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Faculty of Biosciences, Fisheries and Economics
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The fate of a key Arctic copepod in future ocean acidification

Integrating molecular, organismal, and evolutionary thinking in the face of climate change

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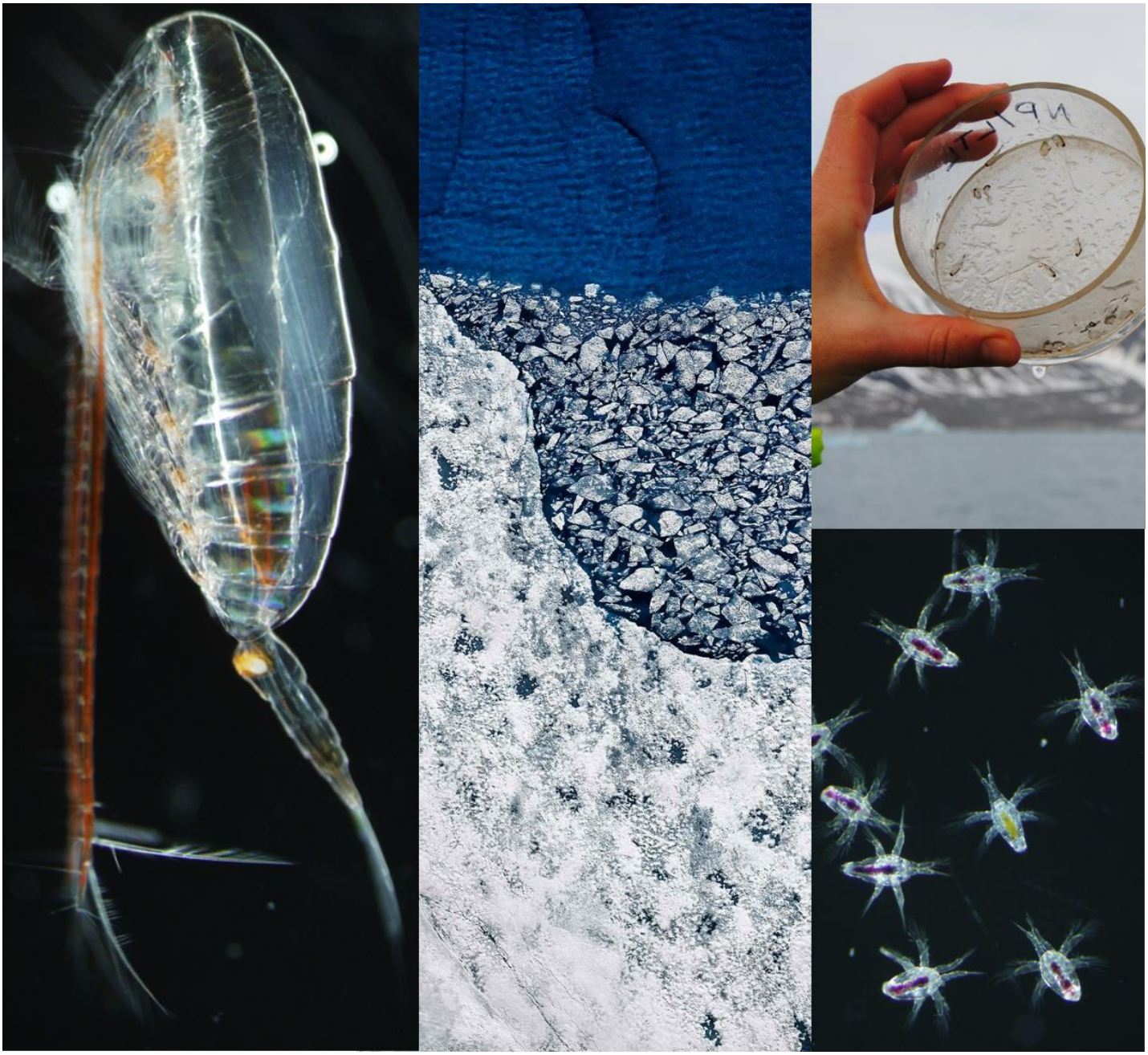
Allison Michelle Bailey

A dissertation for the degree of Philosophiae Doctor – March 2017



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Calanus glacialis females (left) and nauplii stage N4 (lower right) by Allison Bailey
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Upper right: Ella Guscelli

The fate of a key Arctic copepod in future ocean acidification:

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CONTENTS

SUMMARY

Uptake of anthropogenic carbon dioxide (CO_2) into the world's oceans is causing an increase in seawater acidity and decrease in pH. This alteration of seawater chemistry, called ocean acidification, is predicted to have harmful effects on a variety of marine organisms. With the greatest and fastest changes in pH expected to occur the Arctic seas over the next 100 years, the need for understanding the effects of low pH on Arctic marine organisms is pressing. This thesis examined the effects of projected levels of ocean acidification on the physiology of a key component of the Arctic marine ecosystem, the copepod *Calanus glacialis.* To best understand the response of this species to future environmental change, diverse molecular, organismal, and evolutionary methodologies were used to investigate the effects of low pH on *C. glacialis* throughout its lifespan (nearly all developmental stages, including young stages), in combination with other stressors (food availability) and across geographically distant sub-populations (to predict their capacity for adaptation or acclimation in the future).

Young stages of marine organisms are often the most vulnerable to environmental stressors, and are commonly predicted to be the bottle-necks in ecological responses to ocean acidification. The effects of low pH on the young, naupliar stages of *C. glacialis* were tested in a long-term exposure experiment, allowing for the parallel quantification of fitness-related, organismal-level traits (development, growth, and respiration rate) and gene expression responses. The results did not indicate a sensitivity of young stages to low pH; the nauplii developed successfully from egg to naupliar stage N6 at all four pH treatments investigated (pH 7.5, 7,7, 7.9 and 8.05), developing, respiring, and building biomass (dry weight, carbon and nitrogen mass) at the same rates in all treatments. The gene expression of stage N6 nauplii supported the organismal-level tolerance observed. The nauplii regulated a small portion of their gene expression in response to pH, and the genes that were regulated did not indicate an energetically costly response or stress response to low pH. Interestingly, gene expression patterns provided insight into the molecular basis of tolerance to low pH, showing a general down-regulation of stress-related genes, potentially a characteristic of stress tolerance.

While previously unstudied in the context of ocean acidification, significant effects of low pH were found in young copepodite stages of *C. glacialis* (C2-C4). An experiment investigating the interaction of food level and low pH revealed potentially important energetic costs of low pH in young copepodite stages (C2-C3), but not older stages (C5). While feeding and biosynthesis were similar at ambient and low pH (pH 8.0 and 7.6), the metabolic costs of feeding were 2.5× higher for young copepodites in low pH, potentially indicating increased costs of protein biosynthesis. While these results are based on short-term exposures, if these increased metabolic costs were to be sustained under future ocean acidification, they would significantly reduce the amount of energy these copepodites could use for growth. This, in turn, could potentially incur negative impacts on the population level. In another experiment, the response of *C. glacialis* copepodites to a wide range of low pH levels was found to vary both by developmental stage and by population. Again, the younger copepodites (C3 and C4) were more sensitive to low pH than older copepodites (C5s). In the most strongly affected stage, C4, ingestion decreased and respiration increased in response to low pH in two of the three populations investigated (Kongsfjorden and Billefjorden, on Svalbard), while there was no response in copepods from Disko Bay, Greenland. The energetic costs of the alterations in ingestion and respiration reduce the amount of energy these copepodites have for growth by 19-50 %, again with potential population-level effects if sustained in future ocean acidification. However, the population-specific responses indicate that *C. glacialis* may have the ability to adjust its tolerance to pH over time, to better survive in its local habitat. The natural seasonal variability of pH in Disko Bay is likely considerably higher than that in Kongsfjorden and Billefjorden, and could be driving local adaptation or acclimatization of that population towards the wider pH tolerance that was observed. If adaptation or acclimatization is possible in *C. glacialis*, they may be able to alleviate the detrimental effects seen in the young copepodite stages over time in future ocean acidification.

Results from this thesis and other studies indicate that the early copepodite stages (C2-C4) of *C. glacialis* are affected detrimentally by exposure to seawater pH predicted for Arctic seas in 2100 and 2300 (pH 7.7-7.5), while young stages (egg-C1) and older stages (C5s and females) appear to be more tolerant to realistic ocean acidification pH levels. Though effects on a few stages can be important, the results also indicate a potential to adapt or acclimatize to gain tolerance to pH levels

that are much lower than those predicted for future ocean acidification (down to pH 6.4). This suggests that the few negative effects of low pH that were observed could potentially be alleviated in the future. *C. glacialis* populations face a myriad of environmental changes driven by global warming and ocean acidification in the Arctic, and may experience declines due to the interactions of these multiple stressors, though likely not primarily due to ocean acidification.

LIST OF PAPERS

Paper I:

Allison Bailey, Peter Thor, Howard I. Browman, David M. Fields, Jeffrey Runge, Alexander Vermont, Reidun Bjelland, Cameron Thompson, Steven Shema, Caroline M. F. Durif, Haakon Hop (2016) Early life stages of the Arctic copepod *Calanus glacialis* are unaffected by increased seawater pCO_2. ICES Journal of Marine Science. doi:10.1093/icesjms/fsw066

Paper II:

Allison Bailey, Pierre de Wit, Peter Thor, Howard I. Browman, Reidun Bjelland, Steven Shema, David M. Fields, Jeffrey A. Runge, Cameron Thompson, Haakon Hop (In review) Regulation of gene expression underlies tolerance of the Arctic copepod *Calanus glacialis* to CO_2-acidified seawater. *Ecology and Evolution*

Paper III:

Peter Thor, Allison Bailey, Claudia Halsband, Ella Guscelli, Elena Gorokhova, Agneta Fransson (2016) Seawater pH predicted for the year 2100 affects the metabolic response to feeding in copepodites of the Arctic copepod *Calanus glacialis*. PLoS ONE 11(12):e0168735. doi:10.1371/journal.pone.0168735

Paper IV:

Peter Thor, Allison Bailey, Sam Dupont, Piero Calosi, Janne Søreide, Pierre De Wit, Ella Guscelli, Lea Loubet-Sartrou, Ida Deichmann, Martin Candee, Camilla Svensen, Andrew L. King, Richard G.J. Bellerby (In review) Potential for rescue from future ocean acidification by extant physiological differences among distinct Arctic copepod populations. *Global Change Biology*

INTRODUCTION

1 Climate change and ocean acidification

Since the Industrial Revolution 200 years ago, human activities have increased the levels of carbon dioxide (CO_2) in the atmosphere (IPCC, 2013). Having largely ranged from 172-300ppm over the past 800,000 years (Lüthi *et al.*, 2008), the annual mean global atmospheric CO_2 concentration surpassed 400 ppm in 2016, (Dlugokencky & Tans NOAA/ESRL), a rate of change unprecedented in the last 22,000 years (Masson-Delmotte *et al.*, 2013). Future CO_2 scenarios are largely dependent upon global political choices to reduce carbon emissions, and are modelled as Representative Concentration Pathways (RCPs), spanning from a strong reduction in CO_2 emissions (RCP2.6) to sustained high and increasing emissions, also called "business-as-usual" (RCP8.5; Riahi *et al.*, 2011). In the RCP8.5 projection, the average atmospheric CO_2 concentration is expected to become 936 ppm in 2100 and 1960 ppm by 2300, whereas RCP2.6 scenario shows a peak during the middle of the century, with 420 ppm in 2100 decreasing to 360 ppm by year 2300 (Figure 1; Cubasch *et al.*, 2013).

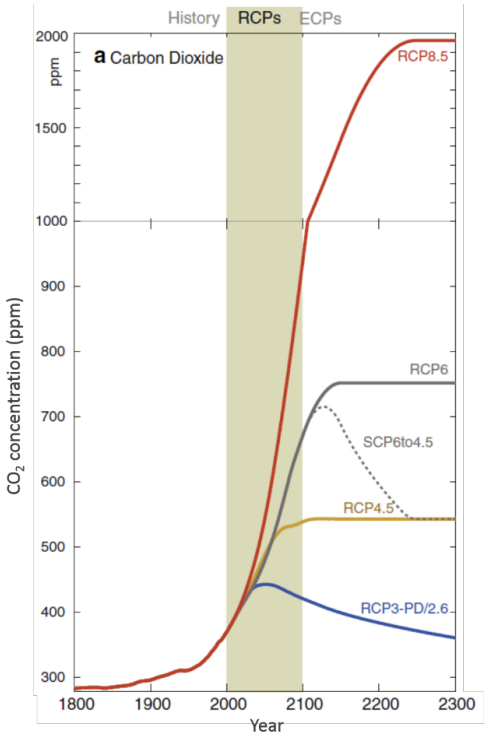

Figure 1. Historical and projected atmospheric CO_2 concentrations. For the period 2000-2100, the projected increase in CO_2 is based on representative concentration pathways (RCPs) while projections beyond 2100 are based on extended concentration pathways (ECPs; Collins *et al.*, 2013). Pathway names include the projected radiative forcing values for 2100 (in W m^{-2}), with higher values indicating more warming. Figure from IPCC 2013, Ch. 1, Box 1.1, Fig. 2a (Cubasch *et al.*, 2013). At 1 atm of pressure, atmospheric CO_2 concentration (in ppm) is roughly equivalent to the partial pressure of CO_2 (in µatm), and in this thesis and much of ocean acidification research, RCPs are expressed in terms of µatm CO_2.

As anthropogenic carbon enters the global carbon cycle, it is partitioned into naturally existing sinks. Though the majority (45 %) of the anthropogenic carbon remains in the atmosphere, the world's oceans absorb 26 % and the terrestrial biosphere absorbs 29 % (Le Quéré *et al.*, 2009). In the atmosphere, increased carbon dioxide concentrations are warming the Earth and changing the global climate (IPCC, 2013), with striking effects on terrestrial and marine ecosystems (Parmesan, 2006; Doney *et al.*, 2012). In the global oceans, increased CO_2 concentrations are significantly changing seawater carbonate chemistry, most markedly by decreasing the pH and carbonate ion (CO_3^{2-}) concentrations. This process is referred to as anthropogenic ocean acidification, or OA (Orr *et al.*, 2005; IPCC, 2013) and has been referred to as the "other CO_2 problem" (Doney *et al.*, 2009).

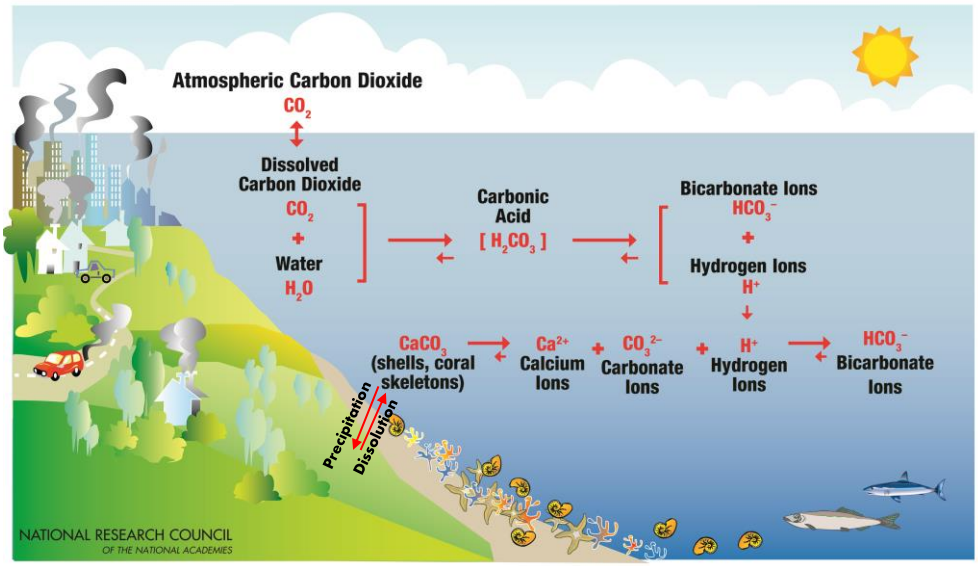

Figure 2. The simplified chemistry of ocean acidification. Figure reprinted with permission from the National Academy of Sciences, Engineering, and Medicine, courtesy of the National Academies Press, Washington, D.C. (National Resource Council, 2010). *https://www.nap.edu/catalog/12904/ocean-acidification-a-national-strategy-to-meet-the-challenges-of.* Arrows indicating the precipitation and dissolution of calcium carbonate from bedrock to the ocean are added by A. Bailey.

When CO_2 is dissolved in seawater it reacts with water molecules to form carbonic acid (H_2CO_3), which quickly dissociates and releases a hydrogen ion (H^+) and a bicarbonate ion (HCO_3^-; Figure 2). Some of the hydrogen ions combine with carbonate (CO_3^{2-}) ions to form additional bicarbonate ions. This results in a decrease in (CO_3^{2-}) and an increase in HCO_3. Since pH is measured as the negative logarithm of the H^+

concentration, increased H^+ concentrations reduces pH. In sum, ocean acidification decreases pH and carbonate ion (CO_3^{2-}) concentrations and increases hydrogen ion (H^+), bicarbonate ion (HCO_3^-), and dissolved inorganic carbon (DIC) concentrations (Caldeira & Wickett, 2003; Orr *et al.*, 2005). Since saturation state depends on the CO_3^{2-}, concentration, ocean acidification results in decreased saturation state (Ω) of calcium carbonate minerals such as aragonite and calcite (Gattuso & Hansson, 2011) which are fundamental components of the shells and exoskeletons of many calcifying marine organisms (Orr *et al.*, 2005). A saturation state < 1 causes net dissolution in exposed calcium carbonate structures (Feely *et al.*, 2004; Orr *et al.*, 2005).

