NOAA Alaska Fisheries Science Center Ocean Acidification Research: FY 2010 Progress Report and FY 2011 Plan

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Summary

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

Lacking basic knowledge of the species-specific physiological response to ocean acidification for most Alaskan marine species, a broad research effort is directed toward several taxa including shellfish, calcareous plankton, coldwater corals, and fish and prioritization of taxa more likely to be affected by ocean acidification (Sigler et al. 2008). The assessment focuses on commercially and ecologically important Alaskan species most likely to be directly affected by ocean acidification, especially larval and juvenile stages. Commercially important calcareous species (shellfish) are first priority because of their economic value 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 effects on fish and marine mammals are indirect effects through predator-prey relationships. Direct effects on fish and marine mammals are less likely because their calcareous tissues are internal. Third priority is coldwater corals whose ecological importance includes sheltering marine organisms (e.g. rockfish). Fourth priority is commercially important fish species; some research will screen for early life history effects. We will add a focus on marine mammals if we find effects on prey of planktivorous (baleen whales) or piscivorous (pinnipeds, toothed whales, porpoise, and dolphin) species.

Focal species groups

- Shellfish
- Calcareous plankton
- Coldwater corals
- Fish

In FY 2010, studies were conducted on king crab, euphausiids, coldwater corals, pollock and cod (Table 1). Some preliminary results are available for king crab, coldwater corals and pollock (Table 2). No results are available for euphausiids because no gravid females were captured for the planned experiments. In addition, ocean acidification laboratories were established in Newport, Juneau, and Kodiak, and an ocean acidification chemistry laboratory was established in Juneau. Multiple laboratories were established in order to match expertise resident at each

laboratory (e.g., the ability to culture pollock larvae at the Newport laboratory). Finally, a NMFS-wide ocean acidification meeting was held August 30-September 1, 2010 in Seattle.

Table 1. FY 2010 research projects. The status of each project is reported in the next section.

Principal Investigators	**Project Title**
Foy	Alaska king crab growth and survival
Eisner, Carls	Survival of calcareous plankton
Stone	Calcium carbonate mineralogy of Alaskan corals
Carls, Rice	Calcium carbonate measurements
Hurst	Growth and survival of Pacific cod and Walleye pollock
Dalton	Alaska king crab abundance forecasts
Foy	Travel, planning workshop
Carls	Travel, planning workshop
Hurst	Travel, planning workshop
Dalton	Travel, modeling workshop

Table 2. Preliminary results for effects of reduced pH for FY 2010 projects. The status of each project is reported in the next section.

Species	**Increase**	**Decrease**	**No change**
Red king crab larvae	mortality	Morphometrics (carapace length, carapace height, telson spine length, total length, rostrum length)	
Golden king crab adult	mortality		
Age-1 pollock	blood CO_2, bicarbonate, cortisol	none	growth, condition
Pollock eggs	highest size-at-hatch at intermediate pH (7.9, 7.6)	none	time to hatch

Coral species	**Skeleton composition**
15 gorgonian corals	All contained high levels of magnesium calcite (the most soluble form), some of which were polymorphous
19 hydrocorals	All polymorphous, some contained high levels of magnesium calcite
6 scleractinian corals	All aragonite, some polymorphous
4 pennatulaceans	All high magnesium calcite
1 sponge	High magnesium calcite

In FY 2011, the king crab, pollock and coral research will continue. The king crab research was expanded to include a genomics approach which has the potential to better understand how ocean acidification affects king crab. The euphausiid work was discontinued because the PIs so far have been unsuccessful at obtaining sufficient gravid individuals but this research may resume if future collections are successful.

Table 3. FY 2011 funded research proposals. Each project is described in the Research Plans section.

Principal Investigators	**Abbreviated titles**
Foy	Alaska king crab growth and survival
Foy	Alaska king crab genomics
Carls, Rice	Calcium carbonate measurements, king crab
Dalton	Alaska king crab abundance forecasts
Hurst	Growth and survival of larval pollock
Stone	Calcium carbonate mineralogy of Alaskan corals

NMFS FY 2010 Ocean Acidification Research Progress Reports

Report 1: Alaska King Crab Growth and Survival

Principal Investigator(s): Robert Foy (AFSC), Chris Long (AFSC), Kathy Swiney (AFSC)

Goals: In 2010, the objectives were to develop infrastructure to support ocean acidification research and to conduct initial research on the effects of increased CO_2 on larval red king crab. An additional objective was to begin studies of increased CO_2 on adult golden king mortality and $CaCO_3$ content in the shell.

Approach: The Kodiak Laboratory infrastructure was developed to assess effects of ocean acidification included hiring laboratory technicians, purchase of water chemistry analytical equipment, and design of a CO_2 delivery and multiple treatment system. An Agilent spectrophotometer was purchased to quantify pH with increased precision over existing probes. A Metrohm titrator was purchased to assess alkalinity in the experimental system. A CO_2 delivery and multiple treatment system was designed and constructed to deliver 3 pH levels for experimentation on larval or juvenile stages of crab.

