Introduction

This proposal aims to understand 1) the direct impacts of ocean acidification (OA) on vulnerable early life stages of economically and ecologically important coastal zooplankton and 2) how OA-induced changes in zooplankton populations could cascade through coastal food webs. To address these goals, we will employ a combined field-laboratory-modeling approach. We propose to characterize the pH variability experienced by zooplankton in an ecosystem that is already experiencing seasonally low (<7.6), but highly variable, pH conditions (Puget Sound, WA); test in the laboratory how pH conditions impact survival and development of select species; and model the ecosystem impacts of OA by extending an existing trophic model of Puget Sound food webs.

Mid-latitude coastal oceans and estuaries are among the most productive ecosystems in the world, and calcifying zooplankton such as crustaceans, bivalves, echinoderms, and pteropods are critical components of their food webs. Coastal organisms are likely to be subjected to a double-jeopardy in terms of OA: in these systems, anthropogenic-driven OA is exacerbated by inputs of low-pH waters from upwelling and high amounts of respiration (Doney et al. 2009). OA threatens to undermine primary consumer taxa, such as molluscs, crustaceans, and echinoderms (Fabry et al. 2008; Guinotte &Fabry 2008; Cooley and Doney 2009a). Any change in the demographics of these calcifying organisms due to OA will have significant impacts on ecosystems, with implications for economically valuable fisheries and global carbon cycling. Little is known about how coastal zooplankton will respond to OA and how those responses will cascade through food webs.

Global mean surface ocean pH has declined by about 0.1 units from pre- industrial levels and is predicted to decline another 0.2-0.3 units by the end of this century (Caldeira & Wickett 2003). However, ***much steeper than average declines in pH have already been reported in the Pacific Northwest where the combination of summer upwelling of low-pH waters, low alkalinity, and increased anthropogenic CO_2 create some of the most corrosive conditions in the world's surface oceans*** (Hauri et al. 2009; Wootton et al. 2008). Surface pH levels that had not been predicted to occur for many decades have already been measured throughout the coastal upwelling areas of the northern California Current (Feely et al. 2008).

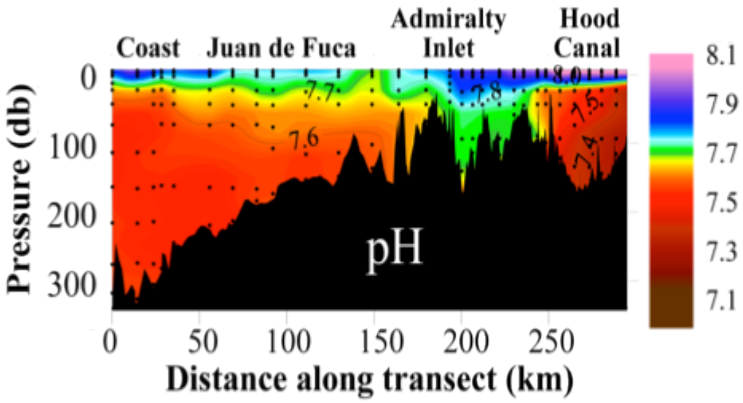

Fig. 1. pH along a transect in Puget Sound from the WA coast through Hood Canal, summer 2008. Very low pH water (<7.5) was found below 50-m depth in Hood Canal. From Feely et al. 2010.

In Puget Sound, WA, the second largest estuary in the USA, low-pH deep waters that originate as intruding coastal upwelling water from a deep marine canyon increase in pCO_2 when phytoplankton sinks out of the productive surface layer and is respired at depth. ***pH levels <7.6 are already common in Puget Sound in summer*** (Fig. 1, Feely et al. 2010). In summer 2008, extremely low pH values (<7.4) and aragonite saturation states (Ω_a< 0.5) were observed in Hood Canal, a sub-basin of Puget Sound. Levels of CO_2 needed to lower the pH of surface seawater from 7.8–8.0 to the lowest values observed in Puget Sound (7.3–7.4) are ~2,000 ppm, which substantially exceed current and predicted future average pCO_2 levels (Caldeira &Wickett 2003) and illustrate the role that local processes can play in exacerbating OA impacts. The impact of such low pH has already been felt by regional shellfish harvesters. For the last several years, failed recruitments and declining oyster populations in WA coast estuaries and Puget Sound have been partially attributed to OA. Hatcheries have reported unusually high mortalities of early-stage Pacific oyster larvae associated with upwelled, corrosive deep water that is characterized by low pH (7.6-7.8), high pCO_2 (400–600 μ atm in hatchery areas), and low aragonite saturation states (Ω_a), (e.g. Feely et al. 2008).

Surface water pCO_2 is controlled by a variety of environmental conditions including temperature, circulation, riverine inputs, tidal circulation, and biological activity. Different processes are likely to dominate at different locations, but all can contribute to spatial and temporal variation in productive coastal environments (Dai et al. 2009). Where surface waters support large phytoplankton production and strong

vertical clines exist, large diel or episodic fluctuations in pCO_2 conditions may occur in surface waters (e.g. Fig. 2). Predicting ecosystem response to OA will be particularly difficult in coastal ecosystems which experience such large variability in physical and chemical conditions over short spatial and temporal scales.

Large vertical gradients in pH and pCO_2 occur across the thermocline in stratified areas (e.g. Fig. 1), so vertical movement of organisms through the thermocline or mixing events which bring deep water toward surface can lead to large variation in the pH conditions experienced by organisms. Many crustacean zooplankton such as euphausiids (krill), crabs, and many copepods are among the diel vertical migrants who move from deep water to the surface layer at night. It is not known whether these species have adaptations that allow them to tolerate low pH conditions, at either short or long time scales. As average pH continues to decline, such adaptations may protect these taxa from deleterious effects of OA, but there is no evidence to date that tolerance to diel variability promotes tolerance to chronic long-term decreases (Fabry et al. 2008). Furthermore, as the average pH declines due to anthropogenic increases in CO_2, the below-thermocline minimum pH is also likely to decline, exposing organisms to more severe conditions during a portion of their diel migrations. *The question remains as to whether the average pH, minimum pH, or duration of low pH that a species is exposed to determines its ability to mitigate the effects of OA.*

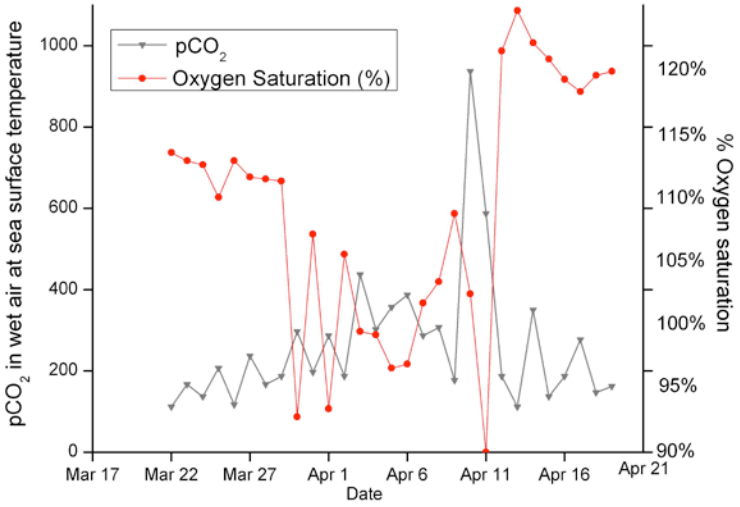

Fig. 2. Time series of surface water pCO_2 and oxygen saturation at the NOAA PMEL Twanoh buoy in Hood Canal, Puget Sound (47.37N 123.00W) in 2010. (http://www.pmel.noaa.gov/co2/coastal) Note the very high spike (>900 pp,) in surface pCO_2 apparently associated with a deep mixing event on April 11.

To address the need to understand the impact of OA on coastal ecosystems, we propose a study that will test the consequences of OA on ecosystem processes in a highly variable, pelagic marine system, Puget Sound, WA. We will focus on the impacts of OA on the development, growth, and survival of early life stages of native marine mollusc and crustacean species and, ultimately, will explore how those changes may cascade through marine food webs. The seasonal occurrence of highly corrosive conditions in Puget Sound presents a unique opportunity to study the effects of OA under field conditions that are presently being experienced by organisms, with implications for the future of coastal regions worldwide.