Despite extensive exchange of CO_2 over the ocean-atmosphere interface, the partial pressure of CO_2 (pCO_2) in seawater is rarely equivalent to that of the atmosphere (Takahashi *et al.*, 2009; Riebesell *et al.*, 2010; McElhany & Busch, 2013). CO_2 is absorbed at the ocean surface based on atmospheric partial pressure, temperature, and wind speed and is transported via vertical mixing and ocean currents into deeper waters (DeVries *et al.*, 2017; Fletcher, 2017). However, many other factors modify seawater pCO_2 in addition to the equilibrium with atmospheric pCO_2. Respiration increases seawater CO_2 concentration, while photosynthesis decreases it. These processes can add seasonal variation in seawater pH of 0.05-0.45, largely due to the spring phytoplankton bloom (Rhein *et al.*, 2013; Shadwick *et al.*, 2013; Kapsenberg *et al.*, 2015). pCO_2 can also be high in deep or eutrophic waters, where respiration exceeds photosynthesis, and in deep conveyor belt currents that have accumulated decades of respiratory CO_2 without venting to the atmosphere (Broecker *et al.*, 1982; Broecker & Clark, 2003). The temporal variability of photosynthesis can also add a strong diurnal and seasonal signal to seawater pCO_2 (Kayanne *et al.*, 1995; Bates, 2001; Hofmann *et al.*, 2011). Finally, at some sites, hydrothermal vents and carbon dioxide seeps induce local, sharp increases in CO_2 concentration (Childress *et al.*, 1993; Hall-Spencer *et al.*, 2008).

A complex carbonate buffering system controls the relationship of pH to pCO_2 in seawater. Seawater with a given pCO_2 can have a range of different pHs, depending on the alkalinity and temperature of the seawater. Alkalinity, a cumulative measure of the many weak bases that are present in natural seawater, buffers a decrease in pH for a given increase in CO_2 (Riebesell *et al.*, 2010). Seawater with high alkalinity can better buffer CO_2, whereas fresher water with low alkalinity has a lower buffer capacity and a lower pH for the same pCO_2. Alkalinity varies naturally across in the world's oceans, and is related to the degree of freshwater mixing (fresher water has lower alkalinity) and weathering of carbonate-containing bedrock (Key *et al.*, 2004). Temperature also influences seawater pH, with higher temperatures leading to lower pHs than the same seawater at lower temperatures. Seawater pCO_2 and pH, therefore, vary both spatially and temporally.

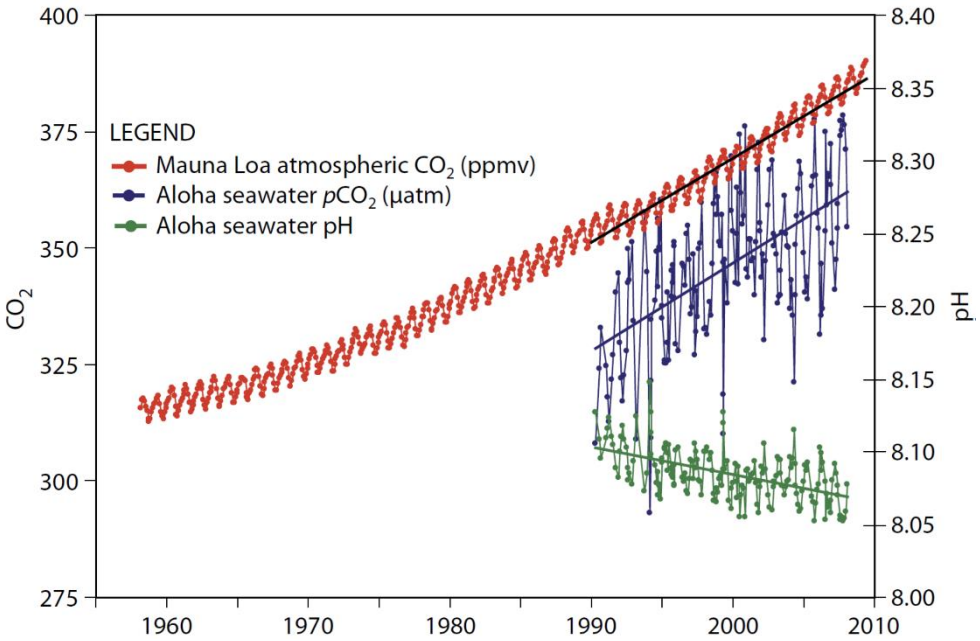

Figure 3. Concurrent trends in atmospheric CO_2 (ppmv), seawater pCO_2 (µatm) and seawater pH, in the Pacific (Hawaii). Adapted from Feely *et al.*, (2009; Fig. 1): Mauna Loa data: Pieter Tans, NOAA/ESRL, http://www.esrl.noaa.gov/gmd/ccgg/trends. HOT/ALOHA data: David Karl, University of Hawaii, http://hahana.soest.hawaii.edu.

Despite this large spatial and seasonal variability, seawater pCO_2 is increasing and pH is decreasing in concert with the rise in atmospheric CO_2 levels (Feely *et al.*, 2009; Rhein *et al.*, 2013; Figure 3). Since the beginning of the Industrial Revolution, the global average surface seawater pH has decreased by ~0.1 (from 8.18 to 8.09), roughly a 30 % increase in hydrogen ion concentration (Caldeira & Wickett,

2003; Doney *et al.*, 2009; Stocker *et al.*, 2013). Models forced with the RCP emissions scenarios project a further pH decrease of 0.31, 0.20, 0.15, or 0.07 by 2100 (for RCP8.5, RCP6.0, RCP4.5, and RCP2.6, respectively; Stocker *et al.*, 2013). Associated projections of global mean surface pH in 2100 range from 7.76 to 8.05, depending on the emission scenario (RCP8.5: 7.76, RCP6.0: 7.89, RCP4.5: 7.96, and RCP2.6: 8.05; Stocker *et al.*, 2013). If all fossil fuels are burned, a total potential decline of pH by 0.77 in surface waters is possible by 2300 (Caldeira & Wickett, 2003). These long-term trends are global mean projections for surface water, with true changes varying geographically (Figure 4).

The projected rates of change of seawater pH in the coming centuries are faster than any that have occurred in the last 300 million years (Caldeira & Wickett, 2003). Interestingly, it is this fast rate of change that is allowing the pH of the oceans to undergo such drastic changes; it outpaces the rate at which the carbonate buffering system (the geologic weathering of carbonate rich sediments) can moderate changes in pH (Caldeira & Wickett, 2003; Feely *et al.*, 2004; Widdicombe & Spicer, 2008). Thus, while atmospheric carbon levels have been higher in the past, the ocean pH change has not been as dramatic (Caldeira & Wickett, 2003). The longevity of anthropogenic CO_2 in the atmosphere and the magnitude of anthropogenic CO_2 absorbed by the oceans, combined with the slow rate of weathering of carbonate sediments, means that the low pH levels of ocean acidification are expected to persist in the world's oceans for thousands of years (Caldeira & Wickett, 2003).

1.1 Arctic ocean acidification

The Arctic is predicted to experience the first and fastest signs of climate change (IPCC, 2013), and in the last three decades it has warmed three times faster than the global mean (Comiso & Hall, 2014). Similarly, the largest changes in pH are predicted to occur in the Arctic over the coming centuries, in part due to its naturally cold and freshwater-influenced (low alkalinity) waters, and exacerbated by climate change (Steinacher *et al.*, 2009; Denman *et al.*, 2011; IPCC, 2013; Figure 4). The solubility of CO_2 (and other gases) is higher in cold water than in warm water, which allows the cold waters of the Arctic to absorb more CO_2 per volume than other seas (Bellerby *et al.*, 2013). Low-salinity and low alkalinity Arctic waters have lower capacity to buffer acids, and thus larger decreases in pH occur for a given change in

pCO_2 than in lower latitude seas (Steinacher *et al.*, 2009; Bellerby *et al.*, 2013). Additionally, climate change, is expected to accelerate ocean acidification in the Arctic, via multiple mechanisms (Steinacher *et al.*, 2009). Firstly, climate change has prompted an unprecedented loss of multi-year pack-ice in the Arctic, reducing the temporal and spatial extent of this barrier between the atmosphere and ocean, potentially allowing for greater net fluxes of CO_2 from the atmosphere into the ocean (Bellerby *et al.*, 2013, Bates *et al.*, 2006; Arrigo *et al.*, 2010; Parmentier *et al.*, 2013; Barber *et al.*, 2015). Secondly, increasing seawater temperatures in the future will also lower seawater pH. Thirdly, climate change is projected to increase the inflow of fresh meltwater into Arctic seas, both from melting sea ice, glacial melt, and increased river runoff from the large rivers that feed the Canadian and Russia shelves (Peterson *et al.*, 2002; Yamamoto-Kawai *et al.*, 2009; Denman *et al.*, 2011; Fransson *et al.*, 2016). This inflow will further lower the buffering capacity of the seawater, resulting in lower pHs and lower carbonate saturation states (Bates *et al.*, 2009; Chierici & Fransson, 2009; Azetsu-Scott *et al.*, 2010).

Observations from the last two decades supports the predictions that the Arctic will undergo faster rates of ocean acidification than the rest of the world (Ericson *et al.*, 2014; Qi *et al.*, 2017). Ericson *et al.* (2014) found significant trends in the anthropogenic carbon content of the central Arctic Ocean from 1991-2011, with rates of increases that were 160 % of that seen in the global ocean (Sabine *et al.*, 2004). Projected seawater pH in the Arctic is challenging to model, due to the uncertainties of the physical responses to climate change (i.e. changes in ice cover, primary production and mixing), but model projections under RCP8.5 forcing predict pH 7.7 for the Pacific Arctic in 2100 (Bellerby *et al.*, 2013; Deal *et al.*, 2014) and < 7.7 for the Arctic ($>$ 70 °N; IPCC, 2013; Figure 4a). Investigating ocean acidification in the Arctic Ocean and marginal seas is of utmost importance, yet relatively few studies have been conducted in high latitude seas.

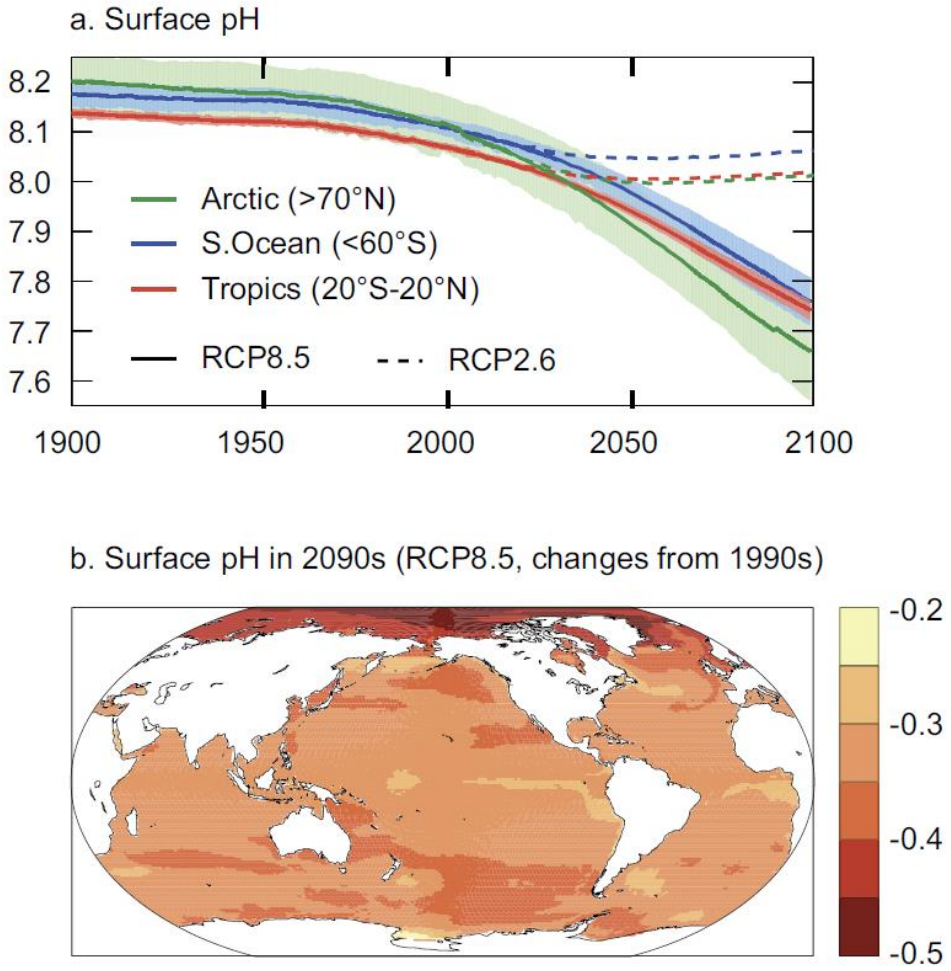

Figure 4: Projected ocean acidification under high CO_2 emissions, RCP8.5, showing the largest changes in the Arctic. (A) Mean surface pH (solid line) and range of 11 CMIP5 Earth System Models (shaded area), for the Arctic Ocean (green), the tropical oceans (red) and the Southern Ocean (blue). Dashed lines indicate pH predicted under RCP2.6. (B) Global map of the median model's change in surface pH from 1850 to 2100 under RCP8.5. From IPCC (2013), Ch. 6, Fig. 6.28 (Ciais *et al.,* 2013).

2 Implications of ocean acidification for marine organisms

Changes in seawater carbonate chemistry due to ocean acidification are predicted to present many marine organisms with considerable physiological challenges in the coming centuries (Feely *et al.*, 2004; Orr *et al.*, 2005; Doney *et al.*, 2009). While the degree to which these changes will affect organism fitness appear to vary by species, ocean acidification will cause a shift in the environmental conditions to which marine organisms have adapted for the past 20 million years (Turley *et al.*, 2006), indicating that it will likely elicit physiological changes in organisms (Stillman & Paganini, 2015).

2.1 Chemical mechanisms of physiological impact

While seemingly simple, ocean acidification is in fact a combination of multiple chemical changes that may have physiological implications for marine organisms. These changes include decreased calcium carbonate saturation state, increased H^+ concentration (and thus decreased pH), increased concentration of CO_2 , and altered chemical states of biologically important chemicals. While these parameters often covary, their relative changes can decouple depending on seawater alkalinity, salinity, and temperature. Further, each of these parameters can affect the physiology of marine organisms in distinct ways (Pörtner *et al.*, 2004; Stapp *et al.*, 2015; Waldbusser *et al.*, 2015a, 2015b). In light of this, ocean acidification has recently become considered a "multi-stressor" in and of itself (Waldbusser *et al.*, 2015a). Some of the better-understood chemical mechanisms of ocean acidification and their physiological impacts are discussed below.

Calcifying organisms are among those expected to be most vulnerable to ocean acidification, due to the reduction in calcium carbonate saturation state (Orr *et al.*, 2005; Wittmann & Pörtner, 2013). **Decreasing calcium carbonate saturation states** (Ω) are expected to make it more difficult for calcifiers to build their solid calcium carbonate structures, as dissolution is favoured over mineralization at saturation states < 1 (Fabry *et al.*, 2008; Cohen & Holcomb, 2009; Chan & Connolly, 2013). Utilizing ion pumps to actively concentrate

carbonate and reduce pH at the sites of calcification (Tortell, 2000; Ries *et al.*, 2009; Reinfelder, 2011; Toyofuku *et al.*, 2017) makes it energetically costly for some calcifying organisms to maintain their calcified structures under ocean acidification (Wood *et al.*, 2008; Findlay *et al.*, 2009; Ries *et al.*, 2009; Waldbusser *et al.*, 2015a, 2015b), though not in others, with disparate responses existing even within the same taxon (i.e. corals, McCulloch *et al.*, 2012). Energetic limitations cause in calcifiers either to reduce calcification, as was found in a coral meta-analysis by Chan and Connolly (2013) and in bivalves (Beniash *et al.*, 2010), or maintain it, but devote less energy to other processes such as growth (Wood *et al.*, 2008) or produce deformed calcified structures (Cohen & Holcomb, 2009), though some are able to calcify without extra energetic costs. While the effects of reduced calcium carbonate state on marine calcifiers was one of the first and most logical effects of ocean acidification investigated, recent studies have complicated the causal link between seawater carbonate concentrations and calcification rates (Findlay *et al.*, 2009; Ries *et al.*, 2009; Cyronak *et al.*, 2015, 2016; Waldbusser *et al.*, 2016), underlining the complexity of seawater carbonate chemistry changes, their physiological effects, and species-specific responses.

The adverse effects of ocean acidification on marine organism are, however, not restricted to calcifying organisms. All marine organisms, including non-calcifying species, are affected by the **decreased pH** associated with ocean acidification, varying only in their capacity to regulate it. The pH of internal fluids affects many physiological processes, including ion transport, enzyme activity and protein function, oxygen transport as well as photosynthesis (Pörtner, 1990; Casey *et al.*, 2010; Gattuso & Hansson, 2011; Whiteley, 2011; Wittmann & Pörtner, 2013). Maintaining a favourable extracellular (pH_e) and intracellular pH (pH_i) is thus vital to the optimal physiological functioning of an organism. Maintaining pH homeostasis in low pH seawater is accomplished through complex acid-base regulation, involving ion transporters, buffers, and circulation (Wheatly & Henry, 1992; Pörtner *et al.*, 2004; Whiteley, 2011). Ion pumps are energetically expensive (Pörtner *et al.*, 2000); if their use is up-regulated at low pH, the energetic cost of these pumps can comprise large portions of cellular energy budget (Seibel & Walsh, 2003; Pörtner, 2008, 2010; Melzner *et al.*, 2009; Stumpp *et al.*, 2011a; Whiteley, 2011; Pan *et al.*, 2015). With higher energetic costs of maintenance, less energy remains for growth, development, and reproduction (Wood *et al.*, 2008; Beniash

et al., 2010; Stumpp *et al.*, 2011a). Organisms can also buffer internal pH via internal buffers or the uptake of bicarbonate (Claiborne *et al.*, 2002; Pörtner *et al.*, 2004; Whiteley, 2011). If ion regulation and buffering are insufficient to maintain pH homeostasis, acidosis of internal fluids will ensue, with ramifications for a wide range of cellular functions (Pörtner *et al.*, 2004). Metabolic responses to acidosis can include increased metabolic rate, due to energetically costly compensation mechanisms (Wood *et al.*, 2008; Lannig *et al.*, 2010; Thomsen & Melzner, 2010), or metabolic depression, a possible short-term survival strategy in extreme stress (Reipschläger & Pörtner, 1996; Michaelidis *et al.*, 2005), with some studies revealing a metabolic rise in intermediate acidosis, and metabolic depression at extreme acidosis (Baker & Brauner, 2012). The regulation of internal acid-base balance (pH), and the associated energetic costs, are the most wide-reaching effects of ocean acidification on marine organisms.