To assess CO_2 effects on larval red king crab condition and mortality, larvae were entered into treatments once they hatched. Five replicates were established at each of the pH treatments. Mortality was assessed daily. For the larval condition experiment, zoea were preserved for baseline condition (morphology, dry mass, calcification, and CHN content). Three hundred zoea were reared in two pH treatments (pH 8.1 and 7.7). Three zoea from each beaker were imaged under a microscope and their morphology (CW, lateral spine length and dorsal spine length) determined using image analysis software. A sub-sample of 20 individuals from each beaker were dried and massed. The remaining zoea were dried and samples taken for calcification (25 mg wet weight) and CHN analysis.

Eight female golden king crab were randomly assigned to one of two treatments 1) ambient, pH ~8.1 (control, pCO_2 ~ 375 ppm), 2) pH 7.7 (pCO_2 ~ 840 ppm), for a total of 4 females per treatment.

Work Completed/Significant Accomplishments/Problems: Data for shell $CaCO_3$ of larval red king crab are pending at the Auke Bay Laboratories and CHN analysis will be contracted in 2011. The average daily standard deviation in pH was 0.012 for the treatments and 0.009 for the control. Mortality of larvae in the pH 7.7 treatment was significantly higher than in the pH 8.1 control. In addition, an ordination of morphometric measurements (carapace length, carapace height, telson spine length, total length, and rostrum length) found a significant difference between the treatment and control pH.

The female golden king crab controls all successfully molted. Two of the treatment crab died during the experiment while 1 did not molt and eventually was sacrificed.

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

Long, W.C., K.M. Swiney, and R.J. Foy. In review. Effects of ocean acidification on late embryogenesis, and larvae condition and survival in red king crab. Marine Biology.

Harris, C. 2010. The effect of ocean acidification on juvenile red king crab growth, survival, and calcification. Undergraduate Senior Thesis. Alaska Pacific University. Anchorage, Alaska.

Page, H., R. J. Foy, W.C. Long, K.M. Swiney. 2010. Effect of ocean acidification on juvenile Tanner crab, *Chionoecetes baridi*, survival and growth. Ernest F. Hollings Undergraduate Scholar 2010 Symposium, August 2010. Washington, DC.

Blakeslee, M., and W.C. Long. 2011. Design and operation of a flow-through seawater acidification system for Ocean Acidification research at the NMFS Kodiak Laboratory. Alaska Marine Science Symposium, January 2011. Anchorage, Alaska.

Foy, R.J. 2010. Ocean Acidification: What is it and how important is it for crab species around the Kodiak Archipelago. Alutiiq Museum Lecture Series. October, 2010. Kodiak, Alaska.

Foy, R.J. 2010. Effects of ocean acidification on crab stocks in Alaska. Alaska Marine Conservation Council Lecture Series. October, 2010. Kodiak, Alaska.

Report 2: Alaska King Crab Abundance Forecasts

Principal Investigator(s): Mike Dalton (AFSC) and André Punt (UW)

Goals: Provide socioeconomic forecasts using bioeconomic models for Alaskan king crab that incorporate results of species-specific physiological studies.

Approach: Refined existing king crab stock assessment models. Incorporate coupled economic component.

Work Completed/Significant Accomplishments/Problems: A graduate student research assistant under the supervision of Drs. Punt and Dalton acquired the Bristol Bay Red King Crab (BBRKC) stock projection model, studied it and the fishery, and reviewed literature on ocean acidification and crab biology. She is close to finalizing her master's thesis proposal on this topic for the UW School of Aquatic and Fishery Sciences. Dr. Punt recoded the BBRKC stock assessment model, and is currently developing a simpler version of that model, with fewer size classes, for use in this project. In closely related work, Dr. Punt developed a simplified (5-size class) version of the Eastern Bering Sea Snow Crab model and Dr. Dalton developed a size-structured bioeconomic model which was fully coupled to the new simplified snow crab population dynamics model.

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

Report 3: Calcium Carbonate Measurements

Principal Investigator(s): Mark Carls, Lawra Vanderhoof, Lawrence Schaufler

Goals: The purpose of this project was to provide detailed analytical and empirical laboratory support for a variety of research needs across all AFSC laboratories. The principal goals were to 1) operate and maintain the analytical capacity for detailed carbon chemistry measurements to add precision, accuracy, and quality control to routine approximate experimental measures and 2) operate, maintain, and improve a carbon dioxide dosing system for experimental research in collaboration with other investigators.

