. Orr (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* 65: 414-432.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs, C. Maloy (2010) The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuar. Coast. Shelf Sci.* 88: 442-449.

Feely, R. A., S. C. Doney, S. R. Cooley (2009) Ocean acidification: Present conditions and future changes in a high CO_2 world. *Oceanogr.* 21(4): 36-47.

Feely, R. A., C. Sabine, J. M. Hernandez-Ayon, D. Ianson, B. Hales (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science* 320(5882): 1490-1492.

Feely, R. A., C. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, F. Millero (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* 305: 362-366.

Ferrari, M. C. O, R. P. Manassa, D. L. Dixson, P. L. Munday, M. I. McCormick, M. G. Meekan, A. Sih, D. P. Chivers (2012) Effects of ocean acidification on learning in coral reef fishes. *PLoS ONE* 7(2): e31478.

Fissel, B., M. Dalton, R. Felthove, B. Garber-Yonts, A. Haynie, A. Himes-Cornell, S. Kasperski, J. Lee, D. Lew, L. Pfeiffer, J. Sepez, C. Seung (2012) Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/Aleutian Islands area: Economic status of the groundfish fisheries off Alaska, 2011. NOAA Alaska Fisheries Science Center, Resource Ecology and Fisheries Management Division, Economic and Social Sciences Research Program: Seattle, WA. 309 pp.

Foo, S. A., S. A. Dworjanyn, S. G. B. Poore, M. Byrne (2012) Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification performance of early embryos. *PLoS ONE* 7(8): e42497.

Franke, A. and C. Clemmesen (2011) Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). *Biogeosci.* 8: 3697-3707.

Frommel, A. Y., R. Maneja, D. Lowe, A. M. Malzahan, A. J. Geffen, A. Folkvord, U. Piatkowski, T. B. H. Reusch, C. Clemmesen (2011) Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nature Clim. Change* 2: 42-46.

Gildberg, A. (2004) Digestive enzyme activities in starved pre-slaughter farmed and wild-captured Atlantic cod (*Gadus morhua*). *Aquacult.* 238: 343-353.

Grant, S. M., J. A. Brown, D. L. Boyce (1998) Enlarged fatty livers of small juvenile cod: a comparison of laboratory-cultured and wild juveniles. *J. Fish Biol.* 52: 1105-1114.

Grover, J. J. (1991) Trophic relationship of age-0 walleye pollock (*Theragra chalcogramma*) collected together in the eastern Bering Sea. *Fish. Bull.* 89: 719-722.

Hare, C. E., K. Leblanc, G. R. DiTullio, R. M. Kudela, Y. Zhang, P. A. Lee, S. Riseman, D. A. Hutchins (2007) Consequences of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. *Mar. Ecol. Prog. Ser.* 352: 9-16.

Hartline, B. K. (1980) Coastal upwelling: physical factors feed fish. *Science* 208: 38-40.

Haugan, P. M. and H. Drange (1996) Effects of CO_2 on the ocean environment. *Energ. Convers. Manage.* 37: 1019-1022.

Hayashi, M., J. Kita, A. Ishimatsu (2004a) Acid-base responses to lethal aquatic hypercapnia in three marine fishes. *Mar. Biol.* 144: 153-160.

Hayashi, M., J. Kita, A. Ishimatsu (2004b) Comparison of the acid-base responses to CO_2 and acidification in Japanese flounder (*Paralichthys olivaceus*). *Mar. Poll. Bull.* 49: 1062-1065.

Hjort, J. (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. *J. Cons. Int. Explor. Mer.* 20: 1-228.

Hopkinson, B. M., Y. Xu, D. Shi, P. J. McGinn, F. M. M. Morel (2010) The effect of CO_2 on the photosynthetic physiology of phytoplankton in the Gulf of Alaska. *Limnol. Oceanogr.* 55(5): 2011-2024.

Houde, E. D. (1997) Patterns and trends in larval-stage growth and mortality of teleost fish. *J. Fish Biol.* 51(Supplement A): 52-83.