Closely related to pH is the ocean acidification-related **increase in CO_2 concentration**. Biological membranes are highly permeable to CO_2; increases in seawater pCO_2 results in increased pCO_2 in internal fluids, a condition known as hypercapnia (Pörtner *et al.*, 2004). However, the consequences of increased CO_2 concentration differ for autotrophs and heterotrophs. While providing additional building blocks for photosynthesis in autotrophs, increased CO_2 can affect the ability of heterotrophs to rid themselves of the excess, metabolically-produced CO_2 in their tissues. As a by-product of aerobic respiration, this CO_2 is usually dissipated passively out of the body by utilizing a concentration gradient from higher internal CO_2 to lower external CO_2 (Hochachka & Somero, 2002; National Resource Council, 2010). Accordingly, multicellular marine heterotrophs often have pH_i that is ~0.4-0.6 pH lower than external seawater (Wheatly & Henry, 1992; Claiborne *et al.*, 2002). Increased CO_2 concentration in seawater can weaken this gradient and therefore make the dissipation of CO_2 less effective or the equilibration point occur at higher internal levels. This, in turn will affect an organism's internal acid-base homeostasis, with ramifications for a range of cellular functions associated with low pH_i (see above).

Finally, ocean acidification can affect marine organisms by altering the **state of important chemicals**. This includes changes to the charge and conformation of signalling chemicals used by marine organisms, affecting their interaction with chemosensory receptor proteins (Wyatt *et al.*, 2014; Roggatz *et al.*, 2016), with implications for the organism's

ability to detect predators (Dixson *et al.*, 2010), conspecifics and appropriate habitats (Lecchini *et al.*, 2016). Ocean acidification also affects the bioavailability of essential nutrients (nitrogen, phosphorus), and metals, both toxic (i.e. cadmium) and essential (i.e. iron) via changes in solubility and partial charge (Millero *et al.*, 2009; Shi *et al.*, 2010, 2016; Hardege *et al.*, 2011; Lewis *et al.*, 2016). Low pH effects on biologically important chemicals may affect a wide range of marine organisms, with effects documented in gastropod molluscs, polychaete worms, crustaceans, and fish (Wyatt *et al.*, 2014).

2.2 Impacts of ocean acidification

Experimental exposure to low pH (also called experimental ocean acidification) has been shown to cause changes in physiological performance in a wide range of taxa, though there is considerable variability in the direction and magnitude of effects, even within taxa (Hendriks *et al.*, 2010; Kroeker *et al.*, 2010, 2013; Wittmann & Pörtner, 2013). In heterotrophic organisms, direct physiological impacts of experimental ocean acidification include metabolic depression or elevation, slowed development, decreased feeding, sensory disorientation, behavioral changes, and mortality, though some species do not experience deleterious effects (Kroeker *et al.*, 2010, 2013; Wittmann & Pörtner, 2013; Lefevre, 2016).

2.2.1 Predicting sensitivity

The effects of increased seawater pCO_2 vary both within and between taxonomic groups (Ries *et al.*, 2009; Kroeker *et al.*, 2010, 2013; Wittmann & Pörtner, 2013). Thus, the understanding of what underlies either sensitivity or tolerance to increased pCO_2 continues to be ambiguous. “Sensitive” species are those that show detrimental physiological responses to pCO_2 levels relevant for future ocean acidification, while “tolerant” species do not. Being able to generalize the common features of sensitive and tolerant organisms, either by specific physiological strategies, geographic origin, taxonomy, or life stage will contribute to understanding of the stressor on animal physiology and focus conservation efforts (Melzner *et al.*, 2009).

While taxonomic generalizations are currently an imperfect generalization of sensitivity, crustaceans appear to be generally more tolerant than molluscs, corals and echinoderms (Ries *et al.*, 2009; Kroeker *et al.*, 2010, 2013; Wittmann & Pörtner, 2013). Distinguishing by physiological characteristics makes a somewhat clearer picture. In general, organisms with higher metabolic rates or activity levels, higher capacity to buffer the pH of internal fluids, and less or no calcified structures tend to be less affected by increased seawater pCO_2 (Pörtner *et al.*, 2005; Pörtner, 2008; Widdicombe & Spicer, 2008; Melzner *et al.*, 2009). This is in line with the species shown to have been sensitive to extinction during past periods of high pCO_2 by fossil record (Pörtner *et al.*, 2005; Pelejero *et al.*, 2010; Knoll & Fischer, 2011; Hönisch *et al.*, 2012). Geographic, or habitat-based generalizations are also theoretically useful. Organisms which inhabit environments with low natural variability in pH (i.e. the deep sea, Seibel & Walsh, 2003) are expected to be more sensitive than those species that regularly experience large pH fluctuations, like coastal or estuarine areas (Pane & Barry, 2007; Clark *et al.*, 2009; Almén *et al.*, 2014; Aguilera *et al.*, 2015). Also, species inhabiting regions with low food availability (such as the deep sea or polar areas) are expected to be more sensitive to pH changes, as their limited metabolic scope restricts their ability to support extensive ion regulation (Pörtner, 2010; Whiteley, 2011). Finally, ontogenetic differences in tolerance may exist, with young stages suggested to be less tolerant than older stages due to their incompletely developed ion regulation structures or mechanisms (Kurihara, 2008; Dupont & Thorndyke, 2009; Melzner *et al.*, 2009).

To conclude, we do not know enough yet to predict with certainty which organisms will be affected by ocean acidification from information on taxonomy or physiological generalizations (Lefevre, 2016).

2.2.2 Responses to environmental stressors

Nearly all environmental drivers have ranges within which organisms can “exist indefinitely” (Hutchinson 1957). Most typically, an organism’s tolerance range encompasses the range of environmental variability that it normally experiences in the habitat to which it has adapted over time (Lynch & Gabriel, 1987). In this context, an

environmental stressor can be defined as an environmental driver that is outside of its normal range of variation that reduces the performance or fitness of an organism (Vinebrooke *et al.*, 2004; Schulte, 2013). Quantifying physiological performance or fitness over a range of an environmental driver produces what is known as a reaction norm (Figure 5; Schlichting & Pigliucci, 1998), also referred to as performance or tolerance curves (Lynch & Gabriel, 1987). These indicate that for many environmental drivers, there is a range within which performance and fitness is high, often with an optimum, while both high and low values of the environmental driver reduce organismal performance and extremes are potentially lethal (Huey *et al.*, 2012; Sokolova *et al.*, 2012).

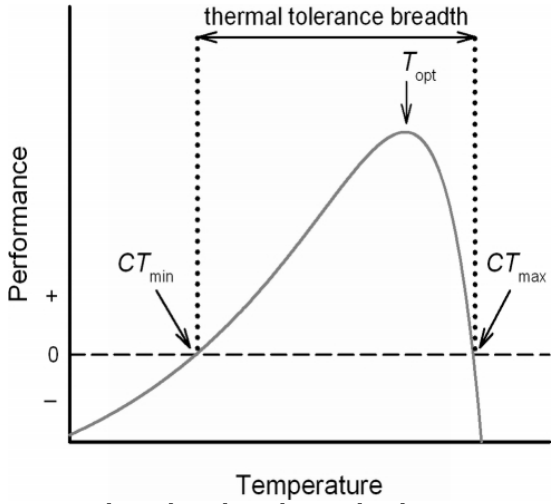

Figure 5. A reaction norm describes the relationship between an environmental driver (here, temperature) and a phenotypic trait of interest (here, described as performance). If the trait can be defined as having a positive range and a negative range, critical maximum and minimum values (CT_{max} and CT_{min}) of the environmental trait can then be identified, thus defining a tolerance range of environmental conditions for that trait. Comparing reaction norms between populations or species is a way of comapring tolerance to a given envrionmental driver. Figure from Krenek *et al.* (2012; Figure 1).

The persistence of a species at a given environmental condition is ultimately connected to the fitness of individuals in the population, collectively the population growth rate (Daniels & Allan, 1981; Chevin *et al.*, 2010). Fitness, or the lifetime reproductive output of an individual, is difficult to measure in many long lived species. Therefore, fitness-related traits, physiological performance measures that are correlated to fitness, are often used as proxies when investigating tolerance to environmental stressors (Calow & Forbes, 1998). Fitness-

related traits can include survival, growth rate, and fecundity. The energy budget of organisms, the balance between energy intake and its allocation to different functions, is also a fitness-related trait (Sokolova *et al.*, 2012), and can be linked to population growth (Kooijman & Metz, 1984; Calow & Sibly, 1990). Scope for growth is a measure of this budget, defined as the energy gained by feeding minus the energy lost from respiration. Therefore, metabolic rate and feeding rate can also be considered fitness-related traits in the context of environmental stressor responses. Identifying the response of multiple fitness-related traits to an environmental driver will provide information about what the range of tolerance is to that driver.

When long-term environmental change in species' habitat pushes it towards the limits of its tolerance range, it is faced with four options: **migrate, acclimatize, adapt, or go extinct**. Some species' distributions are shifting poleward as they track their thermal tolerance envelopes a warming climate (Beaugrand *et al.*, 2002; Parmesan, 2006; Richardson, 2008). **Migration** in response to ocean acidification, in contrast, is of limited efficacy, as there are few clear spatial gradients of pCO_2 or pH. While there are generally higher carbonate saturation states near the equator, the direction of this latitudinal gradient will work against that of simultaneously warming temperatures, which will push species poleward (Riebesell *et al.*, 2010, Ch. 10). An organism can **acclimatize** to a changed environment by altering its physiology in order to adjust its tolerance to the new range of the environmental driver. Laboratory-based acclimatization, called, acclimation, has been documented in several species in response to experimental ocean acidification (Deigweiher *et al.*, 2010; Form & Riebesell, 2012), but the degree to which marine organisms can utilize acclimatization to tolerate ocean acidification in the future is still not fully understood. **Adaptation** is an evolutionary process that occurs when the allelic composition of an entire population changes in result of selection for tolerant genotypes, increasing the tolerance of the population to the driver of the selection. While adaptation can occur over hundreds of thousands of years and lead to the emergence of new species, adaptation over relatively short time scales is known as microevolution and can confer species with physiological mechanisms of dealing with environmental stressors, even rescuing populations from extinction (Dobzhansky & Dobzhansky, 1937; Hendry & Kinnison, 1999; Bell & Gonzalez, 2009; Hoffmann & Sgro, 2011; Dam, 2013). For adaptation to be possible, however, a portion of the population must be tolerant, the tolerance must be heritable, and the lifespan of the organism must be short relative to the rate of change, allowing for many generations of

selection to respond to a given change. If the environmental change is highly detrimental to the performance and fitness of an organism, and its rate of change outpaces both the dispersal ability, rate of possible acclimatization and the rate of adaptation, then the population will face **extinction** (Bell & Collins, 2008).

3 Ecophysiology in the time of climate change: Challenges in predicting effects on organisms and ecosystems

In the era of the anthropocene (Crutzen, 2006), understanding and predicting future effects of human activities on ecosystems is of utmost importance for the optimal direction of conservation efforts. The quality of these predictions relies on our ability to understand the multiple processes that will simultaneously affect the ecosystems and replicate them in our experiments. Efforts to predict the effects of future environmental change, including ocean acidification and climate change, on ecosystems are met with several considerable challenges.

Firstly, unlike some anthropogenic pressures on natural ecosystems (like oil spills, deforestation, trawling, and overharvesting), which occur over relatively short time periods (days to years), climate change and ocean acidification are environmental changes that are occurring gradually over decades and centuries. The slow rate of change expected under ocean acidification (Orr *et al.*, 2005; Riebesell *et al.*, 2010, Ch. 3), and its duration over the next several thousand years (Caldeira & Wickett, 2003), is such that acclimatization and population-level genetic adaptation will undoubtedly contribute to the response of marine organisms to the change (Pörtner, 2008; Melzner *et al.*, 2009; Dam, 2013; Munday *et al.*, 2013; Reusch, 2014; Sunday *et al.*, 2014). However, both the slow rate of change and the long duration of such changes are impractical, if not impossible, to replicate realistically in the lab. Therefore, approximations are necessary, such as faster changes in pH over shorter exposure periods. These approximations must always be interpreted in light of their relevance to the time scale of the environmental change, as the immediate response to a quick change in an environmental stressor differs from the response to chronic stress, and eventual acclimation and adaption. Short-term

studies, while important for understanding physiological mechanisms behind a stressor, may fail to detect an increasingly deleterious effect with time (i.e. Yamada & Ikeda, 1999; Pörtner *et al.*, 2005) or, alternately, a potential acclimatization with time (e.g. Dupont *et al.*, 2013). In general, long-term exposures often show milder effects than short-term exposures due to acclimation (e.g. Miller *et al.*, 2012; Ko *et al.*, 2013). Potential for evolutionary adaptation to ocean acidification has been indicated in several species, including mussels (Parker *et al.*, 2012), coccolithophores (Lohbeck *et al.*, 2012), calanoid copepods (De Wit *et al.*, 2015; Thor & Dupont, 2015), sea urchins (Pespeni *et al.*, 2013), and polychaetes (Calosi *et al.*, 2013). Importantly, models predicting the response of species to environmental change can strongly overestimate the effects on future populations if the potential for acclimatization and adaptation are not taken into account (Chevin *et al.*, 2010; Dam, 2013). Long-term, multigenerational studies will improve predictions of how whole populations will respond over time scales that are relevant to the environmental change.

Secondly, changes occurring over decadal and century time scales will necessarily be happening in concert with other changes, both known and unknown. In the marine environment, expected long term changes include warming, freshening, pollution, altered bloom timing, and decreases in oxygen in addition to ocean acidification (Doney *et al.*, 2012). Multiple stressor scenarios are more realistic, if correctly predicted, than single. Investigating multiple stressors is especially important in light of the synergistic interactions that are possible between stressors, where their combined effect on a physiological trait is more than the additive effects of the two stressors acting independently (Widdicombe & Spicer, 2008; Kelly *et al.*, 2016). For example, elevated pCO_2 has been found to increase an organism's sensitivity to warming (Metzger *et al.*, 2007; Pörtner & Farrell, 2008). When logistically possible, testing multiple stressors in a fully crossed, factorial experimental design provides more realistic predictions of responses as well as comparisons of the relative physiological effect of the stressors.

Thirdly, and finally, predicting the effects of an environmental change on ecosystems necessitates understanding its effects on the whole spectrum of biological organization, from communities to molecules. While organisms are often the unit upon which the effects of stressors are studied, trophic interactions and interspecies competition can be strong mediators of how a species is affected by a stressor (Harrington *et al.*, 1999; Connell & Ghedini, 2015). Therefore, studies at

the community and ecosystem level, either in nature or in mesocosms, are the most realistic for predictions of future change (e.g. Riebesell *et al.*, 2010, Ch. 6). However, these methods are often prohibitively logistically challenging and subject to large stochastic variability. Studies at lower levels of biological organization can therefore be useful for detecting the various causal mechanisms of effects on an ecosystem (Pörtner & Farrell, 2008). In particular, studying the effect of stressors at a molecular level can help build generalized theories on the physiological mechanism of a stressor's effects and give insight into the physiological basis of tolerance. These can then be applied to predict responses in other, untested species. Recent advancements in high-throughput technology for nucleotide sequencing and protein detection have made it accessible to investigate the molecular aspects of ecology in novel ways (Kültz *et al.*, 2007; Stillman & Armstrong, 2015). Instead of focusing on a few, targeted genes or proteins, these methods make it possible to quantify the entire genome, transcriptome, or proteome of an organism. The transcriptome and proteome are reflections of the physiological state of an organism at a specific state in time, reflecting, respectively, the genes the organism is transcribing from its genome into mRNA, and the proteins that have been translated from mRNA into utilizable protein and enzymes. Both processes react sensitively and rapidly to biotic and abiotic drivers and can provide information on the cellular processes involved in an organism's physiological response to a stressor. These methods are increasingly utilized in studies on global change, providing information on the molecular basis of acclimation, plasticity, adaptation, and sub-lethal effects (Sutherland *et al.*, 2012; Pespeni & Palumbi, 2013; Pespeni *et al.*, 2013; Windisch *et al.*, 2014; De Wit *et al.*, 2015; Seneca & Palumbi, 2015; DeBiasse & Kelly, 2016; Huth & Place, 2016; Papetti *et al.*, 2016). Integrating results from multiple levels of biological organization provides the most complete understanding of species and ecosystem responses to environmental change.

How studies of the effects of environmental change are conducted has implications for the realism and applicability of the findings. The three challenges discussed above, exposure time (from short to long; shock to adaptation), the number of stressors (from one to multiple) and the level of biological organization (from the molecular level to that of the gene, cell, organ, organism, species, community and ecosystem), are axes of experimental consideration where experimental feasibility and ecological realism are at odds (Figure 6). Studies must choose the way in which they deal with the trade-off between experimental feasibility and ecological realism along each of

these three axes. Nonetheless, studies at different places along these axes can all be valuable to building towards the final goal— the entire ecosystem, adapted to multiple stressors.

