RESEARCH MOTIVATION

Antarctic krill and climate change in the Western Antarctic Peninsula (WAP). Antarctic krill, specifically *Euphausia superba*, are a key species in Antarctic food webs (Everson 2000, Atkinson et al. 2004). They feed on diatoms, microzooplankton, and copepods (Boyd et al. 1984, Hopkins 1985, Price et al. 1988, Pakhomov et al. 1997, Perissinotto et al. 2000) and are the major food source for many top predators including baleen whales, seals, and sea birds including penguins (Smetacek and Nicol 2005). They are commonly found in dense aggregations, or swarms, which are often associated with bathymetry (canyons, continental shelves, and slopes) or frontal zones (Hamner and Hamner 2000, Nicol 2006 and references therein, Ribic et al. 2008). Through feeding and excretion processes, krill are major sources of regenerated nutrients, which support phytoplankton growth (Smetacek et al. 2004, Tovar-Sanchez et al. 2007, Whitehouse et al. 2011). However, krill in the WAP region, the northernmost part of the mainland of Antarctica extending into the Southern Ocean, have declined two-fold since the mid-1970s due to profound changes along the WAP in the past decades (Atkinson et al. 2004; Fig. 1). The west coast of the northern WAP is changing from a cold, dry polar climate to a warmer, humid subantarctic climate (Montes-Hugo et al. 2009) as a result of a 6°C increase in mid-winter surface atmospheric temperatures (> than 5x the global average) in the past 50 years (Skvarca et al. 1999, Vaughan et al. 2003; Fig. 2). This rapid warming is increasing the heat content of seawater over the shelf (Martinson et al. 2008) and reducing both amount and duration of sea ice (Cook et al.
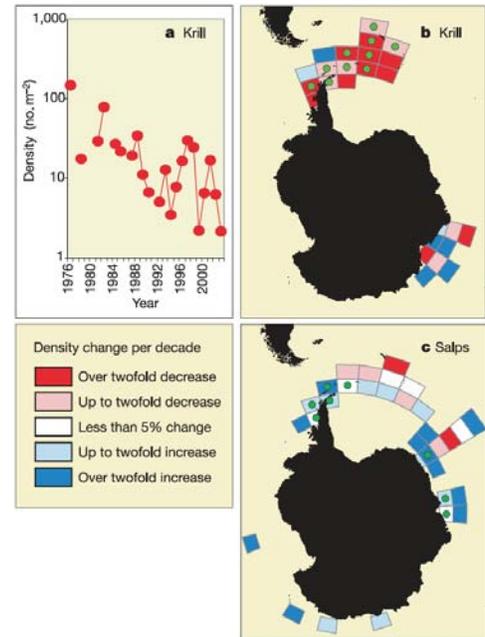

Figure 1. Temporal change of krill and salps. a, Krill density in the SW Atlantic sector. Temporal trends include b, post-1976 krill data; c, 1926–2003 circumpolar salp data. (from Atkinson et al. 2004).

2005, Stammerjohn et al. 2008; Fig. 2). Concurrent changes in the biomass and composition of krill food source have occurred (Hosie et al. 2000). WAP summertime chlorophyll *a* (Chl *a*) has declined by 12% over the past 30 years (Montes-Hugo et al. 2009), and, in the northern WAP region, there has been a non-uniform shift in the phytoplankton size fraction from large diatoms to small cells (< 20 µm) (Moline et al., 2004; Montes-Hugo et al., 2009), on which krill can not efficiently feed (Meyer and El-Sayed 1983, Boyd et al. 1984, Quetin and Ross 1985, Weber and El-Sayed 1985, Haberman et al. 2003). Consequently, the reduction in biomass and size of phytoplankton in the region has been reflected not only in krill declines, but also in the 90% decline of Adélie penguins in the past 30 years in the northern WAP (Ducklow et al. 2007, Trivelpiece et al. 2011).

Reproductive output and recruitment success of *E. superba* in the WAP region has been linked to sea ice extent and duration (Kawaguchi and Satake 1994, Siegel and Loeb 1995, Kawaguchi et al. 2009), and recruitment success is the major driving factor for krill population size (Siegel and Loeb 1995, Atkinson et al. 2004). The reproductive cycle for *E. superba* alternates between a fall/winter resting period, and a maturation and spawning period in spring and summer (Quetin and Ross 2001). Spawning is most intense in late January and February (Quetin and Ross 2001). Krill reproduction is an energetically demanding process (Ross and Quetin 1986, Nicol et al. 1995), but female krill have been observed to lay up to 3588 eggs in a single batch and are capable of producing nine batches in a single season (Ross and Quetin 2000). No correlation has been found between water column temperature and krill stock/spawning stock size (Siegel and Loeb 1995). In

contrast to reproduction, the observed growth of postlarvae krill decreases at high temperaures (>1ºC) (Atkinson et al. 2006). To our knowledge, the Atkinson et al. (2006) study was one of only a handful of studies to directly determine instantaneous growth rates (IGR) of postlarvae krill and was the first to determine the growth rates of *E. superba* under different temperature conditions. Thus, there remains considerable uncertainties regarding krill growth and the effects of environmental factors on growth rates.

Ocean acidification and effects on organisms. In addition to rapid warming in the WAP region, there are predictions that by the end of this century the Southern Ocean will be the first region to be affected by seawater chemistry changes associated with enhanced CO_2. Human activities have driven the rapid 40% increase in atmospheric carbon dioxide (CO_2), from preindustrial levels of 280 ppmv (parts per million volume) to current levels of nearly 393 ppmv (Solomon et al. 2007, ESRL, NOAA; Fig. 3). Current CO_2 concentrations are projected to double by the end of the 21st century (Houghton et al. 1996, Solomon et al. 2007). Nearly one third of emitted anthropogenic CO_2 is absorbed by the oceans (Sabine et al. 2004, Sabine and Feeley 2007), resulting in reductions in seawater pH and alterations in carbonate chemistry (i.e., reductions in carbonate, CO_3^{2-}, ions). The current rapid rate of oceanic CO_2 uptake, one million metric tons of CO_2 per hour (Brewer 2009), is paralleled by rates of acidification at least ten times faster than any change seen in the fossil record over the past 65 million years (Ridgwell and Schmidt 2010). These rapid changes are expected to cause adverse effects, propagating from individual species of both calcifying and non-calcifying marine organisms to entire ecosystems (Riebesell 2004, Raven et al. 2005, Fabry et al. 2008, Doney et al. 2009).