Hunt, G. L., K. O. Coyle, L. B. Eisner, E. V. Farley, R. A. Heintz, F. Mueter, J. M. Napp, J. E. Overland, P. H. Ressler, S. Salo, P. J. Stabeno (2011) Climate impacts on

eastern Bering Sea foodwebs: a synthesis of new data and an assessment of the Oscillating Control Hypothesis. *ICES J. Mar. Sci.* 68(6): 1230-1243.

Hunt, G. L., P. Stabeno, G. Walters, E. Sinclair, R. D. Brodeur, J. M. Napp, N. A. Bond (2002) Climate change and control of the southeastern Bering Sea pelagic ecosystem. *Deep Sea Res. II* 49(26): 5821-5853.

Hurst, T. P. (2004) Temperature and state-dependence of feeding and gastric evacuation in juvenile Pacific halibut. *J. Fish Biol.* 65: 157-169.

Hurst, T. P., E. R. Fernandez, J. T. Mathis (2013) Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). *ICES J. Mar. Sci.*70(4): 812-822.

Hurst, T. P., E. R. Fernandez, J. T. Mathis, J. A. Miller, C. M. Stinson, E. F. Ahgeak (2012) Resiliency of juvenile walleye pollock to projected levels of ocean acidification. *Aquat. Biol.* 17(3): 247-259.

Ianelli, J. N., S. Barbeaux, T. Honkalehto, S. Kotwicki, K. Aydin, N. Williamson (2010) Assessment of the walleye pollock in the Eastern Bering Sea. EBS Walleye Pollock Stock Assessment 2010. National Marine Fisheries Service Alaska Fisheries Science Center. 104 pp.

Ianelli, J. N., T. Honkalehto, S. Barbeaux, S. Kotwicki, K. Aydin, N. Williamson (2012) Assessment of the walleye pollock stock in the Eastern Bering Sea. EBS Walleye Pollock Stock Assessment 2012. National Marine Fisheries Service Alaska Fisheries Science Center. 104 pp.

Intergovernmental Panel on Climate Change (IPCC) (2007) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Avery, M. Tignor, and H.L. Miller, eds. Cambridge University Press, Cambridge: United Kingdom and New York, NY. 996 pp.

Ishimatsu, A. and A. Dissanayake (2010) Life threatened in acidic coastal waters. In: *Coastal Environmental and Ecosystem Issues of the East China Sea.* (pp. 283-303)

A. Ishimatsu and H.-J. Lie, eds. Tokyo, Japan: TERRAPUB, Nagasaki University. pp. 306.

Ishimatsu, A., M. Hayashi, T. Kikkawa (2008) Fishes in high-CO_2, acidified oceans. *Mar. Ecol. Prog. Ser.* 373: 295-302.

Ishimatsu, A., T. Kikkawa, M. Hayashi, K. S. Lee, J. Kita (2004) Effects of CO_2 on marine fish: larvae and adults. *J. Oceanogr.* 60: 731-741.

Jobling, M. (1988) A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquacult.* 70(1-2): 1-19.

Johansson, N. and J. E. Kihlström (1975) Pikes (*Esox lucius* L.) shown to be affected by low pH values during first weeks after hatching. *Environ. Res.* 9: 12-17.

Kamler, E. (2002) Ontogeny of yolk-feeding fish: an ecological perspective. *Rev. Fish Biol. Fish.* 12: 79-103.

Katoh, F., A. Shimizu, K. Uchida, T. Kaneko (2000) Shift of chloride cell distribution during early life stages in seawater-adapted killifish, *Fundulus heteroclitus*. *Zool. Sci.* 17: 11-18.

Kendall, A. W., L. S. Incze, P. B. Ortner, S. Cummings, P. K. Brown (1994) Vertical distribution of eggs and larvae of walleye pollock, *Theragra chalcogramma*, in Shelikof Strait, Gulf of Alaska. *Fish. Bull., U.S.* 92: 540-554.

