NOAA Northwest Fisheries Science Center Ocean Acidification Research:

FY 2010 Progress Report and FY 2011 Plan

Note: Public version
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Contents

Projects Summary

Ocean acidification research by the Northwest Fisheries Science Center (NWFSC) follows the priorities established by the NOAA ocean acidification implementation team (Feely et al. 2010). Research is directed towards conducting studies to investigate the impacts of ocean acidification on living marine resources. Studies focus on assessing the physiological effects on living marine resources and the resulting ecosystem impacts of these effects. NMFS Science Centers work together to ensure that NMFS ocean acidification research provides an integrated research program with publishable scientific results. In 2010, a NMFS-organized workshop was held in Seattle WA for NMFS and academic researchers to communicate research results and plans and for NOAA oceanographers to present results from related ocean acidification research. In addition, the three northern NMFS Science Centers (Northwest, Alaska and Northeast) which are responsible for coldwater regions have been collaborating closely since 2009 due to similarities in ecology, research priorities and approaches.

Recent oceanographic studies have shown that the Pacific Northwest is particularly vulnerable to ocean acidification, and substantial declines in West Coast oyster production related to pH conditions indicate that ocean acidification is likely already having negative effects on important marine resources in the region. To understand how ocean acidification will affect Northwest marine ecosystems, research at the NWFSC addresses the six themes outlined in the NOAA ocean acidification research plan: 1) monitoring, 2) ecosystem response, 3) modeling, 4) data synthesis, 5) human dimensions and 6) outreach (Feely et al. 2010). Research so far has focused primarily on CO_2 exposure experiments (theme 2) and on food web modeling (theme 3). The NWFSC has also developed a number of outreach projects to inform the larger community about ocean acidification (theme 6). In FY11, we propose to continue projects in species response, ecosystem modeling and outreach, while initiating projects in coupled biological/chemical monitoring (theme 1), linking ecosystem models to impacts on human communities (theme 5) and providing public databases of species vulnerability (theme 4). In 2010, the NWFSC Research Council identified OA as a Near-Term Research Priority, highlighting it as an area of special research focus at the Center.

Research in FY10 focused on development of a cutting edge system for conducting experiments evaluating the response of species exposed to future ocean chemistry conditions (Report 1a). This system has several unique features which enable it to mimic historical, current, and future marine conditions:

- Independently controls carbon chemistry, temperature and dissolved oxygen to look at interactions between the effects of acidification, climate change and eutrophication.
- Allows for dynamic control of all parameters to mimic natural patterns in pH, temperature and oxygen at tidal, diurnal and other scales. In coastal environments these parameters can be extremely variable.
- Simulation of pre-industrial conditions by removal of CO_2 from seawater. This ability is useful to determine whether species are adapting in changes in seawater chemistry that have already occurred.

- Over parameterization of carbon chemistry system by continuous monitoring of pH (electrode) and pCO_2, with on-site analysis of discrete samples for pH (spectrophotometric) and alkalinity, followed by periodic validation with DIC and alkalinity measurements from an external lab (NOAA-PMEL).
- Multiple species evaluated simultaneously. The system has a large volume of water for each treatment (>2,500 liters), so several species can be exposed at the same time, including larger species like adult Dungeness crab.

In FY10, capacity for six treatments plus one back up were constructed, allowing for 3x2 experiments (e.g. 3 pH levels at 2 different dissolved oxygen levels). In FY10, the NWFSC also purchased equipment for spectrophotometric pH analysis and automated alkalinity titrations (Report 1b) In FY11, the NWFSC, will operate and provide modifications to the Montlake OA system (Proposal 1a) Sometime after FY11, the NWFSC plans to expand the number of treatments, permitting 3-way interaction experiments, as the OA system is moved from its current location at the NWFSC Montlake lab to a new facility at the NWFSC Mukilteo field station. This move is dependent on NOAA plans to completely rebuild the Mukilteo facility, with OA as one of the primary areas of research focus.

Species targeted for experimental focus are those of high ecological, economic or conservation importance that are also expected to be vulnerable to the effects of acidification. Preliminary experiments were conducted in FY9 and FY10 at the Montlake lab on Pacific oysters, pinto abalone, geoducks and krill (Report 2a). In FY11, the NWFSC plans to continue research on these species, plus initiate studies on Dungeness crabs, Olympia oyster, copepods and rockfish (Proposal 2a). These species-response studies are done in collaboration with university, commercial, tribal, and other governmental and non-governmental biologists, allowing the NWFSC to take advantage of local expertise and external funding while using the large capacity and unique capabilities of the NWFSC OA experimental system. The mollusk work has been conducted in collaboration with researchers at the University of Washington (UW) and Taylor Shellfish, the zooplankton work with another group at UW, the crab work with Suquamish Tribal biologists, and the rockfish with Seattle Aquarium. The pinto abalone study was partially funded by a NOAA Species of Concern grant. In addition to the preliminary work conducted on krill at the Montlake lab, a separate preliminary study on several zooplankton species was conducted by NWFSC researchers using the AFSC OA research system at the Newport, Oregon lab (Report 2b). The NWFSC proposes to continue work on zooplankton at the Newport lab in FY11 (Proposal 2b).

Exposure experiments provide information on how a few species will directly respond to acidified conditions. However, we are more broadly interested in how entire ecosystems might be affected by OA and how food web interactions will create indirect effects on species of ecological, economic or conservation concern. In FY10, the NWFSC was involved in three ecosystem modeling projects that looked at potential effects of OA (Report 3). In one study, the Atlantis model was used to evaluate how OA might affect an area of the California current. In another study, several Ecopath/Ecosysem models along Northeast Pacific shelf were evaluated to look at the potential effects of both climate change and acidification. A third study developed scenarios of OA in Puget Sound based on a mineralogy database of all species in the Puget Sound, and used these scenarios in an Ecopath/Ecosim model to explore the ecosystem response to OA. In FY11, NWFSC researchers will complete work on manuscripts on the NE Pacific shelf and the Puget Sound work, and will extend the Puget Sound research with new modeling analyses (Proposal 3). In addition, a new model is being initiated that focuses on Dungeness crab using the Species Life-cycle Analysis Module (SLAM) modeling framework (Proposal 3). Rather than a comprehensive ecosystem model, the crab model uses a "minimum realistic" approach that only incorporates those life-stages, environmental drivers and other species considered critical to understanding crab dynamics.

In FY10, the NWFSC led several OA education and outreach projects, two of which were largely funded by a NMFS outreach grant (Report 4). The NWFSC collaborated with the Pacific Science Center to create and fund operation of an interactive OA "discovery cart", staffed by Pacific Science Center interpreters at this internationally recognized science education center that hosts almost a million visitors a year. In collaboration with UW, the NWFSC also helped develop and fund an OA education curriculum that was implemented in local public schools by UW graduate students. NWFSC researchers also put together interactive OA education kits which they presented at various area events. In FY11, in addition to participating in these outreach event opportunities, the NWFSC is planning to help design, produce, and fund a display or interactive exhibit on OA at the Seattle Aquarium (Proposal 4). While we will submit a NMFS outreach grant proposal for the Seattle Aquarium project, this grant will not cover salaries for NOAA employees working on the project.

In FY10, the NWFSC hosted a NMFS-wide OA workshop to exchange information and coordinate research among all of the NMFS Science Centers (Report 5) and a NWFSC employee attended the WHOI ocean acidification short course. In FY11, NWFSC researchers will attend the OA PI's workshop sponsored by NOAA and other agencies to be held at the WHOI in March. Researchers at the NWFSC have organized a symposium on ocean acidification at the 2011 American Fisheries Society meeting to be held in Seattle in September, 2011. In addition, NWFSC OA researchers will participate in other OA workshops and scientific meetings, including a workshop planed between NWFSC, AFSC, NEFSC and IMR in Norway (Proposal 5).

In FY11, if increased funding is available, we propose two new projects related to OA monitoring and field sampling. The first is a pilot project to develop protocols for coupled biological/chemical monitoring in Puget Sound (Proposal 6a). This sampling will take advantage of regular transects conducted in Puget Sound as part of another project. In an effort to characterize the carbon chemistry environment actually experienced by ecologically important organisms in the field, we will collect carbon chemistry data at the same time and in the same place that other researchers collect phytoplankton and zooplankton samples. The second project involves expanding a pH monitoring system currently being established on the west coast into the state of Washington (Proposal 6b). The NWFSC would deploy a new type of moored continuous pH sensor (Seafet) at a few key locations to determine the temporal and spatial dynamics of ocean pH in the near shore environment. The NWFSC proposes three sensor locations: 1) Willipa Bay, where it can be correlated with monitoring of one of the largest natural oyster beds on the West Coast, 2) Outer Washington Coast, where it can be correlated with monitoring conducted by the NOAA National Marine Sanctuary, and 3) off the pier at the NWFSC Mukiteo field station, where it can be connected with sampling and experiments conducted as part of the new OA facility. Deployment of the continuous sensor monitoring system is dependent on receiving the higher funding level.

NWFSC Researchers and Collaborators Working on OA

Researchers and collaborators working on ocean acidification at the NWFSC, along with their areas of focus are listed in Table 2. This list does not include interns, students or others who may be involved in some aspect of NWFSC OA research. The table lists primary outside collaborators and does not list everyone who may be secondary authors on NWFSC OA projects. Five researchers listed in Table 1 spend the majority of their time working on OA projects.

Table 2: Primary researchers and collaborators working on ocean acidification at the NWFSC. Researchers with an asterisk next to their names spend the majority of their time working on ocean acidification projects.

Researcher	Location	Projects
Paul McElhany*	NWFSC- Montlake	NWFSC OA research lead, OA system design, species response experiments (esp. abalone and zooplankton), ecosystem and Dungeness crab modeling
Shallin Busch*	NWFSC– Montlake	Species response experiments (esp. bivalves), carbon system chemistry (spectrophotometric pH and alkalinity), ecosystem modeling
Jason Miller*	NWFSC - Montlake	Development, construction and operation of OA system, Species response experiments (esp. Dungeness Crab)
Mike Maher*	NWFSC - Montlake	Development, construction and operation of OA system, Species response experiments (esp. krill and copepods)
Sarah Norberg*	NWFSC - Montlake	Development, construction and operation of OA system, Species response experiments (general aquaculture and)
Andy Dittman	NWFSC - Montlake	Species response experiments (fishes), sperm motility experiments
Mark Tagal	NWFSC - Montlake	Species response experiments (rockfish aquaculture)
Isaak Kaplan	NWFSC - Montlake	Ecosystem modeling (Atlantis)
Chris Harvey	NWFSC - Montlake	Ecosystem modeling (Ecopath/Ecosim)
Cameron Ainsworth	NWFSC - Montlake	Ecosystem modeling (Ecopath/Ecosim)
Corriegh Greene	NWFSC - Montlake	Puget Sound field sampling proposed pilot project
Bill Peterson	NWFSC - Newport	Krill and copepod experiments in AFSC Newport system

Leah Feinberg	NWFSC - Newport	Krill and copepod experiments in AFSC Newport system
Tom Hurst	AFSC - Newport	AFSC OA system in Newport used for NWFSC zooplankton experiments
Dick Feely	NOAA-PMEL	Collaborator on carbon chemistry analysis
Carolyn Friedman	University of Washington -SAFS	Primary collaborator on mollusk experiments
Steven Roberts	University of Washington -SAFS	Primary collaborator on evaluation of genetics/genomics
Julie Keister	University of Washington - SOO	Primary collaborator on zooplankton experiments
Moose O'Donnell	University of Washington – FHL	Collaborator on OA system design and carbon chemistry analysis
Paul Williams	Squamish Tribal Department of Fisheries	Primary collaborator on Dungeness crab experiments

Collaborations Among NMFS Science Centers

NMFS Science Centers work together to ensure that NMFS ocean acidification research provides an integrated research program with publishable scientific results. In 2010, a NMFS-organized workshop was held in Seattle WA for NMFS and academic researchers to communicate research results and plans and for NOAA oceanographers to present results from related ocean acidification research. In addition, the three northern NMFS Science Centers (Alaska, Northwest and Northeast) which are responsible for coldwater regions have been collaborating since 2009 due to similarities in ecology, research priorities and approaches. Their research has focused on a range of taxa because the biological effects of ocean acidification are mostly unknown; priority has been placed on species considered most vulnerable ecologically and of economic importance. The approach consists primarily of species-specific laboratory studies and population and ecosystem modeling. Scientists from the 3 science centers teleconference monthly to compare notes on diverse topics such as laboratory setup, experimental challenges and water chemistry. A joint proposal formulated among the three science centers in 2010 continues to guide, coordinate and integrate ocean acidification research that will continue and expand during 2011. All studies by the these three NMFS Science Centers address themes highlighted in the NOAA regional ocean acidification implementation plans that have been developed by the NOAA ocean acidification implementation team. In 2011, chemists from these three Science Centers are testing instruments and comparing their results using certified reference materials from the same source. These comparisons give the researchers a standard frame of reference, verify measurement accuracy and assure measurement quality.

