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An Ocean Acidification Research Plan
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Frontispiece photo: Juvenile stage blue king crab. Photographer: Brad Stevens (NOAA-AFSC).

Forecast Fish, Shellfish, and Coral Population Responses to Ocean Acidification in the North Pacific Ocean and Bering Sea

An Ocean Acidification Research Plan for the Alaska Fisheries Science Center

by

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Summary

The North Pacific Ocean is a sentinel region for signs of ocean acidification. Approximately 30-50% of global anthropogenic carbon dioxide (CO_2) emissions are absorbed by the world's oceans. Dissolving CO_2 increases the hydrogen ion (H+) concentration in the ocean, and thus reduces ocean pH. Corrosive waters reach shallower depths more so there than in other ocean basins, especially in Alaska, and so biological impacts will likely occur earlier than in many other places. Ocean acidification reduces the calcium carbonate ($CaCO_3$) saturation point, which stresses calcifying organisms by making calcification more difficult. The Alaska Fisheries Science Center research plan will focus on commercially important fish and shellfish species, their prey (calcareous plankton) and shelter (corals). Ocean acidification will likely impact the ability of marine calcifiers, such as corals and shellfish, to make shells and skeletons from $CaCO_3$. Ocean acidification may also affect fish, marine mammal and seabird species through reduced abundance of calcareous plankton at the base of the food web. Species-specific studies of shellfish, calcareous plankton, corals and fish will be conducted to **understand** physiological effects (growth and survival). The $CaCO_3$ content of calcareous organisms is not well known and a survey of shellfish, calcareous plankton and corals will be conducted to **assess** species vulnerabilities to ocean acidification. The results of the species-specific studies will be incorporated into population and ecosystem models to **forecast** population and ecosystem impacts. Bioeconomic models of Alaskan crab fisheries will be used to **forecast** fishery performance for a range of climate and ocean acidification scenarios.

Contents

Case for Concern in Alaska

Approximately 30-50% of global anthropogenic carbon dioxide (CO_2) emissions are absorbed by the world's oceans (Feely et al. 2004, Sabine et al. 2004). Increased CO_2 uptake by the oceans is expected to reduce surface ocean pH by 0.3 – 0.5 units over the next century, which would be the largest change in pH to occur in the last 20-200 million years (Feely et al. 2004). Ocean acidification reduces the calcium carbonate ($CaCO_3$) saturation point, which stresses calcifying organisms by making calcification more difficult. Dramatic reductions in $CaCO_3$ saturation have been observed in the North Pacific since the Industrial Revolution and saturation depths are decreasing (Feely et al. 2004). Reductions in the North Pacific Ocean are greater than in other oceans due to respiration processes as ocean water circulates along the deep conveyor belt from the Atlantic to Indian and Pacific Oceans (Feely et al. 2004).

> Dissolving CO_2 increases the hydrogen ion (H+) concentration in the ocean, and thus reduces ocean pH. The use of the term "**ocean acidification**" to describe this process was introduced by Caldeira and Wickett (2003). As the pH of the ocean decreases (i.e., becomes less basic), the equilibrium between $CaCO_3$ and the dissolution products (Ca^{2+} and CO_3^{2-}) favors dissolution.

Ocean acidification will likely impact the ability of marine calcifiers, such as corals and shellfish, to make shells and skeletons from $CaCO_3$. This will occur principally because of a reduction in the availability of the chemical constituents needed for calcified shells and plates. Ocean acidification may also affect fish, marine mammals, and seabird species through reduced abundance of calcareous plankton at the base of the food web. Non-calcifying organisms may also be affected through less obvious pathways, such as the availability of nutrients to phytoplankton, the bioavailability of marine toxins to bacteria and phytoplankton, internal CO_2 concentrations of marine animals and reduced demersal egg adhesion or fertilization success of eggs broadcast into the ocean (UK Royal Society 2005). The numerous pathways for effects (both direct and indirect) imply that ocean acidification will impact many marine species.

The two major forms of $CaCO_3$ (aragonite and calcite) have different dissolution properties. Aragonite dissolves more readily than calcite. Therefore, marine organisms that use aragonite (corals, pteropods) are more vulnerable to ocean acidification than marine organisms that use calcite (foraminifera, coccolithophores, crustaceans, echinoderms) (UK Royal Society 2005). Although calcite is less soluble than aragonite, making it less susceptible to pH changes, the incorporation of magnesium into either form increases their solubility (UK Royal Society 2005). In addition, as depth increases, several other physical factors change (pressure increases, temperature decreases, and pH decreases) – all of which promote the dissolution of $CaCO_3$.

The North Pacific Ocean is a sentinel region for signs of ocean acidification. Corrosive waters reach shallower depths more so there than in any other ocean basin, especially in Alaska, and so impacts of ocean acidification on marine calcifiers will likely occur earlier there than in many other places. Waters below the $CaCO_3$ saturation horizon are

corrosive to calcifying organisms. The $CaCO_3$ saturation horizon is relatively shallow in the North Pacific Ocean. For example, the aragonite saturation horizon is about 200 m in the North Pacific Ocean compared to about 2,000 m in the North Atlantic Ocean (Feely et al. 2004). In the North Pacific Ocean, the upward migration of the aragonite saturation horizon from pre-industrial times to the present is between 30 and 100 m (Feely et al. 2004). By comparison, the aragonite saturation horizon has changed little in the North Atlantic (Feely et al. 2004). The saturation horizon is projected to reach the surface of the North Pacific Ocean during this century (Orr et al. 2005). At that point, a wide range of North Pacific species will be exposed to corrosive waters.

Hypothesis

Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will adversely affect (reduce growth and survival of) fish and shellfish, ecologically important prey of fish and shellfish, and coldwater corals.

Increased atmospheric CO_2 and reduced ocean pH could impact fish and shellfish through two distinct pathways: (1) direct physiological stress manifested as reduced rates of growth and survival (Michaelidis et al. 2007); and (2) indirectly through reduced abundance of calcareous plankton that are prey for fish and shellfish (UK Royal Society 2005). Decreased pH may reduce building of $CaCO_3$ structures in coral (Guinotte et al. 2006). Crustaceans require calcium and bicarbonate ions for the mineralization of their exoskeleton after molting and may, therefore, be particularly vulnerable to ocean acidification. Calcification rates of organisms including bivalves, corals, coccolithophores, echinoderms, pteropods and foraminifera decrease in response to increasing atmospheric CO_2 (UK Royal Society 2005, Gazeau et al. 2007), though the effect on coccolithophores appears species-dependent (Iglesias-Rodriguez et al. 2008). Substantial decreases in calcification rates of shallow-water tropical corals (>40%) occur with relatively modest decreases in aragonite saturation state (Langdon et al. 2003, Langdon and Atkinson 2005). The growing edge of the shell aperture of live subarctic pteropods *Clio pyramidata* showed marked dissolution within 48 hours when subjected to a level of undersaturation predicted for Southern Ocean surface waters in the year 2100 (Orr et al. 2005).

Research Priorities

The Alaska Fisheries Science Center (AFSC) ocean acidification research program will focus on commercially important fish, shellfish, and corals.
Research priorities are:

- Understand species-specific physiological responses to ocean acidification.
- Forecast the population and ecosystem impacts of the physiological responses.
- Forecast economic consequences of these impacts.

In addition, the AFSC will collaborate with NOAA's Pacific Marine Environmental Laboratory (PMEL) to monitor ocean pH using the AFSC's ship time. Climate change

may affect both ocean pH and temperature, and to the extent practicable, both factors will be integrated in our studies.

Focal species groups
- Shellfish
- Calcareous plankton
- Coldwater corals
- Fish

Research will focus on the commercially important species most likely to be affected by ocean acidification, especially larval and juvenile stages that are least equipped to cope with additional energetic stress imposed by lower $CaCO_3$ saturation. Prioritization for ocean acidification research ranks commercially important species higher than species with no commercial value and direct effects higher than indirect effects. Commercially important calcareous species (shellfish) are first priority because of their commercial importance and because these species are likely to suffer direct effects of reduced $CaCO_3$ availability. Second priority is calcareous prey (pteropods, euphausiids) of commercially important species (walleye pollock, Pacific salmon) and marine mammals because the most likely effect on fish and marine mammals is an indirect effect through a predator-prey relationship. Third priority is coldwater corals which provide habitat for commercially important species (e.g., rockfish, which is an indirect effect through a habitat relationship). Commercially important fish species are fourth priority because direct effects on fish are less likely, but a limited effort will screen for effects on species from high-value fisheries (walleye pollock, Pacific cod) (direct effect, but less likely). We will not focus on marine mammals unless we find effects on prey of planktivorous (baleen whales) or piscivorous (pinnipeds, toothed whales, porpoise, and dolphin) species, as internal physiology regulates deposition of calcareous tissue in these air-breathing vertebrates.

The remainder of the research plan is organized as follows. Sections 1-4 describe proposed studies for shellfish, calcareous plankton, coral, and fish. Section 5 describes a proposed survey of the $CaCO_3$ content of shellfish, calcareous plankton and corals. Section 6 describes proposed models to forecast population, ecosystem and economic impacts. Sections 7-9 describe research integration, research partners and budgets. Appendix 1 describes ocean chemistry of CO_2 acidification and Appendix 2 describes the proposed budget.

Shellfish

Hypothesis Addressed
Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will reduce growth and survival of commercially important fish and shellfish.

Background
The growth (e.g., Stevens 1990, Donaldson et al. 1992, Loher et al. 2001, Incze et al. 1984) and morphology (e.g., Shirley et al. 1987, Webb et al. 2006) of early life stages and reproductive cycles (Swiney and Shirley 2001) of many crab species has been well-studied and is known to be affected by variable environmental influences, but the effects of ocean acidification are not known. To grow, crustaceans require calcium and bicarbonate ions for the mineralization of their exoskeleton after molting and may,

therefore, be particularly vulnerable to ocean acidification. Calcification rates of calcareous organisms decrease in response to increasing atmospheric CO_2 (UK Royal Society 2005, Gazeau et al. 2007) and increased CO_2 concentrations have induced defective skeletogenesis and morphological changes in sea urchin larvae (Kurihara et al. 2004a). Fertilization rates of sea urchins and egg production and eclosion rates of copepods decreased and nauplius mortality rate of copepods increased with increased CO_2 concentrations (Kurihara et al. 2004a, Kurihara et al. 2004b). Although the calcification process in crabs is poorly understood, a significant decrease in pH or undersaturation of calcite will likely influence the growth and morphology of early life stages and the reproduction of crab.