Approach: During FY10, a full suite of carbon chemistry measurements became fully operational and were applied to local laboratory experiments, local field-based time series data, and samples from the Kodiak Laboratory. Observations include total dissolved inorganic carbon (TC) by Coulometry, colorimetric pH measurement in 10 cm cells, total alkalinity (TA) by open cell titration, and salinity measurement with a precision salinometer. The "guide to best practices ocean CO_2 measurement" (Dickson et al., 2007) was used as the starting point for all standard operating procedures (SOPs). Certified Reference Materials (CRMs) (purchased from Dickson's group) were routinely analyzed with other sets of data (typically referred to as "strings") for quality control and assurance. In addition, an ion chromatograph was operated for analysis of calcium, magnesium, sodium, and other metal ions to analyze crab carapace samples from Kodiak.

In addition to adapting best practices SOPs to the specific instruments in our laboratory, data logging, recording, and storage procedures were developed to assure thorough, long-term sample documentation. These procedures include chain of custody sheets to document original sample collection and transfer to the laboratory; instrument-specific data sheets that include sample analytical information together with quality control samples, CRMs, and standards; spreadsheets designed to record, manipulate, and output data from each instrument; and a database designed to collate and store all data for each sample.

Work Completed/Significant Accomplishments/Problems: Measurements obtained in our laboratory are consistent with CRM values. The pH measurements were 0.05% greater than the CRM value, and total alkalinity was 0.01% less (as of August 4, 2010). Coulometric estimates of total dissolved inorganic carbon were consistently 1.9% less than the CRM value. After extensive experimentation to determine the reason for this, we are quite certain that carbon is lost during an atmospheric removal step required by the glassware as configured by the manufacturer. We will run further tests to determine if this is true and continue testing response against other CRMs to decide if a 1.9% adjustment is warranted for existent data and if modified glassware will provide better agreement with CRM values.

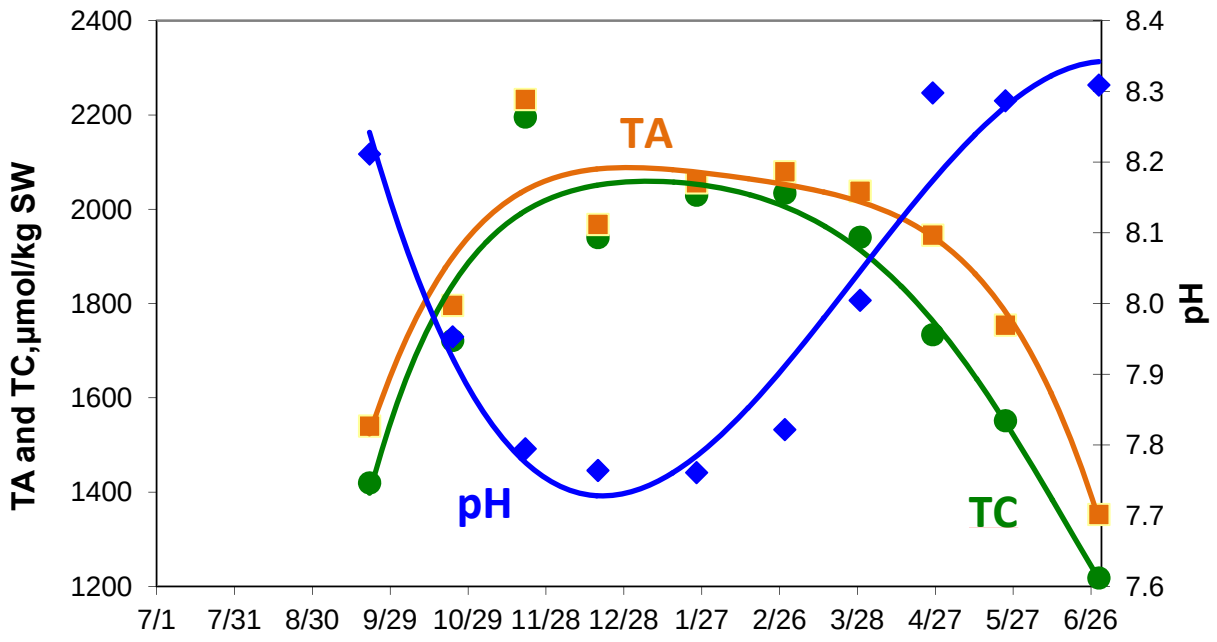
Water chemistry for the first Kodiak experiment (June, 2010) has been analyzed and processing for carapace chemistry has started. July samples are pending. Numerous samples have been analyzed from our laboratory in support of dosing system development, particularly in support of

apparatus sizing, controllability, and reliability. These tests will lead to an improved, second-generation dosing system.

To understand the context of local fauna, a long-term field time series study was initiated. These data will provide valuable insight into experimental design and interpretation and demonstrate clear seasonal trends (Fig. 1). With time, evidence of long-term change may emerge but this may require many years of observation to tease out from the substantial seasonal changes evident in early data. Assuming the first year of data are representative, pH levels decline to a minimum in winter (roughly 7.7) and peak in summer (roughly 8.3). This pattern roughly follows photoperiod and thermal cycles. Seasonal changes in TC and TA were opposite, with maxima in winter and minima in summer. This pattern roughly follows seasonal change in salinity. The amplitude of change at depth was smaller for all three parameters.