FY10 Report 1a: NWFSC OA research facility (Montlake)

System features

Research in FY10 focused on development of cutting edge system for conducting experiments evaluating the response of species exposed to future ocean chemistry conditions. This system has several features which enable it to uniquely mimic historical, current, and future marine conditions:

- Independently controls carbon chemistry, temperature and dissolved oxygen to look at interactions between the effects of acidification, climate change and eutrophication.
- Allows for dynamic control of all parameters to mimic natural patterns in pH, temperature and oxygen at diurnal and other scales. In coastal environments these parameters can be extremely variable (e.g. Figure 1).
- Simulation of pre-industrial conditions by removal of CO_2 from seawater. This is useful to determine whether species are adapting in changes in seawater chemistry that have already occurred.
- Over parameterization of carbon chemistry system by continuous monitoring of pH (electrode), and pCO2, with on-site analysis of discrete samples for pH (spectrophotometric), and alkalinity, followed by period validation with DIC and alkalinity measurements from an external lab (NOAA-PMEL).
- Multiple species evaluated simultaneously. The system has a large volume of water for each treatment (>2,500 liters), so several species can be exposed at the same time, including larger species like adult Dungeness crab.

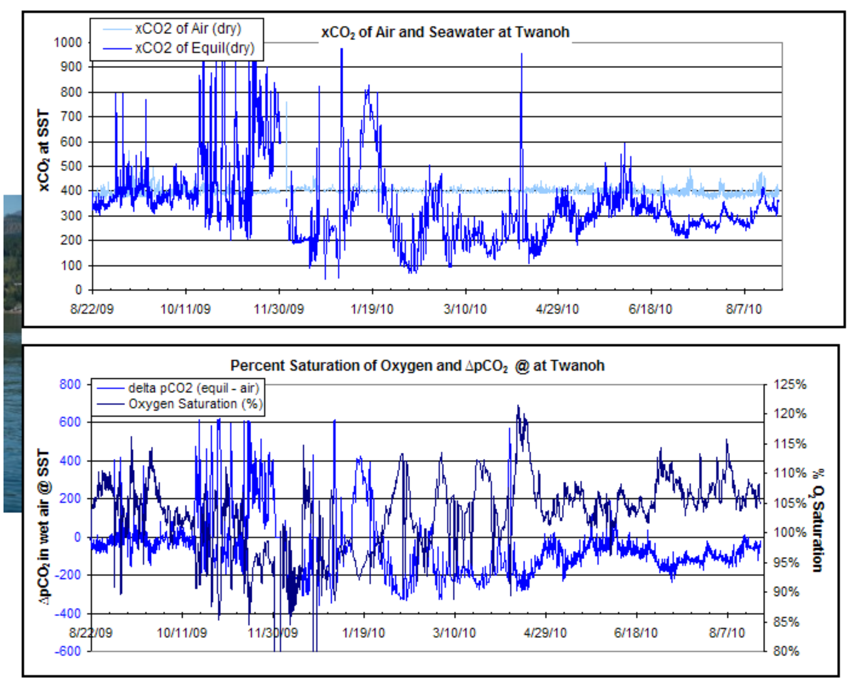

Figure 1: Time series of xCO2 in surface waters of Puget Sound (from PMEL buoy at Twanoh). The NWFSC can mimic these diurnal swings of hundreds of ppm ranging from <100 to >1,000.

System design

The system manipulates carbon chemistry and dissolved oxygen by controlled bubbling of five gases (air, CO_2-free air, pure CO_2, nitrogen, and pure oxygen) (Figures 2 and 3). Temperature is manipulated using heat pumps and emersion heaters. The system is monitored and regulated based on Durafet pH probes, Licor pCO_2 measurements, dissolved oxygen probes, and temperature sensors. The entire system is controlled by custom Lab View programs developed at the NWFSC. Although adding $CO_2$to seawater is a relatively easy process, removing it is more challenging. To obtain pre-industrial CO_2 conditions and to mimic natural patterns that have rapid drops in CO_2, a large reservoir of low-CO2 water is maintained that acts as a source for achieving desired treatment levels.

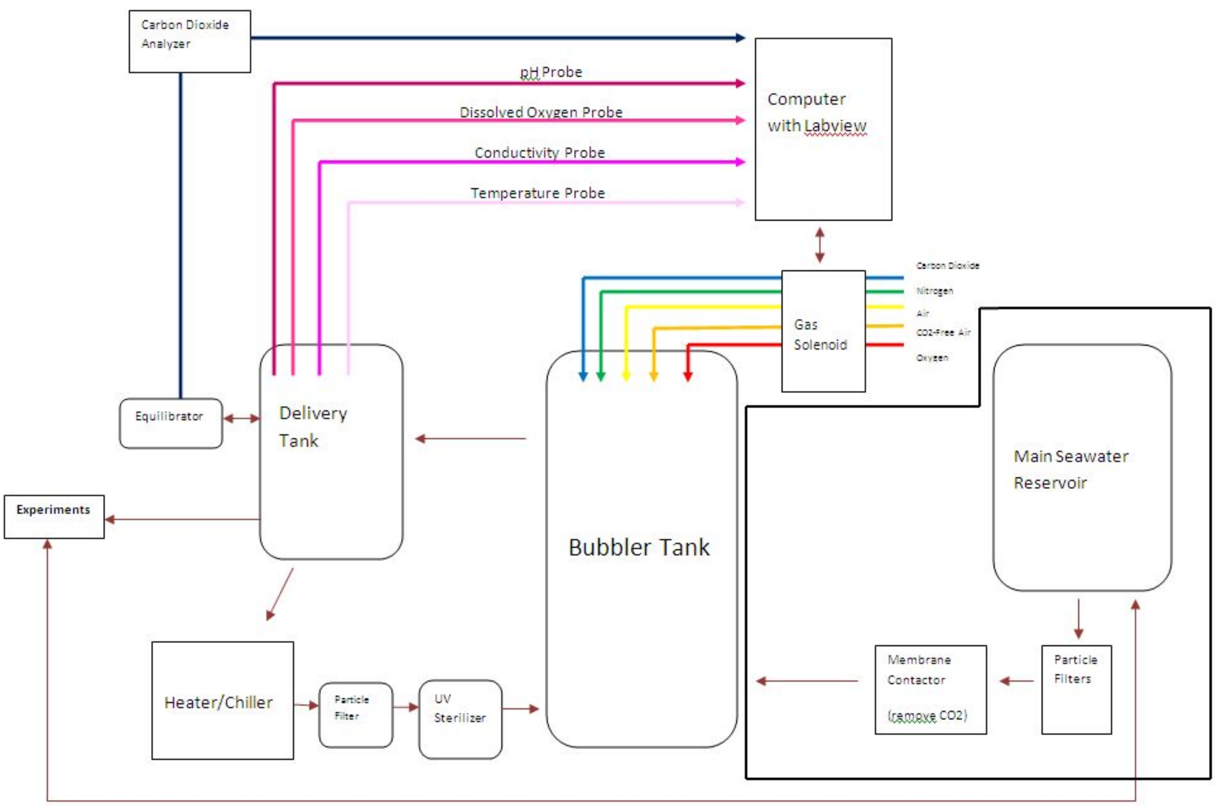

Figure 2: Simplified diagram of NWFSC OA exposure system.

Figure 3: Photo of four treatment systems at the NWFSC Montlake OA research facility. There are three additional treatment systems not in the photo. The round white tanks on benches closest to the camera are the “delivery tanks” where water conditions are measured. The rectangular white boxes in front are the equilibrators. The tall white tanks in back are the “bubbler tanks” where gases are added. The gray boxes on tables are water baths holding the containers where organisms are exposed to treated water.

Organisms are exposed to the treated water in aquaria appropriate to their life history and the experimental design. For many of the larval and zooplankton species, rearing occurs in 4 liter jars developed at the NWFSC (Figure 4). These containers are sealed to prevent interaction with the atmosphere. For smaller numbers of organisms, like the brood from an individual female krill, smaller sealed containers are used (Figure 4). Larger organisms, like adult pre-spawn Dungeness crab can be held directly in the larger water bath containers.

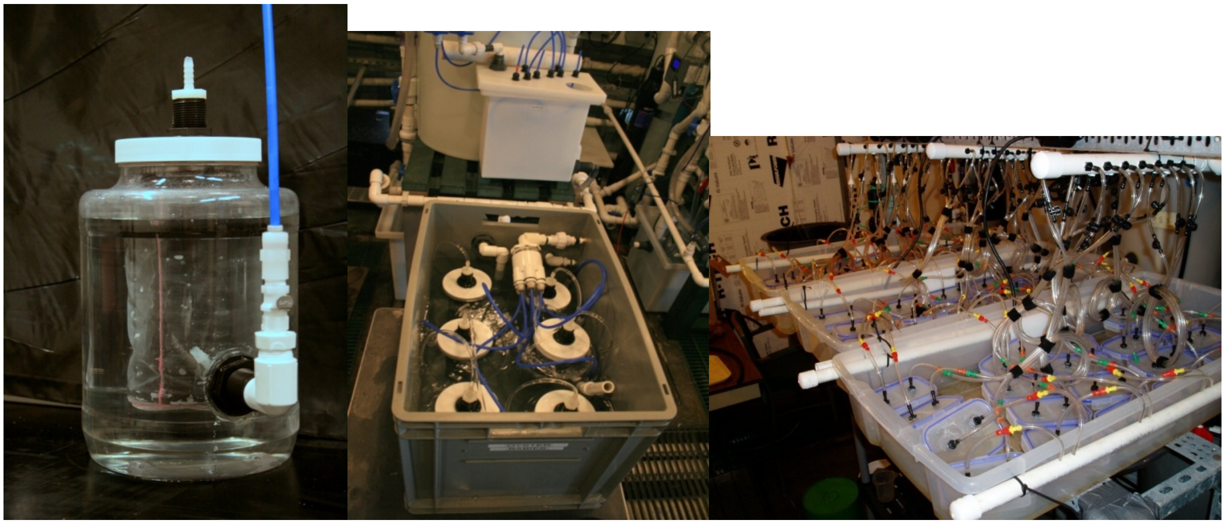

Figure 4: Left panel shows a 4 liter jar used for larval rearing during OA exposure experiments. Center panel shows six of the 4 liter jars nested in a water bath. Right panel shows smaller containers used to hold krill larvae from an individual brood for OA exposure experiments.

FY10 Report 1b: Carbon chemistry analysis equipment

Seawater carbon chemistry can be measured in four ways: pH, pCO_2, total alkalinity, and total inorganic carbon. With knowledge of two carbon chemistry parameters, one can calculate the two others and dependent parameters, such as aragonite or calcite saturation state. During FY10, we developed the ability to measure three carbon chemistry parameters: pH, pCO_2, and total alkalinity. Constraining carbon chemistry calculations with three parameters instead of two is especially important in coastal waters, as equations used to calculate seawater chemistry often do not perform well in coastal environments due to the abundance of biological byproducts that are typically negligible in the open ocean.