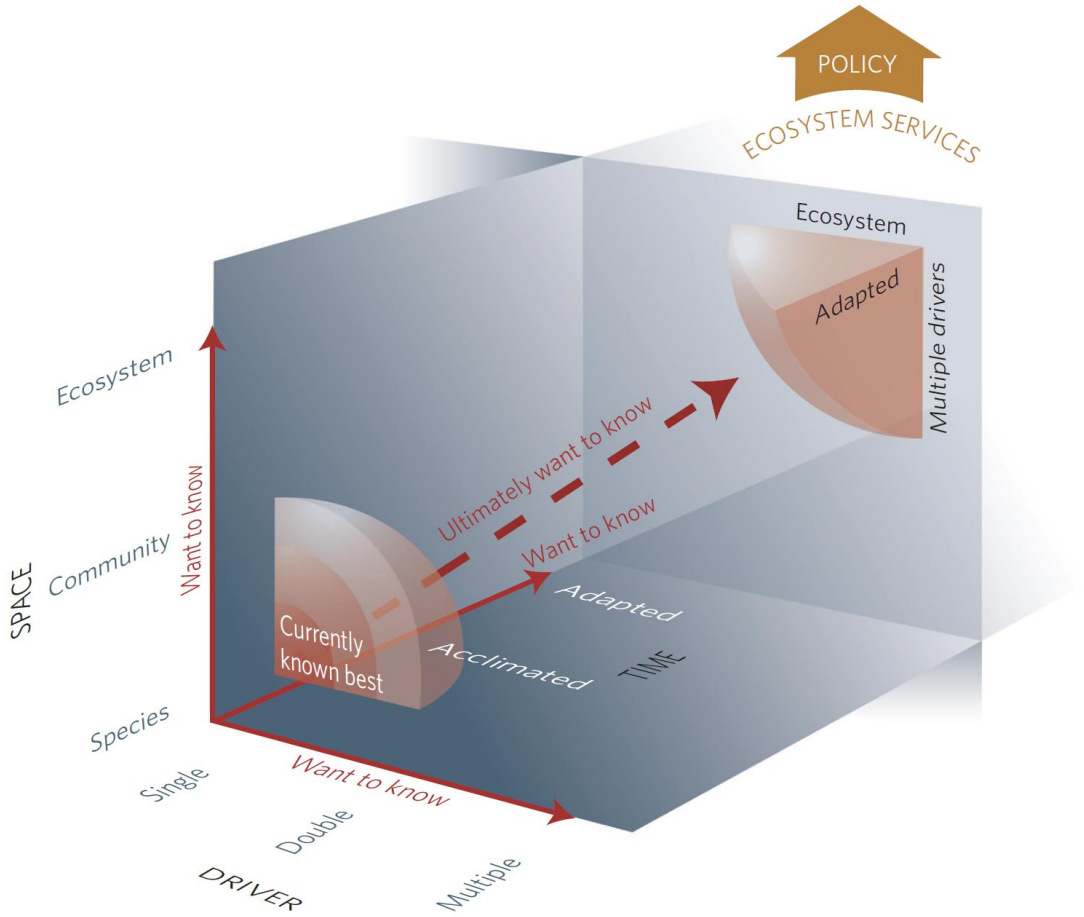

Figure 6. Studies investigating the effects of long term environmental change on ecosystems can be organized within three axes: exposure time, number of concurrent drivers, and space (or, relatedly, level of biological organization). While the ultimate goal of such research is often understanding the response of an entire ecosystem which has had time to adapt to multiple concurrent environmental changes, designing experiments to predict these responses are often simplified components of these axes. Figure from Riebesell and Gatusso (2015, Figure 2).

4 Copepods and potential ocean acidification effects

Copepods are abundant small aquatic crustaceans which inhabit marine and freshwater ecosystems around the world (Mauchline, 1998). They are an evolutionarily successful taxon, potentially constituting the most numerous metazoans on Earth (Humes, 1994; Kiørboe, 2011). In the pelagic zone, copepods dominate the zooplankton biomass (Verity & Smetacek, 1996), playing an essential role in marine food webs by converting phytoplankton sugars into lipids and proteins that are utilizable by higher tropic levels (Falk-Petersen *et al.*, 2009).

4.1 *Calanus glacialis* ecology

The calanoid copepod *Calanus glacialis* (Jaschnov, 1955) is a key component of the Arctic marine ecosystem, comprising up to 80 % of the zooplankton biomass in ice-covered shelf regions (Figure 7; Conover & Huntley, 1991; Blachowiak-Samolyk *et al.*, 2008). Their considerable lipid reserves (50-70 % of their dry weight, reviewed in Lee *et al.*, 2006) act as an energy supply in food-poor periods of the Arctic winter and make them an important prey item for fish, seabirds, and whales (Karnovsky *et al.*, 2003, Hop and Gjøsæter, 2013), thus directly or indirectly supporting much of the upper food chain (Dahl *et al.*, 2003; Falk-Petersen *et al.*, 2004). *Calanus glacialis* has a life span of 1-3 years, depending on environmental conditions (Falk-Petersen *et al.*, 2009), with six naupliar stages (N1-6) and six copepodite stages (C1-C5, plus C6 male or C6 female). In order to survive in the Arctic, with a short-lived food supply and long periods of starvation, *C. glacialis* has adopted a strategy of lipid accumulation and seasonal migration to deep waters, where it overwinters in diapause, a state of suspended development and highly reduced metabolism (Hagen & Auel, 2001; Lee *et al.*, 2006). After overwintering at depth, females ascend and produce eggs, fueled by stored lipid reserves or by feeding on ice algae and the phytoplankton bloom during spring sea ice breakup (Figure 8; Hirche & Bohrer, 1987; Melle & Skjoldal, 1998; Kosobokova, 1999).

In light of the intensity of changes expected with climate change and ocean acidification in the Arctic, and the central role played by *C.*

glacialis in the Arctic marine ecosystem, investigating their response to future changes is of great interest. In a changing climate, the persistence of *C. glacialis* in the Arctic is potentially threatened by the ocean acidification, the mismatch of ascent from diapause and sea ice breakup, loss of sea ice and associated ice algae blooms, and metabolic responses to ocean warming (Søreide *et al.*, 2008, 2010; Slagstad *et al.*, 2011; Grote *et al.*, 2015; Feng *et al.*, 2016).

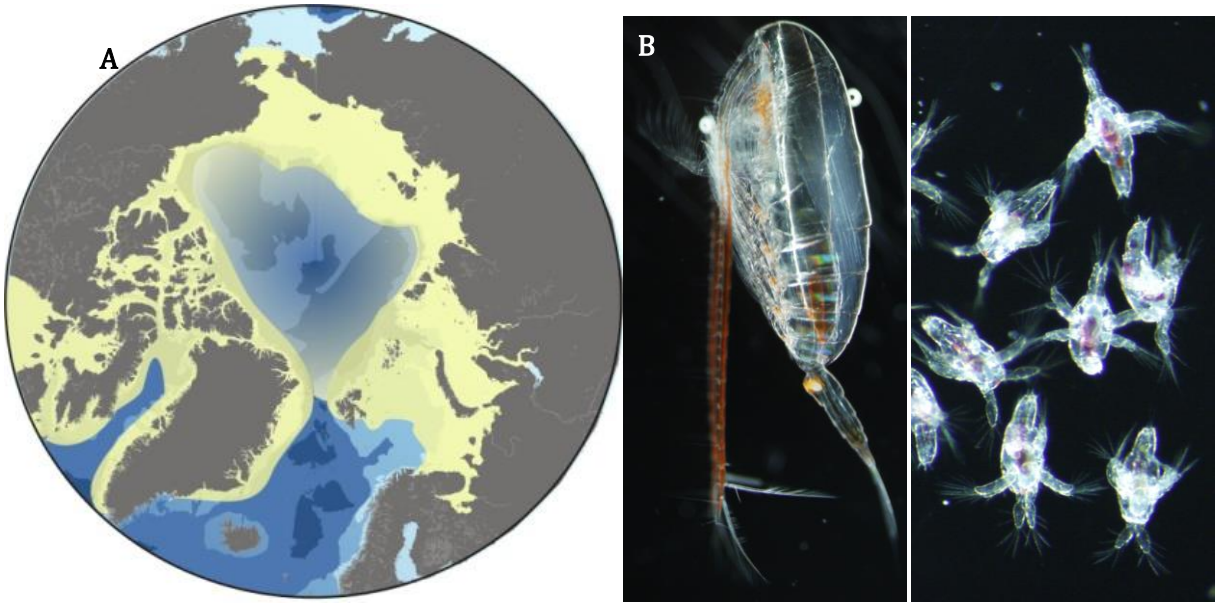

Figure 7. A: Range of *Calanus glacialis* in the Arctic (in yellow) over bathymetry (shades of blue). Map created by Malin Daase, based on Conover (1988); used with permission. B: *C. glacialis* female and N4 nauplii, photographed at different scales. Females are 2.8-4.0 mm (prosome length) and N4 nauplii are 0.48-0.58 mm (total length).

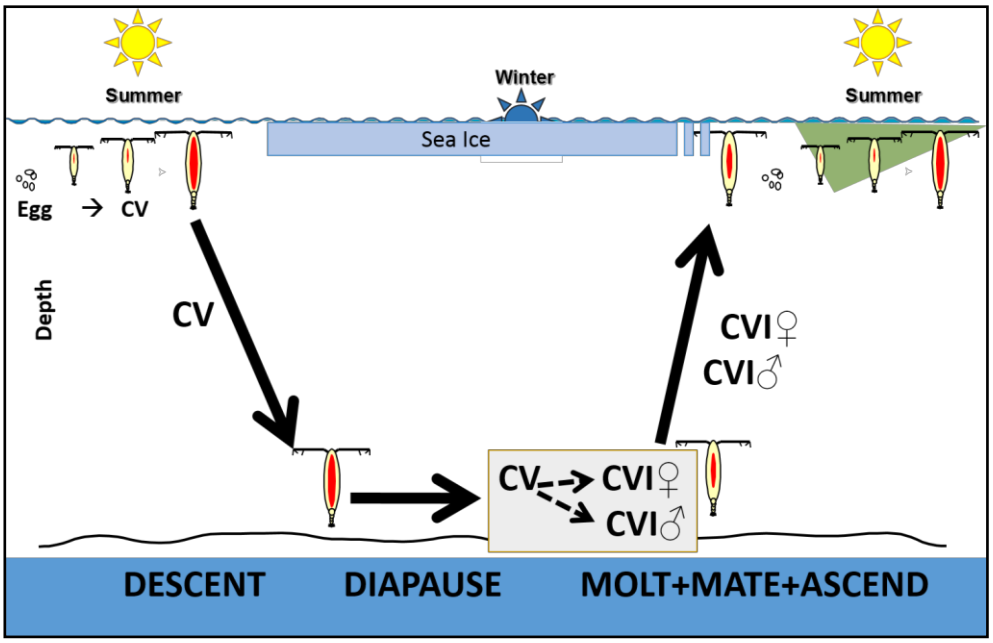

Figure 8. The *C. glacialis* 1-year life history, with ontogenetic descent to diapause at depth. In 2- and 3- year life cycles, copepodite stages C4 and C3, respectively, undergo the first diapause. Adapted from Varpe (2012).

4.2 Potential effects of ocean acidification on copepods

The effects of experimental ocean acidification on copepods appear to vary widely by species. Copepods are non-calcifying, and therefore the effects of low pH are expected to be linked to the energetic cost of acid-base regulation. In short-term incubation experiments of single species, some copepod species show no change in fitness-related traits at lowered pH, for example egg production and hatching rates in *Centropages typicus* and *Temora longicornis* (McConville *et al.*, 2013), and survival, body size, developmental rate, and egg production in *Acartia tsuensis* (Kurihara & Ishimatsu, 2008). However, other species show decreased reproductive output, reduced body size, and increased respiration, such as *Tisbe battagliai* (Fitzer *et al.*, 2012) and *Pseudocalanus acuspes* (Thor & Dupont, 2015). In addition to variable effects on the species level, effects vary by life stages, with eggs and early naupliar stages being most vulnerable (Cripps *et al.*, 2014a; Pedersen *et al.*, 2014a).

For copepods of the genus *Calanus* (congeners of *C. glacialis*), exposure to pH or pCO_2 levels projected for 2300 (~2000 µatm, ~pH 7.5, under the most severe, but realistic emissions pathway, RCP8.5) has not shown strong effects. Egg and nauplii viability of *Calanus helgolandicus* (Mayor *et al.*, 2012), hatching success, body mass, development, respiration and feeding in *Calanus finmarchicus* (Pedersen *et al.*, 2013; Hildebrandt *et al.*, 2015; Runge *et al.*, 2016) and respiration and body mass in *Calanus hyperboreus* (Hildebrandt *et al.*, 2014) are unaffected by pCO_2 levels ≤ 2000 µatm. However, other studies on *C. finmarchicus* and *Calanus* sp. show increased respiration rate and naupliar mortality and decreased developmental rate within realistic ocean acidification ranges of pCO_2 (400-2000 µatm pCO_2; Lewis *et al.*, 2013; Pedersen *et al.*, 2014b). *Calanus* copepods also show detrimental physiological responses to high pCO_2 at levels that are higher than those expected for pelagic waters in ocean acidification scenarios (> 2000 µatm), though may be relevant to carbon capture and storage scenarios, which bring with them the possibility of large quantities of CO_2 leaking from deep sea sequestration sites (Hawkins, 2004; Halsband & Kurihara, 2013). For *C. finmarchicus*, extremely high pCO_2 (> 7000 µatm; pH < 7.0) reduced egg viability to 4 % (Mayor *et al.*, 2007), decreased the survival of nauplii (Pedersen *et al.*, 2014a) and survival over the whole lifespan (Pedersen *et al.*, 2013). *Calanus sinicus* egg production decreased by over half at 10,000 µatm (Zhang *et al.*, 2011). Using higher than realistic treatment levels can be useful to understand the underlying physiological effect of a stressor (Pörtner, 2008). By including a treatment of pCO_2 of 3500 µatm in their experiment, Pedersen *et al.* (2014b) revealed that increased pCO_2 likely causes a linear increase in respiration, and decrease in scope for growth, dry weight and body length in *C. finmarchicus*. Due to the degree of variation in the measurements, this effect may not have been detectable if the analysis was restricted to pCO_2 values realistic for future ocean acidification (< 2000 µatm), though the trends appear to hold even within the < 2000 µatm range, and therefore may play a role in the species response to chronic ocean acidification.

In *C. glacialis*, older stages (C5 and females) exhibit respiration, ingestion, survival, gonad maturation, and egg production that is unaffected by low pH (Weydmann *et al.*, 2012; Hildebrandt *et al.*, 2014, 2015). However, a delay in egg hatching at pH 6.9 (but not at pH 7.6; Weydmann *et al.*, 2012) and an increase in mortality of Arctic copepod nauplii (possibly *C. glacialis*) at the relatively moderate pH levels of 7.8

and 7.6 (700 and 1000 µatm; Lewis *et al.*, 2013) indicate that effects may be stage-specific and that there may be detrimental effects on young stages.

Previous studies on the effects of low pH on *C. glacialis* have, therefore, been restricted to a few, single stages (C5s, females, and eggs), primarily on short-term (1 week) exposures, and on copepods collected from Svalbard fjords. This thesis will fill some of these knowledge gaps, by 1) conducting experiments on naupliar stages, a traditional ecotoxicological approach to focus on what are often the most sensitive stages (Grice *et al.*, 1973; Widdicombe & Spicer, 2008; Dupont & Thorndyke, 2009; Byrne, 2012), 2) conducting a long term exposure, covering half the lifespan of *C. glacialis*, and allowing for continuous development at low pH, 3) complementing organismal-level studies with molecular-level (transcriptomic) physiological responses, 4) investigating the responses of the younger copepodite stages, 5) investigating the interaction of another stressor (food limitation), and 6) comparing separate sub-populations of *C. glacialis* from distant geographical areas to understand the extant variability in its tolerance to low pH.

OBJECTIVES

The aim of this thesis is to better understand the effects of future ocean acidification on the key Arctic copepod, *Calanus glacialis*. In order to do this, experiments were conducted to answer the following questions:

1. How does low pH affect the growth and development of *C. glacialis* nauplii, potentially the most sensitive life stages? (Paper I)

2. Quantify the gene expression response to low pH to ask the following questions: (Paper II)

    - Is gene expression altered in nauplii raised at low pH?
    - What are the cellular processes involved in *C. glacialis'* physiological response to low pH?
    - Does gene expression reveal a stress that is undetected in organismal-level measurements?

3. How does concurrent food limitation affect the response of *C. glacialis* to low pH? (Paper III)

4. Do responses to low pH vary between geographically distant sub-populations? (Paper IV)
    - Does *C. glacialis* have the potential to acclimatize or adapt to future ocean acidification?

5. How does the response of *C. glacialis* to low pH vary by developmental stage? (Papers I, III, IV)

METHODS

The four papers comprising this thesis are based on data from three experiments conducted between 2014 and 2015. Papers I and II are based on the same experiment, while Papers III and IV are on separate experiments. *Calanus glacialis* individuals were collected from three fjords on Svalbard, Norway and one in western Greenland for use in the experiments (Figure 9). For Papers I, II, and III, the most extreme pH treatments used were close to the maximum decrease in pH expected for 2300, following the most severe, but realistic emissions pathway, RCP8.5, while in Paper IV, a larger pH range was tested in order to quantify the physiological reaction norms to low pH.

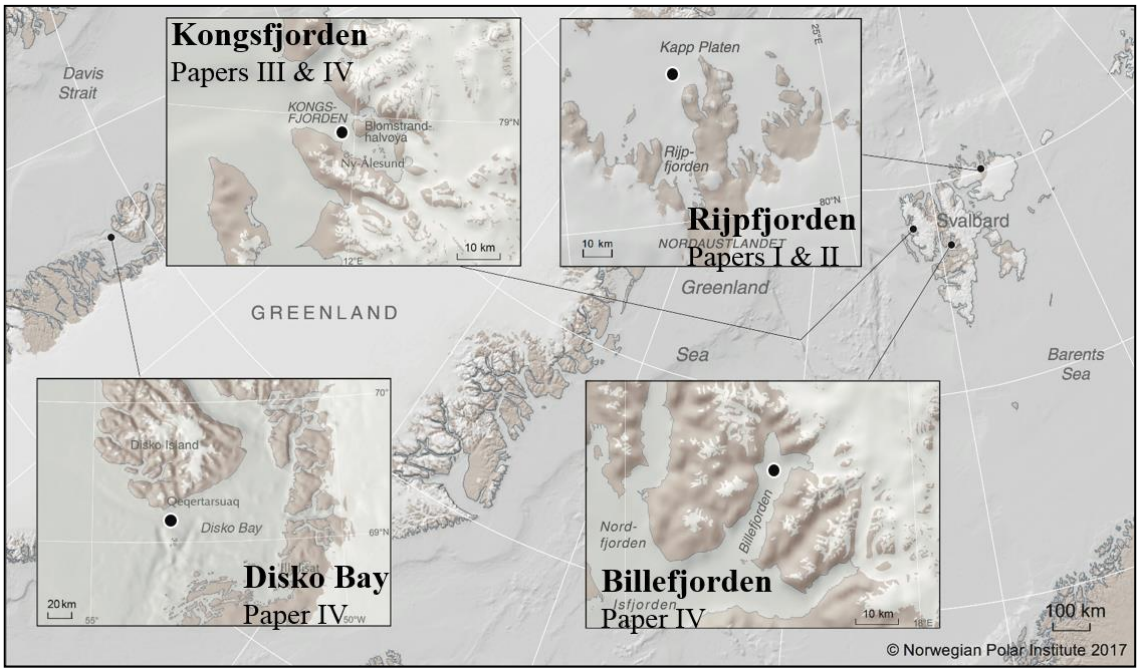

Figure 9. Collection sites for *Calanus glacialis* used in experiments for this thesis. The paper(s) to which it contributed is indicated for each. Map created by Norwegian Polar Institute, with labels added by A. Bailey.