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

Fig. 1. Seasonal trends in pH, total alkalinity (TA) and total dissolved inorganic carbon (TC) in southeastern Alaska marine waters near Juneau, Alaska (2009 to 2010).



Report 4: Survival of Calcareous Zooplankton

Principal Investigator(s): Mark Carls (AFSC), Lisa Eisner (AFSC), Jeff Napp (AFSC), Fletcher Sewall (AFSC), Marissa Capito (AFSC), Alexei Pinchuk (UAF).

Goals: The purpose of this project was to determine if zooplankton vital rates and survival are influenced by reduced pH. Two general hypotheses are under consideration, 1) increasing difficulty in deposition of calcium carbonate into chitonous exoskeletons because of decreasing calcium carbonate saturation levels, and 2) increased physiological demand to compensate for increased intracellular hydrogen ion influx. Two groups were targeted, euphausiids (*Thysanoessa raschii*) and copepods (*Pseudocalanus* spp).

Approach: Equipment was designed and set up for euphausiid experiments, including reduced pH exposures of gravid females and early life stages in closed containers, and for sub-adult/adult exposures in flow-through containers. A manually operated experimental flow-through treatment system was monitored for reliability in producing desired pH levels. Four reduced pH treatments plus controls were generally regulated within an absolute range of ± 0.04 pH unit.

Work Completed/Significant Accomplishments/Problems: Field collections in late spring and early summer focused on obtaining gravid females. Capture of gravid euphausiids was unsuccessful despite multiple attempts with various gear types and search strategies at varying locations, depths, and time of day/night, apparently because peak reproductive activity was earlier than predicted. Subsequent attempts to capture sub-adults yielded primarily larval individuals which were not robust to handling. Previous experience suggests better success with fall/winter/early spring collections of sub-adults and the next collection efforts will be targeted accordingly. Earlier and more extensive sampling will also be completed early next spring to capture gravid females.

Intended measurement metrics euphausiids vary by life stage. Metrics for reproductive euphausiids and early life stages will include hatching success, molting and progression from nauplii to calytopsis or furcilia stages, respiration, and mortality. Metrics for subadult or adult euphausiids will include growth, reproduction, molting, respiration, mortality, lipid content, metals (notably calcium) in chiton, and RNA/DNA ratios.

Our initial efforts at copepod capture, handling and rearing demonstrated that copepods (e.g., *Pseudocalanus* spp.) are readily available locally without boat use, but present significant challenges for study. *Pseudocalanus* spp. produce several (~10) clutches of eggs during summer, and current effort is focused on discovering the precise timing of their reproductive phase, and best methods for obtaining and feeding adult females. The immediate goal is to acquire sufficient quantities of reproductive females that the effects of reduced pH on reproductive output can be assessed. Other metrics will include hatching success, molting and progression to N2 and N3 stages, respiration, and mortality.

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

Report 5: Growth and Survival of Pacific Cod and Walleye Pollock

Principal Investigator(s): Thomas Hurst (AFSC) and Jeremy Mathis (UAF)

Goals: Determine the direct effects of ocean acidification on growth and survival of early life stages of walleye pollock and Pacific cod

Approach: In FY2010, two experiments exposing walleye pollock to forecasted levels of ocean acidification were completed. In the first, age-1 walleye pollock were reared under 4 pH levels for 6 weeks with unrestricted feeding to determine direct effects on growth potential and condition factor. In addition, cortisol levels were measured in the blood and in the kidney. Analysis of blood chemistry examined responses in the acid-base regulation system of the fish.

In a second experiment, walleye pollock eggs (produced from laboratory broodstock) were incubated at 4 pH levels to determine the potential effects of ocean acidification on hatching success, time to hatch, size at hatch, and energy reserves at hatch.

Work Completed/Significant Accomplishments/Problems: For age-1 walleye pollock, the results indicated that elevated environmental CO_2 increased levels of blood CO_2. The fish responded to this shift with an increase in blood bicarbonate. Cortisol levels exhibited a trend toward higher values in the lower pH treatments. Under ad libitum feeding, there was no effect of rearing pH on somatic growth or condition factor.

For walleye pollock eggs, preliminary analysis of the data indicates that time to hatch was unaffected by pH, but highest sizes at hatch were observed in the intermediate pH treatments (7.9 and 7.6).

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

A poster was presented twice:

E. R. FERNANDEZ, J. T. MATHIS, T. P. HURST. The response of juvenile walleye pollock (*Theragra chalcogramma*) to projected increases in ocean acidification. Alaska Chapter Meeting of the American Fishery Society

E. R. FERNANDEZ, J. T. MATHIS, T. P. HURST. The response of juvenile walleye pollock (*Theragra chalcogramma*) to projected increases in ocean acidification. Alaska Marine Science Symposium.