For each of our 7 replicate systems, we use a Honeywell Durafet pH probe to collect continuous pH data (Figure 5). The Durafet probe is based on new technology and is considered the only low-maintenance, stable, yet accurate way to measure pH. We regularly verify the performance of these probes against a standard solution and pH values from a reference lab (NOAA PMEL) and our spectrophotometer. The LiCor CO_2 analyzer can determine the concentration of CO_2 in a stream of dry gas (Figure 5). Using a multiport valve, we can step through gas streams equilibrated to the water in each system and determine the pCO_2 in each treatment system. We test the performance of the LiCor against calculated values of pCO_2 from seawater samples sent to a reference lab (NOAA PMEL).

Figure 5: Left panel shows Honeywell Durafet pH probe. Right panel shows LiCor CO_2 analyzer for measuring pCO_2.

We use two technologies to assess seawater carbon chemistry in discrete samples taken from our OA system or the field (Figure 6). The most accurate technique for measuring seawater pH is to directly access the concentration of H^+ ions in a sample, instead of using electrical charge as a proxy for pH, which is what pH probes do. We use a spectrophotometer and an indicator dye, which is one color when bound to H^+ and another when not bound to H^+, to assess the concentration of H^+ in seawater samples. We are able to accurately and precisely measure pH of reference materials with our spectrophotometer and our measurements of pH are very close to pH values calculated from a reference lab (NOAA PMEL). Total alkalinity gives a measure of the "proton accepting-ness" of seawater, and is measured by titrating a seawater sample of known volume against HCl acid. In FY10, we purchased an automated titrator that develops the data needed to calculate alkalinity. Our team has developed two stand-alone Java programs for our chemistry work: a program called *pHAST* assists in the collection and processing of data from the spectrophotometer and a separate, program called alkcalc that calculates alkalinity from titration data using necessary data fitting techniques.

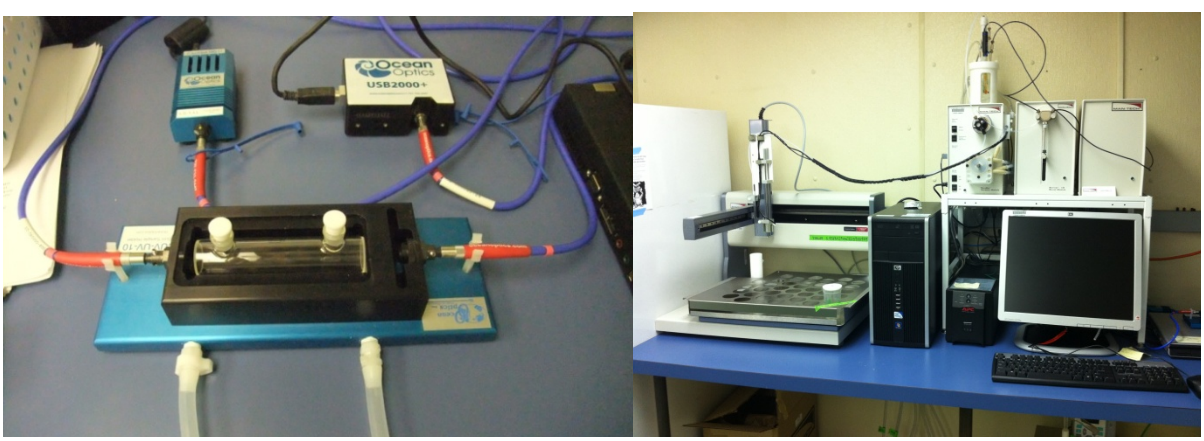

Figure 6: Left panel shows spectrophotomter for measuring pH. Right panel shows auto-titrator for measuring alkalinity.

FY10 Report 2a: Pilot species response studies (Montlake)

Geoduck

A pilot experiment on larval geoduck (*Panopea abrupta*) was conducted in 2009. Freshly fertilized geoduck eggs were collected from the Taylor Shellfish hatchery then exposed to three different pH levels. The CO_2 dosing system was relatively imprecise compared to that in the current NWFSC OA facility (Figure 7). Using images from a scanning electron microscope, we found some evidence of shell dissolution in our study subjects (Figure 8). We also found some differences in the size of the larvae among treatments. However, our inability to precisely control the experimental conditions prevents us from making conclusions about the response of geoduck to OA.

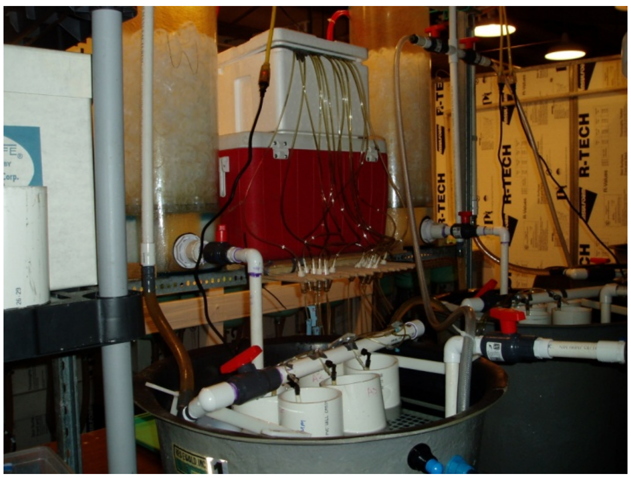

Figure 7: Larval geoduck acidification exposure experiment using the first generation CO_2 dosing system at the NWFSC.

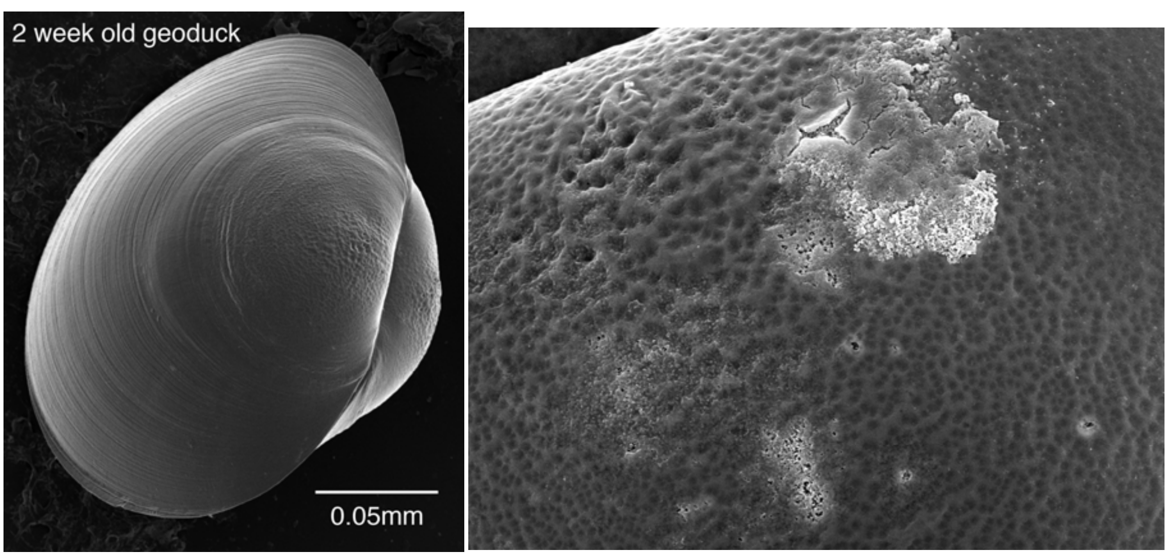

Figure 8: Scanning electron micrographs (SEM) of geoduck from NWFSC 2009 acidification pilot study. The panel on the right shows pitting that was observed in some larval shells.

Krill

A pilot experiment on larval krill (*Euphasia pacifica*) was conducted in 2009. Gravid adult E. pacifica were collected from Puget Sound (Figures 9 and 10). Eggs released by females (Figure 10) were distributed by brood into three different pH treatment levels (Figure 9). The CO_2 dosing system was relatively imprecise compared to that in the current NWFSC OA facility, and the system was not temperature controlled. Compared to control conditions, we found an increase in the number of developmental deformities at an elevated CO_2 level with target pH of 7.5 and mortality of all developing krill at the highest CO_2 treatment (target pH of 6.5). For this study, our sample sizes were extremely small and pH levels were not externally verified.

Figure 9: Left panel shows collection of gravid female krill from Puget sound. Right panel shows krill egg exposure system connected to first generation NWFSC CO2 dosing system.

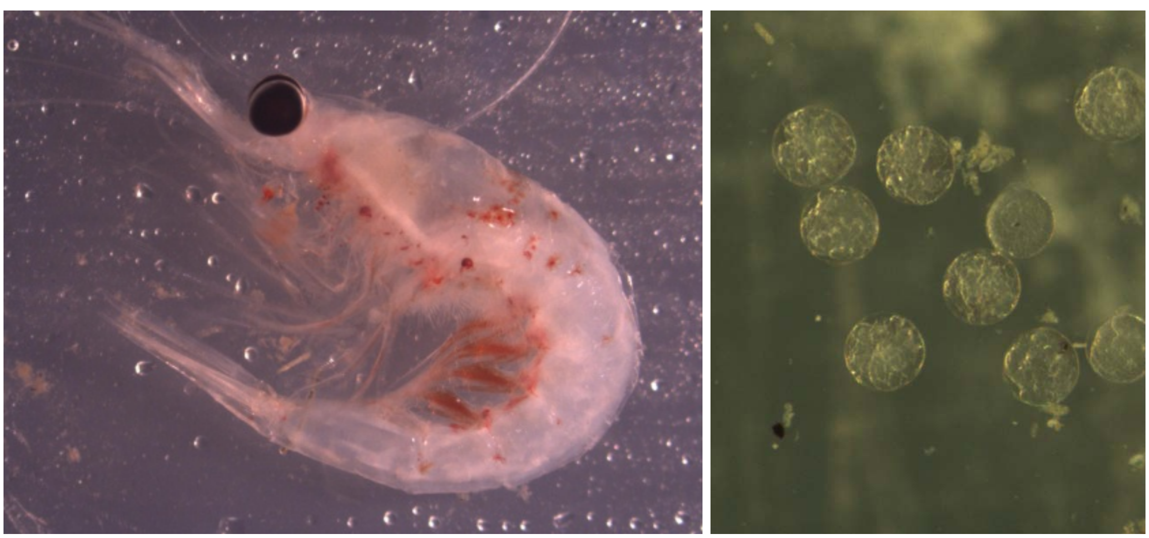

Figure 10: Left panel shows adult gravid female E. pacifica. Right panel shows recently released eggs.

Pacific oyster

In FY10, a pilot acidification study was conducted on Pacific oysters (*Crassostrea gigas*) in collaboration with the Friedman and Roberts labs at the University of Washington. This experiment was the first one in the new NWFSC OA experimental system, and we spent much time and effort learning how to operate the system during an experiment, collect oyster samples and water chemistry samples, and successfully rear larvae. We spawned wild-collected oysters from Puget Sound in the lab, and distributed fertilized eggs among four CO_2 treatments (280, 380, 750, 2000ppm CO_2). Data on fertilization success, developmental progress, and survival were collected and analyzed by Emma Timmins-Schiffman of the Roberts lab (Figure 11). We collected data on shell calcification, and found that oyster larvae living in 2000ppm CO_2 seawater did not develop fully calcified shells (Figure 12). In addition, gene expression of larvae in each treatment was analyzed by Emma Timmins-Schiffman. Results indicate some differences in gene expression among treatments, however, sample sizes were small and confounded by differences in culture density. A ciliate outbreak prevented Elene Dorfeimer, a graduate student in the Friedman lab, from conducting a study on the interaction between oyster larvae and *Vibrio tubiashii*, a pathogen.