5 Naupliar development experiment

The first experiment investigated the effects of low pH on naupliar stages of *C. glacialis*, both at the organismal and transcriptomic level, in a continuous exposure to low pH levels that lasted for 8 developmental stages. Eggs from *C. glacialis* females were cultured at four pCO_2/pH treatments (1700, 800, 530, and 320 µatm pCO_2; pH 7.5, 7,7, 7.9 and 8.05, respectively) for about half their lifespan: from eggs through all naupliar stages (N1-N6) to the first copepodite stage, C1, approximately 45 days. The experiment was run in a cold-water (2 °C) ocean acidification lab at the Austevoll Research Station (Institute of Marine Research) near Bergen, Norway. In addition to the ambient treatment, three stable pH treatments were maintained via a computer-controlled, automatic mixing system. Based on feedback from a pH meter, small amounts of high or low pH stock seawater (for high or low pH treatments, respectively) were added to ambient seawater in 100 L mixing tanks to maintain the desired pH level. The low pH stock water was created by bubbling pure CO_2 into ambient seawater, while the high pH stock water was created by bubbling CO_2-stripped air into ambient seawater. Each of the four pH seawater treatments fed three replicate, 40L flow-through tanks. From stages N3 to C1, the copepods were fed ad libitum by adding a mix of live algae continuously to the tank (minimum of 600 µgC L^{-1}).

5.1 Paper I

Mean development time, dry weight, carbon and nitrogen mass, and C:N ratio were measured by sampling 30 animals from each tank every second day throughout the experiment. The respiration rate (taken as the oxygen consumption rate, as a proxy of metabolic rate) of the nauplii was measured at three different stages (N3, N6, and C1). Dry weight, C and N mass, C:N ratio, and respiration measurements were made on groups of individuals. The effect of pH treatment on the measured traits was analysed for each stage using a mixed effects model with pCO_2 as a fixed effect factor and replicate tank as a random effect.

5.2 Paper II

After over a month of exposure to pH treatment, when nauplii reached stage N6, individuals were collected for transcriptomic analysis. Two pools of 10 individuals were collected from each tank and stored in RNA-later. From these samples, mRNA was isolated, sequenced, and a *de novo* transcriptome compiled. Gene expression was quantified as the counts of contiguous sequences (contigs, putative gene transcripts) in each sample. Differential gene expression was evaluated with generalized linear models, using the pH of the four treatments as a continuous variable. Gene set enrichment analyses provided information on which cellular processes were transcriptomically regulated in low pH.

6 Interaction of food availability, Paper III

The impact of food limitation on the metabolic response of *C. glacialis* copepodites to low pH was tested in a series of experiments conducted at the Kings Bay Marine Laboratory (Ny-Ålesund, Svalbard) during the summer of 2014. The experimental design was a simple 2x2 cross, with two pH levels, low pH (7.6) and ambient pH (8.0), and two algal food levels, no food or ad libitum food (800 µgC L^{-1} of live *Rhodomonas baltica*). The pH treatments were created by bubbling CO_2 gas into ambient seawater until target pH was reached. Copepodites (stages C2-C3 and C5) were exposed to treatment conditions for 7 days at 5 °C in groups of 10 in 620 mL glass bottles. At the end of the exposure, respiration was measured and copepods were stored on RNAlater for later determination of RNA and DNA content. For respiration, C5s were measured individually, while C2-C3s were measured in small groups in 1.8 mL vials. For RNA and DNA quantification, guts were excised and analysed separately for C5s while whole copepods were analysed for C2-C3 individuals. Whole copepod RNA:DNA ratio was used as an indicator of feeding and biosynthesis (Holmborn *et al.*, 2009; Ning *et al.*, 2013). The difference in gut DNA between fed and unfed copepods was assumed to reflect the algal biomass in the gut, and was used as an indicator of feeding. The responses were compared between treatments using permutational analysis of variance (PERMANOVA), with pH, food level and their interaction as explanatory variables and replicate bottle nested within pH and food level.

7 Pan-Arctic comparison of sub-populations, Paper IV

In this set of experiments, copepodites from three distinct fjord systems on Svalbard and Greenland were exposed to a wide range of pHs (6.4-8.1) in order to produce and compare metabolic reaction norms across sub-populations, to indicate the potential for differential acclimatization and adaptation in distinct populations. Experiments were conducted at the Kings Bay Marine Laboratory (Ny-Ålesund, Svalbard) and the Arctic Station Laboratory (Qeqertarsuaq, Western Greenland) during the summer of 2015. Copepods were collected from the Disko Bay, Greenland for experiments in Qeqertarsuaq and from both Kongsfjorden and Billefjorden in Svalbard for experiments conducted in Ny-Ålesund. *Calanus glacialis* copepodites C4 and C5 (and C3 from Kongsfjorden) were exposed to pH treatments (8.1, 7.9, 7.7, 7.5, 7.3, 7.1, 6.9, 6.6, and 6.4) for 8 days at 5 °C before ingestion and respiration were measured. Copepods were exposed in groups of 10 in 620 mL glass bottles, with the seawater exchanged daily to maintain desired pH and food level (10 μg Chl *a* L^{-1} algal paste). The pH treatments were created by bubbling CO_2 gas into ambient seawater until target pH was reached. Respiration was measured in single individuals. Significant reactions norms, and their shape (linear, quadratic, or cubic) were detected by sequentially testing polynomial regression models of increasing order on the relationship between the response (ingestion or respiration) and the pH for each stage and population.

RESULTS & DISCUSSION

8 How does low pH affect the growth and development of *C. glacialis* nauplii, potentially the most sensitive life stages? (Paper I)

We investigated *C. glacialis* nauplii development at four different pH treatments (pH 8.05, 7.9, 7.7, and 7.5). The nauplii developed successfully from egg to N6 at all four pH treatments, developing, respiring, and building biomass (dry weight, C and N mass) at the same rates in all treatments. The range of pH treatments used included an estimate for Arctic seawater pH in 2300 (pH 7.5) and, thus, indicates that these important aspects of naupliar development in *C. glacialis* may not be detrimentally affected in future ocean acidification.

Our knowledge on the effects of low pH on nauplii is, however, restricted to what we measured. Some physiological effect of low pH on *C. glacialis* nauplii could have been present but not detected in our measurements. Firstly, naupliar mortality was not tracked throughout the experiment. As with most marine species which freely spawn high numbers of eggs, mortality in copepod eggs and nauplii is high (Rumrill, 1990; Kiørboe & Sabatini, 1994; Ohman *et al.*, 2004), ranging from 30-90 % at stage C1 for *Calanus helgolandicus* and 42-51 % for *C. finmarchicus* in experimental setting (Cook *et al.*, 2007) and 70-90 % by C1 for *Calanus* spp. in a Norwegian fjord with natural predation (Eiane *et al.*, 2002). The opportunity for selection to occur within the cultures is therefore accordingly high. As our measurements were carried out on the surviving portion of the culture population, they may have focused only the response of low pH-tolerant individuals. However, the size of the populations in the tanks at the end of the experiment did not appear to correlate with pH treatment, based upon rough visual estimation, and high mortality in the low pH treatments is therefore unlikely. Similarly, the survival of *C. finmarchicus* nauplii was unaffected by exposure to far lower pH (7.16), only showing lowered survival at pH 6.94 (Pedersen *et al.*, 2014a). While capturing the intra-population variability is important for understanding the potential for an evolutionary adaptation to future ocean acidification, our results

indicate that at least a majority, if not all, of the *C. glacialis* nauplii were tolerant of a pH range relevant to future ocean acidification. If selection occurred, it didn't represent a large portion of the population, increasing the likelihood that the low pH-tolerant nauplii would also be genetically diverse enough to retain fitness relevant to other aspects of *C. glacialis*' environment.

Secondly, there may have been physiological changes in the nauplii we sampled that were not detected in our measurements. This is not unlikely, as the decrease in pH from 8.05 to 7.5 represents a 350 % increase in H^+ concentration. These physiological changes could have been beneficial for the nauplii, allowing for the maintenance of the development and growth we observed, or detrimental, by reducing other measures of performance. The ability of an organism to maintain fitness-related traits under stress by adjusting underlying physiological processes has been referred to as phenotypic buffering (Reusch, 2014; Sunday *et al.*, 2014). Possible mechanisms of beneficial phenotypic buffering include increased feeding to compensate for potential additional energetic costs, a reallocation of the energy budget to cover growth at the expense of other investments, and altered gene expression to increase naupliar ion regulation capacity. Compensatory feeding is unlikely to have played a role in the nauplii's tolerance to low pH, as the non-feeding stages (N1-N2) did not show a developmental delay and older nauplii did not show higher respiration rates at low pH, which would be expected if more food had been ingested and overall metabolic rate had increased (due to specific dynamic action, (Jobling, 1983)). The lack of increase in feeding at low pH in nauplii is consistent with findings for older stages of *C. glacialis* (Paper IV, Hildebrandt *et al.,* 2015) and *C. finmarchicus* (Pedersen *et al.*, 2014a; Runge *et al.*, 2016). A reallocation of the energy budget has been found in several species in response to low pH, and may have allowed *C. glacialis* nauplii at low pH to maintain respiration rates while experiencing increased costs of acid-base regulation. In the copepod *Tisbe battagliai*, reproduction was maintained at low pH, at the cost of somatic growth and cuticle composition (Fitzer *et al.*, 2012). Pan *et al.* (2015) found a reallocation of metabolic energy towards protein synthesis and ion regulation in sea urchin larvae, which allowed size, metabolic rate, and biochemical content to remain unchanged in low pH. Thus, the maintenance of developmental rate and dry mass in *C. glacialis* nauplii at low pH may have come at the expense of another physiological function (i.e. immune system) or physical trait (i.e. cuticle composition) that was not

measured but is nonetheless important for performance during naupliar stages or later in life.

While respiration, development and growth rates are important organismal-level fitness-related measures (Kooijman & Metz, 1984; Calow & Sibly, 1990), they do not necessarily cover all the key aspects of physiology. For example, Hernroth *et al.* (2012) found that the immune system of the Norway lobster was significantly impaired after exposure to low pH for four months, though more traditional growth and calcification measurements did not reveal any negative effects. Impairment of the immune system at low pH has been found in several species (isopods, Wood *et al.*, 2014; Pacific white shrimp, Burgents *et al.*, 2005; Atlantic blue crab, Tanner *et al.*, 2006; blue mussel, Bibby *et al.*, 2008). Detrimental effects of low pH can detectable in short-term experiments on the molecular level only, underlining the importance of complementing organismal-level measurements with molecular studies (Pan *et al.*, 2015).

Finally, the range of pH used in the experiment may be within the range that the physiology of *C. glacialis* nauplii can tolerate. As they are hatched into the spring bloom, when pH often varies, a wide pH tolerance may be beneficial. Nevertheless, theory suggests that pushing an organism closer towards its physiological tolerance limit with regards to one environmental driver can reduce their tolerance to other stressors, for example the narrowing of thermal tolerance windows in elevated pCO_2 (Pörtner & Farrell, 2008; Pörtner, 2010). Reductions of 2-5 °C in the thermal tolerance of several crustacean species at elevated pCO_2 lend support to this theory (e.g., edible crab, Metzger *et al.*, 2007; spider crab, Walther *et al.*, 2009. Therefore, further studies of metabolic scope and tolerance to other stressors (e.g., hypoxia, freshening, warming) would provide information on a narrowing of the environmental tolerance of *C. glacialis* nauplii concurrent with their tolerance of low pH.

In conclusion, while it is important to be cognizant of the possibility that there were other, unmeasured physiological impacts on nauplii at low pH that may be of importance, all measured endpoints were not affected by low pH. Developmental rate, growth and respiration rate represent some of the most important indicators of organismal performance and lifetime fitness. Thus, finding that low pH (to pH 7.5) did not significantly affect these parameters in *C. glacialis* nauplii is a strong indicator that the naupliar stages will maintain performance in

future ocean acidification. The possibility that our findings reflect some natural selection occurring during the experiment does not weaken this argument. Finally, it does not appear that the naupliar stages represent sensitive life stages in *C. glacialis.* While *C. finmarchicus* eggs-N2 showed higher mortality than N3 when exposed to low pH for one week, this was only observed at pHs < 7.16, beyond the range of realistic ocean acidification. Within similar pH ranges, our nauplii findings are in line with those found in *C. finmarchicus* (Runge *et al.*, 2016).

9 Is gene expression altered in nauplii raised at low pH? (Paper II)

Despite an acclimation time of 35 days, *Calanus glacialis* nauplii were significantly regulating a portion of their transcriptome in response to pH at stage N6. More specifically, the expression of 151 contiguous strands of mRNA ("contigs"; discussed hereafter as genes) was correlated to pH across four pH treatments (pH 8.05, 7.9, 7.7, and 7.5) in the same nauplii cultures as in Paper I. In addition to individual genes which were differentially expressed with pH, we evaluated the coordinated expression changes in groups of genes that were physiologically related, for example as components of the same physiological pathway, using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classifications. These analyses showed that 1092 biological processes (GO), 397 molecular functions (GO) and 29 cellular pathways (KEGG) were significantly up- or down-regulated with pH.

Out of a transcriptome of 59,353 genes, the 151 significantly differentially expressed genes (DEG) comprised 0.25 % of the transcriptome. While this is not a large proportion of the transcriptome, we employed a conservative method of detecting differentially expressed genes which may have kept the number detected low. By evaluating the correlation of expression across four pH treatments with six replicates in each, rather than the more common comparisons of two treatments, we lowered the likelihood of detecting DEGs that were false positives. Importantly, there is no established threshold at which the percent of transcriptome affected by a driver is deemed a "significant" physiological response. Rather, the "genes that matter" for understanding an organism's response to environmental stress are often only a few genes, each with a

large effect on fitness, that show mild but concerted gene expression changes (Feder & Walser, 2005; Evans, 2015). This indicates that small numbers of DEGs can also have a physiological impact. It also underscores the value of combining DEG analyses with gene set enrichment analyses that detect the coordinated regulation of genes rather than only focusing on the DEGs that meet an arbitrary level of significance. Both of these methods indicated that *C. glacialis* nauplii regulated their gene expression with pH.

In light of the finding that, on an organismal level, the nauplii were apparently unaffected by low pH (Paper I), transcriptomic data were able to show that the physiology of *C. glacialis* nauplii was affected on a molecular level by low pH. These data were also used to address the following specific questions: 1) What are the cellular processes that allowed for this tolerance? and 2) Does the gene expression indicate a stress response that went undetected in organismal-level measurements?

9.1 What are the cellular processes involved in *C. glacialis*' physiological response to low pH?

Of the genes and physiological processes regulated with pH, the majority were down-regulated with lowered pH (93 % of DEGs, 92 % biological process GOs, 90 % molecular function GOs, and 55 % of KEGG pathways). Of the numerous physiological process identified as significantly regulated by the gene set enrichment analyses, the most striking finding was that many of the down-regulated functions were components of the universal cellular stress response. The universal cellular stress response is an evolutionarily conserved set of physiological mechanisms that is up-regulated in response to stress across a wide range of taxa (Kültz, 2003, 2005). It includes genes related to energy metabolism, DNA repair and damage sensing, protein folding and degradation, redox, and molecular chaperones (Kültz 2005). Components of all of these stress mechanisms were significantly regulated in *C. glacialis* nauplii that had been raised at low pH, but they were down-regulated, rather than up-regulated, as would have been expected in response to a stress. While metabolic depression (Guppy & Withers, 1999), and corresponding reductions in protein synthesis, have been observed in some marine organisms in response to low pH (sipunculid worm, Pörtner *et al.*, 2000; pacific oyster, Dineshram *et al.*, 2012; several sea urchin species, Evans & Watson-Wynn, 2014), respiration measurements did not show any evidence of metabolic

depression in *C. glacialis* nauplii from the same exposure experiment (Paper 1). Alternately, some evidence exists that indicates that down-regulation of stress-related genes when exposed to a stressor may also be a characteristic of tolerant populations. In a study comparing wild oysters to a selectively-bred line that had developed tolerance to low pH, Goncalves *et al.* (2016) found that the line that had developed tolerance to low pH reacted to decreased pH by down-regulating genes associated with stress response. In contrast, the sensitive wild population up-regulated the same genes. Similarly, heat-tolerant strains of *Daphnia pulex* showed a general down-regulation of genes involved in transcription, translation, DNA replication, DNA repair and core metabolic pathways in response to heat stress, a response not present in heat-sensitive strains (Yampolsky *et al.*, 2014). Such responses may have to do with a constitutively elevated expression of stress-related genes in tolerant strains or species. As an example, the gene expression of an Antarctic fish, whose adaptation to cold waters involves constitutively elevated stress-related genes, shows a down-regulation of those genes when exposed to temperature stress (Huth & Place, 2016). Therefore, down regulation of stress response genes may be a characteristic of tolerant species or sub-populations. We found that it was characteristic of the pH tolerant *C. glacialis* nauplii from Rijpfjorden, Svalbard.