Report 6: Calcium Carbonate Mineralogy of Alaskan Corals

Principal Investigator(s): Robert Stone (NOAA, AFSC), Anne Cohen (Woods Hole Oceanographic Institution), John Guinotte (Marine Conservation Biology Institute), Stephen Cairns (Smithsonian Institution), Les Watling (University of Hawaii)

Goals: Determine the skeletal composition of select corals from each major taxonomic group and species of particular ecological importance (i.e. those that form single species assemblages). The mineralogy data will form the basis for a risk assessment to be completed in FY12. The mineralogy data will be used in conjunction with species distribution data (depth and geographical) and the present and projected aragonite and calcite saturation horizons in Alaska to predict the effects of ocean acidification on coral resources of the North Pacific Ocean

Approach: The diverse collections of archived specimens at the Auke Bay Laboratories and Smithsonian Institution were carefully sorted through and specimens were selected to cover major taxonomic groups, species of particular ecological importance (i.e. those that form single species assemblages), and specimens of the same species from multiple depth and geographic zones. The skeletal composition of seventy specimens total (44 species of corals and one species of sponge) were determined with x-ray diffraction at the Woods Hole Oceanographic Institution.

Work Completed/Significant Accomplishments/Problems: Fifteen taxa of gorgonians were analyzed for skeletal mineralogy. All taxa contained high magnesium calcite but seven of the taxa (47%) were polymorphous. Preliminary analyses indicated no trends with depth or geographical location. Nineteen taxa of hydrocorals were analyzed for skeletal mineralogy. Twelve taxa (63%) were polymorphous and eleven taxa (58%) contained some high magnesium calcite, the most soluble form of carbonate. Preliminary analyses indicated no trend with geographical location but possibly some indication that shallow-water species may be using high magnesium calcite to build their skeletons. Six species of scleractinian corals were analyzed for skeletal mineralogy. As expected, all species were composed of aragonite with possible indications that two specimens of a single species (*Caryophyllia alaskensis*) were polymorphous. Preliminary analyses indicated no trends with depth or geographical location. Four species of pennatulaceans were analyzed for skeletal mineralogy and as expected all were composed of high magnesium calcite. One species of calcareous sponge (*Leucandra tuba)* was analyzed and was composed of high magnesium calcite. To our knowledge this was the first time that the mineralogy of any calcareous sponge has been determined.

Publications/Presentations/Webpages: Preliminary results were presented at the NMFS Ocean Acidification Research Meeting Aug 30 - Sep 1, 2010 (NWFSC, Montlake).

Preliminary results were presented at the planning meeting for Deep Sea Coral research in Alaska, FY2012-2014, Anchorage, Alaska, September 14-15, 2010.

Collaboration among the NMFS Science Centers

NMFS Science Centers work together to ensure that NMFS ocean acidification research provides an integrated research program with publishable scientific results. In 2010, a NMFS-organized workshop was held in Seattle WA for NMFS and academic researchers to communicate research results and plans and for NOAA oceanographers to present results from related ocean acidification research. In addition, the three northern NMFS Science Centers (Alaska, Northwest and Northeast) which are responsible for coldwater regions have been collaborating since 2009 due to similarities in ecology, research priorities and approaches. Their research has focused on a range of taxa because the biological effects of ocean acidification are mostly unknown; priority has been placed on species considered most vulnerable ecologically and of economic importance. The approach consists primarily of species-specific laboratory studies and population and ecosystem modeling. Scientists from the 3 science centers teleconference monthly to compare notes on diverse topics such as laboratory setup, experimental challenges and water chemistry. A joint proposal formulated among the three science centers in 2010 continues to guide, coordinate and integrate ocean acidification research that will continue and expand during 2011. All studies by the these three NMFS Science Centers address themes highlighted in the NOAA regional ocean acidification implementation plans that have been developed by the NOAA ocean acidification implementation team (Table 4). In 2011, chemists from these three Science Centers are testing instruments and comparing their results using certified reference materials from the same source. These comparisons give the researchers a standard frame of reference, verify measurement accuracy and assure measurement quality.

Table 4. AFSC deliverables for FY 2011 ocean acidification research. All studies address themes highlighted in the NOAA regional ocean acidification implementation plans that have been developed by the NOAA ocean acidification implementation team. The Northwest and Northeast plans each include a similar table.