Elevated CO_2 can impact marine organisms both via decreased carbonate saturation that affects calcification rates and via disturbance to acid-base (metabolic) physiology (Pörtner et al. 2004, 2005, Orr et al. 2005). Organisms have different responses to hypercapnia, the CO_2-induced acidification of body fluids. Some organisms in environments predisposed to high fluctuations in pH and CO_2 over short time scales, such as coastal upwelling regions, cannot compensate and suppress metabolism when they encounter low pH (Somero 1985, Seibel and Walsh 2003, Pörtner et al. 2004, Fabry et al. 2008). Other organisms can fully compensate extracellular fluid pH; however, as a result acid-base and ion equilibria reach new steady state values (Seibel and Walsh
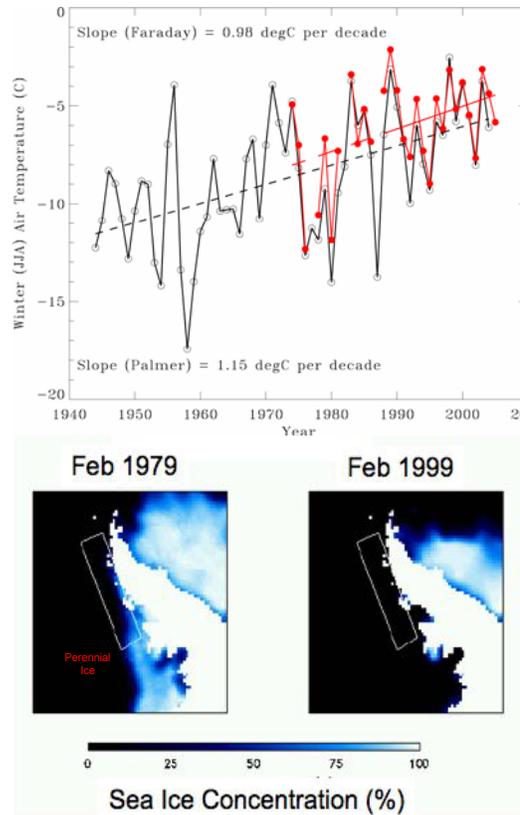

Figure 2. Top; The regional warming of the WAP measured at (black line,1944-2005) the former British Faraday Station) and at Palmer Station (red) indicating a mean winter warming rate of ~1°C per decade. Bottom; Changes in perennial ice in the WAP.
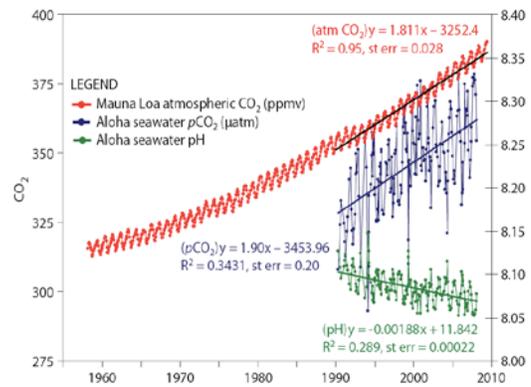

Figure 3. The Keeling curve showing the increase in atmospheric CO2 (red), seawater pCO2 (blue), and a decrease in seawater pH (green) at the HOT series at Mauna Loa over the past 50 years.

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2001, 2003). The result of this adaptation is that there are extra costs of compensation. For instance, organisms may have higher demands for acid-base regulator proteins (e.g., Hu et al. 2011) and would have to work harder to maintain or alter internal acid-base equilibria. Furthermore, their oxygen transport system may be compromised (Pörtner 1990; Pörtner et al. 2004), making them less effective at picking up oxygen (O_2) and forcing them to process more water to extract the O_2 they demand. The two strategies (incomplete or complete compensation) may affect the energetics and performance of an organism differently.

Many studies have demonstrated either no effect of CO_2 on metabolism of organisms (Gutowska et al. 2008) or reduced metabolic activity under elevated CO_2 (Somero 1985, Seibel and Walsh 2003, Pörtner et al. 2004, Fabry et al. 2008, Rosa and Seibel 2008, Maas et al. 2011, Seibel et al. 2012). We (Seibel and colleagues) have shown previously that metabolism of Antarctic pteropods depends on regionally- and time-averaged chl *a* concentrations in the Ross Sea. Seasons with very low Chl *a* are hypothesized to result in low metabolism and subsequent poor recruitment the following season (Seibel and Dierssen 2003, Maas et al. 2012), thus demonstrating metabolic plasticity. High *p*CO_2 can suppress metabolism, measured as oxygen consumption in the Antarctic pteropod, *L. helicina*, by 20%, but the response to CO_2 is dependent on the baseline level of metabolism. We hypothesize that reduced regional Chl *a* levels in 2008 suppressed metabolism of pteropods and masked the effect of ocean acidification. This effect of food limitation was not, we postulate, merely a result of gut clearance and specific dynamic action, but rather represents a sustained metabolic response to regional conditions. Thus, pteropod populations may be compromised by climate change, both directly via CO_2-induced metabolic suppression, and indirectly via quantitative and qualitative changes to the phytoplankton community.

Most studies testing CO_2/pH effects on crustacean zooplankton thus far have focused on growth, development, or mortality of various life stages (Watanabe et al. 2006, Kawaguchi et al. 2010, Bechmann et al. 2011), and a majority of them show decreased hatching success, irregular larval development, or decrease in larval size under conditions of high CO_2 (elevated above ambient) or low pH (below ambient). The subtle effects on the physiology and metabolism of marine zooplankton due to ocean acidification is extremely overlooked. In one study, however, amphipods exposed to high CO_2 concentrations (low pH), exhibited an increase in the expression of the metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh* gene), suggesting that metabolic changes occurred in response to acidification (Hauton et al. 2009). Increases in the expression of metabolic enzymes, ventilatory frequency and effort have also been demonstrated for some fish and elasmobranchs (Burleson and Smatresk 2000, McKendry et al. 2001, McKenzie et al. 2002), and brittle stars (Wood et al. 2008). Such increases may be attributed to enhanced metabolic costs.

We (Saba and colleagues) conducted a preliminary short-term (24 h) CO_2 perturbation experiment along the WAP during Palmer LTER cruise in the austral summer (January 2011) to determine the feeding and nutrient excretion rates of adult Antarctic krill (*Euphausia superba*) at ambient and elevated CO_2 concentrations (~672 ppm) (Saba et al., in review). Under elevated CO_2 conditions (~672 ppm), ingestion rates of krill averaged 82 µg C individual^{-1} d^{-1} and were 2.7 times
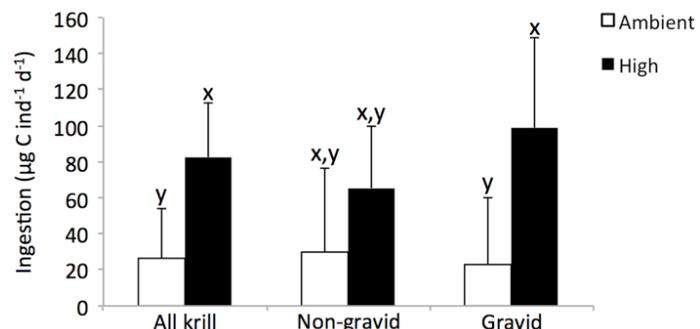

Figure 4. Chlorophyll a ingestion rates of krill exposed to ambient and high ($\overline{X}$ = 672 ppm) concentrations of CO_2. Chlorophyll a was converted to C using equation $C = 80*chl^{0.6}$ from Hewes et al. (1990). Error bars = 2 x SE. x and y denote significance (x > y, one-way ANOVA, $p < 0.05$).

higher than krill ingestion rates at ambient present day CO_2 concentrations (Fig. 4). Additionally, excretion rates of dissolved organic carbon (DOC), ammonium, and phosphate by krill were 1.5, 1.5 and 3.0 times higher, respectively, in the high CO_2 treatment compared to ambient CO_2 concentrations. Excretion of urea, however, was ~17% lower in the high CO_2 treatment, suggesting differences in catabolic processes of krill between treatments. These differences were paralleled with significant observed changes in krill chemical composition, particularly with the relative proportions of body carbon and nitrogen. We hypothesize that increased feeding in krill exposed to high CO_2 reflects the increased energetic cost of maintaining internal acid-base equilibria. These compensation costs reverberated throughout the entire organism by not only increasing rates of feeding and nutrient release, but also creating stoichiometric changes within the krill caused by differential partitioning of C and nitrogen (N) and the utilization and catabolism of proteins or other N-rich compounds (reducing body N, increasing N excretion).