Sublethal effects of ocean acidification (e.g., morphological, growth, disease susceptibility) may have a biochemical or molecular basis preceded by chemical changes in nuclei, cells and extracellular fluids (Thomas 1990). Genes may be turned on or off and depending upon the strength of the stress, the level of gene expression will vary accordingly. Identifying the kinds and amounts of messenger RNA produced in a cell or organism and their relationship to sublethal stressors may provide insight into how that cell or organism responds to ocean acidification.

Current Shellfish Research

In 2006, AFSC scientists investigated the effects of decreased seawater pH on the growth, survival, and calcium content of larval blue king crab. Blue king crab larvae were exposed to treatments of 7.65 and 7.45 pH units by adding HCl to seawater and then compared to an ambient control pH of 7.95. For each treatment, 13 larvae were raised in a 1 L glass beaker from hatch to the first juvenile instar (C1), which took approximately 79 days. Each treatment was replicated four times. Survival rates, growth, and calcium content of surviving C1 crabs were significantly lower in the reduced pH treatments compared to the control. Although pH did in fact negatively impact blue king crab larvae, the treatment levels did not encompass realistic past or predicted values in ocean pH.

In 2007, similar experiments were conducted with the following modifications to the 2006 experiments: 1) tested pH values simulated less acidic conditions consistent with 1970s seawater conditions and theoretical predictions of ocean pH at the end of this century (ambient plus 0.1, and ambient minus 0.1, 0.2, and 0.3 pH units); 2) treatment effects were measured through the second juvenile instar (C2); 3) dissolved inorganic carbon was measured from each treatment container to estimate the availability of carbon for shell growth. In addition, morphological changes in larval development that may not be due to the effects of pH on calcite undersaturation were assessed. Although crustaceans use calcite to harden their chitinous exoskeletons, the amount of calcite that exists in larval king crab shell structure is unknown. Crab were sacrificed and photographed for image analysis at zoea 1, zoea 4, glaucothoe, C1 and C2 stages. Morphological variability and abnormalities were assessed. Carbonate content of shells was also measured (see later section "$CaCO_3$ survey").

Proposed Shellfish Research

CO_2 Delivery System: Micro-scale Closed System and Meso-scale Flow-through System

Acidification experiments to date have manipulated pH levels using the addition of HCl and NaOH to ambient seawater. Although there is precedence for the use of these chemicals to reflect changes in seawater carbonate chemistry (Ishimatsu et al. 2004, Langer et al. (2006), simulating ocean pH by inputting CO_2 gas into seawater would better represent *in situ* conditions. Elevated CO_2 in seawater has been used to test for acidification effects on copepods, juvenile gastropods, larval and juvenile sea urchins, and pteropods (Kurihara et al. 2004a, Shirayama and Thornton 2005, Watanabe and Yamaguchi 2006). For 2008 and onward, a CO_2 delivery system has been constructed. Closed temperature controlled microcosms will be used for larval (Kreisel system (Calado et al., 2003)) and juvenile shellfish studies. A larger flow-through tank system will be used for adult studies. Mixtures of CO_2 consisting of 79% nitrogen, 21% oxygen, and one of the five following concentrations of CO2: 280, 400, 500, 750, and 1,000 ppmv (ppmv = parts per million by volume) will be used. The mixtures were chosen to reflect past, current, and predicted future levels of CO_2 concentrations in seawater. The performance of the CO_2 delivery system will be monitored because the chemical changes induced in the affected seawater will depend on salinity, dissolved inorganic carbon, and initial pH.

Life History Stages – Egg, Larvae, and Juvenile development

Some life stages may be more vulnerable to ocean acidification than others due to spatial and temporal variance and physiological changes, so we propose to examine all life stages of crabs including eggs, larvae, juveniles, and primiparous and multiparous females. We propose to measure gonadal development, embryological development, eclosion, larval fitness, and reproductive cycles of females reared in various levels of acidified seawater via a flow-through CO_2 delivery system. To examine effects on gonadal development, females will be sampled monthly from multiple treatments during the time period when they are expected to develop. Gonadosomatic indexes (GSI) will be calculated and biochemical analyses performed and compared to a control group (no CO_2 delivery). CO_2 effects on embryonic development will be carried out similar to other experiments of Tanner crabs (Swiney 2008), blue king crabs (Stevens 2006a), and golden king crabs (unpublished data). Previous data on the eclosion of Tanner crabs (Swiney 2008), red king crabs (Stevens and Swiney in review), blue king crabs (Stevens 2006b) and golden king crabs (AFSC unpublished data) will be used to assess the impacts of CO_2 treatments. To test effects on larval fitness, newly hatched larvae will be collected and reared in a cold room and larval starvation survival will be calculated. Juvenile stages will be studied because juveniles molt and form new shells numerous times during this period of high growth. In all experiments, shell $CaCO_3$ content will be measured (through link to the study "$CaCO_3$ survey"). Further, temperature effects will also be tested given the predicted increase in ocean temperatures corresponding with increased CO_2 levels.

In Situ Studies

Kodiak Laboratory researchers propose to monitor the variability in available $CaCO_3$ and related parameters in the nearshore areas around Kodiak Island and if significant, the potential effects on crab movement and use of the nearshore habitat. A series of water collection sites will be chosen to represent the local oceanography, as well as important king crab habitat. Researchers at the Kodiak Laboratory Diving Program have monitored the Womens Bay red king crab stock since the 1960s. King crab utilize the shallow near-shore environment during several critical stages of their life history. Adult red king crab move inshore to reproduce and to release larvae (Stone et al. 1992). Blue and red king crab zoeal and glaucothoe stages are planktonic, though the glaucothoe stage spends much of its time on substrates in preparation for settlement. Golden king crab zoea are lecithotrophic and appear to be demersal rather than planktonic. Juvenile crab settle into and occupy the protected niches of the shallow subtidal areas as solitary individuals (Sundberg and Clausen 1977). Juvenile (1-3 year old) blue king crabs have been found among shell hash in the Pribilof Islands (Armstrong and Palacios 1985). Red king crab juveniles settle on structurally complex substrates such as bryozoans and hydroids (Stevens and Kittaka 1998, Stevens 2003). Juveniles graze on particulate matter and algae and are cannibalistic. Older juvenile red king crab continue to occupy the nearshore areas and aggregate into dense groups of hundreds to thousands of individuals referred to as pods (Powell and Nickerson 1965, Dew 1990).

Crustacean Health

Microarrays may provide a tool to indicate cellular and sublethal effects of ocean acidification; microarrays are very sensitive and data rich in that expression levels of hundreds to thousands of genes can be determined. For example, microarray technology may permit effective monitoring of marine organism response to temperature stress (Gracey et al. 2004, Somero 2005, Teranishi and Stillman 2007), hypoxia (Brouwer et al. 2004), handling stress (Krasnov et al. 2005), biomineralization (Quinn et al. 2006), behavior (Sneddon et al. 2005) and pollution (Sheader et al. 2006, Venier et al. 2006). Calibrating the technology against laboratory experiments (see life history stage studies above) may allow population health assessment in response to ocean acidification for field samples.

We propose to apply microarray technology to measure marine organism response to pH in a collaborative effort involving the Stillman Laboratory (San Francisco State University), and the AFSC's Shellfish Assessment Program (SAP) and Fisheries Resources Pathobiology (FRP) Team. A microarray protocol for surveying over 26,000 expressed sequence tags (ESTs which are used in identifying gene transcripts) has been developed for the porcelain crab, *Petrolisthes cinctipes*, a small anomuran. We plan a two-phase study involving the blue king crab, *Paralithodes platypus*. In phase 1, we propose to compare expression profiles between the porcelain crab and the blue king crab. During phase 1, we expect to determine the affinity of expressed blue king crab mRNA with the *Petrolisthes* "chip". Highly conserved genes such as those for hemocyanin, heat shock proteins, ecdysterone and others will likely demonstrate good cross-reactivity. Should phase 1 demonstrate very little or poor affinity, we propose phase 2 development of a chip specifically for the blue king crab, which also would provide a

foundation for developing similar chips for other crustaceans, especially red king crab (*Paralithodes camtschaticus*) and euphausiids.

Calcareous Plankton

Hypothesis Addressed
Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will reduce growth and survival of ecologically important prey of commercially important fish and shellfish.

Aragonite has a shallower saturation horizon than calcite, so plankton that require aragonite (pteropods) are more vulnerable and may be affected sooner than those that require calcite (coccolithophores, foraminifera) (UK Royal Society 2005, Feely et al. 2004). *Limacina helicina* are prey for pelagic fish in subarctic and arctic regions (Orr et al. 2005) and may contribute substantially to forage fish diets in the eastern Bering Sea. For example, during the fall Bering Aleutian Salmon International Survey (BASIS), *L. helicina* comprised up to 25% of prey wet weight for age-0 pollock in oceanic influenced waters that move through the Aleutian passes into the southeastern Bering Sea (L. Eisner pers. obs.). Predicted effects of climate change on pink salmon growth in the Gulf of Alaska are that a 10% increase in water temperature will lead to a 3% drop in mature salmon body weight (physiological effect) and a 10% decrease in pteropod production will lead to a 20% drop in mature salmon body weight (prey limitation) (Aydin et al. 2005). In the eastern Bering Sea, juvenile salmon were not found in areas with high concentrations of coccolithophores (E. Farley, AFSC, pers. comm.). Low grazing rates by microzooplankton on coccolithophores have been reported for the eastern Bering Sea (Olson and Strom 2002).

Monitoring of calcareous plankton distributions will continue in the Bering Sea and Gulf of Alaska from satellite observations (Iida et al. 2002) and shipboard sampling by research vessels and ships of opportunity. These distributions may be compared to water mass properties (e.g., temperature, salinity, nutrients and pH) and distribution of zooplankton and fish to understand factors promoting blooms and potential food web implications. In addition, we plan to conduct at sea experiments to test for effects of ocean acidification on calcareous plankton (e.g., pteropods; Orr et al. 2005) through collaboration between external and in-house experts.

Corals

Hypothesis Addressed
Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will reduce growth and survival of coldwater corals.

Deep-sea corals are widespread throughout Alaska, including the continental shelf and upper slope of the Gulf of Alaska, the Aleutian Islands, the eastern Bering Sea, and extending as far north as the Beaufort Sea. Coral distribution, abundance, and species assemblages differ among geographic regions. Gorgonians, pennatulaceans, and black

corals are most common in the Gulf of Alaska while gorgonians and stylasterids are the most common corals in the Aleutian Islands. True soft corals are common on Bering Sea shelf habitats. Overall, the Aleutian Islands have the highest diversity of deep corals in Alaska, and possibly in the North Pacific Ocean, including at least 50 species or subspecies of corals that may be endemic to that region. In the Aleutian Islands, corals form high density "coral gardens" that are similar in structural complexity to shallow tropical reefs and are characterized by a rigid framework, high topographic relief, and high taxonomic diversity (Stone 2006). Coral communities in Alaskan waters are highly diverse and include six major taxonomic groups: true or stony corals (Order Scleractinia), black corals (Order Antipatharia), true soft corals (Order Alcyonacea) including the stoloniferans (Suborder Stolonifera), sea fans (Order Gorgonacea), sea pens (Order Pennatulacea), and stylasterids (Order Anthoathecatae). One hundred and forty-one unique coral taxa have been documented from Alaskan waters and include 11 species of stony corals, 14 species of black corals, 15 species of true soft corals (including 6 species of stoloniferans), 63 species of gorgonians, 10 species of sea pens, and 28 species of stylasterids (Stone and Shotwell 2007).