Our overarching hypothesis is that early life stages of zooplankton in coastal regions experience pH conditions that vary on the scale of hours to days, and that, in the context of increasing OA, this variability can influence their development in ways important for understanding current and future ecosystem response to pCO_2 increases.

Our specific hypotheses are:

H1: The pH experienced by zooplankton in coastal ecosystems varies on timescales that are relevant to larval development. Research on the effects of pH on zooplankton indicate that early life stages (first 2-3 weeks) are the most susceptible to low pH. During this period, many zooplankton move through several developmental stages. In coastal systems, these early stages are likely to experience pH changes of >0.5 pH units over diel and multi-day cycles through behavioral migrations or mixing/advection.

H2: Zooplankton growth, development, and survival are affected by pH. The few experimental studies conducted indicate clear negative impacts of OA on some calcifying organisms (both those with calcified shells and that use $CaCO_3$ as a strengthening agent; see overview below). Work on more species is needed to understand variability in species response.

$H3_a$: The mean seawater pH experienced by zooplankton over their early life stages influences their development. If organisms respond only to the average pH of their environment, we would expect experimental results to be similar between stable (non-variable) and time-varying pH treatments with similar mean pH.

$H3_b$: Short-period (diurnal to multi-day) variability in pH conditions can influence early development in zooplankton, and the response to variability differs among species. Little is known about the impact of pH variability on species response to OA. However, given that development includes many organizational processes that may be influenced by pH and occur during discrete windows of time, we expect that the pattern of pH variability that zooplankton experience over their early life stages could affect their development. Some species may be detrimentally affected by short exposures to low pH even if the mean environmental pH is high, whereas others may not be affected by episodic low pH if periods of high pH occur within critical developmental windows.

H4: OA-induced changes in zooplankton populations significantly impact food webs and fisheries. The direction and magnitude of OA's influence on zooplankton production may have impacts that cascade through the food web to important fishery species. The influence of changes in zooplankton population abundance on high trophic levels is likely to differ among impacted taxa. For example, model experiments done by NOAA collaborator on this project (Busch) found that reduced production of calcifying crustaceans in Puget Sound increased urchin fishery yields while decreasing yields of herring, shrimp, and rockfish (see Fig. 7, Busch et al. 2010).

To address these hypotheses we will:

- Characterize the small-scale spatial and temporal variability in pH that occurs in the field during the early developmental stages of representative molluscan and crustacean zooplankton.
- Conduct laboratory experiments in which early life stages of zooplankton are exposed to pH conditions that mimic the natural variability experienced in the field and those predicted in the future. Our experiments will target select mollusc (oyster, abalone, and geoduck) and crustacean (copepod, euphausiid, and crab) larvae which are critical components of marine food webs and commercial fisheries, and will result in data relevant to demographic modeling and understanding physiological tolerance.
- Incorporate results of field and laboratory work into a trophic model of Puget Sound to examine potential implications of OA on pelagic food webs and commercial fisheries .

Effects of OA on zooplankton

Changes to water chemistry due to OA may be physiologically challenging for marine invertebrates (Pörtner & Langenbuch 2005, Fabry et al. 2008, Pörtner 2008). Stressful environments can induce a myriad of impacts on developing and adult organisms, ranging from physical deformations to reduced reproductive output. The most common response metric for organismal studies on OA is calcification. Organisms that produce calcium carbonate shells are considered particularly vulnerable to OA for physiological and physical reasons: increases in pCO_2 leads to increases in H+ ions and decreases in carbonate ions. These changes in water chemistry make $CaCO_3$ structures more likely to dissolve and may make it harder for calcifying organisms to create the environment needed for deposition of calcium carbonate (e.g., Cohen et al. 2009). The impact of OA on the processes of calcification and shell dissolution varies widely among both distantly and closely related species due to differences in the mechanisms used for calcification, whether calcification is an internal or external process, the type of calcium carbonate that animals produce (including the mineralogy of precursor crystals), and the presence or absence of tissues (e.g., periostracum) overlaying calcium carbon structures (e.g., Cohen et al. 2009, Miller et al. 2009, Ries et al. 2009). Many calcifiers have reduced calcification with increased pCO_2, though others show no response or increases in calcification (Doney et al. 2009).

Physiological stressors often have their most profound impacts on developing organisms, including calcifiers such as corals, urchins, molluscs, and crustaceans (Green et al. 2004, Kurihara et al. 2004, Cohen et al. 2009). In most marine and estuarine calcifiers, natural mortality is highest before maturation. The larval stage is highly vulnerable to biotic and abiotic stressors, and their response to stress is likely to be

exacerbated in a high pCO_2 environment. From a purely physical point of view, the small size of the larval shell may contribute to vulnerability of marine calcifiers to OA. Here we focus a review of the impacts of OA on larval marine invertebrates on our focal groups within the molluscs and crustaceans. Most mollusc larvae have aragonitic shells, while the chitinous exoskeletons of crustaceans are impregnated with high Mg calcite (Roer & Dillaman 1984, Weiss et al. 2002). However, larvae of both molluscs and crustacea use amorphous calcium carbonate—the form of $CaCO_3$ most susceptible to dissolution (Feely et al. 2004)—as the precursor for the $CaCO_3$ components of their shells (Roer & Dillaman 1984, Weiss et al. 2002, Lee et al. 2006).

Molluscs: Organisms that produce $CaCO_3$ shells are considered particularly vulnerable to OA because OA leads to a reduction in the carbonate ion needed for calcification, so increases the energy needed to precipitate $CaCO_3$. Calcifying organisms vary in their ability to control biomineralization processes (Ries et al. 2009). Most calcifiers have shown reduced calcification rates with increase pCO_2 (Weiner and Dove 2003), even when calcite or aragonite concentrations are above saturation (Fabry et al. 2008), but increased calcification in some adult stages of crustaceans has been found (Ries et al. 2009).

Preliminary work on larval abalone done by Friedman (a PI on this proposal) and others (Crim et al. 2010, Zippay & Hofmann 2010) indicates that OA decreases development, settlement, calcification, and thermal tolerance; increases mortality; and causes no change in expression of two genes known to influence shell development (engralled and ap24). Related, published work on the impacts of OA on larval stages of other gastropods (snails) shows increases in mortality, decreases in growth and thermal tolerance, changes in heart physiology, and abnormal behaviors with OA (Ellis et al. 2009, Zippay & Hofmann 2010). The three published studies on developing clams have shown that OA on increases shell dissolution and mortality, decreases growth, and delays metamorphosis (Green et al. 2004, Green et al. 2009, Talmage & Golber 2009). Much work has been done on the impacts of OA on oysters, and most, but not all, indicate that developing oysters have higher mortality, decreased calcification, and increased developmental abnormalities with low pH (Kurihara et al. 2007, Kurihara 2008, Miller et al. 2009, Hettinger et al. 2010). An elegant study examining the pH tolerance of *C. virginica* larvae found that the larvae could tolerate pH 6.25–8.75, that typical growth rates were achieved at pH 6.75–8.75, but that the optimal pH range was 8.25–8.5034. Preliminary work on the Olympia oyster indicates that exposure to OA early in development can change growth patterns for up to 4 months (Hettinger et al. 2010).

Crustaceans: The biological effects of OA extend far beyond its effects on calcification (Fabry et al. 2008). Acidification can also effect reproduction, development, and growth. For crustaceans, which have chitinous exoskeletons and mainly rely on $CaCO_3$ to harden their exoskeletons after molting (Roer & Dillaman 1984), the greatest impact of acidification is likely to occur through stresses on other physiological processes effected by pH imbalance.

Very few studies have been conducted on crustacean zooplankton. We are unaware of any published studies on the impacts of OA on crab zoea. A single study on lobster zoea showed no change in development or survival with OA, but decreased exoskeleton mineralization in larval lobsters at 1200 ppm pCO_2 (Arnold et al. 2009), a level that can occur in nearshore regions. OA decreases the hatching success of krill (Kurihara 2008), and may cause abnormal development (Nicol 2008; Kawaguchi, Australian Antarctic Division, pers. com.). The impact of OA on copepod spawning, hatching success, and development is mixed (Dupont & Thorndyke 2009, Kurihara et al. 2004, Kurihara & Ishimatsu 2008, Watanabe et al. 2006).