We collected a series of water samples for pH analysis using the spectrophotometer. Analysis of these samples demonstrated that: 1) the pH of the system stayed relatively stable over the course of the experiment, 2) the pH of the water in the each larval culture vessel was not changed by the oyster larvae and was similar across replicates and to the water in the delivery tank, and 3) water pH in each culture vessels was not altered by introduction of dead food (algae paste).

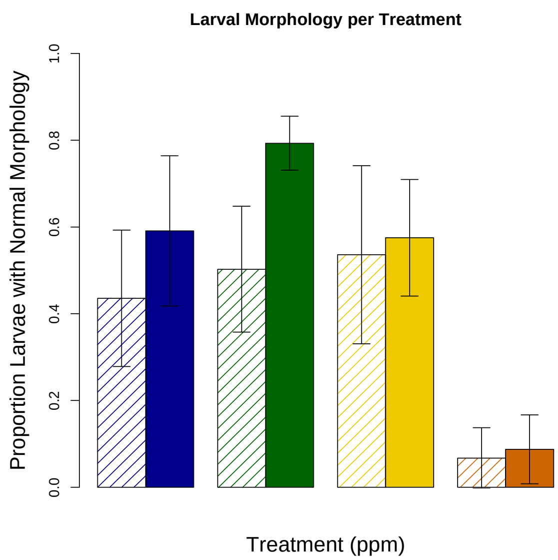

Figure 11: Percent normal morphology per treatment: blue = 280 ppm CO_2 green = 380, yellow = 750, and orange = 2000. Data collected at 24hr post-fertilization is displayed with hashed bars and at 48hr is displayed with solid bars. The differences among datasets were significant with respect to both treatment and day (two-way ANOVA, p=5.35e-12 and 0.0041, respectively). Tukey's HSD showed that the treatment differences were highly significant between the 2000 ppm treatment and all other treatments.

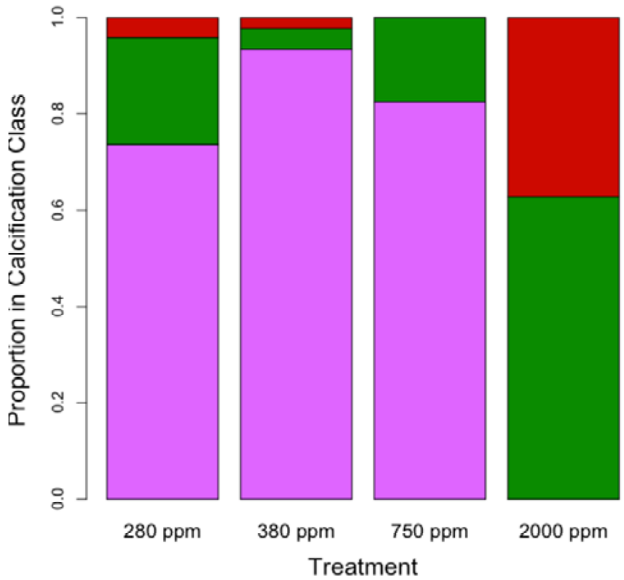

Figure 12: Proportion of larvae in each treatment that are fully calcified (pink), partially calcified (green), and not calcified (red) 24hrs post fertilization.

Pinto abalone

In FY10, a pilot acidification study was initiated on pinto abalone (*Haliotis kamtschatkana*) in collaboration with the Friedman and Roberts labs at the University of Washington. Freshly fertilized abalone eggs were obtained from captive brood stock held at the NWFSC Mukilteo lab by the Friedman lab. For this pilot work, our aims were to learn how to maintain healthy abalone cultures in our system, properly collect abundance data, and process samples for size analysis and SEM. We were able to successful culture the abalone to settlement. However, while we were able to keep some animals alive, we cut the experiment short because of a ciliate outbreak in the system. The ciliate outbreak seemed to respond to both abalone density and treatment conditions (pH), leading us to believe that the pathogen itself might respond to OA and/or the interaction between abalone and ciliates might be influenced by OA.

To accurately assess the impact of OA on population demographics, one must be able to distinguish live larvae from dead larvae. We worked with a Liza Ray, a UW graduate student, to test the efficacy of a number of vital stains on the larval abalone, and, unfortunately, found that the five stains we tested are not accurate enough for our work.

Prior research in other laboratories indicates that in low pH conditions, larval abalone may fail to grow or maintain their shells. We found abalone without complete shells living in 840ppm CO_2 seawater at age 7 days but not age 4 days, indicating that shell maintenance may be compromised. To build the tools to explore this hypothesis further, we worked with Carla Stehr (NWFSC) to learn how to process larval abalone for imagining with a scanning electron microscope (Figure 13). Imaging this species is challenging, as one must find a technique that removes the periostracum from larval shell without damaging the shell itself. We tested a number of techniques for preserving both soft tissue and the shell.

Dave Metzger, a UW graduate student, collected samples for DNA and RNA from the abalone pilot study. He used these samples to test RNA extraction procedures, and determined the best method to store samples and the number of larvae that would yield enough RNA for quantitative PCR and "next gen" sequencing experiments.

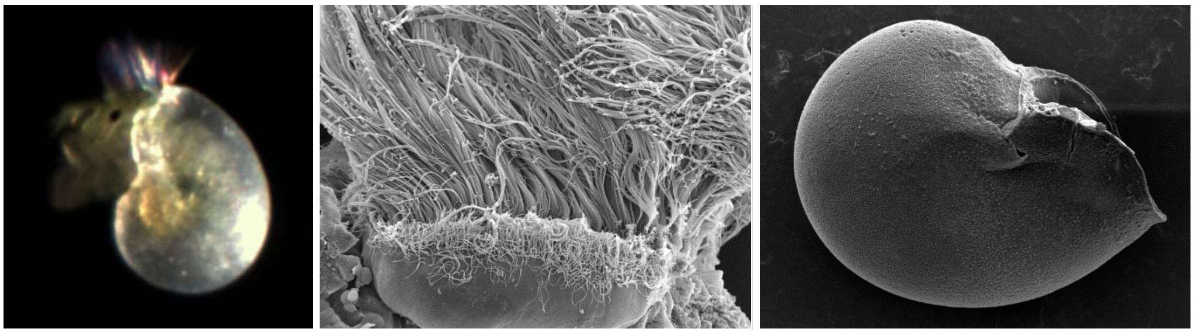

Figure 13: Images of larval abalone. Left panel is of a live individual and taken with a light microscope. Center and right panels were taken with a scanning electron microscope and show cilia and the larval shell.

FY10 Report 2b: Pilot species response studies (Newport)

In FY10, gravid female krill (Euphasia pacifica) and two copepod species (Calanus pacificus and Calanus marshallae) were collected off the Oregon coast (Figure 14). The eggs released were placed in the AFSC OA exposure system in Newport in target pH treatment levels of 7.1, 7.6, 7.8 and 8.0. Developing zooplankton were observed until day 8. Preliminary results suggest no effect of the treatments on E. pacifica (though there was a strong maternal effect). Lower pH seemed to slow development for C. pacificus, but did not affect C. marshallae. However, sample sizes were small and the pH levels for the treatments have not yet been verified (stored samples waiting processing), so all results should be considered preliminary.

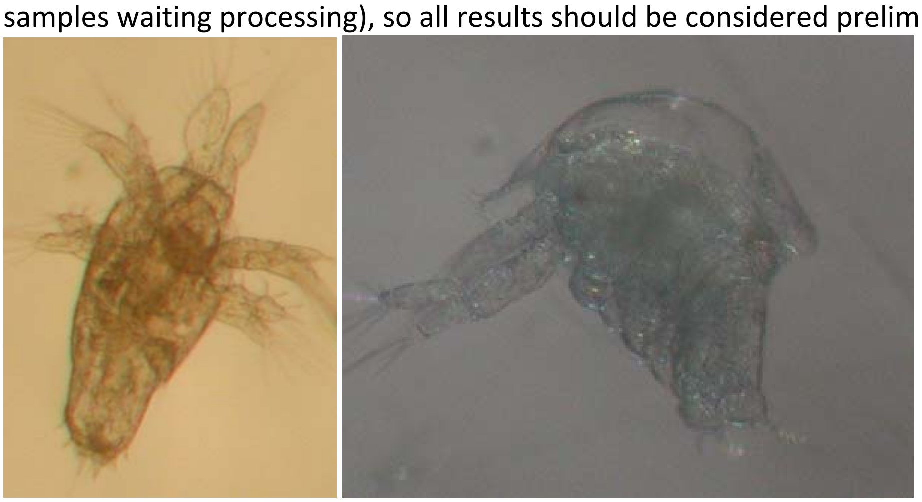

Figure 14: Left panel Calanus pacificus nauplii stage at 8 days. Right panel Euphasia pacific nauplii at 8 days.

FY10 Report 3: Ecosystem models of OA effects

Three ecosystem modeling projects examining the potential impact of ocean acidification have been conducted at the NWFSC. These are summarized below using journal abstracts.

Northern California Current with Atlantis

Title: Fishing catch shares in the face of global change: a framework for integrating cumulative impacts and single species management
Authors: Isaac C. Kaplan, Phillip S. Levin, Merrick Burden, and Elizabeth A. Fulton
Journal: Can. J. Fish. Aquat. Sci. **67**: 1968–1982 (2010)
Abstract: Any fishery management scheme, such as individual fishing quotas (IFQs) or marine protected areas, should be designed to be robust to potential shifts in the biophysical system. Here we couple possible catch scenarios under an IFQ scheme with ocean acidification impacts on shelled benthos and plankton, using an Atlantis ecosystem model for the US West Coast. IFQ harvest scenarios alone, in most cases, did not have strong impacts on the food web, beyond the direct effects on harvested species. However, when we added the impacts of ocean acidification, the abundance of commercially important groundfish such as English sole (*Pleuronectes vetulus*), arrowtooth flounder (*Atheresthes stomias*), and yellowtail rockfish (*Sebastes flavidus*) declined up to 20%–80%, owing to the loss of shelled prey items from their diet. English sole exhibited a 10-fold decline in potential catch and economic yield when confronted with strong acidification impacts on shelled benthos. Therefore, it seems prudent to complement IFQs with careful consideration of potential global change effects such as acidification. Our analysis provides an example of how new ecosystem modeling tools that evaluate cumulative impacts can be integrated with established management reference points and decision mechanisms.

Northeast Pacific Shelf with Ecopath/Ecosim

Title: Potential impacts of climate change on Northeast Pacific marine food webs and fisheries
Authors: C.H. Ainsworth, J.F. Samhouri, D.S. Busch, W.L. Cheung, J. Dunne , T.A. Okey
Journal: ICES Journal of Marine Science, in press
Abstract: Climate change is altering the world's oceans. The impacts of changes in water temperature, carbonate chemistry, and other variables on species are complex, but not completely unknown. However, cumulative impacts of these effects have rarely been studied. Here we simulate changes in (1) primary productivity, (2) species range shifts, (3) zooplankton community size structure, (4) ocean acidification and (5) ocean deoxygenation both individually and together using five Ecopath with Ecosim trophodynamic models of the northeast Pacific Ocean. We used a simplistic but standardized method to represent climate effects that relied on time series forcing functions; that is, annual multipliers of species productivity. We focused on changes in fisheries landings, biomass, and ecosystem characteristics (diversity and trophic indices). Fisheries landings generally declined in response to cumulative effects, and often to a greater degree than would have been predicted based on individual climate effects, indicating possible synergies. Total biomass of fished and unfished functional groups showed an overall decline under cumulative effects, though unfished groups were less negatively affected. Some functional groups (e.g., pelagic and demersal invertebrates) were predicted to respond favorably

under cumulative effects in some regions. Cumulative climate effects consistently caused biodiversity decline. The challenge of predicting cumulative impacts of climate change is enormous, but must be met if we are to adapt and manage rapidly changing marine ecosystems in the 21st century.