Extra compensation costs of enhanced CO_2 may cause long-term shifts in respiration and metabolic equilibria and eventually hamper growth and reproduction of organisms (Seibel and Fabry 2003, Pörtner et al. 2004, 2005, Fabry et al. 2008, Melzner et al. 2009). We do not yet know whether or not krill have the capacity to fully compensate under elevated CO_2. Additionally, the underlying mechanisms for increased energetic costs of krill at elevated CO_2 in our (Saba and colleagues) study are unknown, but may represent an additional stress that may hamper growth and reproduction and negatively impact an already declining krill population. For instance, increased growth and metabolism was also observed in brittle stars at elevated CO_2, but the cost - muscle wastage - was substantial (Wood et al. 2008). Stoichiometric changes in krill as well as shifts in krill excretia products at elevated CO_2 could be indications of biochemical changes that we were unable to determine in our study. Krill compensating for higher energy requirements at high CO_2 will increase feeding and nutrient release rates, which, under favorable food conditions, may provide sufficient energy to maintain growth and reproduction. However, in time periods (i.e., winter) or locations (i.e., northern WAP) with low food availability, and more importantly with continued reductions in phytoplankton biomass as a result of rapid climate change along the WAP (Montes-Hugo et al. 2009, Schofield et al. 2010), krill may not be able to sustain increased energetic costs. They will allocate more energy to compensating for elevated CO_2 and reduce the energy dedicated to other processes such as growth and reproduction. Responses of krill exposed to chronic elevated CO_2 are unknown, but metabolic suppression of krill would likely occur. *E. superba* have a very pH sensitive respiratory protein that could impair oxygen transport depending on blood buffering (Bridges et al. 1983). The total amount of oxygen that can be carried in the blood in support of routine activities is reduced by half in E. superba under an increase in pCO$_2$ to 1000 ppm (drop in arterial pH from 8.1 to 7.9) (Bridges et al. 1983). This will, in the absence of compensation of the extracellular respiratory acidosis under elevated CO_2, require alteration of blood oxygen binding properties during short-term acclimation or a suppression of total metabolism, which would also act to hamper growth and reproduction. Additionally, rapid warming in the WAP region will act to bring theses different responses to extremes, as metabolic requirements will increase with temperature (Ikeda 1985), further impairing Antarctic krill. Indeed, some invertebrate species have exceptionally narrow thermal windows and are affected by temperature elevations as small as 1-2ºC above habitat temperature (Pörtner et al. 2007).

*Synergistic effects of CO₂ and temperature on organis*ms. As CO**₂** enters the atmosphere it causes global warming due to the greenhouse effect and also diffuses into the ocean causing acidification. Warming oceans lead to increased stratification and reduced gas solubility leading to reduced oxygen content as well. These three variables act synergistically, in some cases, to impair animal performance (Fabry et al. 2008, Rosa and Seibel 2008). Fig. 5 represents the interaction of these variables and their effect on metabolism (ATP demand) and the critical oxygen level (P_{crit}), below which routine metabolism cannot be maintained. Total energy (ATP) demand is influenced by

seawater oxygen partial pressure (pO_2) below critical values. The solid blue line represents the effect of increasing ATP demand on the P_{crit}. The P_{crit} at a basal rate of metabolism is indicated by the horizontal line. The white region indicates that anaerobic metabolism contributes to maintain total ATP flux. For example, an animal can maintain a basal rate of metabolism even below the P_{crit} by increasing anaerobic glycolysis. Alternatively, total ATP demand could be suppressed. In the gray region anaerobic metabolism is insufficient to maintain ATP flux. Metabolism must be suppressed below that point to avoid impaired function. As pO_2 approaches P_{crit}, respiratory adjustments may be made to maintain the rate of oxygen (O_2) provision to sites of consumption. Adjustments include increased heart rate, enhanced gill surface area, increased blood pigment levels and elevated ventilation. The arrows in either direction from 'routine' metabolism, between 'basal' and 'VO_{2max}' (the maximum rate of aerobic metabolism), indicate the maximum aerobic scope for activity. Oxygen concentrations in polar regions are not

Figure 5. Interactions of global warming, ocean acidification, and reduced oxygen on the metabolism (ATP demand) of animals.

expected to reach limiting values due to climate change. However, as warming along the WAP elevates ATP demand and as CO_2 impairs O_2 transport, the P_{crit} is elevated and organisms may be pushed toward physiological limits. Thus, the synergism between these variables may be responsible for impaired function in polar regions. A long-term dataset available for the energetic condition of *E. superba* will provide a baseline upon which to accurately assess the impact of high CO_2 and temperature on energy requirements of these krill.
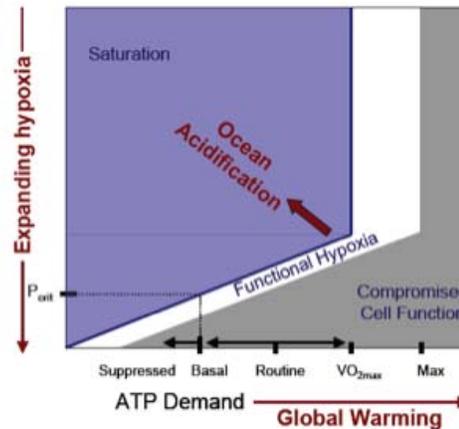
A better understanding of these phenomenon through the elucidation of the physiological processes behind it is critically important, yet no studies have measured krill acid-base regulation, metabolism, growth, or reproduction in the context of ocean acidification or synergistic "greenhouse" conditions of elevated CO_2 and temperature. Prolonged exposure experiments are necessary to pinpoint the underlying physiological mechanisms of CO_2 and temperature induced responses, determine potential costs of increased energetic demand, determine potential acclimation of krill to high CO_2, and understand the associated feedbacks on the ecosystem and biogeochemical cycles. Our proposed study addresses these needs.

RESEARCH OBJECTIVES, STRATEGY, AND HYPOTHESES

Aim 1: Examine the independent and synergistic effects of CO_2 and temperature on acid-base regulation and metabolic physiology of adult *Euphausia superba*. *We will conduct both short (48 hours) and prolonged (21 days) exposure experiments at ambient CO_2 and temperature and those predicted by year 2100 (750 ppm CO_2 [Houghton et al. 1996, Solomon et al. 2007]; +2°C more than ambient temperature [Meredith and King 2005, Murphy and Mitchell 2005]). Specifically, we will monitor over time krill internal acid-base regulation (blood pH, hemocyanin/O_2 equilibration), oxygen binding ability, oxygen consumption (respiration), and rates of feeding and nutrient excretion. Conducting short and prolonged incubations will provide insight into the tolerance and potential acclimation of Antarctic krill to impending ocean conditions.*

Hypothesis 1a: Krill metabolism is compensated completely in response to elevated CO_2, but at an energetic expense
- Enhanced rates of respiration, feeding, and nutrient excretion

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- Little to no long-term change in blood pH and oxygen binding ability

Hypothesis 1b: Krill do not compensate completely, leading to metabolic suppression
- Reduced rates of respiration, feeding, and nutrient excretion
- Reduced blood pH and oxygen binding ability
- Responses will be magnified in high CO_2/high temperature treatment

Hypothesis 2: Krill may initially respond as described in Hypothesis 1a, but under chronic exposure to elevated CO2 and temperature, the response may shift to that described in Hypothesis 1b (metabolic suppression).