Kikkawa, T., A. Ishimatsu, J. Kita (2003) Acute CO_2 tolerance during the early developmental stages of four marine teleosts. *Environ. Toxicol.* 18(6): 375-382.

Kikkawa, T., J. Kita, A. Ishimatsu (2004) Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages. *Mar. Poll. Bull.* 48: 108-110.

Kleypas, J. A., R. A. Feely, V. J. Fabry, C. Langdon, C. L. Sabine, L. L. Robbins (2006) Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide

for future research. Report of a workshop held 18-20 April 2005, St. Petersburg, FL. pp. 1-96.

Kurihara, H. (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* 373: 275-284.

Kurihara, H. and A. Ishimatsu (2008) Effects of high CO_2 seawater on the copepod (*Acartia tsuensis*) through all life stages and subsequent generations. *Mar. Poll. Bull.* 56: 1086-1090.

Kurihara, H., S. Simode, Y. Shirayama (2004) Effects of raised CO_2 concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). *Mar. Poll. Bull*. 49(9-10): 721-727.

Lambert, Y. and J.-D. Dutil (1997) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? *Can. J. Fish. Aquat. Sci.* 54(Suppl. 1): 104-112.

Landes, A. and M. Zimmer (2012) Acidification and warming affect both a calcifying predator and prey, but not their interaction. *Mar. Ecol. Prog. Ser.* 450: 1-10.

Larsen, B. K. and H. O. Pörtner (1997) Extra- and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*). *Mar. Biol.* 128: 337-346.

Laurel, B. and D. M. Blood (2011) The effects of temperature on hatching and survival of northern rock sole larvae (*Lepidopsetta polyxystra*). *Fish. Bull.* 109(3): 282-291.

Laurel, B., L. A. Copeman, T. P. Hurst, C. Parrish (2010) The ecological significance of lipid/fatty acid synthesis in developing eggs and newly hatched larvae of Pacific cod (*Gadus macrocephalus*). *Mar. Biol.* 157 (8): 1713-1724.

Lekva, A., A.-C. Hansen, G. Rosenlund, Ø. Karlsen, G.-I. Hemre (2010) Energy dilution with α-cellulose in diets for Atlantic cod (*Gadus morhua* L.) juveniles: Effects on growth, feed intake, liver size, and digestibility of nutrients. *Aquaculture* 300: 169-175.

Lewis, E. and D. Wallace (1995) Program development for CO_2 system calculations. Carbon Dioxide Information Analysis Center: Oak Ridge, TN.

Lloyd, R. and W. R. White (1967) Effect of high concentration of carbon dioxide on ionic composition of rainbow trout blood. *Nature* 216: 1341-1342.

Lupica, S. J. and J. W. Turner (2009) Validation of enzyme-linked immunosorbent assay for measurement of faecal cortisol in fish. *Aquacult. Res.* 40(4): 437-441.

McNeil, B. I. and R. J. Matear (2006) Projected climate change impact on ocean acidification. *Carbon Balance Manage.* 1: 2.

Mathis, J. T., J. N. Cross, N. R. Bates, S. B. Moran, M. W. Lomas, C. W. Mordy, P. J. Stabeno (2010) Seasonal distribution of dissolved inorganic carbon and net community production on the Bering Sea shelf. *Biogeosci.* 7: 1769-1787.

Mazeaud, M. M., F. Mazeaud, E. M. Donaldson (1977) Primary and secondary effects of stress in fish- some new data with a general review. *Trans. Amer. Fish. Soc.* 106: 201-212.

Melzner, F., S. Göbel, M. Langenbuch, M. A. Gutowska, H. O. Pörtner, M. Lucassen (2009b) Swimming performance in Atlantic cod (*Gadus morhua*) following long-term (4-12 months) acclimation to elevated seawater pCO_2. *Aquat. Tox.* 92: 30-37.