Title: Evaluating uncertainty in estimates of how climate change may impact Northeast Pacific marine ecosystems *(Winner of "best presentation" award for the FUTURES section)*
Authors: D.S. Busch, C.H. Ainsworth, J.F. Samhouri, W.L. Cheung, J. Dunne , T.A. Okey
Abstract: Climate change will affect Earth's physical environment strongly and influence its biota in complex ways. While empirical studies provide valuable information on species' responses to environmental conditions, modeling exercises can be used to gain insights into the indirect and cascading effects of climate change on whole biological communities. Such modeling exercises can, however, contain a large number of uncertainties, all of which can influence their results. We present a study that addresses how uncertainty in both our knowledge of species responses to climate change (effect size and process uncertainty) and our ability to model them (model uncertainty) influences our understanding of the impacts of climate change. This work is an extension of a modeling exercise undertaken by Ainsworth et al. (in review), which attempts to predict the ecosystem-level impacts of climate change on Northeast Pacific shelf ecosystems using simulations with five Ecopath with Ecosim food web models. The Ainsworth et al. study applies five climate change impacts to these food web models: change in annual mean level of primary production, shifts in distribution of fish and invertebrates, alteration of the size structure of plankton communities, ocean acidification, and deoxygenation of surface waters. We consider and explore the effects of the three types of uncertainty using a variety of analytical techniques. Results of this study focus on data that are relevant to resource managers, namely biomass of groups that provide important ecosystem services (e.g., harvested species, forage fish) and measures of ecosystem health (e.g, diversity, reorganization).

Title: The importance of community interactions for predicting climate change impacts
Authors: J.F. Samhouri, C.H. Ainsworth, D.S. Busch, W.L. Cheung, J. Dunne , T.A. Okey
Abstract: Climate change is altering the world's oceans. The direct impacts of projected changes on species are complex, but not completely unknown. Warmer, more acidic, and less oxygenated waters will alter metabolic processes, modify demographic rates such as survival and fecundity, and produce shifts in the biogeographic ranges of individual species. Less appreciated than these direct climate change impacts are the indirect consequences of climate change resulting from interactions among species. The objective of this study was to compare the predicted impacts of climate change induced range shifts, ocean acidification, and ocean deoxygenation on individual species to those predicted for the same species in a food web context. We used two approaches to simulate the impacts of each of these effects on marine species in five Northeast Pacific marine food webs. First, we simulated the impact of each climate change effect on each species individually. Second, we used Ecopath with Ecosim models to simulate the impact of each climate change effect on all species simultaneously, allowing for dynamic trophic interactions among the food web members. The difference between the biomass predicted for each species using the first and second approach highlights

the relative importance of species interactions in determining the impact of climate change in these systems. As new information becomes available on how climate change affects species biology, modeling approaches such as this one will become increasingly valuable for understanding how species interactions shape community- and ecosystem-level responses to climate change.

Puget Sound with Ecopath/Ecosim

Title: Potential impacts of ocean acidification on the Puget Sound food web
Authors: Shallin Busch, Chris Harvey, Paul McElhany
Journal: Ocean Sciences Meeting 2010, Portland, Oregon
Abstract:
Over a third of the carbon dioxide released by anthropogenic activities during the last 200 years has been absorbed by the oceans. Some of this carbon dioxide has been stored in the sediments, but much of it remains in solution in seawater. Increasing atmospheric carbon dioxide and, as a result, increasing the amount of carbon dioxide dissolved in seawater decreases both the pH of seawater (due to increases in carbonic acid) and the abundance of carbonate, the molecule that most organisms use to make calcium carbonate structures. Ocean pH has already decreased by 0.1 units since the industrial revolution began. Experiments and theoretical work indicate that the chemical changes to sea water induced by increased atmospheric carbon dioxide will affect the physiology of a diverse suite of organisms. For example, some organisms that have calcium carbonate structures (e.g., bivalves, coralline algae) may suffer from higher rates of calcium carbonate dissolution and increased energetic costs for building and maintaining calcium carbonate structures. Such phenomena could decrease individual and population-level growth rates and increase mortality rates. In converse, seagrasses seem to be carbon-limited under current atmospheric carbon dioxide levels, and are likely to increase growth and reproduction rates under future climates. Changes in species growth and survival rates can alter the biomass of species groups within an ecosystem, creating a new balance among primary producers, prey, and predators. Using an Ecopath with Ecosim model developed to approximate the food web of Puget Sound's Central Basin, we explore the potential consequences of ocean acidification on an estuarine food web. To determine which species groups would be susceptible to ocean acidification and whether the species response would be positive or negative, we surveyed 135 papers on species responses to the carbon dioxide titers likely to occur within the next few centuries. We developed four test scenarios, altering the productivity, habitat-building capacity, and/or predation/consumption of 1) seagrasses, 2) molluscs, 3) crustacea, and 4) echinoderms. Due to uncertainty in how severe the species groups' response will be, we tested three effect levels for each scenario (low, moderate, and high). Our results suggest how changes to a limited number of species groups can impact other members of the food web, indicating that ocean acidification can influence species that it does not directly affect. Given the high uncertainty in forecasting the impacts ocean acidification at the ecosystem level, we consider our results as suggestions of what may occur in the future, and hope that they will help guide future modeling efforts and field monitoring programs.

FY10 Report 4: OA outreach

The following is the final report for a 2010 NMFS internal education funding grant. Materials and contract were funded by the grant as indicated. NWFSC staff time and some development materials were funded by NMFS FY10 OA funds.

Project Title: Ocean Acidification and Puget Sound: A 'Less Basic' Education
Period Dates: April 2010 – June 2011
Introduction
Northwest Fisheries Science Center staff collaborated with University of Washington faculty to develop a hands-on curriculum explaining the science of ocean acidification (OA) and the potential effects of decreasing pH levels on marine ecosystems. Lessons were distributed to University of Washington Ocean and Coastal Integrated Sciences (UW OACIS) GK-12 fellows for inclusion into local high school marine science curriculum. GK-12 fellows are a group of graduate students obtaining a masters in education with special emphasis on teaching marine science at the high school level. As part of their graduate program, GK-12 fellows are required to pair up with and teach along side professional high school teachers.

Northwest Fisheries Science Center also partnered with the Pacific Science Center (PSC), a premier public science education facility in Seattle, WA, to design and construct an interpreter-led, interactive floor exhibit focused on increasing community awareness of OA with an emphasis on the potential environmental and economic effects to Puget Sound.
Purpose: (objectives of the project)
The goals of the project:

- Develop and provide lesson plans and materials that are effective in teaching about the science behind OA to local school teachers and students.
- Create an interactive display in a free-choice science learning facility that will increase awareness of the science of OA and the potential devastating effects OA can have on the marine ecosystem.

Approach: (the work that has been performed)
<u>University of Washington (UW), SAFS, OACIS GK-12 Program</u>
An overview of the lesson plan and experimental setup was presented to incoming GK-12 fellows in September 2010 for incorporation into the marine science curriculum during the 2010/2011 school year. Funding provided by NMFS was used to purchase materials for 6 OA classroom science kits. Each kit contains basic materials needed for an OA experiment including: plastic beakers, a sports drink bottle, aquarium air pump, digital thermometer, hanging scale, sugar, yeast and pH testing strips. Live Pacific oyster spat are also provided to the classrooms and students are instructed on how to conduct their experiment.
<u>Pacific Science Center Discovery Cart Program</u>
From April to September 2010, NWFSC staff collaborated with the science education staff at the PSC to develop an OA discovery cart, a collection of hands-on interactive activities shared by trained interpreters with visitors. The OA cart is dedicated to providing the general public with a tangible lesson about the science behind OA and its potentially detrimental effects on marine

ecosystems and our local community. Visitors engage in several step-by-step activities that explain fundamental concepts needed to understand how CO_2 in the atmosphere affects pH levels and calcium carbonate concentrations in the ocean. The cart complements another recent PSC display that features real-time CO_2 measurements from local monitoring sites such as the top of the Space Needle and buoys around Puget Sound.

Results: (accomplishments to date)

OA classroom kits for the GK-12 fellows have been assembled and will be distributed starting the week of November 1st for use with the marine science curriculum during the 2010/2011 school year.

The PSC OA discovery cart has been completed and is available for public viewing. Approximately 8,700 visitors will be engaged within the 580 hours of designated OA cart delivery time at the PSC.

Evaluation: (the extent to which the project goals and objectives have been attained)

Goal	Extent to which it has been attained
Proposed project provides training and professional development opportunities for formal and informal educators and graduate students to learn about OA science and also new ways to deliver accurate, up-to-date scientific information in an engaging way.	OA curriculum and teaching aids developed by NOAA Fisheries and NWFSC staff were presented to professional high school teachers and graduate school fellows as well as informal education development and interpretive staff at a public free-choice learning center.
Through these partnerships, NWFSC will be developing lesson plans, activities, and other educational information that can be made available on NWFSC's website.	The new OA educational resources have been incorporated into several presentations given by NWFSC staff. We do plan to post new OA education resources to our website soon.
NWFSC will partner with PSC, a free-choice learning facility, to develop an OA discovery cart based on NOAA Fisheries Science. This collaboration will increase NOAA Fisheries staff's capacity for sharing scientific information in both formal and informal settings.	An OA discovery cart (based on OA and climate research currently underway at the NWFSC) was successfully completed in August 2010. Collaboration with PSC staff has opened up avenues for future educational work with the PSC on the impacts of OA on the marine ecosystem and NOAA Fisheries involvement in working to mitigate these effects. PSC staff has helped to develop novel and interactive methods for sharing NWFSC and NOAA Fisheries science with general audiences.
By partnering with UW's OACIS GK-12 program, NWFSC will increase the use of NOAA Fisheries research/data and establish connections between NOAA Fisheries and local schools to enhance the science curriculum available for high school students.	NWFSC research will be presented to 8 or more high schools throughout Seattle and the San Juan Islands, enhancing the pre-college marine science curriculum and exposing professional educators and potential future scientists to the work conducted by NOAA fisheries.

Changes/Problems Encountered:
No problems with either the OA cart or the OA classroom kits have occurred. We will continue to work with UW and the PSC to evaluate these new education resources and their effectiveness in teaching the complex science behind OA and evolve them as necessary.

FY10 Report 5: NMFS OA workshop

The following is the final summary report for the 2010 NMFS OA workshop hosted by the NWFSC.

Workshop dates: August 30 – September 1, 2010
Workshop Location: NOAA Northwest Fisheries Science Center, Seattle, WA

Steering Committee
Paul McElhany (lead, NWFSC), Rusty Brainard (PIFSC), Margaret Miller (SEFSC), Beth Phelan (NEFSC), Mike Sigler (AFSC), Russ Vetter (SWFSC)

Attendees
The workshop had approximately 60 participants, primarily researchers from the six NOAA Fisheries Science Centers. In addition, there were some regional academic collaborators (from UW, Scripps, and HIMB) and several external speakers (from WHOI, MBARI and NOAA-PMEL). There were three participants from CEFAS in the UK.

Workshop Goals
The workshop had several goals related to advancing NOAA Fisheries capabilities in ocean acidification research. These goals included

- Update science centers on each others OA projects
- Exchange information on latest developments in OA research
- Continue or establish collaborations among science centers and among disciplines
- Coordinate research to address critical gaps and avoid redundancy
- Further develop strategies for implementing NOAA OA research plan

Workshop Accomplishments
This workshop was the first opportunity for researchers at all six NOAA Fisheries Science Centers to meet and discuss ocean acidification research. The workshop provided a productive environment for learning about current OA research inside and outside of NOAA and for improving the research plans at each of the NOAA Fisheries Science Centers.

Workshop Content
The workshop was organized into six sessions. Exploration of each topic generally began with an opening overview talk by an external expert. These talks were then generally followed by brief presentations from researchers at each of the science centers on research they were conducting or had planned relevant to the topic. Following the presentations, workshop participants broke into small groups for focused discussion on specific issues related the topic.