More experiments on these trophically-important taxa are needed to determine inter-species differences in response to OA and the potential ecosystem impacts. Furthermore, if we are to understand the implications of OA on coastal organisms, experiments must incorporate the pH variability inherent to coastal ecosystems. We are unaware of any species-response experiments designed to test how such variability influences species response to OA. Thus, the work proposed here represents a major step forward in the science of OA and in our ability to understand the consequences of OA on marine organisms.

In situ small-scale pH variability

Unique components of this proposal are measurement of variability in water chemistry at spatial and temporal scales relevant to early life stages of zooplankton, and the collection of linked water chemistry and zooplankton community datasets. The pH conditions that zooplankton experience can vary on the scale of

hours or days. ***We hypothesize that this short-period variability is critically important to the development of larval stages of zooplankton, which have development times on similar (few day) scales***. ***However, few studies have characterized the pH and pCO_2 variability on these small scales that are relevant to zooplankton.*** To correctly parameterize our controlled laboratory experiments with natural pCO_2 conditions, we must first assess the conditions that the species in Puget Sound experience. The resolution of our time and space scales, therefore, are determined by biological considerations.

Zooplankton may be exposed to multiple pH environments as deep low-pH mixes with surface water or as they actively move through a range of pCO_2 conditions in their normal random or directed swimming. To capture the relevant scales of variability, we require high temporal and spatial resolution. Spatially, the meaningful variability occurs on the scale over which individual zooplankton move, either randomly or in directed swimming separate from advection in which they stay within a water mass. Vertically, sharp gradients in pH occur over the 20–100's of meters that many zooplankton vertical migrate across each day (e.g., Fig. 1). Horizontally, zooplankton may move 10's to 100's of meters per day (depending on taxa). Small-scale horizontal variability in temperature and oxygen occurs in our study area over scales <50-m (Devol unpub. data), indicating that similar variability in pH and pCO_2 is likely. Because the spatial scale of pH variability has not been well characterized *in situ*, we have designed a sampling approach to capture that variability to constrain our experimental protocols.

Measuring carbonate chemistry

The oceanic inorganic carbon system has four measurable parameters: pH, dissolved inorganic carbon (DIC), total alkalinity (TA), and the partial pressure of CO_2 (pCO_2). In addition, there are a number of carbon system parameters that cannot be measured directly with available technology, but that are particularly relevant to OA studies. Important parameters include the saturation state of aragonite (Ω_a) and calcite (Ω_c) and the concentration of the CO_3^{2-}. When Ω decreases, so does the amount of CO_3^{2-} available to form shells. Seawater is saturated when Ω=1. When Ω_a or Ω_c >1, the seawater is supersaturated and shell formation is favored. Conversely, when Ω_a or Ω_c <1, the seawater is undersaturated and shell dissolution is favored (Corliss and Honjo 1981).

Describing the marine carbon system requires measurement of at least two of the four carbon measurement parameters (pH, pCO_2, total alkalinity, and DIC) (Dickson 2010). We will use a variety of parameter pairs for various components of this project due to logistical considerations, recognizing that not all pairs are equally accurate (Dickson 2010, Hoppe et al. 2010). When continuous water flow is available (e.g., experimental system described below), we will directly measure pCO_2 with a Licor gas analyzer. TA data is the best pair to pCO_2 data for constraining the carbonate system, and will be measured during experiments using an automated TA titration system (Dickson 2010). Where only discrete samples are available (e.g., on cruises and for calibrating field sampling), pCO_2 measurements are not feasible and the pH and TA pair will be used. pH will be measured spectrophotometrically with a pH sensitive indicator dye within hours of collection. Excessive biological activity can potentially confound measurements of TA in nearshore environments (Dickson 2010). To account for this problem, measuring DIC in a portion of the samples is necessary to establish any relationships between titration results and calculated TA values. DIC will be measured with coulometry at NOAA's Pacific Marine Environmental Lab (PMEL). All methods will follow the best practices guidelines for measuring sea water carbon chemistry (Dickson et al. 2007).

Field location

Our field site will be Dabob Bay, WA, a sub-basin of Puget Sound. Dabob Bay is a deep (>200 m), silled basin that extends off the northern end of Hood Canal in Puget Sound. Several commercial oyster hatcheries are located there which, in combination with the low-pH conditions the area is already experiencing, makes it a region of intense interest for OA research. Because Dabob Bay receives low freshwater inputs, the species composition is dominated by marine species of copepods and euphausiids which are important taxa throughout the California Current and have congeners that are globally distributed. Field observations and collection of experimental organisms will all occur at this field site.

Field measurements

Profiling buoy

We will collect high-frequency, long-term water chemistry data using pH and pCO_2 sensors on a

profiling buoy to determine the full range and variance in conditions experienced by zooplankton through their spawning seasons (see Table 1). Autonomous profiling moorings are maintained by the ORCA (Oceanic Remote Chemical Analyzer) project in Puget Sound (www.ocean.ORCA.washington.edu). Beginning in May 2010, one of these moorings will be moved to Dabob Bay to address OA issues. Each buoy consists of a toroidal floatation package (~2500-kg buoyancy) on which an electric winch capable of profiling to depths of 150-m is mounted (Fig. 3). Solar panels (to power the buoy), a navigational beacon, and a meteorological sensor package are mounted on the floatation package. Suspended from the conducting cable on the winch is a SeaBird 19 series CTD that forms the basis of the underwater package. Details of the buoy configuration and mooring technique can be found in Dunne et al. (2002).

Water column profiles of temperature, salinity, dissolved oxygen, chlorophyll fluorescence, NO_3, and photosynthetically active radiation (PAR); surface pCO_2; and current speed/direction are collected by the mooring. Profiling is controlled by a microcomputer (BitsyX) that is programmed with profiling times and depths. After a profile is completed the microcomputer uploads the data from the CTD. The microcomputer also records 10 min averaged meteorological data. At preprogrammed times the data is telemetered by cellular internet or 802.11b WIFI to a central computer at the UW where CTD files are processed, graphed, and posted to the internet (http://orca.ocean.washington.edu). During the summer, sufficient solar energy is available to profile every 2 hours; during the winter, 2 profiles a day at most are collected. To minimize biofouling, the underwater sensor package is parked well below the euphotic zone when not profiling. Every 4 to 6 weeks the underwater sensor packages are replaced with cleaned reconditioned units.

To detect high resolution temporal patterns in carbon chemistry throughout the water column, we propose to add SAMI2™ pH and pCO_2 sensors to the buoy profiling package. The SAMI2 sensors (Sunburst Sensors, Missoula, MT, DeGrandpre et al. 1995, Martz et al. 2003, Seidel et al. 2008), have been successfully deployed for high resolution time series of carbon parameters in other studies (e.g. Martz et al. 2009, Rutgersson et al. 2009, Kuss et al. 2006). pH and pCO_2 are not the best two parameters to constrain the carbon system, so we will take water samples for pH, TA and DIC at the buoy to validate the estimates during cruises, organism collections, and when the buoy is serviced every 4-6 weeks. At those times, we will take discrete samples with a Niskin bottle and processes them for oxygen, chlorophyll, nutrients and the chemistry using standard protocols (Grasshoff 1999, Knap et al. 1996 JGOFS protocols).