Unlike most tropical corals, all Alaskan species are azooxanthellate and satisfy all of their nutritional requirements by suspension feeding presumably on small zooplankton and phytoplankton. They are ahermatypic or non-reef building corals, but many form structures. The degree to which they provide structure depends on their maximum size, growth form, intraspecific fine-scale distribution, and interaction with other structure-forming invertebrates. In Alaska, many commercial and non-commercial fish species are associated with deep corals. Most associations are believed to be facultative rather than obligatory. Fish and crabs, particularly juveniles, use coral habitat as refuge and as focal sites of high prey abundance (Stone et al. 2005). Some shelter-seeking fishes such as rockfish use coral habitat as spawning and breeding sites.

The skeletal composition has been determined for only a few Alaskan coral species. The sclerites of octocorals are calcitic, but the axes may be composed of calcite, aragonite, or amorphous carbonate hydroxylapatite (Bayer and Macintyre 2001). The axial rod of the pennatulacean (*Halipteris willemoesi*) is composed of high-magnesium calcite (Wilson et al. 2002), whereas the skeleton of the red tree coral (*Primnoa pacifica*) is composed partially of calcite (Andrews et al. 2002). Many Alaskan stylasterids use calcite to build their skeletons (Cairns and Macintyre 1992). Growth rates for deep-sea corals are typically slow and have been determined for only three species that occur in Alaska. Growth rates range from 5.8 mm per year for the common gorgonian *Calcigorgia spiculifera* to approximately 23 mm per year for *Halipteris willemoesi* and *Primnoa pacifica* (Stone and Wing 2001, Wilson et al. 2002, Andrews et al. 2002).

Effects of ocean acidification on deep-sea corals will be direct (e.g., decreased growth and recruitment) and indirect (e.g., changes to food supply). Corals will be affected differently depending on their skeletal composition (i.e., aragonite vs. calcite), geographical location, and depth. Several studies have shown substantial decreases in calcification rates of shallow-water tropical corals (> 40%) with relatively modest decreases in aragonite saturation state (Langdon et al. 2003, Langdon and Atkinson

2005). Some evidence suggests that deep-sea biota will also be affected by small changes in pH (Seibel and Walsh 2001, Guinotte et al. 2003, Roberts et al. 2006). The aragonite and calcite saturation horizons are already quite shallow in areas of the North Pacific Ocean and predicted to become shallower in the near future (Feely et al. 2004, Guinotte et al. 2006). Mounting evidence suggests that if CO_2 emissions continue as projected, undersaturated regions will develop in the sub-arctic and arctic regions of Alaska by the end of the 21st century (Orr et al. 2005, Guinotte et al. 2006). Scleractinian corals would be most affected by this development, but they are not important structure-forming corals in Alaskan waters. Octocorals, stylasterids, and pennatulaceans, however, are important structure-forming components of benthic ecosystems in Alaskan waters and will likely experience decreased growth and recruitment rates and ultimately changes to their distribution with cascading effects to the ecosystems they support.

A suite of six studies is proposed to examine the potential effects of ocean acidification on North Pacific Ocean corals. The degree to which corals will be affected will depend on their skeletal mineralogy, geographical distribution, and biology. Fairly good information on coral depth distribution exists, but information on skeletal mineralogy is limited. Coral Study A will provide detailed information on skeletal mineralogy that will be used to construct a risk assessment for North Pacific Ocean corals (Coral Study B). Measurements on the aragonite and calcite saturation horizons currently being collected during PMEL oceanographic studies will be critical to this assessment. Coral Studies C-F will examine potential direct and indirect effects of ocean acidification on corals through the collection of specimens in the field and laboratory measurements (Coral Study C), manipulative experiments in the laboratory (Coral Study D), establishment of baseline studies *in situ* (Coral Study E), and finally, an *in situ* experiment using state-of-the-art technology (Coral Study F).

Coral Study A. $CaCO_3$ Mineralogy of Alaskan Corals

The purpose of this study will be to determine the $CaCO_3$ mineralogy of the skeletons of Alaskan corals in order to predict which coral resources in Alaska are most at risk to the effects of ocean acidification. The skeletons of these calcareous organisms contain aragonite, calcite, or coexisting carbonate polymorphs. Alaskan corals will be affected by ocean acidification differently depending on their $CaCO_3$ mineralogy. One hundred and forty-one unique coral taxa have been documented from Alaskan waters (Stone and Shotwell 2007). The $CaCO_3$ mineralogy is known for only 13 of these species: 11 hydrocorals (Cairns and MacIntyre 1992), 1 gorgonian (Andrews et al. 2002) and 1 pennatulacean (Wilson et al. 2002).

The skeletal composition of representative corals from each major taxonomic group and species of particular ecological importance (i.e., those that form single-species assemblages) will be determined using X-ray diffraction analyses. The analyses will be undertaken either at the Smithsonian Institution or the Woods Hole Oceanographic Institution. The majority of the specimens available for the analyses are currently archived at the National Museum of Natural History. Specimens archived at the AFSC's Auke Bay Laboratories (ABL) will augment these collections.

Coral Study B. Risk Assessment of Alaskan Corals to the Effects of Ocean Acidification

This study will complete a risk assessment of Alaska's coral resources based on $CaCO_3$ mineralogy (Coral Study A), geographical and depth distribution, and predicted aragonite and calcite horizons for the region. The degree to which corals will be affected by decreasing ocean pH will depend on their skeletal $CaCO_3$ mineralogy, and their geographical and depth distribution relative to the predicted aragonite and calcite horizons for the region.

In this study, known coral distributions (i.e., Stone and Shotwell 2007) will be mapped in ArcView-GIS based on their skeletal mineralogy. These distributions will be compared to maps of present and projected aragonite and calcite saturation horizons in Alaska to provide a risk assessment for coral taxa. The AFSC (ABL) will collaborate with the Marine Conservation Biology Institute, PMEL and the Smithsonian Institution on this study.

Coral Study C. Feeding Ecology of Alaskan Deep-Sea Corals

Very little is known regarding the feeding ecology of deep-sea corals including those found in Alaska. Some species depend for nutrition on suspended organic matter, including marine snow heavily laden with senescent phytoplankton and mesozooplankton. Ocean acidification could have an indirect, detrimental effect on deep-sea corals by limiting the quantity and quality of food and nutrients available to these organisms (Guinotte et al. 2006). This study will address that potential effect by examining, for the first time, the feeding ecology of deep-sea corals in Alaska to determine if they prey on plankton species that use $CaCO_3$ to build their tests (i.e., coccolithophores, crustaceans).

Coral specimens will be collected during annual NOAA bottom trawl surveys in the Aleutian Islands where coral abundance and diversity are exceptionally high (Stone 2006). Specimens from at least five families of gorgonians and soft corals will be collected and preserved in 95% ethanol. In the laboratory, gastrozooids will be carefully dissected and the preserved contents identified to the lowest possible taxa. The AFSC (ABL) will seek to collaborate with the University of Alaska Fairbanks on this study.

Coral Study D. Growth and Survival of Alaskan Corals Exposed to Reduced pH Levels in the Laboratory

This study will examine the effects of reduced pH on the growth and survival of faster-growing species of Alaskan corals in the laboratory. Several studies have shown substantial decreases in calcification rates of shallow-water tropical corals (> 40%) with relatively modest decreases in aragonite saturation state (Langdon et al. 2003, Langdon and Atkinson, 2005). There is currently little information to predict whether cold-water corals will experience similar effects to pH conditions they are likely to encounter in the coming decades.

This study will involve manipulative laboratory experiments to determine the effects of reduced pH on the growth and survival of faster-growing coral species (i.e., *Eunepthia* sp., *Anthomastus* sp., *Ptilosarcus gurneyi*). The experimental protocol already established

for ongoing studies with crustaceans will be used in this study with corals. Five treatments will be examined, involving equilibration of seawater with 280, 400, 500, 750, and 1,000 ppmv (ppmv = parts per million by volume) to reflect past, current and predicted future levels of CO_2 concentrations in seawater, following the same dosing methodology as planned for the proposed shellfish studies. This will be a long-term study (minimum one year) due to the relative slow growth rate for these taxa. Specimens will be collected during annual AFSC bottom trawl surveys in the Bering Sea (*Eunepthia* sp. and *Anthomastus* sp.) or during scuba surveys (*Ptilosarcus gurneyi*) and transported live to the laboratory.

Coral Study E. Growth and Recruitment of Shallow-water Scleractinian Corals in Alaska - a Baseline Study for the Future

Scleractinian corals, while not important structure-forming corals in the North Pacific Ocean, do have skeletons composed of aragonite and may be an important sentinel taxa for gauging the biological impacts of ocean acidification in the region. Growth and recruitment rates of cold-water corals are slow and inherently difficult to measure for that reason. This study will establish baseline information for shallow-water populations of three species of the scleractinians (*Javania borealis*, *Caryophyllia alaskensis*, and *Balanophyllia elegans*) in the central Aleutian Islands (Stone 2006) – the only shallow-water populations known in Alaska. This baseline information will provide for detailed measurements on growth and recruitment for scleractinian corals.

Baseline surveys of shallow-water populations of scleractinian corals will be made at two sites in the central Aleutian Islands using scuba. Patches of corals will be marked and geo-referenced and corals will be video-documented within patches. In the laboratory, computer images will be archived, corals will be enumerated, and the size of corals will be measured using computer image analysis tools (Stone and Wing 2001). The surveys will be repeated at approximately 5-year intervals to provide a time series of measurements on growth and recruitment of corals. The AFSC (ABL) plans to collaborate with the University of Alaska Fairbanks on this study.

Coral Study F. Growth and Calcification Rates of Red Tree Corals *in Situ* Using an Ocean pH Observatory

New deep-sea technology (e.g. Free Ocean CO_2 Enrichment system [FOCE]) is currently being developed that will allow scientists to monitor calcification rates of corals *in situ* while carefully manipulating and controlling pH (Bill Kirkwood, Monterey Bay Aquarium Research Institute [MBARI], pers. comm.). The technology is still in the experimental stages but has the potential to be a valuable tool for studying the effects of ocean acidification on corals in Alaska. Shallow-water populations of red tree corals have recently been discovered in several Southeast Alaska fjords and are excellent candidates for studies using this technology. Red tree corals are one of the most ecologically important corals in the North Pacific Ocean and their relatively fast growth rates (Andrews et al. 2002) make them excellent candidates for study. We propose to partner with the Science and Technology Branch of MBARI to deploy the first FOCE system in a shallow-water ecosystem of the North Pacific Ocean.