Hypothesis 3: The extra costs of compensation (e.g. higher demands for internal acid-base regulation) are necessary to protect oxygen transport due to pH sensitivity of hemocyanin-oxygen binding.

Aim 2: Determine potential changes in growth and reproduction of *Euphausia superba* due to elevated temperature and the increased energetic cost of acid-base regulation to compensate for elevated CO_2. *In prolonged exposure experiments (30 days) under varying levels of CO_2 and temperature, we will 1) monitor growth rates in juvenile krill, 2) determine spawning frequency and fecundity in adult gravid female krill, and 3) measure internal acid-base regulation parameters and oxygen binding ability in both life stages of krill. The inclusion of multiple krill life stages in the proposed work will help to identify critical life cycle phases that may be more vulnerable to climate change and will help to predict the fate of Antarctic krill populations in a rapidly changing climate.*

Hypothesis 1: Under conditions of high CO_2 and temperature in perturbation experiments, growth rates of juvenile krill and spawning frequency and fecundity of adult gravid female krill will be reduced compared to krill in ambient CO_2 treatments.

Hypothesis 2: These reductions in krill growth and reproduction under elevated CO_2 are caused by higher demands for internal acid-base regulation or a metabolic suppression.

METHODS

This ambitious proposal is achievable because it is highly leveraged with the already funded Palmer Long Term Ecological Research (PAL LTER) program, a time series that has just entered its 21st year. Investigators in this project have full access to the extensive phytoplankton, krill, and environmental (physical and chemical) data. In addition, we will have access to infrastructure and data collection mechanisms of PAL LTER at little to no cost to this proposal.

Field season plan. We propose to conduct the proposed research at Palmer Station during the summer field seasons in Year 1 (2013/2014) and Year 2 (2014/2015). This land-based field program will provide a stable environment with which to complete the CO_2/temperature perturbation experiments as Palmer Station is already well-equipped with large aquarium tanks, a large walk-in incubator with custom temperature and light regulation, constant and ample supply of inflowing natural seawater, an Aqua-medic pH/pCO_2 system capable of regulating the amount of CO_2 bubbling in up to 24 independent systems, instruments (i.e., spectrophotometer, fluorometer) and laboratory space for running real-time carbonate chemistry and biomass samples, and a vast supply of other equipment and resources necessary to execute the proposed project. Year 1 will focus on the independent and synergistic effects of CO_2 and temperature on acid-base regulation and metabolism of adult krill. In Year 2, we will assess the acid-base regulation of multiple krill life stages (juvenile, and adult gravid females) and measure growth in juvenile krill and spawning in gravid female krill during prolonged (30 d) exposures to elevated CO_2 and temperature.

Year 1: Independent and synergistic effects of CO_2 and temperature on adult krill acid-base regulation and metabolism

Preparation of seawater for perturbation experiments. Multiple 50 L carboys equipped with custom-made gas inflow/outflow and sampling ports will be filled with unfiltered seawater. The seawater will be equilibrated to target CO_2 concentrations by bubbling with ambient air (ambient CO_2) or, for high CO_2 (750 ppm) treatments, by regulating the amount of CO_2 bubbling with an Aqua-medic pH/pCO_2 system. Carboys will either be kept in a water bath (2400 L tank) with flow through seawater at ambient temperature (ranges from -2°C to +2°C during the summer season) or in the adjacent walk-in incubator set to +2°C above ambient temperature (high temperature). Thus there will be four treatments: 1) ambient temperature/CO_2 (control), 2) ambient temperature/high CO_2, 3) high temperature/ambient CO_2, and 4) high temperature/CO_2.

Carbonate chemistry. The water in the carboys will be subsampled daily for pH and total alkalinity (TA; methods outlined in Dickson et al. 2007) to monitor carbonate chemistry and will be used to fill/exchange water in incubation containers during the course of the perturbation experiments. Seawater pH will be determined spectrophotometrically using the indicator dye *m*-cresol purple (outlined in Dickson et al. 2007). TA will be determined with an open-cell, potentiometric titration of seawater with 0.1 M HCl following the potential of a pH electrode (Dickson et al. 2007). Measurements of pH and TA will be quality controlled using certified reference materials (CRMs) obtained from the U.S. National Institute for Standards and Technology (pH) or Andrew Dickson at UCSD Scripps Institute of Oceanography (TA). Additional parameters pertinent to an accurate estimation of seawater pCO_2 will be measured including dissolved silicate (Strickland and Parsons 1968), phosphate (Grasshoff et al. 1999), and salinity (Guideline AutoSal salinometer). Final carbonate system parameters and pCO_2 will be calculated using CO2calc software (Robbins et al. 2010) using a total pH scale (mol/kg-SW), K1 and K2 constants (Mehrbach et al. 1973) with refits (Dickson and Millero 1987), and the acidity constant of $KHSO_4$ in seawater (Dickson 1990).

Collection of organisms and experimental set-up. Krill will be collected via near-surface net tows (2 m diameter net, 1000 μm mesh, non-filtering cod end,) along the northern grid stations near Palmer station (600 line, Fig. 6). These collections will be coordinated with and conducted by the collaborating scientists on board the R/V Laurence M. Gould (Chief scientist, Dr. Hugh Ducklow) at the beginning (early January) and end (early February) of the annual LTER cruise, which will allow us to conduct two, replicate 3-week sets of experimental perturbations. For each of the two sets of experiments, a short-term incubation (48 hour) with multiple timepoints will be set up first to determine the immediate acid-base response (changes in blood and tissue pH, hemocyanin/O_2 equilibration, oxygen binding ability) of krill to elevated CO_2 and temperature. A prolonged (21 day) incubation will immediately follow, during which we will: 1) determine krill oxygen consumption (respiration), feeding rates, nutrient excretion rates, biochemical composition,

Figure 6. Palmer LTER study region along the WAP showing the annual cruise sampling grid (filled squares). White dot, Palmer Station (from Ducklow et al. 2007).

and metabolic enzyme activity at multiple timepoints, and 2) monitor krill internal acid-base regulation (blood and tissue pH, hemocyanin/O_2 equilibration) and oxygen binding ability over time (see schematic, Fig. 7). Upon collection, krill will subsequently be expedited to Palmer Station and sorted. To minimize individual variation in metabolic activity measurements, 2100 individual adult
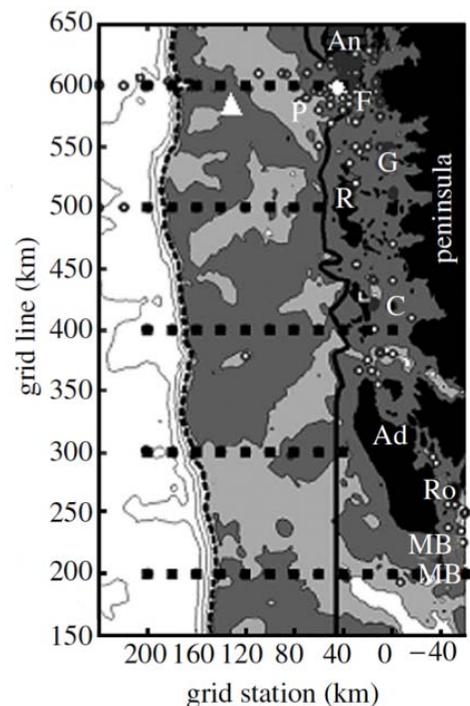
non-gravid females of matched size will be selected for the perturbation experiments. We will randomly select 600 krill for the short-term incubations and 1500 will be placed in a 2400 L holding tank with flow through unfiltered seawater at ambient temperature until the start of the prolonged perturbation experiment.