Theme	**Deliverable Product**	**Description**
1. Ocean acidification monitoring network	OA monitoring section of research report	Report on proposed oceanographic monitoring to detect effects of ocean acidification
2. Ecosystem impacts of ocean acidification	Experiments on king crab	We will conduct experiments on the effect of CO_2 and temperature on early life-stage development of red king crab and prepare results for publication.
	King crab genomics	Apply microarray technology to measure the effect of CO_2 and temperature on early life-stage development of red king crab.
	Calcium carbonate measurements	Regional Ocean Acidification Center: Water and tissue chemistry analysis of king crab experiments.
	Experiments on pollock	We will conduct experiments on the effect of CO_2 and temperature on development of larval walleye pollock and prepare results for publication.
	Mineralogy of Alaskan corals	We will determine the calcium carbonate mineralogy of several Alaskan corals and prepare results for publication.
3. Biogeochemical and ecosystem models	OA meeting	In the 4th quarter of FY11, AFSC scientists including modelers will participate in a meeting on OA research.
4. Human dimensions	Alaska king crab bioeconomic model	Socioeconomic forecasting using bioeconomic model for Alaskan king crab.
5. Synthesis of data and information products	OA meeting	In the 4th quarter of FY11, AFSC scientists including modelers will participate in a meeting on OA research.
6. Public outreach	Print and display materials	We will produce educational materials (posters, handouts) that can be used by AFSC staff to describe the science of ocean acidification to school groups, public fairs, stakeholders groups and others.

NMFS FY 2011 Ocean Acidification Research Funded Proposals

Proposal 1. Alaska King Crab Growth and Survival

Principal Investigators: Robert Foy (AFSC), Chris Long (AFSC), Kathy Swiney (AFSC)

Project Description: In FY11, the goal is to examine the effects of increased CO_2 on aspects of the reproduction and larval development of red king crab. Our experiments divide into three general groups: 1) effects of OA on larval condition and survival; 2) effects of OA on calcification in both the adult females and the larvae; 3) effects of OA on juvenile condition and survival

Increased CO_2 is likely to impose an energetic cost on developing larvae and juvenile that would lead to a decrease in mass and changes in their chemical composition. It is therefore hypothesized that increased CO_2 will have the following effects on red king crab larvae and juveniles:

H1: Decrease survival
H2: Alter the morphology of larvae
H3: Decrease mass
H4: Decrease carbon (lipid) and nitrogen (protein) content

Twenty adult ovigerous females will be collected in the Bering Sea and returned to the Kodiak Laboratory, where they will be held in individual 68 L containers with flow-through sand-filtered seawater at a rate of 1 L/min, so that the tank water will be exchanged ~ 1/hr. Crabs will be reared in temperatures that do not exceed 6ºC and water will be chilled when the ambient temperature nears 6ºC. Throughout the experiment, females will be fed squid and fish twice a week to excess. Females will be ovigerous at time of capture, and will be allowed to hatch their larvae in the laboratory. Zoea will be collected and will be used to examine the effects of increased CO_2 on larvae. Separate larvae from 2010 were grown up to the juvenile stage for subsequent experimentation on older life stages.

Two experiments will be performed on each set of larvae: a survival experiment and a condition experiment. Larvae hatched from multiple healthy ovigerous females will be pooled together and used for the first set of experiments. Five replicate containers will be established at each of three pH treatments (pH 8.1, 7.8, 7.5), for a total n of 15, for each experiment. Results will be analyzed with a one-way ANOVA with pH treatment as the factor. When multiple measurements of the same variable are made in each container, “Container” will be included as a nested factor. An additional experiment will be conducted where zoea are hatched from multiple females held within each of the pH treatments above (pH 8.1, 7.8, 7.5). Five replicate beakers will be established at each of the three pH treatments (pH 8.1, 7.8, 7.5) from zoea hatched from each of the pH treatments above, for a total n of 45 for each experiment (3 maternal treatments x 3 zoea treatments x 5 replicates). Results will be analyzed with a fully-crossed two-way ANOVA, with “Maternal” treatment and “Zoea” treatment as factors.

To assess larval survival, twenty zoea will be placed in a 1L beaker randomly assigned to one of the three pH treatments. The zoea will be checked daily for molts and mortality, and percent survival will be calculated. Survival in each beaker will be fit to a standard survival model using least-squares fitting techniques. To assess larval condition baseline condition (morphology, dry mass, calcification, and CHN content) will be measured from each of the original pools of zoea prior to the start of the experiment. Three hundred zoea will be reared in a 2L beaker assigned to one of the above three pH treatments (as above). The experiment will be run concurrently with the mortality experiment and will be ended on the day mortality exceeds 50% in any beaker in the mortality experiment. Three zoea from each beaker will be imaged under a microscope and their morphology (CW, lateral spine length and dorsal spine length) determined using image analysis software. A sub-sample of 20 individuals from each beaker will be dried and massed and the average zoeal mass determined. The remaining zoea will be dried and samples taken for calcification (25 mg wet weight) and CHN analysis as above. All variables will be analyzed with an ANOVA model as specified above.

To assess juvenile survival, growth, and calcification, 90 juvenile red king crab will be placed in three pH treatments (as for larvae). Periodic checks will be made for mortalities and molts. Weight and carapace measurements (width and length) will be made and image analysis will be conducted on molted shells. $CaCO_3$ analysis will also be conducted at the ABL chemistry laboratory.