Melzner, F., M. A. Gutowska, M. Langenbuch, S. Dupont, M. Lucassen, M. C. Thorndyke, M. Bleich, H. O. Pörtner (2009a) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosci.* 6: 2313-2331.Miles, H., S. Widdicombe, J. I. Spicer, J. Hall-Spencer (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar. Poll. Bull.* 54: 89-96.

Mueter, F. J., C. Ladd, M. C. Palmer, B. L. Norcross (2006) Bottom-up and top-down controls of walleye pollock (*Theragra chalcogramma*) on the Eastern Bering Sea shelf. *Prog. Oceanogr.* 68: 152-183.

Munday, P. L., N. E. Crawley, G. E. Nilsson (2009a) Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral fishes. *Mar. Ecol. Prog. Ser.* 388: 235-242.

Munday, P. L., J. M. Donelson, D. L. Dixson, G. G. K. Endo (2009b) Effects of ocean acidification on the early life history of a tropical marine fish. *Proc. Roy. Soc. B.* 276: 3275-3283.

Munday, P. L., M. Gagliano, J. M. Donelson, D. L. Dixson, S. R. Thorrold (2011) Ocean acidification does not affect the early life history development of a tropical marine fish. *Mar. Ecol. Prog. Ser.* 423: 211–221.

Nacci, D. E., D. Champlin, S. Jayaraman (2010) Adaptation of the estuarine fish *Fundulus heteroclitus* (Atlantic killifish) to polychlorinated biphenyls (PCB's). *Estuar. Coasts* 33: 853-864.

Olla, B. L. and M. W. Davis (1990) Effects of physical factors on the vertical distribution of larval walleye pollock *Theragra chalcogramma* under controlled laboratory conditions. *Mar. Ecol. Prog. Ser.* 63(2): 105-112.

Olla, B. L., M. W. Davis, C. H. Ryer, S. M. Sogard (1996) Behavioural determinants of distribution and survival in early stages of walleye pollock, *Theragra chalcogramma*: a synthesis of experimental studies. *Fish. Oceanogr.* 5(Suppl. 1): 167-178.

Olla, B. L., M. W. Davis, C. B. Schreck (1997) Effects of simulated trawling on sablefish and walleye pollock: The role of light intensity, net velocity and towing duration. *J. Fish. Biol.* 50: 1181-1194.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindeay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. Najjar, G. Plattner, K. Rodgers, C. Sabine, J. Sarmiento, R. Schlitzer, R. Slater, I. Totterdell, M. Weirig, Y. Yamanaka, A. Yool (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437: 681-686.

Pane, E. F. and J. P. Barry (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Mar. Ecol. Prog. Ser.* 334: 1-9.

Pankhurst, N. W. and P. L. Munday (2011) Effects of climate change on fish reproduction and early life history stages. *Mar. Fresh. Res.* 62: 1015-1026.

Patiño, R., C. S. Bradford, C. B. Schreck (1986) Adenylate cyclase activators and inhibitors, cyclic nucleotide analogs, and phosphatidylinositol: effects on interrenal function of coho salmon (*Oncorhyncus kisutch*). *Gen. Comp. Endocrin.* 63: 230-235.

Patiño, R. and C. B. Schreck (1988) Spontaneous and ACTH-induced interrenal steroidogenesis in juvenile coho salmon (*Oncorhyncus kisutch*): Effects of monovalent ions and osmolality in vitro. *Gen. Comp. Endocrin.* 69: 416-423.

Pelejero, C. (2005) Preindustrial to modern interdecadal variability in coral reef pH. *Science* 209: 2204-2207.

Perry, S. F. (1997) The chloride cell: structure and function in the gills of freshwater fishes. *Ann. Rev. Physiol.* 59(1): 325-347.

Perry, S.F. and K. M. Gilmour (2002) Sensing and transfer of respiratory gases at the fish gill. *J. Exp. Zool.* 293(3): 249-263.

Peterson, B. C. and N. J. Booth (2010) Validation of whole-body cortisol extraction procedure for channel catfish (*Ictalurus punctatus*) fry. *Fish Physiol. Biochem.* 36(3): 661-665.