A FOCE system will be deployed at one site in Holkham Bay, Southeast Alaska, where a dense population of red tree corals has been documented. The FOCE system will be deployed using scuba and will fully encompass a minimum of 20 colonies. Each colony will be measured using computer image analysis tools to provide a baseline for growth measurements (Stone and Wing 2001). A nearby (up-current) population will also be delineated to serve as a control (ambient pH) and the same baseline measurements will be made. The experiment will run for one year at which time growth and recruitment (settlement of new colonies) will be measured for both the control and experimental populations. Acoustic doppler current meters are scheduled for deployment at the proposed site in April 2008 as part of an ongoing study on the ecology of red tree corals. These data on water currents will allow us to fully assess the appropriateness of the site for an Ocean pH Observatory. The AFSC (ABL) will collaborate with the MBARI on this study.

Marine Fish

Hypotheses Addressed

Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will reduce growth and survival of commercially important fish and shellfish. Increased atmospheric CO_2 and reduced ocean pH and $CaCO_3$ availability will reduce growth and survival of ecologically important prey of commercially important fish and shellfish.

Successful recruitment to fishery stocks is highly dependent upon the growth and survival of early life stages (eggs, larvae, and juveniles). Because mortality rates of these stages are intrinsically high, even minor changes in survival rates can result in order of magnitude responses in recruitment rates. Slight reductions in growth and development prolong the time that fish stay in these vulnerable stages, thereby increasing mortality rates. Ocean acidification could impact the recruitment dynamics of fishes through two distinct pathways. There could be direct physiological stress associated with low pH environments, manifested as reduced rates of growth and survival (Michaelidis et al. 2007). Alternatively, ocean acidification could impact the production of lower trophic levels, hence altering the foraging environment of the early life stages (UK Royal Society 2005).

The foraging environment of larval and juvenile fishes can be characterized by the abundance and nutritional composition of prey. If prey are not sufficiently abundant or don't meet the specific nutritional needs of a species, fish will experience slower growth rates, higher mortality, and potentially cohort failure (Litzow et al. 2006). Essential fatty acids (EFAs) are produced exclusively by marine phytoplankton and transferred up the food chain to fish through their zooplankton prey. Some of these lower trophic members such as coccolithophores, foraminifera, and pteropods may be particularly vulnerable to ocean acidification as $CaCO_3$ forms their biological structure. Changes in the production rates and community composition of phytoplankton and zooplankton could lead to changes in the fatty acid composition of prey that might not contain the EFAs needed for optimal growth and survival of larval and juvenile fishes.

Researchers at the AFSC's Fisheries Behavioral Ecology Program (FBEP) will develop the protocols and apparatus necessary to conduct experiments with early life stages of marine fishes under projected ocean acidification conditions. These approaches will be applied to examine both the direct physiological and indirect food web effects of ocean acidification on the early life stages of two focal resource species, walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*). The FBEP has extensive experience conducting experimental studies of the growth and survival of early life stages of walleye pollock, Pacific cod, and other marine species. These include demonstration of prey quality effects on larval walleye pollock (Davis and Olla 1992) and evaluation of temperature and prey availability on production of larval Pacific cod.

Physiological Responses (Direct Effects)

To determine the direct effects of ocean acidification on growth and survival of early life stages of walleye pollock and Pacific cod, fish will be reared in the laboratory under a range of acidified ocean conditions ranging from controls (present state of Bering Sea and Gulf of Alaska is pH 8.05) to 0.5 pH units below ambient (predicted for surface oceans, Caldeira and Wickett 2003). Although not expected to occur in this region, an additional treatment of 1.0 pH unit below ambient will also be conducted as a

conservative test of resilience and to evaluate the broader sensitivity of these marine species fish to acidified environments.

For larval fish experiments, adult fish will be captured on spawning grounds and strip-spawned at sea. Fertilized eggs will be packed and shipped by air to the FBEP laboratory in Newport, OR. Eggs will be reared in flow-through containers at the optimal temperatures for hatching. Upon hatching, fish will be transferred to 300 L larval rearing tanks and fed five times per day a combination of commercially available food and live fatty acid-enriched rotifers. For experiments on juveniles, fish will be captured from nearshore nursery grounds in Alaska (Pacific cod) or Washington (walleye pollock), or reared from the eggs of wild caught adults.

Four CO_2 dosing systems will be built and integrated with existing rearing systems. Seawater will be conditioned in a header tank to the target experimental pH condition by cascading water through a CO_2-filled chamber. A pH meter will regulate water flow through the CO_2 chamber to maintain constant pH conditions. The dosing systems will deliver CO_2 conditioned seawater to rearing tanks at a rate of approximately 1,500 mL/min, sufficient to maintain target pH conditions. Fish will be randomly assigned to treatments and at least three replicates will be included in each treatment. Rearing procedures will follow established protocols for each species. After 3-12 weeks of rearing (depending on species, temperature, and life stage) all fish will be captured and enumerated to determine survival rates. Representative samples from all replicates will be measured for body size (length and dry weight) and condition (lipid composition and gas bladder size). The direct effects on marine fishes will be indicated by significant treatment effects on survival rates, growth rates, or condition factor.

Foraging Environment Responses (Indirect Effects)

To determine the potential for ocean acidification-induced changes in lower trophic level dynamics to impact the recruitment of pollock and cod, fish will be reared under laboratory conditions with prey containing different fatty acid compositions. The tested prey compositions would be based on potential ocean acidification related changes in lower trophic level productivity. Definition of baseline and ocean acidification-impacted prey communities will be based on empirical studies of primary and secondary producer responses to changes in ocean pH. In the absence of direct observations of responses of some Bering Sea lower trophic level species, we will rely upon results obtained for taxonomically related species in other high-latitude oceans. These projections of community composition will be combined with expanded libraries of fatty composition of marine phytoplankton and zooplankton from the Bering Sea and Gulf of Alaska to define the fatty acid signatures of baseline and ocean acidification-impacted prey fields for larval fishes. While complete fatty acid composition libraries of lower trophic level organisms have yet to be developed for the Bering Sea, samples appropriate to these analyses have been collected and preserved by scientific staff of the BASIS program. To these existing samples, additional phytoplankton and zooplankton samples will be collected in conjunction with spring ichthyoplankton studies in the Bering Sea (for seasonal matching to production).

Fish will be collected or reared from eggs as described above. The basis for experimental diets for larvae will be intensively cultured rotifers (a marine zooplankton) enriched with specific combinations of fatty acids representing baseline and ocean acidification-impacted prey fields. Rotifers will be harvested from the intensive rearing system and immersed in a solution of fatty acid complex and seawater for 8-12 hours. The rotifers take up the fatty acids and are passed to the fish during feeding. Using a common prey (the rotifer *Brachionus* sp.) enriched with different fatty acid complexes as the prey in the experiment will isolate the effects of trophic transfer of nutritional requirements, removing potential artifacts of prey size, energy content, and catchability that could confound results of experiments based on different prey with inherently differing fatty acid compositions. As above, all survival, growth rates, and condition factors will be assessed after 3-12 weeks of rearing. The effects of ocean acidification-induced shifts in primary producers on marine fishes will be indicated by significant treatment effects on survival rates, growth rates, or condition factor.

Calcium Carbonate Survey

The $CaCO_3$ content of the hard parts of many calcareous species is not well known, and often neither is the mineral form. For example, the most recent quantitative analyses of several shellfish species from the Atlantic Ocean date from the late 19th and early 20th centuries, and indicate that $CaCO_3$ constitutes ~50% or more by weight of the shells of many economically important species such as crabs (Richards 1951). $CaCO_3$ content needs to be determined for Pacific Ocean species, along with the variability in shells among individuals and life-stages, to assess species and life-stage vulnerabilities to acidification-mediated carbonate depletion and to establish a baseline of current values to assess changes as the oceans acidify. We, therefore, will survey calcareous species in the North Pacific Ocean, including shellfish, calcareous plankton and corals, to determine their dependence on $CaCO_3$. In addition, this study will support species-specific laboratory studies by measuring $CaCO_3$ content of experimental animals.

Calcareous organisms will be collected opportunistically during research cruises and dives. The collected organisms will be identified to species, their biometric data recorded (including size, weight and life stage) and their calcified parts stored intact at $0^{\circ}C$ for analysis of carbonate, calcium, and magnesium. The shells of collected organisms will be chemically analyzed at the ABL. The carbonate content will be determined by grinding the dried shell into a coarse powder, and measuring the CO_2 liberated from a weighed aliquot following dissolution in 2 M nitric acid by colometric titration. An aliquot of the digest will then be analyzed by ion chromatography for cations, including calcium and magnesium. In cases where the mineral form of the $CaCO_3$ is doubtful, for a small subsample, the shell will be sectioned dry and examined by X-ray diffraction to determine whether the embedded microcrystals are acicular orthorhombic forms characteristic of aragonite, or trigonal-rhombohedral forms typical of calcite. Since the AFSC does not have an X-ray diffraction facility, these examinations will be performed elsewhere.

Forecast Population, Ecosystem, and Economic Impacts

This project will apply the results of the species-specific physiological studies to forecast population, ecosystem, and economic impacts of ocean acidification. The proposed project has three components:

1. Climate Change/Ocean Acidification Scenario Development.
2. Ocean Acidification and King Crab Stocks in Alaska.
3. Ecosystem Sensitivity to Ocean Acidification and Valuation of Indirect Effects.

Climate Change/Ocean Acidification Scenario Development

Future atmospheric CO_2 levels, which influence ocean pH, will depend on many factors but anthropogenic emissions from the burning of fossil fuels are chief among these. A global energy-economic growth model (Dalton et al. 2008) will be used to project CO_2 emissions from fossil fuels. An earth system model of intermediate complexity (Cao et al. 2007) will take fossil fuel emissions from the economic model as input, track baseline

greenhouse gas emissions from other sources, and project atmospheric CO_2 levels and changes in ocean pH. Assumptions in the scenarios about future technological and demographic change will be based on updates of the Intergovernmental Panel on Climate Change (IPCC) Special Report on Emissions.