Mapping spatial patterns in ocean chemistry and associated zooplankton

To characterize small scale spatial patterns in water chemistry, we will intensively survey the study area around the ORCA buoy. Because the SAMI2 sensors must be stationary for 10-15 minutes prior to obtaining accurate readingwe will instead tow-yo a CTD with DO sensor in a tight 1-km^2 grid centered on the profiling buoy. This grid size was based on the distance a 2-mm copepod may travel in 3 days (a relevant developmental time period), assuming swimming at 1 body length/sec. Studies have shown that DO is tightly correlated with aragonite saturation state over small spatial scales (Juranek et al. 2009) so we will use these spatial patterns of DO as a proxy to describe the spatial scale of carbon parameters. At randomized locations within the sampling grid, we will collect water chemistry, nutrient and oxygen data using a SeaBird CTD and Niskin rosette at least 6 times per day at 3 or more depths. We will measure pH spectrophotometrically on the boat immediately after collection and will bring a water sub-sample poisoned with mercuric chloride back to NOAA for TA analysis via titration. To characterize changes in TA due to biological activity and for quality control, a subset of

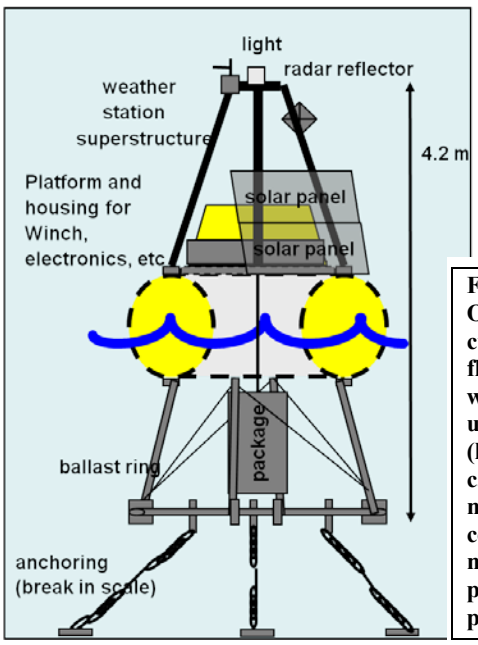

Fig. 3. Schematic of an ORCA buoy showing a cutaway of the toroidal float (wavy blue line is the water surface). The underwater sensor package (labeled 'package') is capable of profiling to 150 m. The profiler can be configured with stops at multiple depths so we can pause to make pH and pCO_2 measurements.

water samples will be sent to the NOAA-PMEL lab for TA titration and DIC (coulometry) analysis.

During these 'spatial' surveys, we will characterize the zooplankton community associated with the water chemistry, and will collect organisms for experiments. [Additional zooplankton collections for experimental work will occur as necessary to obtain animals throughout the spawning season.] A diaphragm pump will be used to collect zooplankton samples for linked water chemistry/zooplankton community data. Four samples per day at three depths (surface, pycnocline, near bottom) will be collected over three days at the locations selected for full water-chemistry analyses. Faster-moving organisms (e.g., euphausiids) that cannot be sampled by pumps will be targeted with stratified net tows (4/day) using a HydroBios® MultiNet, computer-controlled sampling system (similar to a MOCNESS) equipped with 335-µm mesh nets.

Spatial mapping cruises will be conducted in Year 1 in approximately April and June, months that capture spawning activity of our target organisms (Table 1). Zooplankton community samples will be preserved and analyzed in the lab to determine the taxa that are found in low-pH water. Species chosen for experiments may be adjusted if avoidance (absence of a species from low-pH water) is indicated.

Laboratory experiments:

Experimental OA facility

The species exposure experiments will be conducted in the NOAA Northwest Fisheries Science Center (NWFSC) OA facilities. The facility is currently located at NOAA's Montlake lab in Seattle, WA and is slated to move to a larger facility at NOAA's Mukilteo, WA location by late 2010. The facility consists of a series of treatments systems that deliver water with controlled pH/pCO_2, temperature and dissolved oxygen; the system is designed for conducting experiments on multiple species simultaneous (McElhany et al. 2010; Fig. 4). Carbon chemistry and dissolved oxygen levels are maintained with a membrane contactor (LiquiCel) for gas stripping and by controlled bubbling of air, CO_2, CO_2-free air, oxygen and nitrogen into a tank dedicated to bubbling. Treated water then moves to a header tank where the following automated measurements of the water are taken: pH (Durafet® probe), dissolved oxygen, conductivity, and temperature. Water from this tank is flowed through an equilibrator, and the pCO_2 in the equilibrated air is measured with a Licor® gas analyzer. Our equilibration system and Licor® gas analyzer set-up are modeled after those used by NOAA-PMEL. Water from the header tank is delivered to the experiments. Water from the experiments can be released from the system (flow through), passed through two UV/particle filters and deposited in the bubbler tank (closed), or a mix of the two. The system is controlled by a Labview® program developed at the NWFSC that allow us to create complex temporal patterns in pH/pCO_2, dissolved oxygen, and temperature.

Carbon chemistry of our system will be calibrated and verified in a number of ways. We will periodically calibrate our pH probe with spectrophometric pH measurements run by PMEL and at the NWFSC and with a seawater standard from the Dickson laboratory. Water samples from the system will be periodically analyzed

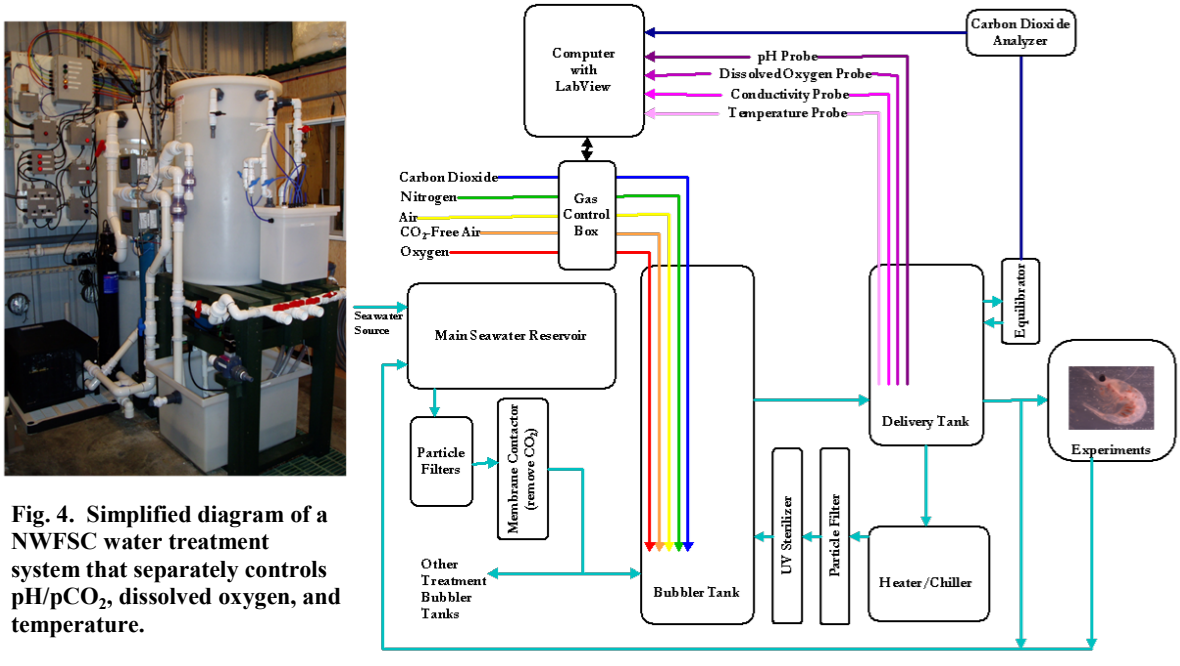

Fig. 4. Simplified diagram of a NWFSC water treatment system that separately controls pH/pCO_2, dissolved oxygen, and temperature.

for DIC and TA at PMEL to verify that our TA measurements are valid, our calculated DIC is consistent with PMEL's measured DIC, and calculated pH values are consistent between PMEL and NWFSC.

The NWFSC currently has one fully operating prototype treatments system and will soon (May 2010) complete six more systems for at total of seven at the Montlake facility. When complete, the Mukilteo facility will contain 21 treatment systems allowing for the factorial design described in this proposal.

NOAA is providing the use of this facility for these proposed experiments without charge. The use of this facility is a substantial contribution to this project, as developing and operating a complex facility of this scale is an expensive undertaking. The use of this facility for this project reflects a commitment by the NWFSC to understand how ocean acidification will affect near shore environments. NOAA is requesting funds to conduct carbon analysis and SEM imaging.