Among the physiological functions that were up-regulated in lower pH was the Na^+/H^+ antiporter. This is an important component of acid-base ion regulation machinery (Claiborne *et al.*, 1999; Casey *et al.*, 2010; Heuer & Grosell, 2014). However, in contrast to the Na^+/K^+-ATPase, which consumes ATP as it actively pumps H^+ out of the cell, the Na^+/H^+ antiporter is passive and generally understood as relying on the ion gradient created by the active Na^+/K^+-ATPase, known as the "motor" of the cellular ion-regulatory machinery (Aharonovitz *et al.*, 1999; Melzner *et al.*, 2009). Na^+/K^+-ATPase energy consumption can comprise large portions of the energy budget of marine organisms, and especially larvae, at control conditions (Leong & Manahan, 1997). Up-regulation of Na^+/K^+-ATPase is predicted in marine organisms in response to low pH, and the expected increase in ATP consumption is one of the main reasons why low pH is expected to incur energetic costs for marine organisms (Pörtner *et al.*, 2004; Pan *et al.*, 2015). Na^+/K^+-ATPase transcription and activity is up-regulated in low pH in some organisms (Catches *et al.*, 2006; Deigweiher *et al.*, 2010; Stumpp *et al.*, 2011b; Tseng *et al.*, 2013; Pan *et al.*, 2015; Huth & Place, 2016), but not

all (Todgham & Hofmann, 2009; Stumpp *et al.*, 2011b; Moya *et al.*, 2012; Evans *et al.*, 2013). However, in *C. glacialis* N6, we found an up-regulation of the energy-neutral Na^+/H^+ antiporter, but not the energy expensive Na^+/K^+-ATPase. Interestingly, the Na^+/H^+ antiporter has been found to be up-regulated at low pH in other species known for their strong ion-regulation capacities, namely fish and squid (Catches *et al.*, 2006; Hu *et al.*, 2013; Huth & Place, 2016). Hu *et al.* (2013) found that squid larvae significantly up-regulated the Na^+/H^+ antiporter, but not Na^+/K^+-ATPase, when exposed to elevated pCO_2. Others have found that some organisms can switch from energy-expensive ion channels to less costly ion transporters (Reipschläger & Pörtner, 1996; Pörtner *et al.*, 2000). Perhaps some species, like *C. glacialis*, can utilize less energy costly mechanisms of acid-base regulation (such as the Na^+/H^+ antiporter) more than other species, therefore conferring them tolerance to low pH.

While gene expression (transcription) does not explain the whole pattern of variation in protein concentration and activity in an organism, molecular studies and gene expression studies can provide unique insight into the physiology of organismal response to environmental stressors, potentially contributing to a molecular understanding of tolerance to low pH. After 35 d of acclimation, *C. glacialis* nauplii showed general down-regulation of stress-related genes, potentially a characteristic of tolerance, and implicated an energy-neutral proton pump in acid-base regulation.

9.2 Does gene expression reveal a stress that is undetected in organismal-level measurements?

Our gene expression results did not indicate the presence of a low pH stress that was undetected in the organismal-level measurements (respiration, development, growth; Paper I). While stress-related genes were differentially expressed with pH, they were down-regulated. The down-regulation of these genes does not indicate an activation of the cellular stress response, something that, when up-regulated, is energetically costly for the cells (Calow, 1991; Kültz, 2005; Sokolova *et al.*, 2012). Up-regulation of the energy-neutral Na^+/H^+ antiporter also does not point to an energetic cost of low pH. In all, the

transcriptomic response of N6 nauplii did not indicate a stressful or energetically costly response to low pH.

10 How does concurrent food limitation affect the response of *C. glacialis* to low pH? (Paper III)

Comparing the metabolic response of fed and starved *C. glacialis* copepodites to low pH revealed that feeding status affected the metabolic response to low pH in C2-C3s, but not C5s. Copepodite C2-C3s that were fed respired more than those that were starved, both at low pH and at ambient pH. Interestingly, however, C2-C3s that were exposed to low pH showed a greater increase in metabolic rate in response to food than did copepods at ambient pH. While this may have been attributable to copepods at low pH ingesting more food, and therefore biosynthesizing more, which increases respiration (see Specific Dynamic Action, below), RNA:DNA ratios indicated that the copepods biosynthesized similar amounts at low and ambient pH. C5s did not show the same response as C2-C3s. In the first of two experiments, C5s increased respiration in response to food, though the increase was similar for copepods at low and ambient pH. In the second C5 experiment, which was initiated two weeks after the first, the C5s did not increase respiration in response to food, and gut DNA indicated that they fed at much reduced rates compared to the first C5 experiment.

Specific Dynamic Action

The increase in respiration associated with, but not necessarily caused by, feeding is referred to as specific dynamic action (SDA; Jobling, 1983). While SDA is typically quantified using repeated measurements of oxygen consumption before, during, and following a feeding event, the difference in respiration of fed and unfed copepods in this experiment is another quantification of the same phenomenon. Our results indicate that low pH causes an increase in SDA of 250 % in young copepodites (C2-C3), compared to ambient pH. A similar increase in SDA at low pH was found in a boreal population of the calanoid copepod *Pseudocalanus acuspes* (Thor & Oliva, 2015).

Tirsgaard *et al.* (2014) found that Atlantic cod exhibited an extended SDA at elevated pCO_2, though the total magnitude of SDA was similar to that at control pCO_2. Contrary to our findings, Pan *et al.* (2015) found that the metabolic rate of purple sea urchin larvae only increased in starved individuals, but not fed individuals.

What causes the increase in SDA?

In crustaceans, the magnitude of SDA has been found to depend on meal size and composition, body size, activity level, and abiotic environmental drivers such as salinity and temperature (Whiteley *et al.*, 2001 and sources reviewed within), though a mechanistic understanding of why and how SDA changes with environmental factors is not clear. The majority of the metabolic cost of SDA is attributed to the energetic cost of biosynthesis (50-116 %) and the assimilation and transport of food molecules in the gut (18-28 %), while the capture of food and the production of digestive enzymes and urea constitute only minor contributions (~1 %; Kiørboe *et al.*, 1985). An increase in SDA is therefore likely to be due to a higher cost of biosynthesis (per unit of macromolecule) or a higher cost of assimilation and transport.

Protein biosynthesis accounts for the majority of the biosynthesis costs in SDA in ectotherms (Houlihan *et al.*, 1990; Brown & Cameron, 1991; Lyndon *et al.*, 1992; Carter *et al.*, 1993; Thor, 2002). If low pH increased protein synthesis or protein turnover, as it did in the purple sea urchin (Pan *et al.,* 2015), the total cost of protein biosynthesis would increase, as was seen in the liver cells of the Atlantic cod (Stapp *et al.*, 2015). Alternately, the cost per unit of biosynthesis could increase. Exposure to low pH has been found to increase the costs of protein biosynthesis in gill arches of two Antarctic fish species (Deigweiher *et al.*, 2010) but not in developing sea urchins (Pan *et al.*, 2015). Another alternative is that low pH increases the cost of the assimilation of food in the gut. Impaired digestion and absorption efficiency have been found in several marine organisms exposed to low pH or high pCO_2, including sea urchins (Stumpp *et al.*, 2013) and mollusks (Navarro *et al.*, 2013; Zhang *et al.*, 2015). Absorption of protein, lipid, and carbohydrate monomers across the gut wall is likely dependent on acid-base balance in the gut. The absorption of proteins and carbohydrates is dependent upon the Na^+ gradient across the gut

epithelium (Kiørboe *et al.*, 1985). As the intestine is an important site of acid-base regulation, and Na^+ is involved in several proton exchangers, this gradient may be altered in elevated pCO_2 (Claiborne *et al.*, 2002; Heuer & Grosell, 2016). Finally, pH effects on the quality of the algae as food may also have affected SDA, though the algae was only exposed to low pH for one day (Thor *et al.*, 2002). Alterations in cost of protein biosynthesis, digestion and absorption (and potentially food quality) at low pH may contribute to the increased SDA seen in C2-C3s.

Life history implications of increased SDA

The young copepodites spent significantly more energy on SDA at low pH (2.5×) than at ambient pH. By burning a larger percentage of the energy gained from ingested food on its absorption and use in biosynthesis, less of the ingested energy is available for growth and storage at low pH. If these energetic costs of low pH persisted during future ocean acidification, and were not lessened by acclimatization, they could have detrimental effects on the ability of young copepodite stages to store lipid for diapause, and ultimately, have negative effects at the population level (Kooijman & Metz, 1984; Calow & Sibly, 1990)

Stage-specific effects

The lack of effect of pH on SDA in C5s may be due to the differences in physiology between C5s and C2-C3s. Copepods that reach C5 by mid-summer will overwinter in diapause (Falk-Petersen *et al.*, 2009) while younger stages will continue to develop until at least C4 before preparing for diapause. These differences are likely reflected in their biosynthetic physiology, with the C5s focusing their energy on storing lipids for diapause, and the younger stages devoting more energy to protein biosynthesis for structural somatic growth (Thor, 2002; Graeve *et al.*, 2005; Yebra *et al.*, 2006). While a SDA response to food was observed in C5s at both pHs in the first C5 experiment, it was not observed in the second, potentially because the C5s at that point in time (late July) had begun preparing for diapause and had stopped feeding. *Calanus glacialis* can begin descent to diapause as early as July in Kongsfjorden (Daase *et al.*, 2013).

Implications for multiple stressor inclusion

Notably, pH alone did not have a significant effect on respiration in any of the developmental stages in this study. Respiration rates in starved copepods were particularly similar across pHs, indicating that food-limitation does not reveal an otherwise compensated-for metabolic stress in low pH (as was found in sea urchin larve; Pan *et al.,* 2015) in *C. glacialis* copepodites. Importantly, without the inclusion of two food levels, the energetic effects of low pH in these stages may not have been detected, though they appear to be present and significant A lack of pH effect on respiration is similar to the findings on younger stages *C. glacialis*, N3-C1 (Paper I), and in longer exposures of C5s (Hildebrandt *et al.*, 2014), though neither of these studies compared fed and unfed copepods or quantified SDA. Similarly, Tirsgaard *et al.* (2015) found that while resting metabolic rate was unaffected by elevated pCO_2 in Atlantic cod, the fish exhibited an altered SDA at elevated pCO_2. Likewise, Seibel *et al.* (2012) found that metabolic effects of low pH on an Antarctic pteropod would have been masked if differences in food and baseline respiration hadn't been taken into consideration. This underlines the possibility of important energetic effects of experimental ocean acidification being hidden by experimental design.

In conclusion, investigating the interaction of food level and low pH revealed potentially important energetic costs of low pH in young copepodite stages (C2-C3), but not older stages (C5).

11 Do responses to low pH vary between geographically distant sub-populations? (Paper IV)

Both population-specific and stage-specific effects of low pH on respiration and ingestion were found in *C. glacialis* copepodites in Paper IV. C4s from Svalbard (both Kongsfjorden and Billefjorden populations) significantly increased their respiration and decreased their ingestion with lowered pH. However, there was no response in either measure in the Greenland population (Disko Bay). At pH 7.87, these changes resulted in reductions in scope for growth scope by 19 % in the Kongsfjorden population and 50 % in Billefjorden. Reduction in scope for growth of this magnitude could, if sustained over time, lead to

reductions in fitness and population growth (Kooijman & Metz, 1984; Calow & Sibly, 1990).

In general, the significant responses to pH seen in the Svalbard C4s were absent in C5s and C3s. No significant change in respiration or ingestion with pH was found in C5s, from any population. This is in line with previous findings on respiration and ingestion in C5s from the Fram Strait (Hildebrandt *et al.*, 2014, 2015). In C3s from Kongsfjorden, ingestion showed a curved response to pH (max at ~pH 7.4) and respiration did not respond to pH. C3s were only measured from Kongsfjorden, and therefore population-specific responses in this stage could not be evaluated. Thus, the response of *C. glacialis* copepodites varied between geographically distant populations in stage C4, the stage that was most affected by low pH, whereas in C5s, all populations responded similarly and were not affected by pH.

11.1 Does *C. glacialis* have the potential to acclimatize or adapt to future ocean acidification?

Documenting population-specific responses to low pH are important for making realistic predictions about the effect of future ocean acidification on a species as a whole and the regional ecosystems it inhabits (Sanford & Kelly, 2011; Dam, 2013; Kelly & Hofmann, 2013; Reusch, 2014; Sunday *et al.*, 2014; Calosi *et al.*, 2016). Firstly, it reveals the weakness of using response parameters from one sub-population to model responses to environmental change across the species' geographic range. Secondly, population-specific responses may indicate local adaptation (or local acclimatization). Local adaptation is the adaptation of populations to their local environment via natural selection, and can occur if populations are sufficiently genetically isolated, allowing selection to outweigh immigration of non-adapted genotypes, and the environmental conditions vary between regions (Sanford & Kelly, 2011). While marine species, and plankton in particular, has typically been assumed to be panmictic, with high gene flow between populations, recent studies on marine invertebrates indicate that genetic separation of populations can occur on multiple spatial scales (Peijnenburg & Goetze, 2013), and there is increasing evidence of local adaptation (Sanford & Kelly, 2011; Dam, 2013). Local adaption is indicated when populations' physiological differences are

advantageous for their local conditions (Kawecki & Ebert, 2004). Demonstrating that local adaptation has occurred in spatially and environmentally distinct environments may be used as an argument that similar adaptive evolution could occur in the future in response to gradual environmental change. This substitution of space for time (the synchronic approach; Hendry & Kinnison, 1999) is a useful method that allows investigating the potential for adaptation to gradual environmental change in long-lived species (Schlichting & Pigliucci, 1998; Hendry & Kinnison, 1999; Sanford & Kelly, 2011; Dam, 2013; Sunday *et al.*, 2014). Despite its importance, population-specific responses have only recently been investigated in ocean acidification research (Calosi *et al.*, 2013, 2017; Pansch *et al.*, 2014; Wood *et al.*, 2014, 2016; Thor & Oliva, 2015).

There are several possible explanations of the population-specific responses to low pH we observed in *C. glacialis* C4 in this study, where Svalbard populations of *C. glacialis* C4 showed much higher sensitivity to low pH than their Disko Bay counterparts did. While this may have been due to undesired differences between experiments on Greenland and Svalbard, including differences in the water chemistry of seawater used (for example, alkalinity), body size, or differences in the progression of seasonal population development in the three fjords, it is also possible that the *C. glacialis* C4s populations had adapted (or acclimatized) to different local environments. While high resolution, year-round pH data is not available for any of the three fjords, Fransson *et al.* (2016) found seawater pH in Kongsfjorden to be high (pH $>$ 8.0), both in summer and winter (2013- 2014) whereas studies from Disko Bay show large weekly and inter-annual variation in seawater pH (7.5-8.3), with extended periods in spring where the entire water column was as low as pH 7.5 (2011-2012; Riisgaard & Nielsen, 2015; Thoisen *et al.*, 2015). While the Fransson *et al.* (2016) study does not have high temporal resolution through the year, a shallow, near-shore site in Kongsfjorden (measured every hour from 2015-2017) also shows a narrower seasonal pH range than Disko Bay, despite coastal environments typically having more variable pH than pelagic environments (Duarte *et al.*, 2013; AWI-IPEV & COSYNA, 2017). These data suggest significant differences in the natural pH environments of the Kongsfjorden and Disko Bay populations, with Disko Bay showing variable and low pH, and Kongsfjorden higher and more stable pH (Figure 10). While these populations are likely a mix of local and advected individuals (Wassmann *et al.*, 2015; Hunt *et al.*, 2016), there

are probably local and regional differences in pH environment. Disko Bay C4s appear better adapted to their local pH environment than do the Kongsfjorden or Billefjorden C4s. Exposure to lower pH seawater may have been the selective force driving local adaption towards a more low pH-tolerant population of *C. glacialis* in Disko Bay.

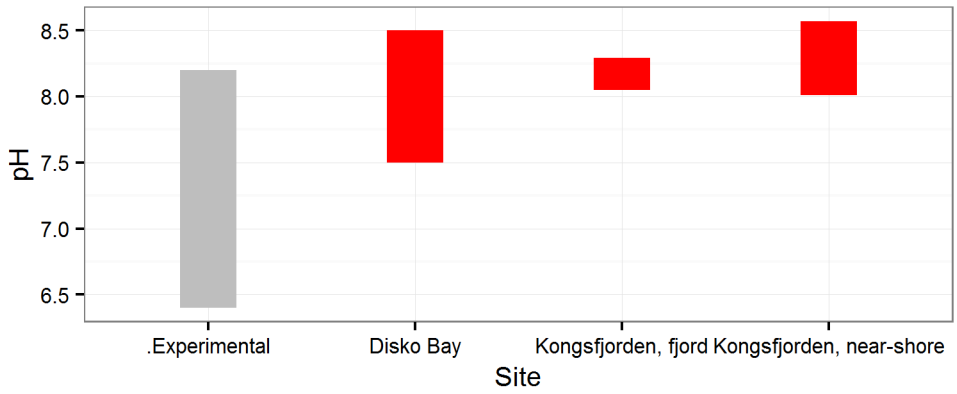

Figure 10. A comparison of the natural seasonal pH ranges of Disko Bay, Greenland and Kongsfjorden, Svalbard, as reported by Thoisen *et al.* (2015) for Disko Bay, Fransson *et al.* (2016) throughout Kongsfjorden where depth was > 60m depth, and AWI-IPEV & COSYNA (2017) close to shore in 11m depth. Seasonal pH data is lacking for Billefjorden. The experimental pH range tested in Paper IV is added in grey.