Ocean Acidification and King Crab Stocks in Alaska

A two-area bioeconomic model of Alaska's king crab fishery will be used to evaluate fishery performance in each of the climate change/ocean acidification scenarios. The structure of the proposed model will be based on previous work (Dalton 2001, Dalton and Ralston 2004). In addition, the proposed model will incorporate an explicit population growth function that will be calibrated to experimental results on king crab growth and survival with varying pH and water temperatures. A first version of the population model will quantify growth and survival information from early life history stages. As results from additional experiments become available, the population model will be extended to quantify effects of acidification on later stages of a crab's life. Decision rules for fishing effort which depend on expected abundance, climate, and prices will be estimated (and tested) using time series data from Alaska's fish tickets, observers, and information on vessel costs from the Bering Sea and Aleutian Islands (BSAI) Crab Economic Data Collection Program and the Alaska Department of Fish and Game.

Three sets of model simulations will be used to forecast and evaluate fishery performance in different scenarios under alternative harvest control rules. These simulations will follow a two-factor design. First, simulations will be conducted holding pH constant while imposing climate change on the model in the form of a warming trend (initially using the historical spatial pattern of climate as a baseline and later incorporating variations to the spatial pattern as projected from oceanographic models). A second set of simulations will impose acidification on the model in the absence of climate change. As a first approximation, the pH level will be assumed spatially uniform at each point in time. Results from these two sets of simulations will be combined under a null hypothesis that effects of changes in temperature and acidification on crab stocks will be additive. Finally, a third set of simulations will test the null of additivity by imposing trends in both temperature and acidification on the model simultaneously.

Ecosystem Sensitivity to Ocean Acidification and Valuation of Indirect Effects

Future ocean pH levels, climate, and the combined direct and indirect ecological effects of ocean acidification and climate change, are uncertain. The AFSC has developed trophic (i.e., food-web) models of the Bering Sea and Gulf of Alaska ecosystems (e.g., Aydin et al. 2007) that have been used with climate change scenarios. These models predict substantial impacts of reduced production of calcareous prey (pteropods) on growth of commercially important species (pink salmon) (Aydin et al. 2005). An exploratory analysis using these models with climate change/ocean acidification scenarios can be conducted at relatively low cost and could yield important insights regarding model sensitivity and the range of ecosystem effects. In particular, this component of the project will examine how direct effects of changes in ocean pH and temperatures can be transmitted across trophic levels to create indirect effects in the

ecosystem models. In addition, an economic model will be developed to evaluate impacts of these indirect effects.

Research Program Integration and Summary

Possible consequences of predicted ocean pH changes may be particularly acute in the North Pacific Ocean and especially Alaskan waters. The shellfish, calcareous plankton, coral and fish studies described in the previous six sections were developed to form a coherent ocean acidification research plan. Species-specific effects identified by these studies are apt to have ecosystem-level consequences; these study results will be used to inform models and forecast population, ecosystem, and economic impacts.

Shellfish

The species-specific physiological responses of king crab to ocean acidification will be measured and then these results will be incorporated into a bioeconomic model to forecast population and economic impacts. In addition, a $CaCO_3$ survey of North Pacific shellfish will be conducted to assess species vulnerabilities to ocean acidification. The survey will measure $CaCO_3$ concentration and form (e.g., aragonite). The species-specific physiological response (growth, survival, reproduction) of larval, juvenile and reproductive female king crab species to mixtures of CO_2 consisting of 79% nitrogen, 21% oxygen, and one of the five following concentrations of CO_2: 280, 400, 500, 750, and 1,000 ppmv (ppmv = parts per million by volume) will be tested. The mixtures were chosen to reflect past, current and predicted future levels of CO_2 concentrations in seawater. The performance of the CO_2 delivery system will be monitored because the chemical changes induced in the affected seawater will depend on the salinity, dissolved inorganic carbon, and initial pH. The $CaCO_3$ content of the shells of the experimental animals in the different treatments will be measured. Microarrays will be used to indicate sublethal effects. These study results will be incorporated into a two-area bioeconomic model of Alaska's king crab fishery; fishery impacts will be evaluated for a range of climate change/ocean acidification scenarios. Assumptions in the scenarios about future technological and demographic change will be based on updates of the IPCC Special Report on Emissions.

Calcareous Plankton

The species-specific physiological responses of calcareous plankton to ocean acidification will be measured, monitoring of calcareous plankton distributions in the Bering Sea and Gulf of Alaska will continue and these results will be incorporated into an ecosystem model to forecast ecosystem impacts. In addition, a $CaCO_3$ survey of North Pacific calcareous plankton will be conducted to assess species vulnerabilities to ocean acidification. Monitoring of calcareous plankton distributions will continue in the Bering Sea and Gulf of Alaska from satellite observations and shipboard sampling by research vessels and ships of opportunity. These distributions may be compared to water mass properties (e.g., temperature, salinity, nutrients and pH) and distribution of zooplankton and fish to understand factors promoting blooms and potential food web implications. In addition, we plan to conduct at sea experiments to test for effects of ocean acidification

on calcareous plankton (e.g., pteropods) through collaboration between external and in-house experts.

Coldwater Corals

A $CaCO_3$ survey of North Pacific corals will be conducted to assess species vulnerabilities to ocean acidification and these results will be evaluated in a risk assessment; the species-specific physiological responses of representative coral species to ocean acidification will be measured. The degree to which North Pacific Ocean corals will be affected by ocean acidification will depend on their $CaCO_3$ concentration and type and their depth distribution. Fairly good information on coral depth distribution exists, but information on $CaCO_3$ composition is limited. A $CaCO_3$ survey will be conducted then used to construct a risk assessment for North Pacific Ocean corals. Measurements on the aragonite and calcite saturation horizons currently being collected during PMEL oceanographic studies will be critical to this assessment. Species-specific physiological responses will measured for representative coral species in the field and laboratory. The species will be chosen based on the risk assessment. For an *in situ* experiment, a Free Ocean CO_2 Enrichment system being developed by MBARI would be deployed in a dense population of red tree corals accessible using scuba in southeast Alaska and growth and recruitment over one year would be compared between experimental and nearby control sites.

Fish

The direct and indirect responses of two fish species will be measured and these results will be incorporated into an ecosystem model to forecast ecosystem impacts. Ocean acidification could impact the recruitment dynamics of fishes through two distinct pathways, reduced growth and survival through direct physiological effects and alternatively, by altering the production of lower trophic levels and thus the foraging environment of the early life stages. Experiments will be conducted to examine both the direct physiological and indirect food web effects of ocean acidification on the early life stages of two commercially important fish species, walleye pollock and Pacific cod. These experiments will extend previous research on prey quality effects on larval walleye pollock and evaluation of temperature and prey availability on production of larval Pacific cod to encompass new research to measure the effects of ocean acidification.

Cross-laboratory Collaboration, Timeline and Budget

Cross-laboratory collaboration is critical for this effort. The research plan budget and timeline are described in Appendix 2. Cross-laboratory collaboration and timeline are organized as follows:

Shellfish

The shellfish research will be conducted by the AFSC's Kodiak Laboratory, Auke Bay Laboratories, and Socioeconomic Program. Shellfish research will focus on king crab species during years 1-3 and will be conducted by the Kodiak Laboratory. $CaCO_3$ content of the crabs will be measured by the ABL. During years 3-4, the AFSC's

Socioeconomics Program will apply the king crab study results to forecast impacts for king crab fisheries.

Calcareous Plankton
Calcareous plankton research will be conducted through collaboration between the Recruitment Processes and BASIS Programs and external partners during years 1-5.

Coral
The coral research will be conducted by the Kodiak Laboratory, ABL and external partners. The focus at the Kodiak Laboratory will shift to coral species during years 4-5. The ABL will analyze the $CaCO_3$ content of the corals. The $CaCO_3$ survey will be conducted by the ABL during years 1-5 and will start with coral species during years 1-2.

Marine Fish
Fish research will focus on cod during years 1-3 then shift to pollock during years 3-5 and will be conducted by the Newport Laboratory.

Research Partners

- The AFSC will collaborate with the PMEL (Feely and Stabeno) on monitoring of ocean pH and at sea measurement of species specific physiological response of plankton.
- Several NOAA components participated in an ad hoc ocean acidification planning meeting in January 2008 including OAR (PMEL, Atlantic Oceanographic and Meteorological Laboratory), NMFS (Alaska, Northwest, Southwest, Pacific Islands, Southeast and Northeast Fisheries Science Centers), NOAA Coral Reef Conservation Program, NESDIS Coral Reef Watch, NOS (National Centers for Coastal Ocean Science) and the NOAA Program Coordination Office.
- The AFSC will collaborate with academic and nongovernmental partners including but not limited to the University of Alaska, San Francisco State University, Smithsonian Institution, Woods Hole Oceanographic Institution, National Museum of Natural History, Marine Conservation Biology Institute and the MBARI.

Internal and External Funding Sources

- The AFSC will continue using a limited amount of AFSC funds to study the effect of ocean pH on shellfish. The use of internal funds in this research area began in FY 2006.
- The AFSC is requesting $700 K per year from NOAA for ocean acidification research (Appendix 2).
- The AFSC will seek funding from the North Pacific Research Board (NPRB) if ocean acidification research is identified as a research priority by NPRB.

Citations

Andrews, A. H., E. E. Cordes, M. M. Mahoney, K. Munk, K. H. Coale, G. M. Cailliet, and J. Heifetz. 2002. Age, growth, and radiometric age validation of a deep-sea, habitat forming gorgonian (*Primnoa resedaeformis*) from the Gulf of Alaska. Hydrobiologia 471: 101-110.

Armstrong, D. A., and R. Palacios. 1985. Early life history of juvenile blue king crab, *Paralithodes platypus*, around the Pribilof Islands. Proceedings of the International King Crab Symposium, Anchorage, AK, University of Alaska Sea Grant Program.

Aydin, K. Y., G. A. McFarlane, J. R. King, B. A. Megrey, and K. W. Myers. 2005. Linking oceanic food webs to coastal production and growth rates of Pacific salmon (*Oncorhynchus* spp.), using models on three scales. Deep Sea Res. II: Topical Studies in Oceanography 52: 747-780.

Aydin, K., S. Gaichas, I. Ortiz, D. Kinzey, and N. Friday. 2007. A comparison of the Bering Sea, Gulf of Alaska, and Aleutian Islands large marine ecosystems through food web modeling. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-178, 298 p.

Bayer, F. M., and I. G. Macintyre. 2001. The mineral composition of the axis and holdfast of some gorgonacean octocorals (Coelenterata: Anthozoa), with special reference to the family Gorgoniidae. Proc. Biol. Soc. Washington 114: 309–45

Brouwer, M., P. Larkin, N. Brown-Peterson, C. King, S. Manning, and N. Denslow. 2004. Effects of hypoxia on gene and protein expression in the blue crab, *Callinectes sapidus*. Mar. Environ. Res. 58: 787–792.