Selected species

The species we have chosen to study are all key components of pelagic and/or benthic food webs and may be representative of related taxa that are found in temperate oceans world-wide. Many support valuable fisheries and aquaculture industries. The crustacean species were chosen for their importance in food webs and, hence, in ecosystem modeling. The chosen mollusks are economically important and this group may show the first, and most severe, impacts of ocean acidification in the region.

Crustaceans: Copepods comprise 70-90% of the zooplankton biomass throughout most of the world's oceans. As a group, they are the most important marine secondary producers world-wide, and they serve as a dominant component of the prey of nearly all larval fish and many sea birds and marine mammals. Euphausiid and crab larvae can dominate the zooplankton at times, particularly following spring spawning events. Information on how ocean acidification will alter populations of crustaceans is important for understanding and predicting ecosystem impacts, and is currently missing from the literature.

Copepods: We will target two species of copepods, focusing on the species that is most available at the time of collection. *Calanus pacificus* is the dominant copepod in Puget Sound by weight, and an important prey item for larval fish. It has a broad range from British Columbia to Baja, California (Fleminger, 1964). Its congeners *C. finmarchicus*, *C. helgolandicus*, and *C. marshallae* are among the most important zooplankton taxa in food webs throughout temperate oceans. *Acartia clausi* is a smaller-bodied copepod that is globally ubiquitous in coastal temperate waters.

Euphausia pacifica, the dominant euphausiid throughout much of the North Pacific and Puget Sound, is a critical component of pelagic food webs due to its role as prey for many fish, seabirds, and whales. *E. pacifica* is a congener of the Antarctic krill (*E. superba*) which has shown effects of OA on hatching and development in laboratory studies. Females will be collected from the field and spawned in the laboratory.

Cancer magister (Dungeness crab) is abundant in the California Current and Puget Sound. The zoea and megalopae are important prey of many fish, including juvenile salmon. On the Oregon coast, the Dungeness crab fishery is the most valuable commercial fishery, producing >$26 million dollars in ex-vessel (to the boat) fisheries revenue in 2009; its average annual fishery revenue in Washington State is ~$20 million dollars. Brooding females will be hand-collected from the field; the stage-I zoea will be hatched in the laboratory.

Molluscs: The molluscs we will study represent ecologically and economically important taxa which are either in decline (pinto abalone and native oysters) over the last several years or face growing exploitation (geoduck clams) that has raised concerns over the health of the species and its future role in nearshore marine ecosystems. As benthic filter-feeders, oysters and clams play important roles in nutrient cycling, habitat complexity, and estuarine food webs, and maintaining water clarity while abalone are important benthic grazers which enhance diversity by opening large patches of subtidal habitat for recolonization by other organisms. Population declines of these taxa can lead to significant changes in benthic habitat and community structure (Kennedy 1996; Miner et al. 2006). Possible causes for these declines include disease, overharvest, habitat loss, and exposure to OA. Larvae will be obtained from local hatcheries.

Haliotis kamtschatkana *(*northern or pinto abalone) is an important subtidal herbivore which is designated a '*State Species of Greatest Conservation Need*,' and a '*Federal Species of Concern'* (Rothaus et al. 2008).

Panopea generosa (the geoduck clam, sometimes *P. abrupta* in the literature, see Vadopalas et al. 2010) is an ecologically important infaunal filter-feeding species which supports an important commercial fishery in Washington and Alaska. It is the largest single-species biomass pool in the Puget Sound (Harvey et al., in prep) and also the species that generates the most revenue for the Puget Sound fishing industry.

Ostrea lurida (the Olympia oyster) is an ecologically and historically important intertidal/shallow subtidal species that is a *State Candidate Threatened Species* and is commercially farmed near our field study site.

Table 1. Experimental species and their associated life history parameters.

	Taxa	Common name	Spawning period	Planktonic Duration (days)*
Molluscs	*Haliotis kamtschatkana*	Pinto abalone	June-Oct.	7
	Ostrea lurida	Olympic oyster	June-Aug	14
	Panopea generosa	Geoduck	Feb-June	21
Crustaceans	*Euphausia pacifica*	Krill	April-June	20
	Acartia clausi	Copepod	March – Sept	15
	Calanus pacificus	Copepod	March – Oct	22
	Cancer magister	Dungeness crab	March-April	120

*Approximate larval period from hatch to settlement (for meroplankton), maturation (for copepods), or furcilia stage III (for euphausiids), which all vary with food and temperature.

Experimental conditions with variable pH

Our objective with the controlled laboratory experiments is to test the response of zooplankton to increasing OA under time-constant and time-varying conditions. Time-varying conditions are selected to mimic the natural diel or multi-day variability in pCO_2 conditions experienced by zooplankton during their early development. Incorporating variability in water chemistry in these experiments is an important advance compared to experiments that expose animals to static conditions.

Our experimental design for Year 1 includes four mean pCO_2 levels: 280 ppm pre-industrial level, 387

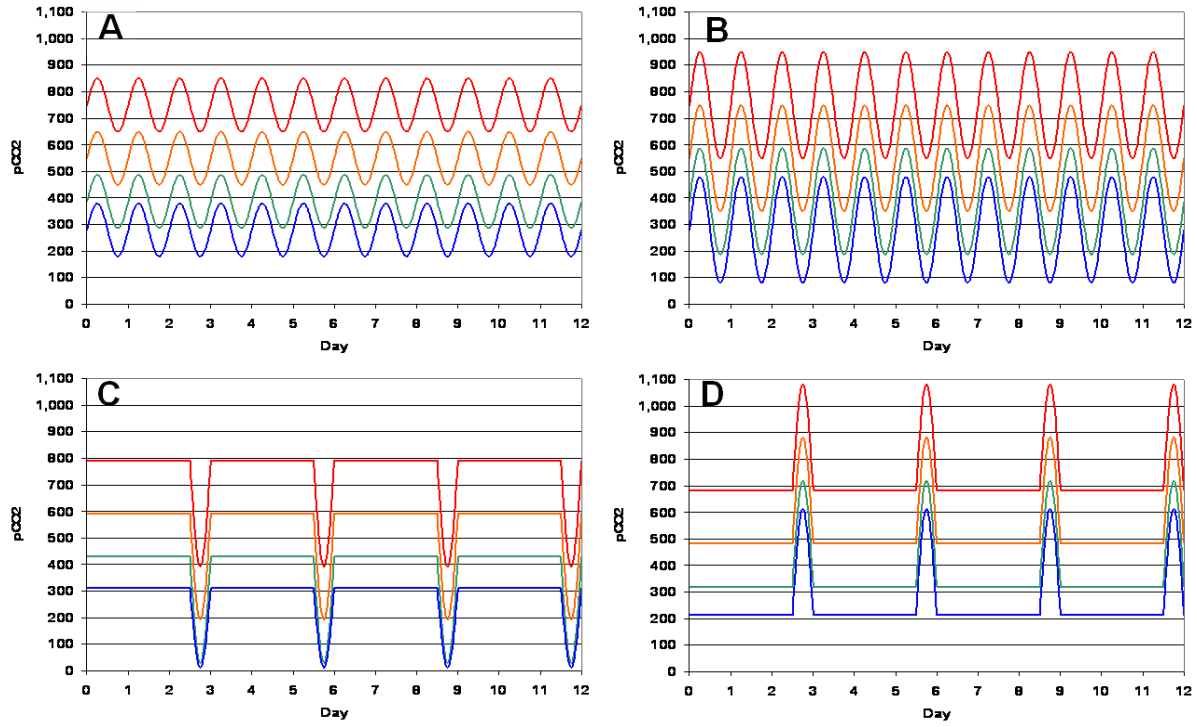

Fig. 5. Experimental treatment patterns of variability. The experiment is a factorial design with five mean pCO_2 levels by five variability patterns (20 total treatments; stable treatments are not shown here). Mean pCO_2 levels are indicated by color, with blue = 280 ppm (pre-industrial), green = 387 ppm (current), orange = 550 ppm (2050 prediction), and red = 750 ppm (2100 prediction). Panels A and B show treatments where pCO_2 varies in a diurnal sine wave. Panels C and D show a spiked change in pCO_2 with one 12-hour sine function spike each three days.

ppm current, 550 ppm predicted by 2050, and 750 ppm predicted by 2100. We chose these mean values based on the recommendations of the 'Guide to Best Practices in Ocean Acidification Research and Data Reporting' (Barry et al. in press). In our initial year, we will focus on determining species-responses to these stable (time-invariant) pCO_2 levels. In Years 2&3, we will impose five patterns of variability (4x5=20 treatments) (Fig. 5) on those taxa which showed significant response to decreased pCO_2 in Year 1. The patterns of variability will mimic typical, generic patterns of variability caused by diel cycles of photosynthesis/respiration, vertical diel migrations, and wind-mixing or upwelling events.