The low pH-tolerance of Disko Bay *C. glacialis* could, however, also be attributed to local acclimatization. As the copepods were collected for this experiment and immediately subjected to the pH treatments, their physiological status is not only a reflection of their genotype, but also a reflection of both the environment in which they developed and the environment to which their parents were exposed (via plasticity, maternal effects, and epigenetic factors; Kawecki & Ebert, 2004; Sanford & Kelly, 2011; Bonduriansky *et al.*, 2012). Separating the effects of genetic and environmental differences between geographically distinct populations is possible using a common-garden experimental approach. In a common-garden experiment, the physiological traits of sub-populations of a species are compared in identical environmental conditions. According to Falconer and Mackay's (1996) equation, the total variance in a phenotypic trait (V_P) is attributable to genetic variation (V_G), environmental variation (V_E) and the interaction of genotype and environment ($V_{G\times E}$): $V_P = V_G + V_E + V_{G\times E}$ + error. If environmental variation is experimentally forced to zero, the remaining phenotypic variation can be attributed to genetic differences in the populations (and measurement error). However, in

light of the significant influence that developmental and parental environment can have on an organism's physiology, common-garden experiments must be carried out after the organisms are allowed to reproduce in identical environmental conditions for at least two generations (Kawecki & Ebert, 2004; Dam, 2013). This will to prove more conclusively that population-specific responses are linked to genetic difference. This, however, is impractical for organisms such as *C. glacialis*, which have a life span of 1-2 years or more, and we must acknowledge that the population-specific responses we observed may be either local adaptation or local acclimatization.

If our data show local adaptation, adaptation may occur in the future in response to ocean acidification in populations that are currently sensitive to low pH (i.e., possibly Kongsfjorden and Billefjorden). Evolutionary rescue refers to the process of a population adapting evolutionarily to an environmental change at a rate fast enough to save it from extinction (Bell & Collins, 2008; Bell & Gonzalez, 2009). Adaptation may arise from extant genetic variation, *de novo* mutation, or the immigration of tolerant genotypes (Gonzalez *et al.*, 2012). The large population sizes of marine zooplankton allow for high standing genetic diversity, increasing the likelihood of evolutionary rescue (Bell & Gonzalez, 2009; Peijnenburg & Goetze, 2013). Some populations of *C. glacialis* show considerable tolerance to low pH (i.e. Disko Bay), raising the possibility of tolerant sub-populations serving as sources of tolerant genotypes via gene flow, also increasing the chance of local evolutionary rescue (Gonzalez *et al.*, 2012). Genetic differentiation has been found across zooplankton populations in distinct gyres and water masses (Peijnenburg & Goetze, 2013), and in *C. glacialis* populations in the Pacific Arctic (Nelson *et al.*, 2009), though the connectivity of currents in the Arctic would allow for considerable gene flow in certain directions (Figure 11). We do not know the full extent of the variability in low pH tolerance in all *C. glacialis* sub-populations around the Arctic, so the beneficial directions of gene flow are currently difficult to map. However, the fjords on the western coast of Svalbard are influenced by the West Spitsbergen current, the northernmost extension of the North Atlantic current, bringing warm, Atlantic Water from the south (Figure 11). Kongsfjorden is heavily influenced by this inflow (Willis *et al.*, 2006, 2008), while Billefjorden is largely protected by a sill, and the *C. glacialis* population here is considered isolated (Arnkværn *et al.*, 2005; Nilsen *et al.*, 2008). Thus, immigration into western Svalbard fjords is likely to come from the south in the Barents Sea.

Regardless of whether our observations reflect local adaptation or local acclimatization, the findings point to the potential for *C. glacialis*, to gain tolerance to low pH over time (down to pH 6.4), either through adaptation in the population over many generations, or through plastic responses within a generation (acclimatization) and across generations (transgenerational plasticity via epigenetics or maternal effects). Depending on how the rate of environmental change in the future compares to the rate of phenotypic change possible in *C. glacialis*, the negative effects of low pH (seen in C2-C4s; Papers III and IV) could be alleviated in the future.

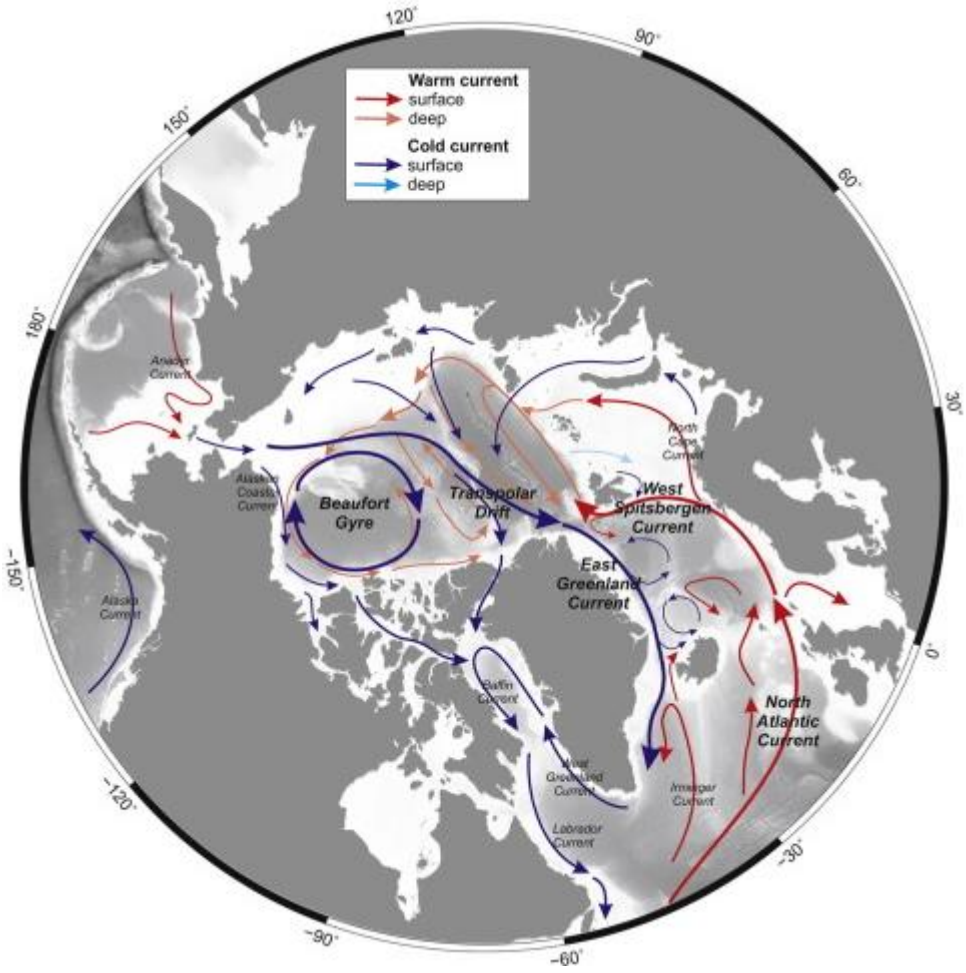

Figure 11. Major currents in the Arctic and sub-Arctic seas, which influence the connectivity of *C. glacialis* populations. Figure from Hunt *et al.* (2016; Figure 1B) .

12 How does the response of *C. glacialis* to low pH vary by developmental stage? (Papers I, III, IV)

The larvae of marine organisms may be the most sensitive life stages to the effects of ocean acidification due to their incompletely developed ion regulation structures or mechanism. In copepods, there has been some evidence of this sensitivity in studies that explicitly compared effects on different stages (Cripps *et al.*, 2014a; Pedersen *et al.*, 2014a). For *C. glacialis*, there is some evidence of stage-specific effects of low pH, though the youngest stages may not be the most sensitive. Results from this thesis and other studies (Table 1, Figure 12) indicate that the early copepodite stages (C2-C4) may experience some detrimental effects in seawater pH predicted for 2100 and 2300 (pH 7.5-7.7), while naupliar stages and older stages (C5s and females) appear to be more tolerant.

For naupliar and young copepodite stages, the results in this thesis are the first studies conducted so far. For eggs and C5s, our results are in agreement with the findings in other studies (Weydmann *et al.*, 2012; Hildebrandt *et al.*, 2014, 2015). We found no developmental delay in eggs that hatched and developed in pHs down to 7.5 (Paper I), which is in agreement with Weydmann *et al.*'s (2012) findings that hatching was not delayed at pH 7.6, but was delayed at pH 6.9. Furthermore, our findings indicate that development, growth and respiration of N2 through C1 was unchanged in pHs down to 7.5 (Paper I), though mortality was not measured and may have been affected. For young copepodite stages, the story is different. The results of Paper III indicate that C2-C3s experience an increased metabolic cost associated with feeding in pHs predicted for the next 100 years (pH 7.6), potentially decreasing the amount of energy they can utilize for growth. Paper IV similarly showed an increase in respiration and decrease in ingestion in C4s from Svalbard, also decreasing their scope for growth considerably (by 19-50 %) in the range of pH predicted for 2100. In the same paper, the ingestion of C3s responded to pH, but didn't decline until below pH 7.6. Together, these data indicate that young copepodites of *C. glacialis*, at least from Svalbard, may experience an energetic cost at seawater pHs realistic for the next century, with important implications for growth. In contrast to the young copepodites, our results and those of others provide extensive evidence that C5s are tolerant to large decreases in pH. Their respiration and ingestion is unaffected in pHs down to 6.4-7.2 in more than three geographically distinct populations (Papers III and IV; Hildebrandt *et al.*, 2014a, 2015), and the respiration

remains unaffected even when exposed to low pH for over 60 days (Hildebrandt *et al.*, 2014).

What might explain this observed pattern of stage-specific sensitivities?

12.1 Nauplii

The tolerance of *C. glacialis* nauplii to pH down to 7.5 is impressive. The maintenance of developmental rate may reflect that it is a relatively static rate, influenced almost entirely by temperature in copepods (Huntley & Lopez, 1992). Therefore, naupliar developmental rate may reflect energetic limitations to a lesser degree than the developmental rate of copepodites. However, if developmental rate was fixed, an energetic cost would reveal itself in smaller body size at each stage (Miller *et al.*, 1977), which also was not observed in the nauplii.

C. glacialis eggs are often released under the sea ice and accumulate at the sea-ice interface (Werner & Hirche, 2001), where they hatch and develop, potentially remaining there to feed on sea ice algae. The narrow sea-ice interface can show extreme variations in carbonate chemistry (CO_2, salinity, pH), both in periods of sea ice growth and melt (Fransson *et al.*, 2013). Compared to the relatively stable pH environment of the open ocean, the variable carbonate chemistry experienced by *C. glacialis* indicates that it may have higher tolerance than copepods not associated with sea-ice.

Further, we have shown that important population-level differences exist in the response of *C. glacialis* to low pH. As the source population for the naupliar experiment (Rijpfjorden, Papers I and II) is different from those used in the experiments on copepodites (Kongsfjorden, Billefjorden, and Disko Bay, Papers III and IV), differences in response between populations may be misinterpreted as differences between life stages. Further investigations on copepodite stages from Rijpfjorden and naupliar stages from Kongsfjorden, Billefjorden, and Disko Bay would shed light on whether our conclusions on stage-specific responses hold true between populations.

12.2 Copepodite CV

The tolerance of stage C5 to low pH may be linked to their capacity for diapause. In preparation for diapause, C5s accumulate much larger lipid stores than previous stages: while they can be twice

the length of the younger C4s (Arnkværn *et al.*, 2005), they can have nearly eight times the lipid mass (Falk-Petersen *et al.*, 2009). The preferential allocation of biosynthesis to lipid or protein biomass may help explain differences in stage's response to low pH (Paper III).

Further, physiological changes associated with diapause may explain the tolerance of C5s to low pH, as these changes often increase stress tolerance in arthropod diapause (MacRae, 2010). In *C. finmarchicus*, diapause brings about changes in lipid metabolism, endocrine signalling, and protection from cellular stress and protein degradation (Tarrant *et al.*, 2008; Aruda *et al.*, 2011). Heat shock proteins, which are characteristically up-regulated in response to stress conditions, are also up-regulated during diapause in several arthropod taxa, including *C. finmarchicus* (Aruda *et al.*, 2011). If these were up-regulated in C5s in the ocean acidification experiments, it may have given them an increased ability to deal with stress from low pH.

Finally, polar copepods have been shown to acidify their extracellular fluids during diapause (Sartoris *et al.*, 2010; Schründer *et al.*, 2013; Freese *et al.*, 2015), potentially contributing to a buoyancy regulation mechanism based on accumulation of heavy ions. In *C. glacialis*, C5s regulate their pH_e from ~7.8 in the summer down to 5.5 during winter diapause (Freese *et al.*, 2015). While this low pH_e in diapause is accompanied by reduced metabolic rate, C5s may accumulate a stage-specific physiological capacity to both regulate pH and tolerate low pH even before diapause.

If physiological preparation for diapause confers tolerance to stressors, including low pH, then this fits with the tolerance seen in C5s but not in C4s (in Svalbard). However, the tolerance observed in Disko Bay C4s would appear to challenge this explanation of differences between C5s and C4s. In some cases, though, C4 *C. glacialis* can also undergo diapause, thus entering a 2-year life cycle. The proportion of the population utilizing a 2-year life cycle in *C. glacialis* is linked to the length of the feeding season (Scott *et al.*, 2000; Falk-Petersen *et al.*, 2009). If diapause preparation confers a tolerance to low pH, the "tolerant" C4s in Disko Bay may be C4s that were preparing for diapause, due to a different (and potentially harsher) environmental regime there compared to Svalbard in 2015.

The capacity for diapause may also explain species-specific differences in the tolerance of copepods to low pH. While Cripps *et al.* (2014a) elegantly showed strong stage-specific differences in mortality in *Acartia tonsa* exposed to low pH, their results show that copepodites

had the lowest mortality, while nauplii, eggs, and adults showed higher rates. However, *A. tonsa* does not undergo the seasonal ontogenetic descent to diapause in older stages, instead producing diapause eggs (McAlice, 1981).

12.3 Young copepodite stages

In contrast to nauplii and C5s, young copepodites (C2-C4) that are either incapable of or not preparing to undergo diapause, are likely focused on protein biosynthesis, and are developing in the relatively stable pelagic zone in the summer, following the ice melt. Beyond these physiological and life history factors, their apparent sensitivity to low pH (in Svalbard populations) is not straightforward to explain. However, regardless of which stage is affected, detrimental effects that are restricted to certain stages nonetheless have the potential to elicit population-level ramifications (Byrne, 2012).

In conclusion, early copepodite stages (C2-C4) of *C. glacialis* may experience some detrimental effects in seawater pH predicted for 2100 and 2300 (pH 7.5-7.7), but naupliar stages and older stages (C5s and females) appear to be more tolerant, potentially due to the variable environments they experience and the physiology associated with overwintering in diapause. Male *C. glacialis* have yet to be studied in the context of ocean acidification.

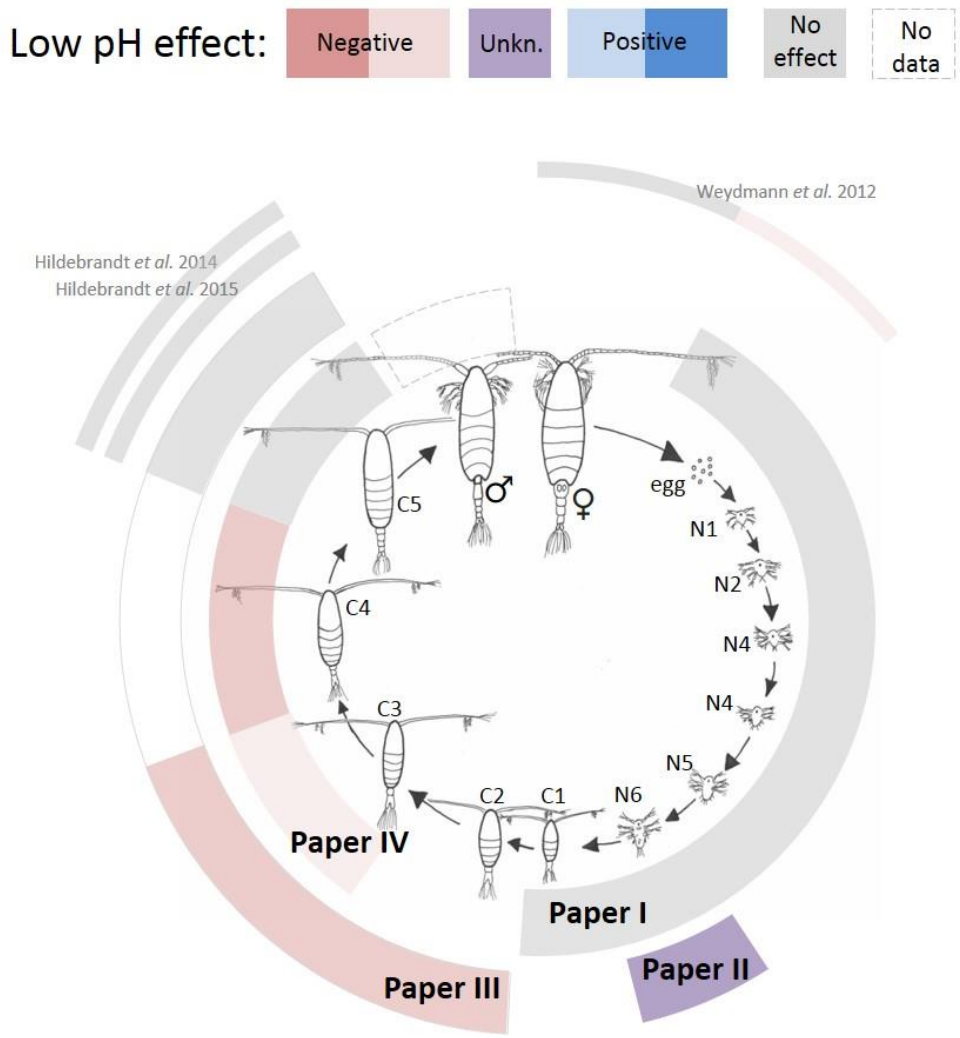

Figure 12. Stage-specific effects of experimental ocean acidification (low pH) on *Calanus glacialis*, showing that the young copepodite stages show the only detrimental effects. Developmental stages include eggs, followed by naupliar stages N1-N6, copepodite stages C1-C5, and adult stages male or female. Contiguous arcs indicate individual studies, with their placement indicating the developmental stages they investigated. The color indicates whether significant effects were found in investigated traits and the potential fitness consequences of the effects (red=negative, blue=positive, purple=unknown fitness effect). Grey indicates that pH did not significantly affect the investigated trait. Light shades indicate effects were only seen at pH levels more extreme than those predicted under future ocean acidification. For details on the traits and pH levels measured in each paper, see Table 1.