For each of the larval and juvenile experiments above crab condition will also be measured using genomic methods of microarray technology to measure crab response to the various CO_2 saturation treatments. Preliminary studies have identified that a microarray protocol for surveying over 26,000 expressed sequence tags for the porcelain crab, *Petrolisthes cinctipes*, can successfully be used for analyzing gene expression patterns in the Alaskan king crab. Samples from each treatment and control will be preserved and sent to San Francisco State University for microarray analysis. Several gene expression products (proteins, mRNA, etc) that can be monitored as indicators of stress level, condition, health, etc. in king crabs will be assessed to determine sublethal effects of ocean acidification on early life history stages of red king crab.

Expected Products: In FY11, publications from FY10 experiments on king crab will be completed and submitted for review. Also in FY11, an experimental system capable of multiple pH treatments combined with 3 temperature treatments will be designed and constructed. An experiment on larval and juvenile red king crab will be conducted in Spring 2011 to assess affects of increased CO_2 on survival, growth, and calcium content. A publication will be completed in 2012. Microarray analysis will also be conducted to detect changes in protein expressed among pH treatments. An initial analysis will be conducted to identify sample sizes required to detect genetic changes as a result of increased CO_2.

Upon completion of the experiments in FY11, results on growth and survival for larvae and juvenile red king crab will be provided by September 2011 to be used to inform a bioeconomic

model of Bristol Bay red king crab based on the impacts of ocean acidification (see AFSC project by Mike Dalton and André Punt).

Proposal 2. Alaska King Crab Genomics

Principal Investigators: Robert Foy and Jonathon Stillman (University of California Berkeley and San Francisco State University; a subcontract will go to Stillman @ UC Berkeley)

Introduction: Prior to 2010 the goals of this project (not funded with NOAA OA funds) were to determine how genomics could be used to measure affects of ocean acidification and other stressors. How organisms respond to environmental change can be assessed using "functional genomics" approaches, which attempt to study how genomes are regulated under specific environmental conditions. Gene regulation, whether a gene is expressed or repressed, is commonly indexed by the level of the corresponding mRNA for that gene in a sample. Gene expression products (mRNA) can be monitored as indicators of stress level, condition, health, and other physiological parameters in crab species to determine sublethal effects of increased CO_2 on early life history stages of red king crab. Studies of all of the mRNA molecules in a sample, the "transcriptome" for that sample, can be made using a number of methods, the most commonly used of which include microarrays (gene chips), and next generation sequencing (NGS) approaches, of which there are several platforms presently available (e.g., 454, Illumina, SOLiD). NGS approaches have great promise for studies of gene expression, but they are expensive and require considerable bioinformatics expertise. Commercially produced microarrays are available for a number of model organisms, but not organisms that are closely related to king crabs. Thus, microarray analyses of red king crab samples would require either construction of a new microarray specific to red king crabs (both time consuming and expensive) or utilization of a microarray that has already been developed for a close relative.

In 2008-2009 larval and adult red king crab samples were sent from the Kodiak Laboratory to test whether gene expression could be assessed using genomics resources (cDNA microarrays) that have been developed for porcelain crabs, genus *Petrolisthes.* The Stillman Laboratory has developed a large cDNA microarray for the porcelain crab *Petrolisthes cinctipes* from one of the largest genomics resources for any crustacean (Stillman et al., 2008, Stillman and Tagmount, 2009, Tagmount et al., 2010). Initial analyses suggested that there was sufficient sequence homology for a large number of genes that the porcelain crab microarray would work for analyses of RKC samples. The porcelain crab microarray is the most comprehensive microarray or "gene chip" for any species of crab (Tagmount *et al.*, 2010). The Stillman Laboratory is presently using this microarray for studies of how porcelain crabs respond to changes in their thermal environment (Stillman and Tagmount, 2009), and have just been funded by the NSF to use the microarray in studies of how larval and adult porcelain crabs respond to ocean acidification (http://www.nsf.gov/news/news_summ.jsp?cntn_id=117823&org=NSF&from=news).

To test the *Petrolisthes cinctipes* microarray on red king crab, four microarrays (Slide 153, 154, 157, and 158) were prepared using red king crab samples to compare how well different reverse transcriptase enzymes worked with the samples, how well the preservation methods worked, and how the hybridization compared to porcelain crab larvae (Table A2-1). Reduced stringency hybridization conditions were used with lower temperature and lower stringency washes to

account for the fact that there would not be 100% sequence identity between red king crab and porcelain crab samples. Cy3 or Cy5 labeled cDNAs were hybridized at 37°C in 50 µl of hybridization buffer (50% deionized formamide, 5XSSC, 0.1% SDS). Two washes were performed in 2 XSSC, 0.1% SDS, 10 min each, two washes in 1 XSSC, 10 min each, and one wash in 0.5 XSSC, 10 min.