Participants then reported the results of the small group discussion to the broader group. The topics covered included:

- Overview of OA research at the science centers
- Species response experiments
- Measuring seawater carbon chemistry in the lab and field
- Experimental exposure system design
- Modeling ecosystem response to OA
- Monitoring to detect biological response to OA
- Future NOAA Fisheries OA research, including connection to NOAA-wide plan

Current Status of NOAA Fisheries OA Research

The workshop provided an excellent snapshot of current NOAA Fisheries research on OA. Species exposure facilities are under construction at the NWFSC, NEFSC and AFSC. These labs are also developing in-house capabilities for measuring seawater carbon chemistry. Species exposure experiments will focus on local species of economic and/or ecological importance. The NWFSC and AFSC are also modifying ecosystem food web and single species population models to predict the impact of OA at multiple trophic levels and on fisheries. The PIFSC and SWFSC are focusing on coupled biological/chemical/physical monitoring to detect any potential ecological changes from OA. These centers are also collaborating with academic and other researchers on species exposure experiments. The SEFSC is in the early stages of planning for OA research. This is an incomplete summary of research at each of the labs, but gives some flavor of the current research projects discussed at the workshop. All of the science centers discussed potential research projects (as funding allows) addressing each of the themes in the NOAA-wide OA research plan (i.e. monitoring, species response, modeling, data management, human dimensions and outreach).

Next Steps

The workshop concluded with a discussion of some next steps for NOAA Fisheries OA research. Discussion topics included

- Need for a NOAA-wide annual workshop on OA. Participants considered this 2010 workshop successful and recommended continuing as an annual event. A NOAA-wide workshop would likely need to be developed through the NOAA OA program office. Workshop participants discussed the merits of a NOAA Fisheries only vs. NOAA-wide workshop and recognized the value of including all relevant disciplines in a NOAA-wide meeting, despite the fact that the workshop would likely be substantially larger in scope and number of participants.
- Need for identification of OA research leads at each of the NOAA Fisheries Science Centers. Identifying research leads will aid in coordination of research both within and among science centers.
- Formation of a NOAA Fisheries carbon chemistry QA/QC working group. For scientifically sound field and lab research on OA, it is essential that carbon chemistry be measured accurately. The QA/QC working group will develop protocols to help ensure the validity

of measurements at each of the science centers. Mark Carls of the AFSC agreed to take the lead on this project.

- More frequent sharing of ongoing research. Several mechanisms were discussed for continuing inter-lab communications promoted at the workshop. These mechanisms included continuing monthly OA calls among the NWFSC, NEFSC and AFSC and development of on line communications tools (e.g. NOAA wiki, research blog, etc.). Further work on this action item is required by the science center OA research leads.

FY11 Proposal 1: NWFSC OA research facility (Montlake)

System modifications

The NWFSC OA facility consists of a total of seven experimental treatment systems; six systems for 3x2 factorial experiments plus one additional treatment system for prototyping new methods and as a backup for the experiments. The majority of construction for the system occurred in FY10. However, additions and modification are required in FY11. The primary addition includes shelving, water baths, larval rearing jars with filter system (see FY10 Report 1), peristaltic pump system for controlled feeding, controlled lighting system over the exposure jars, and other systems related to rearing animals. Other modifications include more membrane contactors and a heating system for the reservoir to improve removal of sufficient CO_2 for dynamic experiments. Materials and labor for these additions and modifications are included in the proposed FY11 budget.

System operation and maintenance

Funds for operation and maintenance of the OA system are included in the proposed budget. Costs include staff time to continuously monitor and repair the system, as well as any replacement parts. Programing will also be required to modify the system for specific experiments. Maintenance expenses also include any repair costs for the spec pH and alkalinity titration equipment, though operation expenses for chemical analyses on any particular set of experiments is include in the cost of that experiment (see FY11 Proposal 2a).

FY11 Proposal 2a : Single species response experiments (Montlake)

General approach

The NWFSC OA experimental system is designed for simultaneous, multi-factor experiments on multiple species. NWFSC researchers have identified a number of priority species and important factorial treatment combinations to explore the potential impacts of ocean acidification in North Pacific coastal ecosystems. While it would be ideal to conduct multi-factor experiments with all study species, doing so would be logistically impossible in a year’s time frame. We expect to conduct some experiments on most of the target species, but likely not all species will experience all treatment combinations. In the following sections, we list the target species, the potential treatment combinations and possible response metrics. Because we cannot perfectly control our access to larvae, due to both the vagaries of both hatchery operation success and field sampling, we are unable to predict the sequence in which larvae of the target species will be available for experiments. Furthermore, because groups of species will be evaluated at the same time with a given set of treatments, it is not possible to completely predict exactly which species will be tested in which experimental condition in FY11. However, the Experimental Prioritization section below contains a discussion of the first set of experiments that will be conducted in the system and a rough prioritization for subsequent experiments.

Species

We selected our study species based on their ecological, economic, and/or conservation importance and their suspected vulnerability to direct effects of ocean acidification. In addition, we limited our study species to those that can be reared in a laboratory environment. All of our work will focus on early life history stages (egg through juvenile), which are considered most vulnerable to the effects of acidification. With Dungeness crab, we plan to examine both larval stages and the molting process in juveniles.

The NWFSC's core group of OA researchers (McElhany, Busch, Miller, Maher, and Norberg) will all participate in some capacity on experiments on all the species. In addition, we have established collaborative relationships with regional experts on each species groups and will have heavy involvement of these researchers during our experiments.

Mollusks

- **Geoducks** (*Panopea abrupta*) are the most economically valuable species in Puget Sound and have the greatest biomass of any species in Puget Sound. They are also commercially raised in aquaculture. Geoduck produce aragonite shells, so may be more susceptible to OA than mollusk species that produce calcite shells. For our experiments, we will obtain fertilized eggs from Taylor Shellfish's hatchery and expose them to treatment conditions within a few hours of fertilization. The NWFSC has conducted pilot OA studies on geoduck.
- **Pacific oyster** (*Crassostrea gigas*) provide a valuable West coast shellfishery. In recent years, Pacific oysters have experienced near total failure in larval survival in both hatchery and wild populations along the west coast. These failures have been associated with low pH conditions, and have had severe impacts on oyster aquaculture. For our experiments, we will spawn wild-collected oysters in the lab, fertilizing eggs in treatment conditions. As logistics allow, adults may be conditioned in treatments prior to spawning. The NWFSC has conducted pilot OA studies on Pacific oysters.
- **Olympia oyster** (*Ostrea lurida*) is a native Northwest oyster with both economic and ecological value. Fertilized eggs will be obtained from a commercial hatchery and exposed to treatment conditions within a few hours of fertilization. We may also attempt to collect animals from the field for spawning in the lab.
- **Pinto abalone** (*Haliotis kamtschatkana*) is a NOAA Species of Concern, Washington State Candidate Species, and Canadian Species at Risk. For our experiments, fertilized abalone eggs from the broodstock maintained at the NWFSC Mukilteo lab by the

University of Washington will be exposed to treatment conditions within hours of fertilization.

All mollusks will be observed to the settlement stage, which, depending on the species and temperature, can be less than a week or more than a month. We will feed the mollusk larvae a mixture of live algae species or an algae paste (see treatment descriptions in next section).

Collaborators: Our primary collaborator for the mollusk work is the Friedman lab at the University of Washington, which has a Sea Grant to look at the interaction between OA and disease (Vibrio) in local mollusk species. We also collaborate with the Roberts lab at the University of Washington, which has a grant to look at gene expression in local mollusks exposed to acidified waters. Genomics provides valuable insight into the effects of OA on our study species, and moves our research team beyond the basic measures of growth, survival, and morphology. The Taylor Shellfish hatchery supports our experiments by providing larvae for many of the bivalve experiments.

Crustaceans

- **Copepods** (*Calanus* species) are the most abundant zooplankton prey item in Puget Sound. Wild adults will be collected and potential gravid females separated into treatments. The species used for the studies will depend on local availability. If we select the same species used at the Newport lab (Proposal 2b), we can look at regional differences in response. If we used different species from Newport, we will have information on potential taxonomic diversity.
- **Krill** (*Euphasia pacifica*) are an important zooplankton food source for marine fish. Gravid females (identified by color) collected from Puget Sound tend to release eggs within 24 hours of capture. Eggs from each brood will be split among the treatments in individual containers. The NWFSC has conducted pilot OA studies on *E. pacifica,* collecting and spawning them in this way.
- **Dungeness crab** (*Cancer magister*) is one of the most important fisheries for Washington and Oregon. Two life stages will be analyzed in the OA experiments. Pre-spawn females will be collected from Puget Sound and placed in treatment conditions until eggs are released. Once females release eggs, the individual broods will be distributed among treatments. This experimental design will provide data with which we can explore interactions between maternal and larval treatment conditions. The second set of experiments will look at molting success as a function of treatment. Results from the AFSC on king crab suggest molting is affected by low pH conditions. Juvenile Dungeness crab (~2 cm), which molt frequently, will be collected from Puget Sound and placed in different treatments for observation of molting survival, frequency, duration and morphology. Results from these experiments will be used as part of the crab life-cycle model to predict population-level effects of OA (see Proposal 3).

Experiments will continue until mortality prevents meaningful analysis. We will feed larval crustaceans algae or artemia, depending on life-stage and size.

We collaborate with the Keister lab at the University of Washington on all crustacean experiments and Paul Williams of the Suquamish Tribal Fisheries Department on the Dungeness crab experiments.

Fish

The number of studies on how OA will impact fish is very limited. Prior research reports that under low pH conditions, otolith size in seabass increases and neurophysiology in two species of reef fish is altered, with effects on olfaction, hearing and behavior. The mechanisms for these responses are not known, but it is suspected they are related to how the fish maintain internal pH.

- **Rockfish** (*Sebastes* species) support important commercial and recreational fisheries, and some rockfish species (or distinct population segments) are listed under the Endangered Species Act. Rockfish are viviparous, releasing live larvae. We have an agreement to obtain gravid rockfish from the Seattle Aquarium and maintain them at the NWFSC Mukilteo Field Station until larvae are released. For the OA experiments, we will place larvae in treatments within hours after release and, if possible, rear them to settlement. Researchers at the NWFSC have successfully reared rockfish to maturity. The species used for the experiments will depend on what is available from the Seattle Aquarium, which varies from season to season. Since rockfish are viviparous, the earliest life stages occur in the maternal environment, with presumably buffered pH conditions. However, substantial larval development still occurs for several months post-release and prior to settlement, making it possible for development to be affected by seawater pH. Rockfish express settling behaviors similar to those expressed by coral reef fish. Research on these coral reef fish has shown that OA disrupts settling behavior related to olfaction and hearing. We plan to examine whether similar disruption occurs in rockfish (see Response Metrics section below).

Treatments

Our experiments will focus on the interaction between OA, temperature, dissolved oxygen, and temporal patterns of exposure. Experimental conditions will be based a series of factorial designs. In general, experiments will be conducted at three mean pCO_2 levels that approximate global mean atmospheric conditions of past, present and future: 280 ppm (pre-industrial conditions), 400 ppm (current conditions), and 1,000 ppm (future conditions). Because we have a closed experimental system with recirculating water, alkalinity is relatively stable during the experiments, meaning that calcium carbonate saturation state is also relatively stable for each pCO_2 level.

The NWFSC OA experimental system currently has six “treatment source tanks”, which can be controlled independently for pH, temperature and dissolved oxygen. Each of these treatment source tanks can provide conditioned water to at least six water baths, each of which can hold six 4-liter larval rearing jars (total of 36 jars/treatment source tank, see FY10 Report 1). Jars can serve as replicates within each treatment for a given species or for simultaneous evaluation of multiple species. Given the capacity of the water baths, experiments tend to be designed with multiples of six. For example, we might have three species with 12 replicate jars each (two water baths). Feeding rate can be controlled independently for the jars in each water bath by programmable peristaltic pumps.