Cairns, S. D., and I. G. Macintyre. 1992. Phylogenetic implications of $CaCO_3$ mineralogy in the Stylasteridae (Cnidaria: Hydrozoa). Palaios 7: 96-107

Calado, R., L. Narciso, S. Morais, A. L. Rhyne, and J. Lin. 2003. A rearing system for the culture of ornamental decapod crustacean larvae. Aquaculture 218: 329-339.

Caldeira, K. and M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature 425: 365-365.

Cao, L., K. Caldeira, and A. Jain. 2007. Effects of carbon dioxide and climate change on ocean acidification and carbonate mineral saturation. Geophys. Res. Lett. 34, L05607, doi: 10.1029/2006GL028605.

Dalton, M. 2001. El Niño, expectations, and fishing effort in Monterey Bay, California. J. Environ. Econ. Manage. 42: 336-359.

Dalton, M., and S. Ralston 2004. The California Rockfish Conservation Area and groundfish trawlers at Moss Landing Harbor. Mar. Res. Econ. 19: 67-83.

Dalton, M., B. C. O'Neill, A. Prskawetz, L. Jiang, and J. Pitkin. 2008. Population aging and future carbon emissions in the United States. Energ. Econ. 30: 642-675.

Davis, M. W., and B. L. Olla. 1992. Comparison of growth, behavior and lipid concentrations of walleye pollock *Theragra chalcogramma* larvae fed lipid-enriched, lipid-deficient and field-collected prey. Mar. Ecol. Prog. Ser. 90: 23-30.

Dew, C. B. 1990. Behavioral ecology of podding red king crab, *Paralithodes camtschatica*. Can. J. Fish. Aquat. Sci. 47: 1944-1958.

Donaldson, W. E., S. Byersdorfer, D. Pengilly, and S. F. Blau. 1992. Growth of red king crab, *Paralithodes camtschaticus* (Tilesius, 1815) in artificial habitat collectors at Kodiak, Alaska. J. Shellfish Res. 11: 85-89.

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

Gazeau, F., C. Quiblier, J. M. Jansen, J. P. Gattuso, J. J. Middelburg, and C. H. R. Heip. 2007. Impact of elevated CO_2 on shellfish calcification. Geophys. Res. Lett. 34: 5.

Gracey, A. Y., E. J. Fraser, W. Li, Y. Fang, R. R. Taylor, J. Rogers, A. Brass, and A. R. Cossins. 2004. Coping with cold: An integrative, multitissue analysis of the transcriptome of a poikilothermic vertebrate. Proc. Natl. Acad. Sci. 101: 16970–16975.

Guinotte, J. M., R. W. Buddemeier, and J. A. Kleypas. 2003. Future coral reef habitat marginality: temporal and spatial effects of climate change in the Pacific basin. Coral Reefs 22: 551-558

Guinotte, J. M., J. Orr, S. Cairns, A. Freiwald, L. Morgan, and R. George. 2006. Will human-induced changes in seawater chemistry alter the distribution of deep-sea Scleractinian corals? Front. Ecol. Environ. 4: 141-146.

Iglesias-Rodriguez, M. D., P. R. Halloran, R. E. M. Rickaby, I. R. Hall, E. Colmenero-Hidalgo, J. R. Gittins, D. R. H. Green, T. Tyrrell, S. J. Gibbs, P. von Dassow, E. Rehm, E. Virginia Armbrust, and K. P. Boessenkooland. 2008. Phytoplankton calcification in a high-CO_2 world. Science 320: 336-340.

Iida T., S. I. Saitoh, T. Miyamura, M. Tortani, H. Fukushima, and N. Shiga. 2002. Temporal and spatial variability of coccolithophore blooms in the eastern Bering Sea, 1998-2001. Progr. Oceanogr. 55: 165-175.

Incze, L. S., D. L. Wencker, and D. A. Armstrong. 1984. Growth and average growth rates of Tanner crab zoeae collected from the plankton. Mar. Biol. 84: 93-100.

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

Krasnov, A., H. Koskinen, P. Pehkonen, C. E. Rexroad III, S. Afanasyev, and H. Mölsä. 2005. Gene expression in the brain and kidney of rainbow trout in response to handling stress. BMC Genomics 2005, 6:1-11.

Kurihara, H., S. Shimode, and Y. Shirayama, Y. 2004a. Sub-lethal effects of elevated concentration of CO_2 on planktonic copepods and sea urchins. J. Oceanogr. 60: 743-750.

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

Langdon, C., W. W. Broecker, D. E. Hammond, E. Glenn, K. Fitzsimmons, S. J. Nelson, T-H. Peng, I. Hajdas, and G. Bonani. 2003. Effect of elevated CO_2 on the community metabolism of an experimental coral reef. Global Biogeochem. Cy. 17(1):1-14.

Langdon, C., and M. J. Atkinson. 2005. Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. 110:C09S07, doi:10.1029/2004JC002576.

Langer, G., M. Geisen. K.-H. Baumann, J. Kläs, U. Riesbesell, S. Thomas, and J. R. Young. 2006. Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochem. Geophys. Geosys. 7, Q09006, doi:10.1029/2005GC001227.

Litzow, M. A., K. M. Bailey, F. G. Prahl, and R. Heintz. 2006. Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. Mar. Ecol. Prog. Ser. 315: 1-11.

Loher, T., D. A. Armstrong, and B. G. Stevens. 2001. Growth of juvenile red king crab (*Paralithodes camtschaticus*) in Bristol Bay (Alaska) elucidated from field sampling and analysis of trawl-survey data. Fish. Bull., U. S. 99: 572-587.

Michaelidis, B., A. Spring, and H. O. Pörtner. 2007. Effects of long-term acclimation to environmental hypercapnia on extracellular acid–base status and metabolic capacity in Mediterranean fish *Sparus aurata*. Mar. Biol. 150: 1417-1429.

Olson M. B., and S. L. Strom. 2002. Phytoplankton growth, microzooplankton herbivory and community structure in the southeast Bering Sea: insight into formation and temporal persistence of an *Emiliania huxleyi* bloom. Deep Sea Res. II. 49: 5969-5990.

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

Powell, G. C., and R. B. Nickerson. 1965. Aggregations among juvenile king crabs (*Paralithodes camtschatica*, Tilesius), Kodiak, Alaska. Anim. Behav. 13(2-3): 374-380

Quinn, P., R. M. Bowers, X. Zhang, T. M. Wahlund, M. A. Fanelli, D. Olszova, and B. A. Read. 2006. cDNA microarrays as a tool for identification of biomineralization proteins in the coccolithophorid *Emiliania huxleyi* (Haptophyta). Appl. Environm. Microbiol. 72: 5512–5526.

Roberts J. M., A. J. Wheeler, and A. Freiwald. 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. Science 312: 543-547.

Richards, A. G. 1951. The Integument of the Arthropods. University of Minnesota Press, Minneapolis.

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

Seibel, B. A., and P. J. Walsh. 2001. Potential impacts of CO_2 injection on deep-sea biota. Science 294: 319-320

Sheader, D.L., T.D. Williams, B.P. Lyons, and J.K. Chipman. 2006. Oxidative stress response of european flounder (*Platichthys flesus*) to cadmium determined by a custom cDNA microarray. Mar. Environm. Res. 62: 33–44.

Shirayama, Y. and H. Thornton. 2005. Effect of increased atmospheric CO_2 on shallow water marine benthos. J. Geophys. Res.-Oceans 110(C9).

Shirley, S. M., T. C. Shirley, and S. D. Rice. 1987. Latitudinal variation in the Dungeness crab, *Cancer magister*, zoeal morphology explained by incubation temperature. Mar. Biol. 95: 371-376.

Sneddon, L. U., J. Margareto, and A. R. Cossins. 2005. The use of transcriptomics to address questions in behaviour: production of a suppression subtractive hybridisation library from dominance hierarchies of rainbow trout. Physiol. Biochem. Zool. 78: 695–705.

Somero, G. N. 2005. Linking biogeography to physiology: Evolutionary and acclamatory adjustments of thermal limits. Front. Zool. 2: 1-9.

Stevens, B. G. 2003. Settlement, substratum preference, and survival of red king crab *Paralithodes camtschaticus* (Tilesius, 1815) glaucothoe on natural substrata in the laboratory. J. Exp. Mar. Biol. Ecol. 283: 1-2.

Stevens, B. G., and J. Kittaka. 1998. Postlarval settling behavior, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus*. Mar. Ecol. Prog. Ser. 167: 197-206.

Stevens, B. G., and K. M. Swiney. In review. Thermal effects on embryo development and hatching for blue king crab *Paralithdes platypus* Brandt, 1850 held in the laboratory, and a method for predicting dates of hatching. J. Shellfish Res.

Stevens, B. G. 1990. Temperature-dependent growth of juvenile red king crab (*Paralithodes camtschatica*), and its effects on size-at-age and subsequent recruitment in the eastern Bering Sea. Can. J. Fish. Aquat. Sci. 47: 1307-1317.

Stevens, B. G. 2006a. Embryo development and morphometry in the blue king crab *Paralithodes platypus* studied by using image and cluster analysis. J. Shellfish Res. 25: 569-576.

Stevens, B. G. 2006b. Timing and duration of larval hatching for blue king crab *Paralithodes platypus* Brandt, 1850 held in the laboratory. J. Crustacean Biol. 26: 495-502.

Stone, R. P., C. E. O'Clair, and T. C. Shirley. 1992. Seasonal migration and distribution of female red king crabs in a southeast Alaskan estuary. J. Crustacean Biol. 12: 546-560.

Stone, R. P., and B. L. Wing. 2001. Growth and recruitment of an Alaskan shallow-water gorgonian, p. 89-44. *In* Willison et al. (eds.) Proceedings of the First International Symposium on Deep-Sea Corals, Ecology Action Centre and Nova Scotia Museum, Halifax, Nova Scotia, pp 88-94.

Stone, R. P., M. M. Masuda, and P. W. Malecha. 2005. Effects of bottom trawling on soft-sediment epibenthic communities in the Gulf of Alaska, p. 461-475. *In* Barnes, P.B. and J.P. Thomas (eds.) Benthic habitats and the effects of fishing. American Fisheries Society, Symposium.

Stone, R. P. 2006. Coral habitat in the Aleutian Islands of Alaska: depth distribution, fine-scale species associations, and fisheries interactions. Coral Reefs 25: 229-238.

Stone, R. P., and S. K. Shotwell. 2007. State of deep coral ecosystems in the Alaska Region: Gulf of Alaska, Bering Sea and Aleutian Islands, p. 65-108. In S. E. Lumsden, T. F. Hourigan, A. W. Bruckner, and G. Dorr (eds.), The State of Deep Coral Ecosystems of the United States. U.S. Dep. Commer., NOAA Tech. Memo. CRCP-3.