The initial patterns tested will be (Fig. 5):

- Constant pCO_2 throughout the experiment,
- Simulated diurnal pattern using a low-amplitude sine wave (photosynthesis/respiration pattern),
- Simulated diurnal pattern using a high-amplitude sine wave (vertical migration pattern),
- Episodic high pCO_2 (simulated mixing/upwelling event, surface-oriented perspective),
- Episodic low pCO_2 (simulated mixing/upwelling event, bottom-oriented perspective).

We will adjust the experimental conditions if the proposed conditions do not reflect those observed in the field data on diurnal cycles of species distributions in relation to pH conditions. To explore inter-annual variability in species response and control for annual variation, we will repeat the constant-pCO_2 treatments each year. Depending on the number of variable pCO_2 treatments suggested by the buoy data, there may be sufficient facility capacity to include temperature and/or temperature variability as additional factors in Years 2&3. Numerous studies have demonstrated interactive effects between temperature and OA (Rosa & Seibel 2008, Gooding et al. 2009, Findlay et al. 2010), so those would be informative additional experiments.

R**esponse variables/Experimental results:** A variety of response variables will be used to capture the impacts of OA on our focal organisms. A minimum of 10 individuals will be targeted for measurements in each treatment at each 2-3 day time step.

- Survival (molluscs and crustacea) – Instantaneous survival $(\#\text{larvae counted}-\#\text{dead})_T/(\text{total } \#_T - \#\text{ removed}_{T-1})$, where T is the experimental duration (time), will be assessed throughout each experiment. Cumulative survival will be calculated as the number of live larvae at the end of the trial relative to the initial number of live larvae per tank.
- Hatching success (crustacea) – Proportion of spawned eggs that successfully hatch will be assessed for copepods and euphausiids.
- Development rate (crustacea) – Duration of each developmental stage (egg, N1, N2, etc.) will be calculated.
- Body size (crustacea) – Growth will be assessed using standard body measurements (prosome and body length for copepods; body length for euphausiids; carapace and anterior spine length for crab zoea).
- Morphometrics (molluscs) – Shell length and height of 50 individuals per treatment will be measured to assess growth rate and conformation of larval shells.
- Scanning Electron Microscopy (molluscs) – To assess shell condition, images will be captured with a LEO 435VP SEM. Larvae will be rinsed in seawater and preserved in modified Karnovsky's solution for 1 hour at room temperature, followed by two washes in 0.1 M sodium cacodylate buffer. Samples will then be post-fixed in 1% OsO4 for 1 hr at room temperature, washed 2x in 0.1 M sodium cacodylate buffer and prepared for standard SEM.
- Calcium content (molluscs and crustacean) – Pooled samples from the final day of each experiment will be used to evaluate total calcification. Ca content will be measured with a Dionex Ion Chromatography system (ICS-2000) after dissolving samples with acid (Jeziorski & Yan 2006).
- Shell strength (molluscs) – A mechanical compression tester will be used to measure the ability to resist crushing or cracking by predators or physical stress.

Genomics: The combination of species-specific responses to acidification (e.g. Ries et al. 2009, Miller et al. 2009) and impacts on multiple physiological processes (e.g. Talmage and Gobler 2009) warrants a comparative, system-level approach to examine organismal responses to OA. We propose to use next-

generation sequencing to characterize the transcriptomic response of a crustacean, *E. pacifica*, and a mollusc, *O. lurida*, to elevated pH conditions. Doing so will build our understanding of the physiological processes affected by OA and provide insight to how OA may cause impacts at the population level. Given the limited genomic resources for these species, the Roche 454 pyrosequencing platform will be used to offer sequence read lengths that are more amendable to assembly and RNA-seq analysis. We have recently completed a similar project with Pacific Herring, another species with limited genomic resources; with the millions of reads produced, we successfully de novo assembled, annotated, performed RNA-seq analysis and identified candidate Single Nucleotide Polymorphisms (SNPs) (Roberts et al. 2010).

For this project, we will perform RNA-seq analysis using the Roche 454 pyrosequencing platform (NGS). Libraries will be made from the larvae of the two species. We will sample larvae prior to mortality in elevated CO_2 conditions and from control systems. Pyrosequencing will be performed at a local core facility, with assembly, annotation, mRNA-seq analysis, and statistical analysis carried out using the Genomics Workbench Server (CLCBio) as recently described (Goetz et al 2010). While NGS provides a global high-throughput means to look at transcriptome variation, it will not be able to characterize individual organism variation in expression. For this we will use quantitative PCR (qPCR) to target genes regulated in RNA-seq analysis and characterize temporal variation (Roberts et al., 2009). An Opticon 2 thermocycler (Bio-Rad) will be used to quantify gene expression; data will be analyzed using Real-Time PCR Miner Software (Zhao & Fernald, 2005).

Population genetics: Examining OA from an evolutionary perspective is essential for understanding its consequences. Carbonate chemistry perturbation will very likely introduce a strong selective pressure that will alter the genetics of local populations. Some selection due to OA will likely occur during larval development. To date we are not aware of any research that has examined how acidification will impact population genetics. To address this, and complement measures on the developmental and physiological responses to OA, we will use SNP markers to characterize populations that show increased mortality to increased pCO_2 exposure. We will take the innovative approach of coupling of transcriptome characterization with SNP marker development.

In addition to providing insight to physiological impacts of elevated pCO_2, the pyrosequencing data will be a valuable source of information for developing genetic markers to directly assess the impact of elevated CO_2 on marine larvae at the population level. SNP markers will be identified *in silico* from the transciptome data using Genomics Worbench (CLCBio). SNPs validated by HRM will be resequenced using standard Sanger sequencing to confirm priming and probe sites. SNP genotyping will be performed using the BioMark 96.96 Dynamic Array. The Dynamic Arrays will be read on a BioMark Real-Time PCR System after amplification and scored using BioMark Genotyping Analysis software (Fluidigm).

Results of preliminary OA experiments: We conducted pilot work on the response of larval geoduck (P. abrupta) and Pacific krill (*E. pacifica*) to OA in 2009 using a pilot OA system. Geoducks were challenged from larval age 13-d to 21-d in waters with a mean pH 7.5,7.8, and 8.1. Survivorship in all treatments was high and marginally significant among treatments (Repeated-measures ANOVA, $F=3.67$ $p=0.06$). Future studies on earlier developmental stages may indicate stronger differences. Approximately 80 specimens from the three treatment groups were observed with SEM; no indication of abnormal shell morphology or dissolution was observed in any treatment. Individuals reared at pH 7.8 were significantly larger than those reared in 7.5 or 8.1 (ANOVA, F-15.84, $p<0.001$), possibly because the larvae's native waters are pH~7.9.

Pilot work on Pacific krill found that OA might decrease hatching success, delay development times, and cause development deformities. Gravid krill females were collected from the Puget Sound and brought into the lab to spawn. The brood from each female was divided equally among three treatment groups. The three pH conditions in the experiment were confounded with temperature differences due to problems with our pilot sea water treatment system: pH 8.2, 21.9˚C; pH 7.2, 15.7˚C; pH 6.5, 15.7˚C. This confound is problematic as temperature is known to affect embryonic development times in krill (Iguchi and Ikeda 1994). At pH 8.2 (21.9˚C), nauplii hatched and swam freely, developing normally to the metanauplius stage. At pH 7.2 (15.7˚C), most of the development that we observed occurred inside the egg. We suspect that the "late hatching" development pathway, previously described Gómez-Gutiérrez (2002), may have been invoked. Some nauplii at this pH showed signs of mutation and died quickly after hatching. At pH 6.5 (15.7˚C), no development occurred.