We plan for three types of factorial designs, each of which includes three mean pCO2 levels and another factor at 2 levels (6 treatments total):

- **pCO2 x temperature** – Marine organism will experience the simultaneous impacts of both OA and climate change. Previous studies have shown species response to OA can be affected by temperature. Temperature treatments will include a current ambient temperature and an elevated temperature.
- **pCO2 x dissolved oxygen** – Low pH and low dissolved oxygen conditions tend to co-occur because both are often driven by respiration processes. For example, water that is deep, upwelled, or influenced by an algal bloom and die-off all generally have low dissolved oxygen and high CO_2. Experiments with three CO_2 levels and two dissolved oxygen levels (saturated and under-saturated) can reveal how these two, co-occurring factors interact to affect survival and growth. Some climate change scenarios predict changes in ocean dissolved oxygen levels.
- **pCO2 x temporal pattern** – OA experiments are typically conducted at constant pCO_2 values that represent global mean atmospheric pCO_2 levels at different points in the future. These experiments are somewhat analogous to testing species response to climate change by exposing them to constant mean global current and future temperatures, regardless of whether they are from the tropics or the arctic or what thermal property actually constrains survival (e.g. daily max temperature, seasonal average low temperature, etc.). In coastal waters, pCO_2 can vary dramatically with depth and on tidal, diurnal, and seasonal scales. In addition, episodic events can lead to temporary, but not unexpected, anomalies in pCO_2. These experiments will explore the impact of this temporal variability on species response to current and future OA conditions. The initial focus will be on diurnal scale patterns. There is a natural day/night pattern in pCO_2 because of photosynthesis/respiration processes. For the experiments, we will use the same mean pCO_2 levels (i.e. 280, 400, 1000), with two temporal pattern levels – one level will be at constant pCO_2 and the other level will be variable. In the first set of experiments, the variable pattern will be a simple diurnal sine wave with the mean shifted to reflect the target mean. Superimposed on the diel cycle are changes induced by variable circulation patterns, precipitation/river inputs and other processes. These co-occurring phenomena can result in pCO_2 levels changing from a low of <200 ppm to a high >800 ppm in a 24 hour period (Figure #). Subsequent experiments will follow some actual pH time series observed in the field, shifted to reflect the pre-industrial or future mean shift (see Proposal 6b for source of time series).

Because food can be delivered to the set of jars in each water bath independently, **feeding rate** can be added to any or all of the 3x2 experiments described above to look at 3-way interactions between CO2, food availability and some other factor. Prior research indicates that, at the individual level, many of the effects of OA may be induced by competing energetic demands.

Response metrics

For all of the experiments, we will collect data on survival, growth rate, the timing of life stage transitions, and morphology. Morphology will be evaluated using both light microscopy and scanning electron microscopy (SEM). For the mollusk species, the morphological evaluations will include an assessment of the extent of calcification. In addition, for the mollusk species, our colleagues at the University of Washington will explore how the treatments affect response to disease challenge (Friedman lab) and gene expression (Roberts lab). The gene expression studies will target genes related to stress or calcification. For rockfish, we will analyze otolith growth with SEM, conduct behavioral studies, and assess the strength of lateral preference ("right finned" or "left finned"). The latter assay allows for simple evaluation of neuro-physiological processes, which appear to be affected by environmental pH in reef fish (Phil Munday, pers com.).

Priority species/treatment experiments

Conducting all species x treatment combinations described above would be extremely informative about interactive effects involving OA and the degree to which responses vary among taxonomic groups, and would yield the data essential for predicting ecosystem response to OA. However, completing all of these studies is not practical given resource and logistic constraints; certainly not in a single year. In this section, we outline the basic approach to prioritization of our experiments. In practice, flexibility will be need when species are available for experimentation can be variable and unpredictable.

In FY11, we plan to complete some experiments involving manipulation of CO_2 on all species listed above. For some species (geoducks, Pacific oyster, abalone, and krill) we have already conducted pilot work, and are confident they can be reared in our experimental system. However, gravid krill can be extremely erratic to collect in Puget Sound, which makes timing for experiments with them challenging to predict. The pinto abalone broodstock has also proven to have erratic spawn timing, requiring flexibility. Although we have not yet reared Olympia oysters in the NWFSC OA facility, they have been reared extensively in aquaculture and are expected to have rearing conditions similar to the other bivalves in our study. The Pacific oyster and Olympia oysters are likely the most reliable spawners during the appropriate season (Table 3). We have not conducted experiments on copepods in the Montlake facility, however, they are readly abundant in Puget Sound as source material and the pilot study on copepods conducted at the Newport lab provides NWFSC expertise on rearing larvae (see Report 2b). Rockfish larvae are perhaps the most challenging group for these experiments. The source brood stock is typically only a few fish (though they each produce a large number of larvae). Although NWFSC biologists have reared rockfish from larvae to maturity, they have not been reared in the OA facility. FY11 may become a pilot year for working out the kinks in rearing protocols on rockfish, but we are hopeful some OA experiments can be conducted.

Table 3: Experimental species and their spawning period.

	Taxa	Common name	Spawning period
Molluscs	*Haliotis kamtschatkana*	Pinto abalone	June-Oct.
	Ostrea lurida	Olympic oyster	June-Aug

	Crassostrea gigas	Pacific oyster	Feb-Sept
	Panopea generosa	Geoduck	Feb-June
Crustaceans	*Euphausia pacifica*	Krill	April-June
	Acartia clausi	Copepod	March – Sept
	Calanus pacificus	Copepod	March – Oct
	Cancer magister	Dungeness crab	March-April
Fish	*Sebastes sp.*	Rockfish	Jan-Sept

Priority treatments will be the pCO_2 x dissolved oxygen and pCO_2 x temporal pattern. We are aware of only one published study (on squid) looking at the interaction of OA and oxygen. We are not aware of any studies on any species mimicking natural temporal CO_2 patterns as we propose. Both temperature and food availability have been shown in other studies to affect response to CO_2. In a second priority set of experiments, we propose looking at how these later two factors interact with OA because we want to know as much as possible about the response of our focal species to OA but also to gain a broader understanding of how OA responses vary among taxa.

Given these considerations, we tentatively propose the following initial two rounds of experiments:

- pCO_2 x dissolved oxygen: geoduck, Pacific oyster, copepod, crab
- pCO_2 x temporal variability (constant vs. diurnal sine wave): Pacific oyster, Olympia oyster, abalone, krill
- Rockfish placed in whatever treatment conditions available at the time of spawning

The basic strategy will be to conduct pCO_2 x dissolved oxygen experiments on winter/spring spawners and pCO_2 x temporal variability experiments on summer spawners. Should time allow, the system would be set to pCO_2 x temperature treatments for evaluation of summer spawners. Once the new research facility is constructed at Mukilteo (after FY11), more treatment/species combinations will be possible.

FY11 Proposal 2b: Zooplankton response experiments (Newport)

In FY11, NWFSC researchers at the Newport lab propose repeating the FY10 krill and copepod study with a larger (statically sufficient) sample size (see FY10 Report 2b). The experiments will be continued for a longer duration into the feeding stages. The experiments will be conducted in the AFSC OA experimental system at the Newport lab operated by Tom Hurst. Modifications have been proposed to this system to improve pH control (see AFSC research plan).

FY11 Proposal 3: Modeling OA effects

Puget Sound with Ecopath/Ecosim

Our previous work exploring the potential impacts of OA on the Puget Sound ecosystem were limited, to some extent, by the structure of the Ecopath/Ecosim model of Puget Sound. In its current form, the Ecopath/Ecosim model aggregates many lower-trophic level species into functional groups with broad taxonomic diversity (e.g., small crustaceans, benthic grazers, macrozooplankton). This grouping style makes building scenarios of OA in the model challenging, especially because lower-trophic levels species are most likely to be impacted by OA. We plan to reorganize the Puget Sound Ecopath/Ecosim model in a way that splits functional groups into sets of species with similar response to OA and to develop and run new scenarios for OA based on this more detailed model structure. This set of exercises will improve our ability to predict the response of the Puget Sound food web, including species groups that provide ecosystem services (e.g., harvest, tourism), to OA. The literature on potential species response to OA is also increasing rapidly. We plan to update the restructured Ecopath/Ecosim model with the results from the latest studies. We will also include potential OA effects on species groups, such as primary producers, not considered in the original study.

SLAM model of Dungeness crab

Dungeness crab are an important West Coast fishery that may be directly or indirectly affected by OA. The goal of this modeling project is to predict how Dungeness crab populations in Puget Sound will respond to OA based on the input of results from laboratory experiments on direct effects of increase CO2 on crab as well as the effect of CO2 on potential crab prey items or predators (from Proposal 2a). Dungeness crab have a complex life-history (Figure 15), where multiple life stages may be affected by OA in different ways. Direct effects may occur during larval development or during molting of juveniles or adults. Indirect effects may result from OA induced changes in the zooplankton prey consumed by some life stages. Habitat mediated indirect effects of OA could occur from changes in eelgrass abundance (preferred juvenile cover) or biomediated effects on benthic substrate.

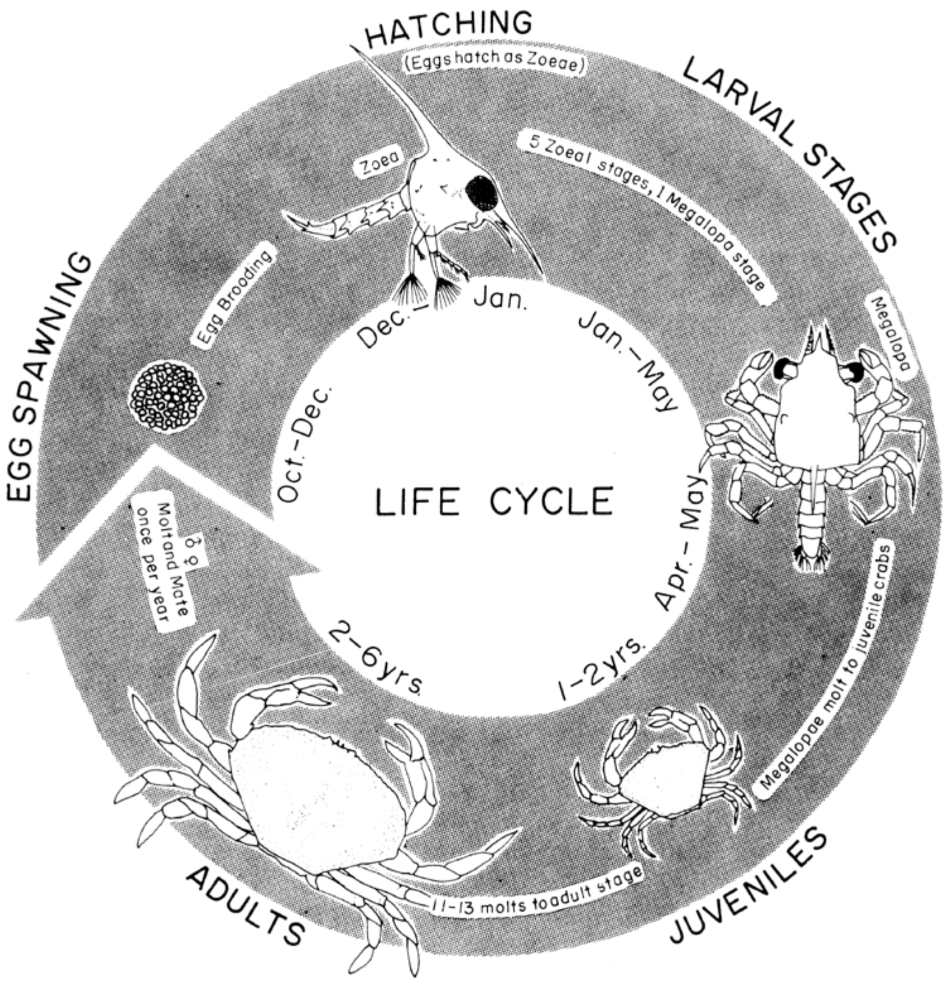

FIGURE 120. Life cycle of the Dungeness crab in California.