   Short-term (48 hour) perturbation. For the short-term perturbation (48 h), 15 of the preselected krill will be placed into each of 40, 5 gallon (~20 L) buckets (600 krill total) filled with unfiltered seawater equilibrated to target temperature and CO_2 (Fig. 7). Thus, there will be 10 buckets for each of the four treatments outlined above. The buckets used for the ambient temperature treatments will be kept in a water bath (2400 L tank) with flow through seawater, and the buckets used for the high temperature treatments will be placed in the adjacent walk-in incubator set to +2℃ above ambient (Fig. 7). The buckets will be sealed, and the water inside the buckets will be exchanged daily with fresh, equilibrated seawater to maintain target pH, food and oxygen concentrations and to flush out wastes (i.e., molts, fecal material, ammonium). Seawater in sealed 20 L containers has been shown to maintain pH for 24 hours (Jim McClintock, pers. comm.). There will be 7 timepoints during the short-term perturbation: T_0, T_{30min}, T_{1h}, T_{2h}, T_{12h}, T_{24h}, T_{48h}. At each timepoint, one krill will be removed from each bucket, totaling 10 krill/treatment; 5 krill will be sampled for blood pH (n=5), and 5 krill will be pooled

Figure 7. Schematic of Year 1 CO_2 and temperature perturbations. ● =20 L bucket with 15 adult krill.

and sampled for hemocyanin/O_2 equilibration curve/ O_2 binding ability (n=1).

   Krill hemocyanin, blood oxygen binding ability, and acid-base parameters. Immediately upon removal from the water, individual krill will be quickly and gently blotted dry using tissue paper. Hemolymph will be extracted from individuals using a microsyringe (Hamilton, 50 µl capacity), the needle of which will be inserted dorsally, directly into the pericardium, via the arthrodial membrane that joins the abdomen to the thorax. The haemolymph will be pooled in order to obtain enough hemolymph for the construction of HcO_2-binding curves (~500 µl). The [Hc] in the hemolymph of individual krill will be estimated using an established spectrophotometric method (Nickerson and van Holde 1971, Hagerman 1983, Baden et al. 1990). Twenty µl of haemolymph will be made up to a final volume of 600 µl with an appropriate saline solution (Schlieper 1972, p 335). The absorbance of the resultant mixture ($\lambda = 335$ nm) will be measured using a spectrophotometer and quartz cuvettes (Hel, path length 1 cm, max. capacity = 1.5 ml). [Hc] will be calculated using the extinction coefficient given by Nickerson and van Holde (1971) and assuming Mr = 75 kDa for krill subunits. To compare the O_2-binding properties of Hc from krill kept under different environmental conditions, pooled hemolymph samples (each 0.5 ml) will be dialysed against a stock crustacean saline solution (buffered at pH = 7.80 using Tris) using the micromethod of Horowitz and Barnes (1983). Oxygen-binding curves for dialysed haemolymph samples will be constructed using a spectrophotometric technique in tonometers (quartz cuvettes fused to pear-shaped flasks (Seibel,

submitted). Dialysed haemolymph samples will be equilibrated against gas mixtures, produced by precision gas-mixing pumps (Sierra Instruments) and changes in absorbance (at $\lambda = 335$ nm) accompanying step-wise oxygenation of deoxygenated samples will be recorded. The half-saturation values (P_{50}) will be calculated using the Hill equation.

Acid-base parameters of the tissue and blood will be measured according the Best Practices Guide for Ocean Acidification (Pörtner et al. 2010). The hemolymph and tissue pH will be measured using microelectrodes requiring only 10 µl sample. Total CO_2 will be measured using a Licor 7000 CO_2 analyzer. The mean intracellular (pHi), total CO_2 and the non-bicarbonate buffer value of tail (abdomen) muscle tissue will be determined by the homogenate method (Pörtner et al. 1990). Briefly, tissue samples will be ground in liquid nitrogen under a nitrogen atmosphere using a mortar and pestle, and the tissue powder will then be thawed in a medium (5x tissue weight) containing potassium fluoride (KF) and nitrilotriacetic acid (NTA). The use of the NBS pH scale for body fluids allows the use of well-established physiological concepts and methodologies (Pörtner et al. 2010). The NBS scale appears the most appropriate at present, due to ionic concentrations and osmolarities of body fluids. The pH electrodes will be equilibrated in seawater prior to calibration and also prior to the actual analyses in body fluids. Acid-base parameters will be analyzed by constructing a pH/bicarbonate diagram, also called Davenport-diagram, which quantifies respiratory (via changes in pCO_2) and non-respiratory processes contributing to changes in the acid-base status. The pH/bicarbonate diagram illustrates the relationships between pH, bicarbonate concentrations and pCO_2 as they result from the Henderson-Hasselbalch-equation of the pCO_2/bicarbonate system (pH = pK + log[HCO_3^-/[CO_2]).

Prolonged (21 day) perturbation. The prolonged (21 d) perturbation will be set up in a similar manner to the short-term perturbations, except that there will be 25 buckets, each with 15 krill, for each of the four treatments: 1) ambient temperature/CO_2 (control), 2) ambient temperature/high CO_2, 3) high temperature/ambient CO_2, and 4) high temperature/CO_2 (Fig. 7). There will be 6 timepoints during the prolonged perturbation (T_0, T_{2d}, T_{5d}, T_{10d}, T_{15d}, T_{21d}) with daily water exchanges with fresh, equilibrated seawater. At each timpoint, one krill will be removed from each bucket, totaling 25 krill/treatment; 10 krill will be used for separate 24 h feeding/nutrient excretion experiments, 5 krill will be used for separate 24 h respirometry incubations to measure O_2 consumption, and 10 krill will be analyzed for acid-base regulation as described above for the short-term incubations [5 krill used for blood pH (n=5) and 5 krill pooled for hemocyanin/O_2 equilibration curve/ O_2 binding ability (n=1)].

Feeding and nutrient excretion rates. For each of the four treatments, 16, 4 L bottles will be filled with the appropriate equilibrated seawater. Three bottles will serve as T_0 controls (no krill added) and will be sacrificed for an initial suite of samples. Three bottles will serve as T_f (24 h) controls, and we will add 1 krill to each of the remaining 10 bottles (T_f treatments). The T_f bottles will be capped to maintain target pH, incubated in the appropriate location to maintain desired temperature (water bath for ambient temperature; +2°C above ambient in incubator for high temperature), and incubated for 24 hours before sacrificed for the final timepoint. In addition to carbonate chemistry related samples referenced above, initial and final samples will be taken for fluorometric chlorophyll (phytoplankton biomass; Parsons et al. 1984), HPLC pigments (phytoplankton composition; Wright et al. 1991), bacterial abundance (flow cytometry; Bouvier et al. 2007), microzooplankton abundance (5% Lugol's; Utermöhl 1931, Hasle 1978), algal identification and abundance (2% formalin; Haas 1982), PC/PN (Carbon-Hydrogen-Nitrogen elemental analyzer, Carlo Erba Instruments, NA 1500 Series 2), ammonia (Grasshoff et al. 1999), dissolved organic carbon (Peltzer et al. 1996), urea (Price and Harrison 1987), nitrate/phosphate/total dissolved nitrogen/total dissolved phosphorus (Grasshoff et al. 1999, Bronk et al. 2000, Sharp 2002).