Peterson, R. H., P. G. Daye, J. L. Metcalfe (1980) Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. *Can. J. Fish. Aquat. Sci.* 37: 770-774.

Petit, J. R., J. Jouzel, D. Raynaud, N. I. Barkov, J.-M. Barnola, I. Basile, M. Bender, J. Chappellaz, M. Davis, G. Delaygue, M. Delmotte, V. M. Kotlyakov, M. Legrand, V. Y. Lipenkov, C. Lorius, L. Pepin, C. Ritz, E. Saltzman, and M. Stievenard (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399: 429-436.

Pickering, A. D., T. G. Pottinger, J. P. Sumpter, J. F. Carragher, P. Y. Le Bail (1991) Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, *Oncorhynchus mykiss*. *Gen. Comp. Endocrin.* 83: 86-93.

Pörtner, H. O. (2008) Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Mar. Ecol. Prog. Ser.* 373: 203-217.

Pörtner, H. O. and A. P. Farrell (2008) Physiology and climate change. *Science* 322(5902): 690-692.

Pörtner, H. O., M. Langenbuch, A. Reipschläger (2004) Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history. *J. Oceanogr.* 60: 705-718.

Prentice, I., G. D. Farquhar, M. J. R. Fasham, M. L. Goulden, M. Heimann, V. J. Jaramillo, H. S. Kheshgi, C. Le Quéré, R. J. Scholes, D. W. R. Wallace, D. Archer, M. R. Ashmore, O. Aumont, D. Baker, M. Battle, M. Bender, L. P. Bopp, P. Bousquet, K. Caldeira, P. Ciais, P. M. Cox, W. Cramer, F. Dentener, I. G. Enting, C. B. Field, P. Friedlingstein, E. A. Holland, R. A. Houghton, J. I. House, A. Ishida, A. K. Jain, I. A. Janssens, F. Joos, T. Kaminski, C. D. Keeling, R. F. Keeling, D. W. Kicklighter, K. E. Kohfeld, W. Knorr, R. Law, T. Lenton, K. Lindsay, E. Maier-Reimer, A. C. Manning, R. J. Matear, A. D. McGuire, J. M. Melillo, R. Meyer, M. Mund, J. C. Orr, S. Piper, K. Plattner, P. J. Rayner, S. Sitch, R. Slater, S. Taguchi, P. P. Tans, H. Q. Tian, M. F. Weirig, T. Whorf, A. Yool (2001) The carbon cycle and atmospheric carbon dioxide. In: *Climate Change 2001: The Scientific Basis, Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell, and C. A. Johnson, eds. Cambridge University Press: New York, NY. 881 pp.

Randall, D. J. and B. Brauner (1998) Interactions between ion and gas transfer in freshwater teleost fish. *Comp. Biochem. Physiol. A: Mol. Integrat. Physiol.* 119(1): 3-8.

Randall, D. J. and C. Daxboeck (1984) Oxygen and carbon dioxide transfer across fish gills. In: *Fish Physiology.* Vol. 10A. W. S. Hoar and D. A. Randall, eds. Academic Press: New York, NY. 456 pp.

Rask, M. (1983) The effect of low pH on perch, *Perca fluvuatilis* L. I. Effect of low pH on the development of eggs of perch. *Ann. Zool. Fenn.* 20: 73-76.

Robinson, W. F. and C. R. R. Huxtable (1988) *Clinicopathologic Principles for Veterinary Medicine*. New York, NY: Cambridge University Press, 440 pp.

Rønnestad, I., W. Koven, A. Tandler, M. Harel, H. J. Fyhn (1998) Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). *Aquacult*. 162: 157-170.

Rooper, C. N., L. J. Haldorson, T. J. Quinn III (1999) Habitat factors controlling Pacific herring (*Clupea pallasi*) egg loss in Prince William Sound, Alaska. *Can. J. Fish. Aquat. Sci.* 56: 1133-1142.