Table 1. Effects experimental exposure to low pH on *C. glacialis*, by developmental stage. Red text indicates a significant detrimental effect of the low pH treatment on the measured trait; black text indicates no significant effect, and purple indicates a significant effect with an unknown relation to organismal fitness. A blank indicates that effects have not been investigated at that stage.

STAGE	THESIS PAPERS	OTHER PAPERS
Eggs		Hatching delayed at pH 6.9 (Weydmann *et al.*, 2012)
N1-C1	Respiration (at stage N3, N6 and C1) and growth and developmental rate unchanged down to pH 7.5 (Paper I) Gene expression altered (N6; Paper II)	
C2-C3	Increased respiration (in presence of food, SDA), but a feeding proxy (RNA:DNA) unchanged at pH 7.6 (Paper III) Decrease in ingestion in Kongsfjorden C3s below pH 7.6 (Paper IV)	
C4	Increased respiration and decreased ingestion in 2 of 3 populations. In other population, no effect on respiration or ingestion down to pH 6.4 (Paper IV)	
C5	Respiration and feeding proxies (RNA:DNA and gut DNA) unchanged down to pH 7.6 (Paper III) Respiration and ingestion unchanged down to pH 6.4 in three populations (Paper IV)	Respiration rates, body mass and mortality unchanged down to 7.2 (Hildebrandt *et al.*, 2014) Ingestion rates and body mass unchanged down to 7.2 (Hildebrandt *et al.*, 2015)
Females		Egg production unchanged down to pH 6.9 (Weydmann *et al.*, 2012)
Males		

CONCLUSIONS & FUTURE PERSPECTIVES

The lipid-rich copepod *Calanus glacialis* is a key component of the Arctic marine ecosystem, an ecosystem which is currently undergoing rapid environmental change. This thesis investigated the potential response of *C. glacialis* to future ocean acidification, which is changing the carbonate chemistry of seawater and is occurring at the fastest rate in the Arctic. To do this, we integrated organismal, molecular, and evolutionary methodologies to understand the response of this species to a long-term change. Organismal measures, such as development, growth, respiration, and ingestion rates, provided an approximation of how fitness may be affected in a lower pH ocean (Papers I, III, IV). Long-term studies provided information on the potential for acclimatization or chronic effects of the pH stressor, while investigating the youngest developmental stages was a traditional ecotoxicological approach that focused on finding bottle-necks in sensitive stages (Papers I and II). On the molecular level, transcriptomics gave insight into potentially hidden stress responses and into the cellular basis of naupliar *C. glacialis*' tolerance to low pH (Paper II). The interaction of multiple drivers was also assessed to better understand potential non-additive responses to concurrent stressors (Paper III). Finally, investigating the potential for evolutionary responses to ocean acidification provided an opportunity to put the results of short-term exposures in a longer-term perspective (Paper IV).

13 Effects of future ocean acidification on *Calanus glacialis*

Our organismal-level results from many developmental stages of *C. glacialis* show that it is pre-emptive and potentially misleading to base environmental change related predictions exclusively on short-term experiments on older and adult stages. We found that detrimental effects of low pH exist in previously unstudied stages (Papers III and IV). However, they appear to be restricted to a few stages in a few

populations (C2-C4s in Kongsfjorden and Billefjorden). Furthermore, while effects were stage-specific, we did not find that the most sensitive stages were the youngest stages (Papers I and II). To best assess a species' response to low pH, continuous exposure of the whole lifecycle, allowing for transitions between stages, from fertilization through reproducing adults, should be tested. This has not been done for *C. glacialis*, though our studies include an investigation of continuous exposure from egg to C1. However, additional effects from continuous exposure may be unlikely; studies on the congener *C. finmarchicus* indicates lifetime exposure to pH levels relevant to ocean acidification do not cause changes in important fitness-related traits on the organismal level (Runge *et al.*, 2016).

The metabolic costs of low pH on young copepodites seen in Papers III and IV were plastic responses to a rapid change in pH and a one week exposure. Exposure which begins at early development usually leads to developmental acclimatization that shifts an organism's tolerance towards the current environmental conditions. Therefore, we may expect that these negative metabolic effects may be lessened or absent if the copepods were exposed to low pH from before egg hatching, and that the decrease in pH occurred over decades instead of minutes. Paper IV also indicates that *C. glacialis* is likely able to mediate its tolerance to low pH, either via acclimatization or adaptation. Other studies have shown that copepods can adapt to low pH quickly; *Pseudocalanus acuspes* increased their tolerance to low pH via adaptation in just 2 generations (De Wit *et al.*, 2015; Thor & Dupont, 2015). If the rate of phenotypic change (a shift in low pH tolerance) in sensitive *C. glacialis* populations can match the future rate of change of the pH conditions in their habitat, the negative effects seen in Papers III and IV will not be reflected in the future populations of *C. glacialis* in year 2100.

Aside from young copepodites in Svalbard, the nauplii, C5s and adults of *C. glacialis* show considerable tolerance to low pH, showing unaltered development through half their lifespan at pH levels down to pH 7.5, and unaltered respiration and feeding in C5s down to pH 6.4. Gene expression of nauplii raised at low pH did not show any indications of a stress response, and instead may have had expression characteristic of other pH tolerant species. Though there are few high-resolution, year-round pH datasets, those that exist indicate that the polar oceans may be environments of high natural pH variability. The

magnitude of seasonal variations in pH vary widely by habitat, from a range of 0.05-0.1 pH units in the open subtropical ocean (Rhein *et al.*, 2013) to 0.3-0.45 pH units in seasonally ice-covered Antarctic waters (Shadwick *et al.*, 2013; Kapsenberg *et al.*, 2015) and up to 0.8 in Disko Bay, Greenland (Thoisen *et al.*, 2015). Thus, Arctic shelf seas, with their strong spring bloom and sea-ice dynamics causing high natural pH variability, may select for *C. glacialis* that tolerate a wide range of pH. Diel vertical migrations and seasonal ontogenetic migrations to depth, where pH is often lower than at the surface, also increases the range of pH to which *C. glacialis* is exposed, in contrast to non-migrating zooplankton (Lewis *et al.*, 2013). Finally, the fact that *C. glacialis* undergo diapause in older stages may contribute to their tolerance of low pH, as diapause is accompanied by acid-base regulation and an acidification of the extracellular fluids (Freese *et al.*, 2015).

While low pH may only have mild direct effects on *C. glacialis* physiology in the future, ocean acidification may affect the species indirectly via effects on their food quality (Rossoll *et al.*, 2012; Bermúdez *et al.*, 2016). The majority of ocean acidification studies on copepods, and *C. glacialis*, are incubations of single species exposed to low pH. Mesocosm studies allow the investigation of low pH effects on a whole food web, including the important trophic interactions and shifts in species composition (Riebesell *et al.*, 2010). In an Arctic mesocosm experiment, simulated ocean acidification altered the algal community composition, with implications for the quantity and quality of *C. glacialis* prey. At pHs predicted for 2100, the abundance of diatoms, a preferred prey, decreased and that of picophytoplankton increased (Brussaard *et al.*, 2013; Leu *et al.*, 2013; Schulz *et al.*, 2013). Picophytoplankton are potentially too small to be effectively grazed by large *Calanus* species, and, accordingly, reduced grazing rates in *Calanus* sp. were reported from the same mesocosms (de Kluijver *et al.*, 2013). Similar results, with a community shift towards the smallest phytoplankton prey, was found in a temperate ocean acidification mesocosm experiment, resulting in lower levels of an essential fatty acids, in the copepod grazer *C. finmarchicus*, at pH levels relevant for year 2300 (Bermúdez *et al.*, 2016). While our results (Paper IV) show that low pH can have direct negative effects on *C. glacialis* grazing, these mesocosm studies indicate that shifts in their prey community may have even stronger effects on their feeding in the future.

14 Concurrent environmental changes in the Arctic

The changes that will occur in the Arctic marine environment in the coming centuries are significant (Overland *et al.*, 2014). Unprecedented rates of sea ice loss (Serreze *et al.*, 2007; Comiso *et al.*, 2008; Stroeve *et al.*, 2012), earlier break up of sea ice in spring (Stroeve *et al.*, 2014), loss of ice algae (Dupont, 2012), ocean warming (Steele *et al.*, 2008), declines in dissolved oxygen (Schmidtko *et al.*, 2017), freshening (McPhee *et al.*, 2009; Timmermans *et al.*, 2011), changes in timing and magnitude of phytoplankton blooms (Arrigo & van Dijken, 2011; Bélanger *et al.*, 2013; Ardyna *et al.*, 2014), and northward range shifts of zooplanktivorous predators (Renaud *et al.*, 2012; Berge *et al.*, 2015) are trends already occurring in the Arctic, and predicted to continue in the coming century. Arctic ocean acidification will therefore certainly occur in conjunction with other environmental changes that are known to affect *C. glacialis*.

As with all ectotherms, temperature has significant effects on the metabolism of *C. glacialis*. Warming temperatures increase the respiratory costs and decrease the ingestion rate of *C. glacialis* C5s and females, resulting in a decrease in scope for growth (Alcaraz *et al.*, 2014). While short-term exposures to increased temperature have the same drawbacks as predictors of a species' response to global warming as short-term pH exposures do for predicting ocean acidification effects, many studies have found that the effects of realistic temperature increases are more detrimental than that of realistic reductions in pH. This is true for both a wide range of non-copepod species (Byrne *et al.*, 2009; Arnberg *et al.*, 2013; Troedsson *et al.*, 2013; Waller *et al.*, 2016); on a sea urchin, shrimp, appendicularian, and lobster, respectively) and for copepods (Mayor *et al.*, 2012; Vehmaa *et al.*, 2013; Zervoudaki *et al.*, 2013; Hildebrandt *et al.*, 2014). In *C. hyperboreus* females, for example, respiration, dry weight, and C:N ratio was significantly affected by temperature but not pH, though both drivers were more extreme than expected in the future (Hildebrandt *et al.*, 2014). Models predict that warming and increased primary production accompanying sea ice retreat in the Arctic will shift *C. glacialis*' distribution, allowing a northward expansion in the Pacific and Russian Arctic (Feng *et al.*, 2016) but potentially causing its die-out in the Barents Sea (Slagstad *et al.*, 2011). However, *C. glacialis* is expected to remain primarily a shelf species, which restricts its ability to move northwards beyond the shelves that ring the deep Arctic Ocean. Thus, in a warming, acidifying, and changing Arctic, *C. glacialis* populations may experience declines due to the interactions of multiple stressors, though potentially not primarily due to ocean acidification.

15 Future perspectives

In future studies, three lines of inquiry into *C. glacialis*' response to ocean acidification stand out as deserving attention.

1) Firstly, to complete the investigation of all life stages of *C. glacialis* with regards to low pH tolerance, conducting studies on males and the fertilization process would cast light onto a potential bottle-neck of ocean acidification effects. The gametes of marine organisms are known for their sensitivity to pollutants, and low pH has been found to affect sperm activity and competitiveness in a range of marine organisms (e.g., Morita *et al.*, 2010; Campbell *et al.*, 2016). While most studies, including ours (Papers I and II), begin pH treatments after fertilization has taken place or expose only females to low pH, Cripps *et al.* (2014b) found that exposing copepod males and females to low pH during fertilization produced unexpectedly large reductions in hatching success. In contrast, female-only exposure, the most common technique, appeared to benefit tolerance of the eggs. However, no other studies have replicated this experiment on other copepod species, highlighting the interest of an investigation of the effects of low pH on *C. glacialis* males and fertilization.

2) Secondly, continuing the investigation of population-specific responses to pH in *C. glacialis* from across the Arctic would increase our understanding of the plasticity of the species and its potential to adapt to local pH conditions. Combining these studies with high-resolution, year-round measurements of seawater pH, such as those recently established in Kongsfjorden (AWI-IPEV & COSYNA, 2017), will make the mapping of tolerance ranges to environmental pH ranges more precise. Inclusion of populations from the Pacific Arctic, which have chronically lower pH than the European Arctic, would also add to the explanatory power of arguments for local adaptation. Finally, in absence of the logistical capabilities to keep *C. glacialis* for three years prior to a true common-garden experiment, measuring genetic population structure in the sites investigated will provide some insight into the contribution of genetic differences to the observed phenotypic differences.

3) Thirdly, using molecular tools to investigate the ion-regulatory machinery of *C. glacialis* throughout its development would be very interesting, potentially providing a physiological explanation for the stage-specific tolerances observed. By using ion-channel-specific inhibitors, the relative contribution of the various ion channels (including Na^+/K^+-ATPase, Na^+/H^+ antiporter, and V-H^+-ATPase) to acid-base regulation could be quantified. This would confirm the validity of our findings on the up-regulation of Na^+/H^+ antiporter in *C. glacialis* nauplii, indicating whether this energy-neutral ion channel is a dominant component of their ion regulation in low pH.

GLOSSARY

ACCLIMATION- see acclimatization.

ACCLIMATIZATION- a process in which individuals can maintain their fitness in a new environment via phenotypically plastic changes in it physiology, morphology, or behavior. When this occurs in a laboratory setting, it is referred to as ACCLIMATION. Three types are categorized, based on the time scale upon which they act:

TRANSGENERATIONAL- across generations.

DEVELOPMENTAL- from early development through life.

REVERSIBLE- within a life stage or period of life.

ADAPTATION- a process in which the genetic composition of a population changes in response to natural selection selecting for genotypes that have the highest fitness in a changing environment. If there are heritable traits that confer higher fitness in the new environment, the alleles underlying those traits will increase in frequency in the population.

CLIMATE CHANGE- changes to the global climate caused by increases in greenhouse gases (including CO_2, CH4). In this thesis, referred to as climate warming and ocean acidification caused by anthropogenic CO_2 emissions.

DE NOVO MUTATION- a change to the nucleotide sequence of the genome of an organism, due to errors in replication or DNA damage. Can have either detrimental, neutral, or beneficial effects on the functioning of the organism.

EPIGENETICS- the transgenerational transfer of heritable phenotypes which cannot be explained by genomic DNA sequence. Includes modifications to the DNA molecules (like attachment of histone or methyl groups) which affect gene transcription. Also occurs during development, leading to differentiation of cell types.

EVOLUTION- a process in which the genetic composition of a population changes over time.

FITNESS- capacity of organisms to transfer genetic information to future generations.

GENE FLOW- the transfer of genes (or alleles) between populations of a species, via migration and immigration of individuals.

GENETIC ISOLATION- low or non-existent gene flow between populations.

GENOME- the complete DNA barcode within each cell of an organism, carrying all genes necessary for life. GENOMICS refers to the study of the sequence, structure, function and evolution of genomes.

GENOTYPE- the genetic basis of a trait.

MATERNAL EFFECTS- refers to all processes by which an organism can be affected by the environment of its mother, rather than the environment itself experiences or its own genotype. Can include differential allocation of lipids, proteins, or mRNA by the mother to the eggs.

NATURAL SELECTION (or SELECTION)- the process in which organisms better adapted to their environment tend to survive and produce more offspring.

NUCLEOTIDE- building blocks of DNA and RNA.

ONTOGENY- referring to the development of an organism through its lifespan.

PANMICTIC POPULATION (or PANMIXIA)- random mating within a population or species, indicating extensive gene flow and no genetic isolation between spatially separate sub-populations.

PHENOTYPE- the physical expression of a trait.

PHENOTYPIC BUFFERING- the alteration of an underlying trait (like gene expression, morphology, energy allocation) which allows a more focal, fitness-related trait to remain unchanged despite changes in environment.

PLASTICITY- the variation of a phenotypic trait in an individual without a change in genotype. Can be displayed either 1) across a range of environmental conditions, or 2) due to acclimation or acclimatization at a certain environmental condition. Includes acclimatization, phenotypic buffering, epigenetic factors, and maternal effects.

PROTEOME- the complete set of proteins in an organism, a reflection of the genes transcribed into mRNA and then translated into protein. PROTEOMICS is the study of proteomes, including how they react to environmental drivers.

SPECIFIC DYNAMIC ACTION- the temporary increase in metabolism after feeding, in animals; often associated with the costs of biosynthesis of protein, lipid, and carbohydrate macromolecules.

TRAIT (or PHENOTYPIC TRAIT)- a measurable characteristic of an organism, be it physiological, morphological, behavioral, or molecular. It is a reflection of the genotype and the environment, and may be either static or variable in response to environmental drivers and ontogeny. FITNESS-RELATED TRAITS are those traits that can be correlated to an individual's fitness, typically including reproductive rate, survival, growth, and energy budget.

TRANSCRIPTOME- the complete set of expressed genes (transcribed as mRNA) in an organism, which will then be translated into protein. TRANSCRIPTOMICS is the study of the transcriptome, including how they react to environmental drivers.

LIST OF ABBREVIATIONS

C	Carbon
$CaCO_3$	Calcium carbonate
Chl *a*	Chlorophyll a
C1-C5	Copepodite stages in the lifecycle of a copepod (can be CI-CV)
CO_2	Carbon dioxide
DEG	Differentially expressed genes
DNA	Deoxyribonucleic acid
GO	Gene Ontology, a classification system, characterizing their molecular function, biological process, and cellular component of proteins and genes
IPCC	Intergovernmental Panel on Climate Change
KEGG	Kyoto Encyclopedia of Genes and Genomes, a classification system organizing genes and proteins into the cellular pathways they are components of
mRNA	Messenger RNA, transcribed from DNA to be translated into protein
N	Nitrogen
N1-N6	Naupliar stages in the lifecycle of a copepod (can be NI-NVI)
OA	Ocean acidification
pCO_2	Partial pressure of carbon dioxide
pH	"Power of hydrogen," a measure of acidity, -log[H^+]
pH_e	Extracellular pH
pH_i	Intracellular pH
ppm	Parts per million
RCPs	Representative Concentration Pathways, scenarios of greenhouse gas emissions based on their total radiative forcing
RNA	Ribonucleic acid
Ω	Saturation state of calcium carbonate
sp.	When following a genus name: an unspecified species of that genus
spp.	When following a genus name: multiple species of that genus
µatm	Micro-atmospheres

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