Ecosystem modeling

To explicitly examine the impact of OA-induced changes on ecosystem structure, we will develop ecosystem food web models to evaluate how the Puget Sound ecosystem is likely to change in response to OA. Numerous papers discussing OA research have called for these types of models (e.g., Fabry et al. 2008), but none that we are aware of are published. For this proposed research program, we will collect data from the model's focal region on 1) natural patterns of variability in carbon chemistry and other parameters relevant to biology, 2) composition of the zooplankton community, and 3) response of a selected number of those species to changes in carbon chemistry that mimic natural variability. Thus, we will be in a position to better parameterize ecosystem models with more realistic predictions about the development and consequences of OA.

We will expand on a trophic model that has already been used to simulate OA impacts on the Puget Sound food web (Busch et al. In preparation and 2010; Harvey et al. in review). Our current simulations manipulate an Ecopath with Ecosim (EwE) model of the Puget Sound Central Basin (Harvey et al. in review) by changing the future productivity of species groups expected to be directly affected by OA (Fig. 6). EwE is a widely used modeling framework—with >150 applications on marine systems world-wide—for predicting changes in species group biomass as a function of productivity and predator-prey interactions (Christensen & Walters 2004). Predicting the species groups that will be affected by OA will involve 1) the compilation of a comprehensive data base of species in Puget Sound, 2) an estimate of the mineralogy (e.g. aragonite, calcite, etc.) for calcareous species, 3) experimental results on individual species response to OA, and 4) application of a qualitative risk assessment for each species group. The Puget Sound species data base with $CaCO_3$ mineralogy will soon be publicly accessible (Busch et al. 2010 In prep.).

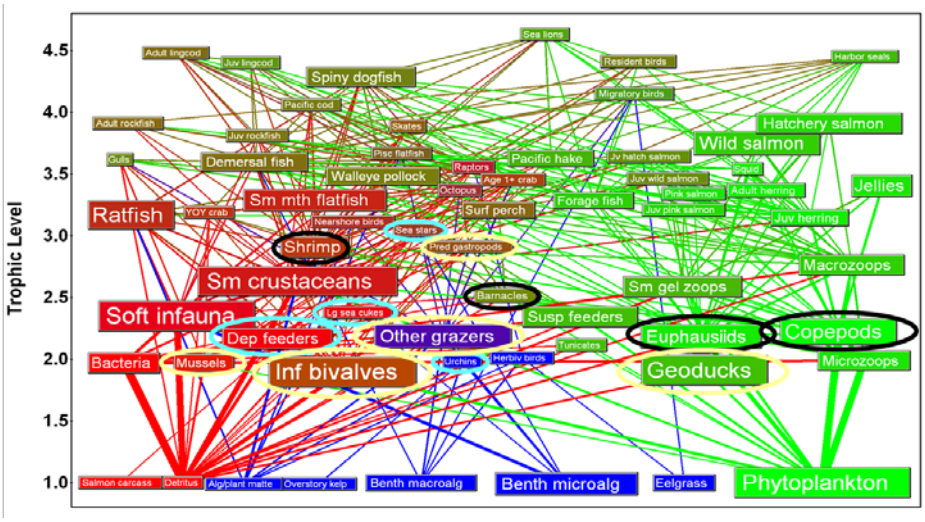

Fig. 6. Food web model of Puget Sound with 65 functional groups (Harvey et al., in prep.). Box size is proportional to biomass. The width of connecting lines is proportional to energy flow from prey to predator. Color indicates the group's energy source: phytoplankton (green); benthic primary production (blue); detritus (red); multiple sources (mixed colors). Functional groups for which productivity was altered in simulations are circled: black–crustaceans, blue – echinoderms, yellow– molluscs.

Simulations with the EwE model show that the Puget Sound ecosystem may be sensitive to future declines in productivity of crustacean zooplankton (the primary food base of upper trophic webs) and benthic bivalves (key components of the benthic food web and the greatest standing biomass in the ecosystem) (Busch et al 2010 and in prep., Harvey et al 2010). Simulation results also indicate non-intuitive indirect effects from OA, such as potential trophically-induced increases in some calcareous species (Fig. 7). This proposed study will provide substantially more robust data for establishing the predictions of future productivity that drive the simulations.

We will modify the existing EwE model in two key ways to better address questions related to OA: we will expand the model's scope from the Central Basin to the entire Puget Sound to better capture highly mobile species and plankton dispersal and we will restructure the food web so that species groupings in the model correspond to groups with differential response to OA, not just to taxonomic or trophic similarity.

We will extrapolate the carbon chemistry variability observed in the field component of this study to the entire Puget Sound. Such an extrapolation will, of necessity, be only approximate. In the most simplified form, an extrapolation could involve repeating the observed patterns in Dabob bay to all of Puget Sound. At the other extreme, we could apply detailed circulation models to explicitly predict fine-scale variation in

carbon chemistry over the entire model area. We propose that an intermediate level of physical detail will be sufficient to realistically predict food web response to OA. We will build on existing high resolution circulations models of Puget Sound (e.g., Babson et al. 2006, Khangaonkar In prep.) and employ algorithms relating carbon chemistry to more easily measured and modeled parameters such as oxygen concentration (Juranek et al. 2009). We will used the modeled patterns of pH variability in Puget Sound in conjunction with results from our experimental studies to project overall changes to productivity for crustacean zooplankton and bivalves in the region. These improved productivity values will be incorporated into our model simulations.

The primary output from our food web model OA simulations will be OA-induced changes in the biomass of species groups (e.g. Fig. 7). This output can be translated into effects on "ecosystem services", generally defined as the human-derived benefits from intact ecosystem processes (Millennium Ecosystem Assessment 2005). For marine systems, these ecosystem services include the provisioning and cultural services of commercial and recreational harvests, the supporting service of clean water that protects human health, and the regulating service of protection from beach erosion. Studies of the potential effects of OA have show substantial costs to ecosystem services (e.g., Cooley & Doney 2009b); however, the full potential costs of OA and cannot be evaluated without considering the indirect effects uncovered via ecosystem modeling.

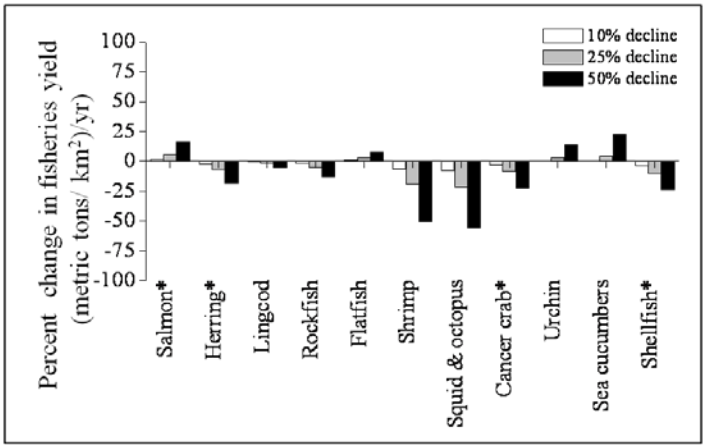

Fig. 7. Simulated 10%, 25%, and 50% reduction in production of OA-sensitive taxa [echinoderms, non-cephalopod molluscs, and selected crustaceans (krill, copepods, shrimp, barnacles)] resulted in large changes in fishery yields at the end of 100-yr simulations in the Puget Sound Ecopath model (Harvey et al 2010). *indicates Central Puget Sound's largest fisheries by weight. From Busch et al. (2010).

Broader Impacts

Results of this study will be of interest to shellfish hatcheries and fisheries managers—early impacts of OA are already being felt by the shellfish aquaculture industry in Puget Sound where recent years of very low recruitment have been associated with acidic (pH<7.8) conditions. Our results will be disseminated to regional stakeholders at local meetings (e.g. Washington Sea Grant's Shellfish Growers Conference; Pacific Coast Shellfish Growers Association meetings) and national conferences (e.g. National Shellfisheries Association; World Aquaculture Society) and to fisheries managers via our NOAA collaborators.