Sabine, C. L., R. A. Feely, N. Gruber, R. M. Key, K. Lee, J. L. Bullister, R. Wanninkhof, C. S. Wong, D. W. R. Wallace, B. Tilbrook, F. J. Millero, T.-H. Peng, A. Kozyr, T. Ono, A. F. Rios (2004) The oceanic sink for anthropogenic CO_2. *Science* 305: 367-371.

Sarmiento, J. L. and N. Gruber (2006) *Ocean Biogeochemical Dynamics*. Princeton University Press: Princeton, NJ. 503 pp.

Schreck, C. B. (2000) Acclimation and long-term effects of stress in fish. In: *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. G. Moberg and J. Mench, eds. CABI Publishing: New York, NY.

Schreck, C. B. (1981) Stress and compensation in teleostean fishes: Response to social and physical factors. In: *Stress and Fish.* A. D. Pickering, ed. Academic Press: London. pp. 295-321.

Schreck, C. B., C. S. Bradford, M. S. Fitzpatrick, R. Patiño (1989) Regulation of the interrenal of fishes: non-classical control mechanisms. *Fish Physiol. Biochem.* 7: 259-265.

Seibel, B. A. and P. J. Walsh (2003) Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. *J. Exp. Biol.* 206: 641-650.

Shirashi, K., T. Kaneko, S. Hasegawa, T. Hirano (1997) Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater. *Cell Tissue Res.* 288: 583-590.

Simpson, S. D., P. L. Munday, M. L. Wittenrich, R. Manassa, D. L. Dixson, M. Gagliano, H. Y. Yan (2011) Ocean acidification erodes crucial auditory behavior in a marine fish. *Biol Lett.* 7: 917-920.

Sink, T. D., R. T. Lochmann, K. A. Fecteau (2008) Validation, use, and disadvantages of enzyme-linked immunosorbant assay kits for the detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiners. *Fish Physiol. Biochem.* 34: 95-101.

Springer, A. M. (1992) A review: walleye pollock in the North Pacific− how much difference do they really make? *Fish. Oceanogr.* 1(1): 80-96.

Stabeno, P. J., N. A. Bond, N. B. Kachel, S. A. Salo, J. D. Schumacher (2001) On the temporal variability of the physical environment over the south-eastern Bering Sea. *Fish. Oceanogr.* 10(1): 81-98.

Stabeno, P. J., N. B. Kachel, C. W. Mordy, D. Righi, S. Salo (2008) An examination of the physical variability around the Pribilof Islands in 2004. *Deep-Sea Res. II* 55: 1701-1716.

Stabeno, P. J., J. D. Schumacher, K. M. Bailey, R. D. Brodeur, E. D. Cokelet (1996) Observed patches of walleye pollock eggs and larvae in Shelikof Strait, Alaska: their characteristics, formation and persistence. *Fish. Oceanogr.* 5(Suppl. 1): 81-91.

Steinacher M., F. Joos, T. L. Frolicher, G. K. Plattner, S. C. Doney (2009) Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model. *Biogeosci.* 6: 515-533.

Sugimoto, T. and K. Tadokoro (1998) Interdecadal variations of plankton biomass and physical environment in the North Pacific. *Fish. Oceanogr.* 7(3-4): 289-299.

Tans, P. and R. Keeling (2013) Recent monthly mean CO_2 at Mauna Loa. Scripps Institute of Oceanography and National Oceanic and Atmospheric Administration/ERSL. Accessed 16 December 2013. http://www.esrl.noaa.gov/gmd/ccgg/trends/.

Tseng, Y.-C., M.Y. Hu, M. Stumpp, L.-Y. Lin, F. Melzner, P.-P. Hwang (2013) CO_2-driven seawater acidification differentially affects development and molecular plasticity along life history of fish (*Oryzias latipes*). *Comp. Biochem. Physiol. A* 165: 119-130.

Varsamos, S., J. P. Diaz, G. Charmantier, C. Blasco, R. Connes, G. Filk (2002) Location and morphology of chloride cells during the post-embryonic development of the European sea bass, *Dicentrarchus labrax*. *Anat. Embryol.* 205: 203-213.