Sundberg, K., and D. Clausen. 1977. Post-larval king crab (*Paralithodes camtschatica*) distribution and abundance in Kachemak Bay, Lower Cook Inlet, Alaska. 1976, p. 1-36. *In* L.L. Trasky, L.B. Flagg, and D.C. Burbank (ed.), Environmental studies of Kachemak Bay and Lower Cook Inlet. Vol. 5. Alaska Department of Fish and Game. Anchorage, AK.

Swiney, K. M. 2008. Egg extrusion, embryo development, timing and duration of eclosion, and incubation period of primiparous and multiparous Tanner crabs (*Chionoecetes bairdi*). J. Crustacean Biol. 28: 334-341.

Swiney, K. M., and T. C. Shirley. 2001. Gonad development of southeastern Alaskan Dungeness crab, *Cancer magister*, under laboratory conditions. J. Crustacean Biol. 21: 897-904.

Teranishi, K. S. and J. H. Stillman. 2007. A cDNA microarray analysis of the response to heat stress in hepatopancreas tissue of the porcelain crab *Petrolisthes cinctipes*. Comp. Biochem. Physiol. Pt. D 2: 53–62.

Thomas, P. 1990. Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring, p. 9-28. *In* S. M. Adams (ed.), Biological Indicators of Stress in Fish. Am. Fish. Soc. Symp. 8: 9-28.

UK Royal Society. 2005. Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society, pp. 57.

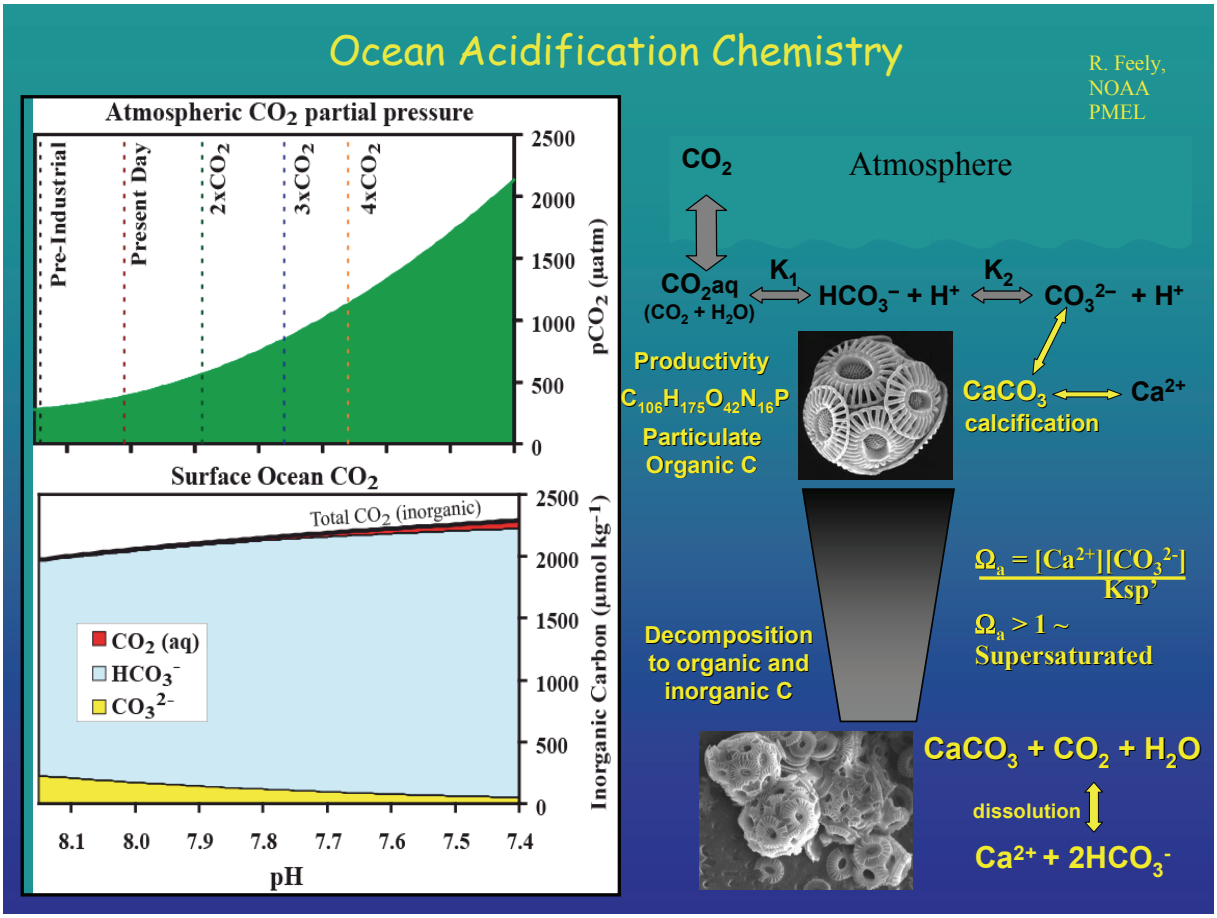
Venier, P., C. De Pitt, A. Pallavicini, F. Marsano, L. Varotto, C. Romualdi, F. Dondero, A. Viarengo, and G. Lanfranchi. 2006. Development of mussel mRNA profiling: Can gene expression trends reveal coastal water pollution? Mutation Res. 602: 121–134.

Watanabe, Y., and A. Yamaguchi. 2006. Lethality of increasing CO_2 levels on deep-sea copepods in the western North Pacific. J. Oceanogr. 62(2): 185-196.

Webb, J. B., G. L. Eckert, T. C. Shirley, and S. L. Tamone. 2006. Changes in zoeae of the snow crab, *Chionoecetes opilio*, with variation in incubation temperature. J. Exp. Mar. Biol. Ecol. 339: 96-103.

Wilson, M. T., A. H. Andrews, A. L. Brown, and E. E. Cordes. 2002. Axial rod growth and age estimation of the sea pen, *Halipteris willemoesi* Kölliker. Hydrobiologia 471: 133-142.

Appendix 1
Ocean Chemistry of CO_2 Acidification



Ocean acidification refers to the increasing acidity of the ocean that results from absorbing CO_2. Since the beginning of the Industrial Revolution, the atmospheric CO_2 concentration has risen from 280 parts per million by volume (ppmv) to the current value of 383 ppmv, and is increasing by 2 – 3 ppmv per year. These increases promote dissolution of CO_2 into the surface layer of the world's oceans. Once dissolved, some of the CO_2 reacts with water to make carbonic acid, which perturbs the carbonate buffer of seawater.

The solubility of atmospheric CO_2 (denoted as $CO_2(g)$) into seawater,

$$CO_2(g) = CO_2(aq) \qquad (1)$$

is determined by the Henry's Law constant, H_{CO2}:

$$CO_2(aq) = H_{CO2} \cdot CO_2(g) \qquad (1)$$

(chemical reactions will be identified by italicized numbers and equilibrium expressions describing them will be denoted by normal typeface here and following). At 35‰ seawater and 20 °C, H_{CO2} is 0.0324 mole per kg per atm, so the current atmospheric CO_2(g) concentration of 3.83×10^{-4} atm results in a dissolved CO_2(aq) concentration of 1.24×10^{-5} mole kg^{-1} water. The solubility decreases slightly with increasing salinity and more strongly with temperature: at 5 °C and 35‰, the H_{CO2} is 0.0521 mole per kg per atm, and the CO_2(aq) concentration increases to 2.00×10^{-5}, a 61% increase. Hence, colder waters nearer the poles contain substantially more CO_2 (aq) than do tropical waters.

The inherent solubility of CO_2 is much greater than the other atmospheric gases. Nitrogen and oxygen have Henry's Law constants of 0.00069 and 0.0014 mole kg^{-1} atm^{-1}, respectively. The seawater concentrations of these two gases are ten- to twentyfold greater than CO_2 only because of their much greater concentrations in the atmosphere (79% and 21%, respectively). The reason is because, unlike nitrogen or oxygen, CO_2 chemically reacts with water.

When CO_2 dissolves into water, some of it reacts to form carbonic acid, H_2CO_3(aq):

$$CO_2(\text{free}) + H_2O = H_2CO_3(\text{aq}). \qquad (2)$$

Because they are indistinguishable analytically, the free CO_2 and the carbonic acid are by convention lumped together and treated as if they were a single entity, with $[CO_2(\text{aq})] = [CO_2(\text{free})] + [H_2CO_3(\text{aq})]$, the square brackets indicating concentrations.

The carbonic acid formed by reaction of CO_2 with water (*2*) can dissociate to form a hydrogen ion and a bicarbonate ion, causing the acidity of the water to increase:

$$H_2CO_3(\text{aq}) = H^+ + HCO_3^- . \qquad (3)$$

The concentrations of these compounds satisfy the following relation at equilibrium:

$$K_1^* = \frac{[H^+][HCO_3^-]}{[CO_2(\text{aq})]} . \qquad (3)$$

The denominator of (3) would ordinarily be $[H_2CO_3]$, but since this is lumped with the free CO_2, K_1^* is called an *apparent* acid dissociation constant, hence the asterisk. This constant has a value of 9.34×10^{-7} at 35‰ seawater and 20 °C, and decreases with lower temperature (e.g., 7.18×10^{-7} at 35‰ seawater and 5 °C) and salinity (e.g., 8.81×10^{-7} at 30‰ seawater and 20 °C). This constant increases with pressure and is ~11% greater at seawater depth of 1,000 m and nearly threefold greater at 10,000 m. Hence, acidity from carbonic acid increases substantially at deeper oceanic depths.

The bicarbonate produced from reaction (3) is itself acidic, and can ionize in water to form carbonate ion:

$$HCO_3^- = H^+ + CO_3^{2-} . \qquad (4)$$

These reaction components also satisfy an equilibrium relationship:

$$K_2^* = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} . \qquad (4)$$

This constant is much smaller than K_1^*, being 6.55×10^{-10} at 35‰ seawater and 20 °C, and has similar dependencies on temperature, salinity and pressure as does K_1^*.

Because the hydrogen ion produced by the first ionization reaction (*3*) tends to shift the second ionization reaction (*4*) to the left to produce more bicarbonate ion (i.e., HCO_3^-), the net result of increased CO_2(g) dissolution is:

$$CO_2\,(aq) + H_2O + CO_3^{2-} = 2\ HCO_3^- . \qquad (5)$$

This reaction is simply the sum of reactions *2*, *3* and *4* (in reverse). Overall, dissolution of CO_2(g) results in higher concentrations of aqueous CO_2, bicarbonate and hydrogen ions, and decreased concentrations of carbonate ions (CO_3^{2-}). Hence, "ocean acidification" really refers to four distinguishable chemical perturbations, only one of which involves acidic hydrogen ions. Changes in the seawater concentrations of each of these chemical species may have biological effects.