Clearance and ingestion rates of *E. superba* on both algae and microzooplankton will calculated according to Frost (1972). Krill nutrient release rates (in ng ind.$^{-1}$ h^{-1}) will be calculated

according to Miller and Glibert (1998), but modified according to (Saba et al. 2009) to correct for nutrient uptake by phytoplankton and bacteria. At the end of the 24 h feeding/nutrient excretion incubation, the length and wet weight of each krill will be measured prior to being placed into individual 5ml cryovials, flash frozen in liquid nitrogen, and stored at -80 °C for later analysis of protein content and metabolic enzyme activity in the home laboratory. Each krill will be homogenized and split for analysis of the following: percent protein (Lowry et al. 1951), citrate synthase (CS; Childress and Somero 1979, Geiger et al. 2001), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) (Walsh et al. 1989). The activity of enzymes CS, MDH, and LDH are proxies for overall metabolism and respiration (Lehninger 1982, Geiger et al. 2001, Donnelly et al. 2004).

O_2 consumption. Five krill for each of the four treatments will be transferred into individual 100 ml glass respirometry chambers (containing over 30 times more water in grams than wet weight of adult krill) from Loligo Systems. The chambers will be temperature-controlled via submersion in a water bath containing either flow through seawater (ambient temperature) or $+2\degree C$ above ambient seawater (high temperature). Oxygen consumption will be measured via flow-through respirometry (Wells and Warinner 1968, see overview in Lighton 2008). A holding tank of seawater (0.22 μm filtered) used to flow through the chambers will be kept at desired temperature, and the pCO_2 of this source water will be maintained at target levels using a pH/pCO_2 STAT system (Loligo). Source water will be pumped through the respirometry chambers at a constant flow rate of 500 ml min^{-1} and will recirculate through the holding tank. The inflow line of each respirometry chamber will contain a pump and galvanic cell oxygen probe/meter, and the outflow line will contain another pump and oxygen probe/meter as well as a pH probe (also from Loligo Systems). Krill will be acclimated to the vessel for 6-12 hours, or until respiration rate stabilizes, to eliminate any stress caused from the transfer process. After this acclimation, oxygen consumption for each krill will be calculated as the difference of oxygen coming into and leaving the chamber over a period of 12-24 hours using the equation: $VO_2 = F * ([O_2]in - [O_2]out)/BW$, where VO_2 = oxygen consumption rate (mg O_2/kg/hour); F = water flow rate (liters/hour); $[O_2]in$ = oxyen content in water inflow (mg O_2/liter); $[O_2]out$ = oxygen content in water outflow (mg O_2/liter); and BW = body weight of experimental animal (kg). At the end of the ~24 hour O_2 consumption incubation, the length and wet weight of each krill will be measured prior to being placed into individual 5ml cryovials, flash frozen in liquid nitrogen, and stored at -80°C for later analysis of dry weight and particulate carbon and nitrogen in the home laboratory. Each krill will be dried and weighed, then homogenized and subsampled for total particulate C and N (n=2 per krill) and particulate organic carbon and nitrogen (POC and PON, respectively; n=2 per krill) after vapor phase acidification with concentrated hydrochloric acid on a Carbon-Hydrogen-Nitrogen elemental analyzer (Carlo Erba Instruments, NA 1500 Series 2) according to Hedges and Stern (1984). Particulate carbonates were calculated as the difference between total particulate C and POC (Hedges and Stern 1984).

Year 2: Effect of CO_2 and temperature on krill growth and reproduction

Preparation of seawater for perturbation experiments. Seawater for the perturbations in Year 2 will prepared the same as that in Year 1 and again, there will be four treatments: 1) ambient temperature/CO_2 (control), 2) ambient temperature/high CO_2, 3) high temperature/ambient CO_2, and 4) high temperature/CO_2. The water in the carboys will be subsampled daily for pH and total alkalinity, dissolved silicate, phosphate, and salinity (methods described above) to monitor carbonate chemistry and will be used to fill/exchange water in incubation containers during the course of the perturbation experiments.

Collection of organisms and experimental set-up. Krill will be collected as described in Year 1 at the beginning (early January) and end (early February) of the annual LTER cruise. To increase our sample size for spawning and growth measurements, which can be variable between individuals

(Ross and Quetin 1983), the first set of 30-day perturbations in early January will be for measuring the spawning frequency and fecundity of adult gravid females and the second set of 30-day perturbations in late January/early February will be for determining growth in juvenile krill. For each of the two perturbation experiments, the collected krill will subsequently be expedited to Palmer Station and sorted, where adult gravid female *E. superba* or juveniles will be sorted. Gravid females, which will release eggs within a week (Ross and Quetin 1983), will be easily identified by their red thelycum (Ivanov 1970) and blue-gray or opaque mass of eggs present in the thoracic cavity (Ross and Quetin 1983). Krill measuring ~25 mm in length are characterized as juveniles (Siegel and Loeb 1994). As in Year 1 experiments, krill of matched size and condition (specifically in adult gravid females) will be selected for the CO_2 perturbation experiments to minimize individual variation in growth, spawning frequency, and fecundity measurements. A subset of collected juvenile and adult gravid female krill will be sacrificed for T_0 measurements of chemical composition, hemocyanin, blood oxygen binding ability, and acid-base parameters (described above) prior to the start of the experiments.

Spawning frequency and fecundity of gravid female krill. The prolonged perturbation to measure the spawning frequency and fecundity of adult gravid female krill will be set up in a similar manner to the Year 1 perturbations, except that there will be 50, 4 L polycarbonate bottles, each with 1 gravid female krill, for each of the four CO_2/temperature treatments. Female krill can produce continuous batches of eggs throughout the summer season (Ross and Quetin 1983). They can lay thousands of eggs in a single batch and are capable of producing multiple batches in a season (Ross and Quetin 2000). These perturbation experiments will each run for 30 days to ensure sufficient time for all krill to produce have a minimum of 1 spawning event. Water will be changed daily as has been described in the Year 1 perturbations. Spawning frequency and fecundity experiments will be conducted according to Ross and Quetin (1983) and Quetin and Ross (2001). Each of the chosen females will be isolated in a 4-L wide-mouth polycarbonate bottle filled with equilibrated seawater pre-screened with 333 μm mesh screen to remove any krill eggs in the incubation water (egg diameter is ~550-800 μm; Harrington and Ikeda 1986). A circle of 1000 μm mesh will be placed 20 mm above the bottom of each bottle to allow spawned eggs to sink without being eaten by the krill. We will check the bottles for eggs every 24 hours for 30 days. If eggs are released, the female will be removed from the bottle, quickly weighed and measured (total length), and placed into a new bottle filled with fresh equilibrated seawater. Eggs will be collected by attaching a funnel with a 20-ml glass vial to the opening of the experimental bottle. As the bottle is inverted, the eggs will sink into the vial. Each batch of spawned eggs will be counted and the diameters of 50 embryos from each spawning female will be measured under a dissecting microscope to the nearest 10-20 μm. The total volume of the batch (eggs from one spawning episode) will be measured to the nearest 0.025 ml in a narrow diameter (0.4 mm ID) calibrated glass tube. At the end of the 30 day incubation, females will be weighed and measured before split for measurements of hemocyanin, blood oxygen binding ability, and acid-base parameters or freezing for later analysis of dry weight and PC/PN.