Walline, P. D. (1985) Growth of larval walleye pollock related to domains within the SE Bering Sea. *Mar. Ecol. Prog. Ser.* 21: 197-203.

Wespestad, V. G., L. W. Fritz, W. J. Ingraham, B. A. Megrey (2000) On relationships between cannibalism, climate variability, physical transport, and recruitment success of Bering Sea walleye pollock (*Theragra chalcogramma*). *J. Mar. Sci.* 57: 272-278.

Whitehead, A. (2012) Comparative genomics in ecological physiology: toward a nuanced understanding of acclimation and adaptation. *J. Exp. Biol.* 215: 884-891.

Yamamoto, A., M. Kawamiya, A. Ishida, Y. Yamanaka, S. Watanabe (2012) Impact of rapid sea-ice reduction in the Arctic Ocean on the rate of ocean acidification. *Biogeosci.* 9: 2365-2375.

Yamamoto-Kawai, M., F. A. McLaughlin, E. C. Carmack, S. Nishino, K. Shimada (2009) Aragonite undersaturation in the Arctic Ocean: effects of ocean acidification and sea ice melt. *Science* 326: 1098-1100.

Appendix A. Components of walleye pollock gel food

Components of "gel food" fed to juvenile and broodstock walleye pollock (*Theragra chalcogramma*) held at the National Marine Fisheries Service laboratory in the Hatfield Marine Science Center in Newport, OR. All components were combined and blended until smooth. This "gel food" mixture was then frozen in Tupperware™ containers until needed. Before distributing as food, the frozen blocks were thawed slightly, then cut to a size that was appropriate for the size of fish to which it was being fed.

Amount	Ingredient
568 g	Herring
28.4 g	Freeze-dried krill
284 g	Squid
864 g	Commercial fish food (Otohime EP)
50 mL	"Phoenix" amino acid solution
2 capsules	Vitamin: TwinLab "Daily One Caps with Iron"
1600 mL	Water
800 mL	Powdered gelatin

Appendix B. UAF Institutional Care and Use Committee approval letter, 2009.
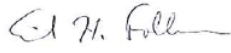
(907) 474-7800
(907) 474-5444 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

May 12, 2009

To: Jeremy Mathis, PhD
        Principal Investigator

From: Erich H. Follmann, PhD
        IACUC Chair

Re: IACUC Assurance Application

The University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) reviewed the following Assurance at their April 21, 2009, meeting. This Assurance was approved pending receipt of a revised assurance addressing the committee's questions. The assurance received on May 4, 2009 and the clarifications received on May 11, 2009 were determined to be satisfactory; therefore I am pleased to issue approval.

Protocol#:   09-25

Title: *The effects of ocean acidification on walleye Pollock physiological processes*

Received: April 15, 2009 (orig)
             May 11, 2009 (final revisions)

Approved: May 12, 2009

Review Due: May 12, 2010

The PI is responsible for acquiring and maintaining all required permits and permissions prior to beginning work on this assurance. Failure to obtain or maintain valid permits is considered a violation of an IACUC assurance, and could result in revocation of IACUC approval.

Appendix C. UAF Institutional Care and Use Committee continuation letter, 2010.

(907) 474-7800
(907) 474-5638 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

UNIVERSITY OF ALASKA
FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

May 28, 2010

To:	Jeremy Mathis
	Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[171620-1] The effects of ocean acidification on juvenile and larval walleye pollock (Theragra chalcogramma)

The IACUC has reviewed the Progress Report by Designated Member Review and the Protocol has been approved for an additional year.

Received:	May 15, 2010
Initial Approval Date:	May 12, 2009
Effective Date:	May 28, 2010
Expiration Date:	May 12, 2011

This action is included on the June 3, 2010 IACUC Agenda.

If you have any questions about how to submit the required information through IRBNet please contact the Office of Research Integrity for assistance (email fyori@uaf.edu or call x7800/x7832).