The ~100 ppmv increase of atmospheric CO_2 since the Industrial Revolution has increased the acidity of the upper few hundred meters of ocean by around 26%, equivalent to a decrease of pH of -0.1 unit ($pH = -\log[H^+]$). If uncontrolled, continued emissions could lead to atmospheric CO_2 concentrations approaching 800 ppmv by the end of this century, which would more than double the acidity of the oceanic surface layer. An increase of this magnitude would halve the concentration of carbonate ions, making life more difficult for organisms bearing shells made of $CaCO_3$.

There are three different types of $CaCO_3$ used by marine organisms to make support structures or shells. The most stable thermodynamically and least soluble in seawater is calcite, which is formed when calcium and carbonate ions precipitate:

$$Ca^{2+}(aq) + CO_3^{2-}(aq) = CaCO_3\,(s) \qquad (6)$$

and which satisfy the following equilibrium expression:

$$K_{sp,\,calc} = [Ca^{2+}][CO_3^{2-}] \qquad (6)$$

(the subscript "sp" stands for "solubility product" and "calc" stands for "calcite"). At 35‰ seawater and 20 °C, $K_{sp,\,calc}$ is 4.3×10^{-7}, and varies little with temperature but directly with salinity. The solubility product constants for aragonite and magnesium-calcite, the two other forms of $CaCO_3$ used by marine biota, are 6.6×10^{-7} and 9×10^{-7}, respectively. All three K_{sp} values increase substantially with pressure, increasing by factors of ~3.7 at 6500 m seawater depth. Hence, shell-bearing organisms have greater difficulty precipitating $CaCO_3$ in lower salinity waters and at greater seawater depth, and organisms forming magnesium-calcite shells are most vulnerable, followed by those forming aragonite shells.

The saturation state of seawater with respect to CaCO3 is measured as:

$$\Omega = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}}\ , \qquad (7)$$

where the concentrations in the numerator are those measured in a seawater parcel. When the product of the calcium and carbonate ion concentrations exceeds K_{sp}, then Ω is greater than 1 and the water is supersaturated. Saturation occurs when $\Omega = 1$. Currently, the surface layer of the ocean is supersaturated with respect to all forms of calcium (or calcium-magnesium) carbonate. The saturation state declines substantially with seawater depth, mainly because of the increased solubility of CO_2 (which is generated *in situ* by respiration) at lower temperatures and the increasing values of K_1*, K_2* and of K_{sp} with increasing pressure. Also, respiration and volcanic releases contribute CO_2 to deep ocean waters, adding to acidity through reactions (*2*) and (*3*), which depletes carbonate ion concentrations through reaction (*4*). In contrast, the calcium ion concentration is a fixed proportion of salinity, and is unaffected by CO_2 changes. These factors combine so that the $\Omega = 1$ saturation horizon occurs at as little as 100 – 200 m in the north Pacific Ocean, to as much as 2,000 – 3,000 m in the north Atlantic Ocean. These saturation horizons are shoaling by 2 – 3 m per year in response to seawater absorption of CO_2. Thus, the surface waters of the North Pacific Ocean and the Southern Ocean surrounding Antarctica are the most vulnerable in the world to the effects of ocean acidification.

Respiration and photosynthesis can cause substantial changes in the partial pressure of CO_2, leading to attendant changes in pH, bicarbonate, carbonate and calcium carbonate saturation state. Respiration adds CO_2 to the water column, whereas photosynthesis removes it. These processes have their greatest effects in high-productivity waters, including continental shelves, upwelling regions, and coastal waters generally, where they can cause local perturbations of CO_2 partial pressure on the order of ± 10%. The CO_2 partial pressure of these waters is consequently much more variable in comparison with the open ocean, and the secular increase of atmospheric CO_2 partial pressure will result in excursions into more frequent and more intense high-acidity conditions. Experimental conditions should therefore recognize the anticipated extremes, and not simply the mean acidity increases expected in the open ocean.

The process of calcification has the interesting result of *increasing* the seawater concentration of CO_2(aq), which can lead to supersaturation and out-gassing of CO_2 back into the atmosphere. Reaction (*5*) shows why: removal of dissolved carbonate ions by precipitation into shells causes bicarbonate ions to react to replace them, which also produces CO_2. This additional CO_2 can then cause additional increases in acidity through reactions *2 – 4*. Hence upwelling polar and sub-polar waters supporting blooms of calcifying plankton such as coccolithophores, foraminifera, pteropods, etc. are most susceptible to acidification effects.

Calcification by pelagic plankton leads to transport of organic and inorganic carbon to the benthos. When these organisms die, the high density of their calcified structures results in rapid sinking, causing benthic sediments above about 5,000 m seawater depths to be rich in $CaCO_3$. This process is often denoted as "the biological pump", and is an important source of organic carbon to the deep benthos. Changes in surface acidity may alter the functioning of this pump, with consequences for carbon transport to the benthos, the buffering capacity of surface seawater, and the ocean's capacity for CO_2 absorption from the atmosphere.

Vertical mixing processes in the ocean eventually will neutralize nearly all of the acidity introduced by absorption of atmospheric CO_2, no matter how high atmospheric concentrations rise. The acid buffering capacity of the whole ocean, augmented by calcareous seafloor sediments that dissolve and thereby neutralize the addition of acid, is much greater than the acidity induced by anthropogenic CO_2 additions. However, these vertical mixing processes are slow, requiring several thousand years. Over the course of the next few centuries, the acidity of the surface layer will rise substantially before falling in response to this long-term neutralization process. The acidification of the oceanic surface layer results from the rapid rise of atmospheric CO_2 and is at least 100 times faster than anytime during the last 700,000 years, and probably much longer. Although atmospheric CO_2 levels have been up to twenty-fold higher in the distant geologic past, the rate of change has been much slower, allowing time for the full buffering capacity of the ocean to neutralize the introduced acidity without causing much change in ocean pH, even in the surface layer. As a result, marine biota have no evolutionary experience with rapidly changing ocean acidity such as will occur during the next century.

Surprisingly, the change in the $CaCO_3$ dissolution rate with seawater depth is not gradual, but undergoes a sharp transition at depths of a few thousand meters. This transition depth is denoted as the *lysocline*, and is the depth at which the downward flux of precipitating $CaCO_3$ is balanced by the dissolution rate. $CaCO_3$ is absent from benthic sediments below the lysocline, but is abundant above it. The depth of the lysocline is ~4,000 m in the North Pacific Ocean, and is somewhat deeper in the Atlantic.

The concept of *alkalinity* is an important aspect of how seawater responds to acidification. This term refers to the capacity of seawater to neutralize acidity. The carbonate buffer system, summarized by reactions *3* and *4*, accounts for 97.5% of seawater alkalinity. This *carbonate alkalinity* is defined as $[HCO_3^-] + 2\cdot[CO_3^{2-}]$, or the sum of the negative charges on all the carbonate and bicarbonate species. Note that while

addition of CO_2 to seawater increases acidity, it has no effect on alkalinity, because every positively charged hydrogen ion that results is balanced by a negatively charged counterion (either bicarbonate or carbonate). However, precipitation of $CaCO_3$ to form shells directly lowers alkalinity because the negatively charged carbonate is removed but the positively charged hydrogen ions remain.

Increasing the acidity of seawater, for example through the addition of hydrochloric acid (HCl), has different effects on the components of the carbonate buffer system compared to increases atmospheric CO_2. This is because addition of HCl lowers alkalinity directly, but CO_2 does not. So, for the same change in acidity, addition of HCl causes a greater decrease in the $[CO_3^{2-}]$ than addition of CO_2. This is because addition of CO_2 also increases the bicarbonate ion concentration (through reaction (*3*)), which partially counteracts the effects of the associated acidity increase on the $[CO_3^{2-}]$ in reaction (*4*). Hence, experimental manipulation of acidity with mineral acids causes more dramatic changes in $[CO_3^{2-}]$ than does manipulation with CO_2.

Complete chemical characterization of the carbonate buffer system in seawater requires measurement of any two of four chemical parameters: pH, CO_2 partial pressure, total alkalinity, and total dissolved inorganic carbon. The last three are denoted as "carbon parameters", because they are related to the amount of inorganic carbon in the system. The pH can be measured accurately to within about 0.002 pH units, but must be done soon after collection because pH is not stable during sample storage. In contrast, the carbon parameters are stable following sample preservation with mercuric chloride, and properly prepared samples are not hazardous materials and hence can be readily shipped to an analysis lab. The carbon parameters can also be measured very accurately, although the methods for attaining high accuracy are rather involved. Measurement of pH and two carbon parameters has the advantage of providing a built-in quality control check, because the pH can also be calculated from the two carbon parameters.

Appendix 2A

Table of proposed budget ($) and timeline for AFSC ocean acidification research

Research area	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Shellfish						
Growth and survival of Alaskan crabs	247,000	245,000	194,000	-	-	686,000
Crustacean health	-	-	20,000	20,000	90,000	130,000
Calcareous plankton						
Survival of calcareous plankton exposed to reduced pH levels in the field	60,000	60,000	60,000	60,000	60,000	300,000
Coral						
$CaCO_3$ mineralogy of Alaskan corals	10,000	20,000	-	-	-	30,000
Risk assessment of Alaskan corals to the effects of ocean acidification	-	20,000	-	-	-	20,000
Feeding ecology of Alaskan deep-sea corals	-	-	-	35,000	35,000	70,000
Growth and survival of Alaskan corals exposed to reduced pH levels in the laboratory	-	-	-	181,000	182,000	363,000
Growth and recruitment of shallow-water scleractinian corals in Alaska	75,000	-	-	-	-	75,000
Growth and calcification rates of red tree corals in situ using an ocean pH observatory	-	-	-	55,000	55,000	110,000
$CaCO_3$						
$CaCO_3$ survey	247,000	244,000	195,000	181,000	182,000	1,049,000
Marine fish						
Direct ocean acidification effects on growth and survival of Pacific cod	61,000	-	-	-	-	61,000
Direct ocean acidification effects on growth and survival of walleye pollock	-	-	48,000	-	-	48,000
Ocean acidification induced prey community changes on growth of Pacific cod	-	111,000	111,000	-	-	222,000
Ocean acidification induced prey community changes on growth of walleye pollock	-	-	-	96,000	96,000	192,000
Forecast impacts						
Forecast population, ecosystem and economic impacts	-	-	72,000	72,000	-	144,000
Total	700,000	700,000	700,000	700,000	700,000	3,500,000

Appendix 2B

Figure of proposed budget (total dollars for years 1 to 5) for AFSC ocean acidification research.