Growth rates of juvenile krill. The prolonged perturbation to measure growth rates of juvenile krill will be set up in a similar manner to the Year 1 perturbations, except that there will be 50, 2 L polycarbonate bottles, each with 1 juvenile krill, for each of the four treatments. Each juvenile krill will be weighed and measured (total length) prior to addition to the bottles. Because Antarctic krill molt every 20-30 days (0ºC; Buchholz 1991), these growth experiments will each run for 30 days to ensure sufficient time for all krill to produce at least one molt. Water will be changed daily as has been described in the Year 1 perturbations. Each bottle will be checked twice daily for molts. We will calculate daily growth rates of juvenile krill using the instantaneous growth rate (IGR) method according to Quetin and Ross (1991), Nicol et al. (1992), Atkinson et al. (2006), and Tarling et al. (2006). Crustaceans grow in length in a series of steps, coinciding with each molt (Atkinson et al. 2006). This is defined as the growth increment, GI, or the percentage change in body

length at molt. The period between molts is the intermolt period, IMP (days). If we observe a krill molt in the bottle, the animal and exuviae will be removed and the length of the uropods on both will be measured. The percentage change in size at molt of each uropod will be noted and the growth rate will be expressed as the mean percentage change of both uropods (Eq. 4 in Tarling et al. 2006). We will then calculate the daily growth rate, DGR (mm d^{-1}), as a function of GI and IMP using Eq. 2 in Tarling et al. (2006): $(L_{pre}$ x GI)/(IMP x 100), where L_{pre} is the length of each krill before molting. IGR experiments usually only last a few days (Quetin and Ross 1991). Our perturbations will last 30 days, allowing us to observe not only the initial growth rates (representative of in situ conditions), but also the growth rate of krill that will experience prolonged variable environmental conditions (Nicol et al. 1992). At the end of the 30 day incubation, juveniles will be weighed and measured before split for measurements of hemocyanin, blood oxygen binding ability, and acid-base parameters or freezing for later analysis of dry weight and PC/PN.

Data and Statistical analysis. Statistical comparisons of the effects of CO_2 and/or temperature on feeding rates, nutrient release rates, oxygen consumption, acid-base regulation, biochemical composition, growth, spawning frequency, and fecundity will be made by 1-way ANOVA, employing the $p = 0.05$ level of significance.

Krill abundance and spatial distribution in and upwelling canyon region

Deep cross-shelf troughs along the WAP provide a possible conduit for the upwelling and circulation of warm, nutrient-rich Upper Circumpolar Deep Water (UCDW) (Klinck et al. 2004), which has been found to support the growth of diatoms and krill (Prezelin et al. 2000). Thus, these canyons are hypothesized to be focal areas for ecosystem processes that result in predictable,

elevated food resources for top-predators such as penguins, influencing their foraging ecology and distribution of breeding and wintering populations. The Adélie penguins breed in locations where deep ocean canyons exist near the land margin and data indicate that during all seasons penguins more consistently foraged over deep cross-shelf canyons (Erdmann et al. 2011; Fig. 8). The Palmer Deep Canyon is located in the northern WAP region, south of Palmer Station (Anvers Island) (see Fig. 6, 8). The head of this submarine canyon is within the location of yearly-averaged high density Adelie penguin foraging. These canyons, while potential "hot spots" for phytoplankton, krill, and penguins, are also areas where deep high pCO$_2$, low pH corrosive water is upwelled. Therefore, it is pertinent to establish how environmental stressors such as elevated temperature and CO_2 will affect krill physiology, metabolism, growth, and reproduction (proposed perturbation experiments), combined with the spatial distribution of

Figure 8. Summer foraging distributions of Adélie penguins in the LTER grid during 24 months beginning on 4/1/2001. The data are based on birds (N=186) tagged with satellite-linked transmitters at summer breeding colonies. These areas correspond precisely with deep, cross-shelf canyons that offer predictable prey concentrations due to a combination of upwelling, bathymetry, & circulation (from W. R. Fraser).

krill near deep cross-shelf canyons (current project to be carried out by the Saba/Schofield Palmer LTER group this upcoming field season, 2012/2013), in order to understand how future conditions will affect the overall fate, not only of this keystone species, but also of the food web of the entire WAP ecosystem.

Using a combination of shipboard sampling aboard the R/V Point Sur and *in situ* observations with a fleet of Slocum Webb gliders (Schofield et al. 2007) equipped with a suite of instruments, including an acoustic doppler current profiler (ADCP), Saba (with PI and co-PI of PAL
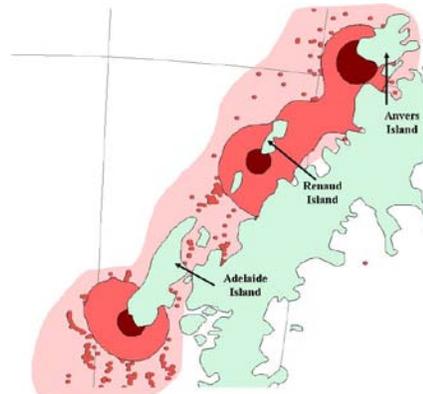
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LTER, Hugh Ducklow and Oscar Schofield, respectively) will study the abundance and distribution of krill in relation to seawater temperature, pH, and pCO$_2$ around a deep cross-shelf canyon (Palmer Deep). The R/V Point Sur will be utilized by Palmer LTER group this upcoming field season (2012/2013). Because of the small size of this ship, relative to the R/V Laurence M. Gould, we have the ability to access the Palmer Deep canyon region for intense sampling. However, this upcoming season (2012/2013) is a trial run and there is no guarantee that it will be available during the field seasons of this proposed project (2013/2014 and 2014/2015). In the event that we do have an opportunity to utilize the R/V Point Sur during the proposed field seasons, we will repeat this effort in Years 1 and 2 of this proposed study at no cost to this proposal. In any event, we will work to combine krill distribution results from the comprehensive robotic/shipboard field effort (from the upcoming 2012/2013 season and potentially seasons 2013/2014 and 2014/2015 of the proposed study) with results from the proposed CO$_2$/temperature perturbations (2013/2014 and 2014/2015) to determine seawater pH and pCO$_2$ that krill are exposed to in their natural environment and estimate the percentage of krill population whose metabolism may be affected by canyon-associated upwelling of warm, corrosive water.

EDUCATION AND OUTREACH: BRINGING MARINE SCIENCE TO THE MIDWEST

The proposed field effort outlined above will be complemented by an extensive education and broader impact effort focused on bringing marine science to both rural and urban high schools in the Midwest (Kansas), which are geographically underrepresented. A multitude of U.S. reports link Science, Technology, Engineering, and Mathematics Education (STEM) education to the future security and economic success of the United States (President's Council of Advisors on Science and Technology 2010, U.S. Department of Commerce 2012) while at the same time, studies show citizens are not being educated for careers in STEM or STEM-related fields (National Academy of Sciences, National Academy of Engineering, and Institute of Medicine 2010). For example, the number of jobs at all levels that require knowledge of STEM is increasing, but in many economic sectors, it is difficult for employers to find job applicants with needed STEM knowledge and STEM problem solving skills (National Governors Association 2007). With regards to assessments of K-12 students' performance in mathematics and science, the U.S. ranks in the middle of the pack internationally (National Science Board 2012). In 2000, the United States ranked 20 out of 24 countries in the percentage of 24-year-olds who had earned a first degree in the natural sciences or engineering (NRC 2007). Studies suggest U.S. institutions would need to quintuple the proportions of 24-year-olds minorities with science and engineering degrees to achieve the NAP recommendation of a 6-10% increase in minorities earning a first university degree in the natural sciences or engineering. The challenge is compounded by the statistics that show underrepresented minorities who majored in STEM at the same rate as others, completed their degrees at a lower rate (NRC 2011) due to cultural and economic barriers to completion (CSEPP 2011).