Figure 15: Life cycle of Dungeness crab (From).

We will project Dungeness crab population dynamics under various OA scenarios using the Species Life-cycle Analysis Modules (SLAM) modeling framework (http://www.nwfsc.noaa.gov/trt/slam/slam.cfm). SLAM is a flexible modeling tool developed at the NWFSC that allows for analysis of complex life-cycles, incorporating environmental drivers and multi-species (or multi-population) interactions. SLAM has been used for a number of projects related to recovery of Pacific salmon populations. The crab model will be constructed using the "minimum realistic" approach in which only those environmental drivers and other species considered crucial for understanding the dynamics of the focal species are included. This approach differs from a single species assessment in that it includes more potential influence factors. The approach differs from an ecosystem model, which attempts to model a large number of species and drivers, then looks for effects on focal species as an emergent property.

Modeling will begin in FY11 (McElhany lead), but will likely not conclude until the Dungeness crab experiments providing critical parameter estimate (Proposal 2a) are complete.

FY11 Proposal 4: OA outreach

OA activity cart at Seattle Aquarium

The Seattle Aquarium is one of Seattle's most visited tourist attractions and is the 7th largest Aquarium in the U.S. by attendance (>800,000 people/year). We will submit a NMFS internal education funding grant to fund development, fabrication, and delivery of displays and activities on OA and how it may impact the Puget Sound ecosystem. These materials will build off of our prior outreach work with the Pacific Science Center, but will be tailored to take advantage of the Aquarium's unique facilities and audience. They will also incorporate new concepts in OA research and advances in how to effectively convey the complex chemistry, physiology, and ecology that underlie OA and make it an issue for concern. The entire OA team volunteered at a science outreach weekend at the Seattle Aquarium in November, 2010, and this experience gave us an excellent perspective on the types of activities and concepts that Aquarium visitors, both young and old, found interesting and engaging. Part of the grant will include training of Aquarium volunteers to deliver the OA materials. These training activities will yield a cadre of science communicators that are well-informed about OA and can effectively spread information about the subject to the great Puget Sound community.

NWFSC OA "outreach cart"

The NWFSC OA has developed a number of education materials with our collaborators (UW, Pacific Science Center, Seattle Aquarium). The majority of these materials are used off-site, such that NWFSC employees do not have ready access to them. We propose to build a set of education materials on OA for use by the NWFSC OA team and other NWFSC employees interested in teaching groups about OA. Fabrication of these materials and development of new ones will allow us to meet requests by the NWFSC education team and others to inform local citizens about the cause and potential consequences of OA, both locally and globally.

College interns

Recognizing the need for more researchers who will focus on OA, mentorship of young scientists is an important part of our OA research program. We will host three undergraduate interns during their mid-winter break. One of them, a senior at St. Olaf's College, will receive credit for working in our lab as part of his college's extern program. The second, a sophomore at Carleton College, was a volunteer with the OA program in summer 2010, and the third, a freshman at University of Hawaii Hilo, has worked with the OA group under various programs since 2009 and continued his work with us under an intermittent, federal Student Temporary Employment Position. For the majority of FY11, we will host a UW undergraduate work-study student from the Keister lab. This student, who participates in the Office of Minority Affair's Early Identification Program, is helping to build the OA experimental system and plans to conduct his "capstone" research with our lab this spring and summer. This summer we will host a Hollings Scholar from the University of Miami. This scholar will develop a research project related to our ongoing OA research, focusing on laboratory experiments and microscopy. We have also submitted a proposal to host a summer intern from the UW and NOAA's Joint Institute for the Study of the Atmosphere and Ocean (JISAO).

FY11 Proposal 5: OA workshops and meetings

Symposium at the annual meeting of the American Fisheries Society (September 2011)
We (Paul McElhany, John Stein, and Shallin Busch) will chair a symposium on ocean acidification at the 2011 annual meeting of the American Fisheries Society. Ocean acidification is an issue of increasing concern for fisheries and other living marine resources, but, given that ocean acidification is a relatively recent area of focused research, it can be a challenging field for a non-expert to summarize and understand. Our symposium will include 19 invited talks from a range of disciplines, including a keynote address likely given by Scott Doney, the White House nominee for NOAA Chief Scientist. Talk topics will include: modeling projected changes in ocean carbon chemistry, marine carbon monitoring, individual species' response to increased CO_2, food web and ecosystem modeling of potential effects on fisheries, and perspectives from the fishing and shellfish aquaculture industries about potential impacts of OA. The symposium will also include a poster session, a tour of the NWFSC OA facility, and, possibly, a movie night.

OA Principle Investigators meeting at Woods Hole Oceanographic Institute
Paul McElhany and Shallin Busch will travel to the Woods Hole Oceanographic Institute in March, 2011 to attend the nation's first ocean acidification-only science and networking meeting. The goal of the workshop is to foster collaborations, reduce redundancy in research agendas, and identify key research goals for the OA community. We will present posters summarizing NWFSC OA research, both experiments and modeling, at the workshop.
Ocean acidification research cooperation between Norway and USA
In October, 2011, OA researchers at Norway's Institute for Marine Research (IMR) will host a workshop to foster collaboration between IMR and the northern NMFS science centers (NWFSC, AFSC, NEFSC). This workshop will build off of the success of a similar workshop held in Seattle in May, 2009, and will emphasize coordination of scientific research and model building on the physiological, ecological and socioeconomic effects of ocean acidification.

Invited research talks
NWFSC OA researchers are often asked to talk about OA research at local, regional, national, and international meetings and workshops. For example, one of us (S. Busch) was recently invited to participate in a panel discussion on ocean acidification at the International Marine Conservation Congress in Victoria, BC in May. Such opportunities to communicate about NWFSC research and OA in general are an important part of communicating our research findings.

FY11 Proposal 6a: Biological/chemical sampling in Puget Sound

We hypothesize that short-period variability is critically important to the development of larval stages of zooplankton. Zooplankton may be exposed to multiple pH environments as deep, low-pH water mixes with surface water or as they actively move through a range of pCO_2 conditions in their normal random or directed swimming. Our laboratory system is designed to include variability in seawater chemistry, but few, if any, studies have characterized the pH and pCO_2 variability on the small scales relevant to zooplankton, especially in temperate waters. We propose to assess how water carbon chemistry and dissolved oxygen in Puget Sound varies at scales relevant to the zooplankton species that are the targets of our experimental work.

We propose to monitor carbon chemistry in Puget Sound at temporal as spatial scales relevant to zooplankton. This year we have the opportunity to participate in a unique series of cruises in Puget Sound designed to characterize how land-based human activities influence the nearshore environment. These cruises, funded by an EPA grant to Correigh Greene, will visit 96 sites throughout the Puget Sound's nearshore environmental four times between April and October. The project already plans to collect samples of phytoplankton, zooplankton, icthyoplankton, and fish. They also plan to collect some basic water chemistry data, but do not have the capacity to measure carbon chemistry well enough to assess OA. We propose to add collection of water samples for spectrophotometric pH and total alkalinity analyses to the cruises, and have been invited to do so. Collection of carbon chemistry data from the cruises would give us a dataset on Puget Sound's nearshore carbon chemistry with excellent spatial and temporal resolution that is also linked to biological samples. The purported link between seawater chemistry and species distribution and abundance could be analyzed in these datasets, and results from such an analysis would likely be very useful for developing a monitoring program for OA.

FY11 Proposal 6b: Continuous pH monitoring

We hypothesize that short-period variability is critically important to the development of larval stages of zooplankton. Zooplankton, including the larval stages of benthic organisms, may be exposed to multiple pH environments as deep, low-pH water mixes with surface water or as they actively move through a range of pCO_2 conditions in their normal random or directed swimming. Our laboratory system is designed to include variability in seawater chemistry, but few, if any, studies have characterized the pH and pCO_2 variability on the small scales relevant to zooplankton, especially in temperate waters. We propose to assess how water carbon chemistry in Puget Sound varies at scales relevant to the zooplankton species that are the targets of our experimental work using continuous pH monitors.

The Seafet pH sensor (Figure 16) was recently developed by researchers at Scripps and will soon be commercially available from the Satlantic company (Todd Martz, pers. com.) The Seafet is a remote deployable marine pH sensor based on the Honeywell Durafet pH probe. The sensor can be deployed for months at a time before it must be retrieved to change batteries and download. The Seafet does have a limited depth range, but it is sufficient for the locations we are proposing.

We propose deploying three sensors in Washington State to obtain continuous pH measures in locations relevant to the focal species in our lab exposure experiments. One sensor will be located in **Willapa Bay** on the outer Washington coast, which contains one of the largest natural oyster beds on the West Coast. Another sensor would be deployed in the **Olympic National Marine Sanctuary**, where sensor data can be associate with monitoring conducted by the NMS. The third sensor would located off the pier at the **NWFSC Mukilteo Field Station**, which will be the location of the new NWFSC OA facility and a source for some of the material used for the species response experiments. If funds were available for a forth sensor, it would be placed in **Dabob Bay**, which is the location of a large shellfish hatchery and natural shellfish population and also contains three pCO2 sensors (surface and two depths). Adding a pH sensor will provide a second carbon system parameter for constraining estimates of saturation state. These sensors will provide important initial information on small scale temporal variation in pH in a variety of marine habitats. The sensors will contribute to a West Coast network of Seafet sensors (the OMEGA network) currently being deploy by a consortium of California research groups (Grechen Hoffman, pers com.) This proposal will extend the network coverage North into Washington (current plans for OMEGA do not extend North of Oregon).

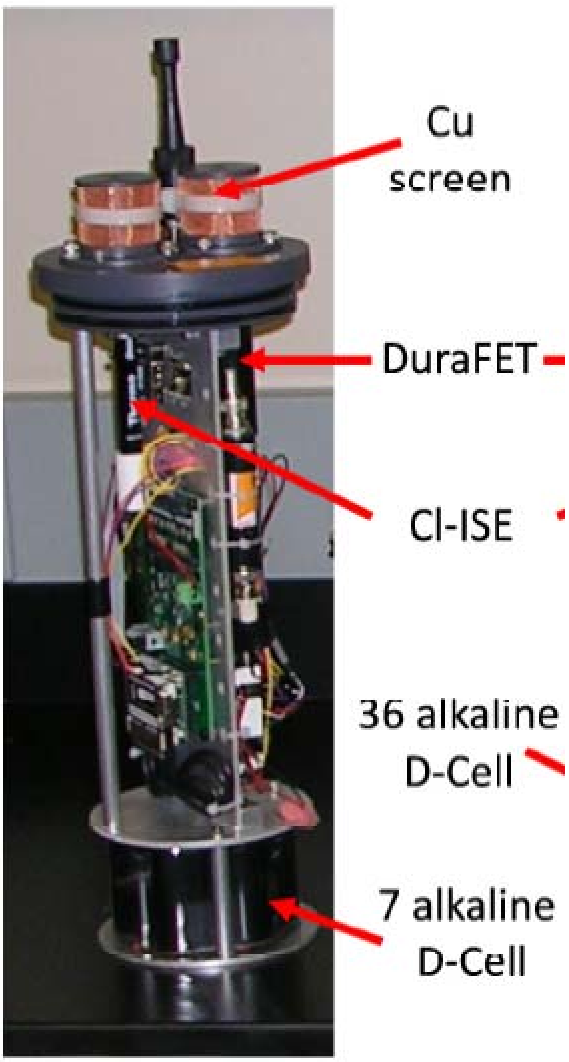

Figure 16: The internal components of the Seafet pH sensor. (photo from Todd Martz)