Our results will be made available for the broader scientific community through the Northwest Association of Networked Ocean Observing Systems (NANOOS). Data from this program will be deposited with the BCO-DMO at Woods Hole Oceanographic Institution. We anticipate that >8 peer-reviewed journal articles will result from this research.

Mentoring and training young scientists is a critical component of this project. Several undergraduates will develop thesis projects within our collaborative program; we will train two Ph.D. students and one post-doc. Four early-career scientists will be supported by this project (Keister, Vadopalas, Roberts, and Busch). Our research findings will be integrated into the curricula of several courses taught by the PIs (e.g. Marine Biology, an undergraduate course with 150 students annually) and posted on websites of grant PIs.

Outreach Plan

Through the curriculum development and delivery activities outlined below, our project will help develop societal understanding of OA through education of teachers and students. The final curriculum will reach underrepresented groups including native American youth via delivery through tribal educators.

To enhance school student understanding of effects of anthropogenic release of CO_2 on the marine environment, we will develop culturally-responsive OA curriculum materials to achieve the Washington State Essential Academic Learning Requirements. We will partner with the UW's Ocean and Coastal

Interdisciplinary Science (OACIS) GK-12 program (annual audience is ~500 students and 8 teachers) to test, expand, and finalize a curriculum. We will recruit GK-12 teachers that have high proportions of students from cultures that are historically underrepresented in the sciences. Teachers will be invited to a 1 day workshop where OA lessons are described and demonstrated. We will modify the lessons according to teacher feedback for use in classrooms. At the end of the year we will invite teachers back for a 1-day workshop to modify lessons as needed. We will repeat the process in year 2, targeting different grade-level. Dr. Vadopalas will help improve curriculum, support teachers, and lead the professional development workshops (~0.25 FTE annually).

In year 3 of the grant, the curriculum will be more widely disseminated. A teacher participant will co-present the materials at a national and/or regional conference for educators (e.g. National Science Teachers Association, Northwest Aquatic and Marine Educators, National Association of Marine Educators). In addition, the materials will be presented in education sessions at scientific conferences (e.g. ASLO, AGU, or National Shellfisheries Association). Also in year 3, the entire OA curriculum unit will be refined for distribution to educators through the Hood Canal Watershed Education Network and ECOnet (environmentally based non-governmental organizations, Girl and Boy Scout camps, Science Centers, YMCA, agency and tribal educators) for incorporation into marine educational events and training.

Using the synergy of our collaborative group, we will create and staff an interactive educational display of our research in years 2 and 3 to share our findings with attendees at events such as OysterFest, the best-attended shellfish celebration on West Coast (>16,000 shellfish connoisseurs attend), the Puyallup State Fair, and/or the regional Puget Sound Restoration Fund Environmental Film Festivals, held semi-annually.

In collaboration with Washington Sea Grant Marine Advisory, in year 3 of the grant we will convene a Sound Science workshop on organismal and ecosystem effects of OA (hosted by C. Friedman). The Sound Science lecture series is a Washington Sea Grant program that brings University researchers into the community for a two-hour lecture to share their work with community residents, tribal members, businesses, and resource agency staff. The OA lecture will be taped for broadcast and individual DVDs will be made available.

Interactive exhibits at the Pacific Science Center (PSC): The PSC in Seattle is one of the nation's premiere free-learning facilities dedicated to advancing public understanding of science and contributing to the development of a scientifically literate society. NOAA scientists conducting OA research have partnered with the PSC to develop two interactive exhibits: a mobile "Discovery Cart" on OA and real-time digital displays of CO_2 data collected from the Space Needle and a buoy off of the WA Coast. We will enhance both of these exhibits. We will work with the PSC to incorporate our OA curriculum and research results into the OA Discovery Cart, which consists of interactive exercises and props presented by a trained interpreter. For example, data on the response of oysters to OA could be presented to illustrate the concept that our valued food resources may change under OA. Second, we will work with the PSC to display the pH and pCO_2 data collected at our proposed site in Dabob Bay with the other NOAA CO_2 data currently on display. Dabob Bay is a popular fishing and recreation locale, so data on environmental conditions there will interest the public. Both the Discovery Cart and CO_2 display will provide information on how visitors can take action to reduce their carbon emissions and mitigate the causes of OA.

Science in action at NOAA's Mukilteo research laboratory: The Mukilteo Laboratory is on the shores of Puget Sound in the heart of Mukilteo, WA, adjacent to a busy Washington State Ferry Terminal. The laboratory is scheduled for a major renovation which will include enhanced opportunities to engage the public in scientific research. The OA experimental facilities and laboratory space will be included in public viewing areas, and all ongoing OA research will be highlighted. Visitors would be able to observe the experiments proposed for this project in action, from manipulation of seawater chemistry to observations of organisms in the experiments, made possible, for example, with a live link between a computer monitor and the microscopes used by scientists. Posters and brief talks by the students and scientists conducting experiments would inform visitors of recent results and engage them in the scientific process.

Expertise and responsibilities of the PIs: Dr. Keister will be responsible for the overall scientific direction and program management, coordinating the field program, and experiments on the crustacean zooplankton in collaborations with McElhany and Busch. Friedman and Vadopalas will be responsible for experiments on molluscan larvae and developing the outreach curriculum. Dr. Friedman's expertise is in marine invertebrate

health; Vadopalas's is in conservation genetics of marine molluscs and environmental education. Roberts, who has extensive experience in the application of genomic approaches to study the biology of fish and shellfish, will lead the genomics analyses. Newton is a biological oceanographer and Executive Director of the NANOOS marine observatory; she will lead the observational hydrography effort on cruises and oversee data and outreach delivery via NANOOS. Devol is an expert marine chemist who will manage the ORCA mooring. McElhany is a marine ecosystem modeler who has taken a lead in developing the NOAA OA experimental facilities; with Essington, he will guide a post-doc in the ecosystem modeling component. Dr. Busch is modeling the impacts of OA on Puget Sound and NE Pacific shelf ecosystems. She and McElhany will collaborate on the OA laboratory experiments and modeling component.

Results of Prior NSF Support:

J.E. Keister: *OCE- 0814698: Collaborative Research: GLOBEC Pan-regional Synthesis: Pacific Ocean Boundary Ecosystems: response to natural and anthropogenic climate forcing. Award period: Sept. 2008 – Sept. 2011; $1,330,630*. This program combines ROMS circulation models with existing time series observations of ocean physics and zooplankton to diagnose the response of four Pacific boundary ecosystems to large-scale natural and anthropogenic climate forcing. Objectives are to determine the mechanisms through which climate change leads to coherent physical and ecosystem changes around the Pacific basin, the extent to which short-period events (e.g. mesoscale structure, upwelling) are locally-controlled, mechanisms of control of zooplankton and phytoplankton dynamics, and an assessment of potential impacts of climate change on regional ocean ecosystems. In its first year, JK prepared a manuscript on advective controls of copepod communities for Global Change Biology and presented papers at two international meetings.

A.H. Devol: *OCE-0647981: Collaborative Research: Anammox and denitrification in the oxygen deficient zone of the Arabian Sea. Sept. 200 –Aug. 2010; $375,113*. This project was developed in response to accumulating evidence that anammox rather than denitrification is the main fixed N removal process in sediments and anoxic water columns. The project provided a unique opportunity to investigate N-cycling processes in the Arabian Sea, arguably the most important OMZ (oxygen minimum zone) in terms of fixed N loss and nitrous oxide fluxes. In 2007, we found that denitrification was the main N loss term in the Arabian Sea (Ward et al. 2009, Bulow et al. in press). Our previous work had found that denitrification in the Arabian Sea was not carbon limited, but in 2007, addition of carbon stimulated denitrification in short term experiments. The size of the N_2 excess in the ETSP was found to equal the size of the DIN deficit (Chang et al. submitted), in contrast to previous reports that the two estimates of DIN loss were not equivalent in the Arabian Sea. The contrast in the relative importance of denitrification and anammox between the Arabian Sea and the ETSP suggests important biogeographical differences or high variability in denitrification rates in both regions. This study has resulted in >5 peer-reviewed papers co-authored by Devol.

Project timeline