On a personal note, both lead-PI (Saba) and co-PI (Seibel) grew up in Kansas. In Saba's experience, there seemed almost a stigma regarding going to college and less than five people from her class of 30 went to college. Consequently, in high school she was the butt of many jokes for the sole reason of wanting to go to college to be a marine scientist. While Seibel was in his first years of high school, he believed that his only options for college were Kansas State or Kansas University and that you picked either school based on your favorite basketball team. It was not until he moved away to California to finish high school that his horizons were broadened. Since then, Seibel has worked to introduce Kansas college students to marine science by taking two of them to sea, one of which is now a geography major pursuing graduate school and the other worked as a wildlife biologist for several years. His efforts have shown that through education and hands-on experience, Kansas students can and will branch out to pursue higher level education in the sciences. What we want to do

is to try to remove any remaining stigma present and introduce the field of marine science on a broader scale to Kansas high schools using an interactive, hands-on approach.

The core educational objectives of this proposal are to 1) instruct students about potential careers in marine science, 2) engage students and promote their interest in the scientific process, critical thinking, and applications of science, mathematics, and technology, and 3) and increase students' and teachers' awareness and understanding of the oceans and global climate change, with special focus on the Western Antarctic Peninsula region. These objectives will be accomplished using a multi-faceted approach that integrates dialogue with high school students and real-time links during the field season with a professional development program that will engage 20 teachers in a workshop before the Year 1 field season to equip them with the tools needed to bring the excitement of the research into their classrooms in real-time. We will develop, coordinate, and evaluate these efforts in collaboration with an education outreach specialist through the Rutgers' virtual teacher/scientist social network: Centers for Ocean Sciences Education Excellence, Networked Ocean World (http://COSEENOW.net). Specifically we will focus our efforts on five key tasks:

1) Educate high school students on potential careers in marine science: Outreach talks at various Kansas high schools will be given by lead PI (Saba), an Environmental Protection Agency (EPA) employee at the Kansas City office with background in marine science, and an employee at the National Oceanic and Atmospheric Administration Fisheries Service (NOAA NMFS). (Year 1)

2) Teacher/scientist mentorship: We propose to select approximately twenty educators from the Eastern Kansas region (i.e., 2 educators from 10 schools, targeting both rural and urban communities) to participate in a 3-day professional development program, hosted by Rutgers University and led by lead PI (Saba) and a Rutgers University COSEE NOW education coordinator. The workshop will be held in August 2013 prior to the start of the first intense field season. During the workshop, the teachers will meet the project team, become familiar with the science, and learn the tools that will enable them to bring it into their classrooms. All the materials developed for this project will be available and promoted through the project website and through COSEE NOW partnerships. The workshop will take place in Kansas in collaboration with Barb Engler at the Burlingame USD 454 school district (see support letter). (Year 1)

3) Engage students and promote authentic marine science research via creating questions, understanding the scientific process, critical thinking, and applying mathematical skills: With the collaboration of Rutgers University COSEE NOW, we will put together a curriculum geared towards general marine science with special focus on Antarctic systems and climate change. The lesson plan will include teaching material already available on the COSEE NOW website (at no cost to this proposal) related to marine science, Antarctic ecosystems, climate change in the Antarctic, and ocean acidification. Please see http://coseenow.net/blog/2010/09/antarctica-melting/ for more information. Additionally, we will undergo interactive teaching during the proposed field season at Palmer Station in Year 1 using skype calls to classrooms from Palmer Station (2x per week) and daily blogging (Saba, Seibel). Students will be assigned projects during this time including a) answering questions directly related to the research motivation and experimental design of the field project (moderated by high school <u>science</u> teachers); b) calculations of krill abundance from measurements taken by a glider equipped with the ADCP (moderated by high school <u>math</u> teachers); and c) blogging, science writing, and creative writing (i.e., poetry) (moderated by high school <u>English</u> teachers) all of which can be posted in real time on the COSEE NOW website. (Year 1 field season)

4) Post-field season symposium (oral presentations and poster session) for participating Kansas high school students to demonstrate what they have learned about the scientific process and how they can apply it to other marine-science related topics. This will also be an opportunity for the students following our research to present related research of their own. Lead PI (Saba) will visit

the Kansas high schools and offer feedback for the students and set up a Youtube channel for the students to post videos of their work. (Year 2)

5) This approach will be evaluated through the project to measure the impact of these programs on the target audiences. Evaluation of the project will be carried out by an independent evaluator, in collaboration with the COSEE NOW program, and will focus on formatively measuring the effectiveness of the instructional materials, including a teacher's guide, implementation strategies, handouts, student worksheets, and any other materials needed to implement the unit and meet the intended learning outcomes. The evaluator will use surveys and interviews, starting with the summer workshop and continuing through the school year, to collect data and feedback on the effectiveness of the teacher professional development training and materials. The project web site will house all of the materials and student worksheets in PDF format. This material will be shared for potential incorporation into the website. During the grant the evaluator will provide interim reports as needed to guide the team's education/outreach efforts, and a final summative evaluation report to NSF. Additionally, results of the outreach project will be presented at an NSTA meeting, followed by a publication of the outreach evaluation in NSTA's high school journal The Science Teacher. (Years 1-3)

Finally, Rutgers and URI will actively engage graduate and undergraduate students in the proposed project. They will be involved in all aspects of the program (field deployments; education & outreach; data collection, analysis, and dissemination) and are expected to be active authors publishing first author publications to further their careers.

RESULTS FROM PRIOR SUPPORT

Dr. Grace Saba has not received prior NSF support.

Dr. Brad Seibel: **NSF- OPP-0538479:** Antarctic field seasons in January 2007 and 2008 focused on adaptations of pteropod mollusks, *Clione antarctica* and *Limacina helicina*, to climate change, including global warming and ocean acidification. This project resulted in one master's thesis and one doctoral dissertation. Additional support was provided to one other graduate student and several undergraduates. Results were presented at the IPCC workshop on ocean acidification as well as in talks at several other international conferences and the NSF-sponsored short-course on ocean acidification. Twelve papers are published or in press in journals such as *Science*, *Proceedings of the National Academy of Sciences USA, PLoS ONE, Geophysical Research Letters, Biogeosciences, ICES Journal of Marine Science, Integrative and Comparative Biology, Polar Biology, Marine Ecology Progress Series, Journal of Experimental Biology*, and *Annual Review of Ecology, Evolution and Systematics*. The primary findings of this project were that the requirement for maintenance of routine locomotion in cold water has led to upregulation of aerobic capacity in wing muscles of *C. antarctica*. This includes an increase in mitochondrial abundance and cristae surface area relative to the temperate congener, *C. limacina*. Mitochondrial proliferation in *C. antarctica* has displaced fast-twitch (mitochondria-poor) muscle fibers that are critical to the multi-geared swim system of *C. limacina*. The routine and fast swim cycles are generated with different temperature dependencies in *Clione* spp. These adaptations may render pteropods vulnerable to increases in temperature. We also found that pteropods are sensitive to ocean acidification but that their response depends on energetic status. In seasons with high regional productivity, metabolism was elevated and responded negatively to acidification. Seasons with low productivity resulted in low rates of metabolism and CO_2 had no further effect. The results reveal a metabolic plasticity that underlies a variable response to CO_2 and may explain some of the diversity of results observed in other animals.

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