The results suggested that the expression of many red king crab transcripts can be detected using the porcelain crab microarray. Raw microarray images indicate a range of hybridization, from samples where only background levels of fluorescence are visible, to spots that are bright in color (Figure A2-1). The fact that all the spots are yellow indicates equal abundance of the Cy3 and Cy5 samples binding to the features) and suggests that the two reverse transcriptase enzymes gave similar quality cDNAs. Examination of all of the features on the array shows approximately equal sample abundance for each transcript in the Cy3 and Cy5 samples across the entire array (Figure A2-2). There is a lot of noise and scatter at fluorescence intensities below about 2000, which is generally considered to be at the threshold for high quality microarray data, so these samples are behaving as expected. Washing with a higher stringency buffer didn't affect the results too much except for features that may have been more "noise" than "signal" as seen by comparison of Slide 153 washed in 1X SSC (low stringency, Figure A2-2) and 0.5X SSC (higher stringency, Figure A2-3). Both RNA preservation methods worked well (Figure A2-4) and both high yield and high quality RNA were obtained from the larval samples. The initial results also suggest that there may be a set of genes commonly expressed across all crab larvae (Figure A2-6). The separation of features in *P. cinctipes* and red king crab larvae hybridized to the same array may be an indication of differences in hybridization between the two species (Figure A2-6). In addition, verification will be necessary to fully characterize the manner in which red king crab transcripts bind to the array.

In general, the initial results from Stillman's Laboratory suggest that we will be able to analyze a wide diversity of transcripts in red king crab. Based on this preliminary analysis of larval and adult red king crab, we believe that the porcelain crab microarray will be able to be used to analyze gene expression patterns in the red king crab.

Project Description: For each of the larval and juvenile experiments outlined in the Foy, Long, and Swiney proposal, crab condition will also be measured using genomic methods of microarray technology to measure crab response to the various CO_2 saturation treatments. Larvae will be hatched from mothers acclimated to three pH conditions. Larvae and juvenile crabs will be raised in three pH conditions. For each experiment we will use 5 biological replicates. The total number of samples to be analyzed by microarray analyses is 60, n=45 in the larval condition experiment (3 mother pH X 3 larval pH X 5 replicates), and n=15 for the juvenile experiment (3 juvenile pH X 5 replicates). For each of the 60 samples we will perform at least two technical replicate hybridizations, for a total of 120 hybridizations.

Microarrays are glass microscope slides that have been specially coated to stick to DNA. In "spotted arrays" such as we will use here, tens of thousands of spots of DNA are printed on the glass slides using a specialized robotic printer (the arrayer) that has very high precision pins that

pick up and deposit minute quantities of DNA molecules. Those DNA molecules come from an arrayed library of PCR products or oligonucleotides, where each molecule in the library represents one from a collection of DNA sequences from a gene that the organism expresses. The porcelain crab array is produced from PCR products of cloned cDNA molecules that were subsampled from a library of nearly 62K clones, and that represent about 30K unique sequences representing up to 19K genes. The microarrays are printed at the University of California San Francisco (UCSF) Center for Advanced Technology (CAT). In preparation for use, each slide is "post-processed" to block any parts of the array that have not been bound to DNA, and any unbound DNA from the printed spots is washed away.

Samples to be hybridized to the arrays are prepared from total RNA extracted from tissue samples. Briefly, the RNA is reverse transcribed into cDNA while incorporating fluorescent dye tags, Cy3 or Cy5. The process of readying the samples for analysis involves many steps and reagents as outlined in Stillman and Tagmount (2009). Two samples, one labeled with each dye, are hybridized to each array. Following hybridization, unbound cDNA molecules are washed away and the slides are scanned in an Axon 4000B microarray scanner. Spots that fluoresce represent sequences of cDNA molecules present in the samples. The fluorescence intensity is an indication of how much of a particular cDNA molecule was present in each sample.

Following microarray analysis of red king crab samples on the Porcelain crab microarray, we will need to verify the results using NGS and quantitative PCR. The reason this verification is necessary is that the microarray method is a hybridization-based, not sequencing-based approach, so it is possible (although unlikely) that hybridization could occur in non-homologous sequences (i.e., sequences from different genes). We will perform one Illumina NGS analyses for larvae and juveniles pooled from each experimental condition at the UC Davis genomics core facility (http://dnatech.genomecenter.ucdavis.edu/uhtsequencing.html). We propose to use a paired end 80bp run for each sample, which should yield adequate data to characterize the transcriptome of red king crab and compare sequences in porcelain crabs and red king crab. Using that sequence data we will design qPCR primers and TaqMan Probes validating differential expression of transcripts across all the samples used in microarray studies.

Expected Products: In FY11, verification results will be provided to fully characterize the manner in which red king crab transcripts bind to the array. A characterization of the transcriptome of red king crab will be provided and compared to sequences in porcelain crabs. Lastly, a publication on crab condition paired with data on mortality and growth as a result of CO_2 treatments will be prepared for likely publication in 2012.

References:

Stillman, J. H., J. K. Colbourne, C. E. Lee, N. H. Patel, M. R. Philips, D. W. Towle, B. D. Eads, G. W. Gelembuik, R. L. Henry, E. A. Johnson, M. E. Pfrender, and N. B. Terwilliger. 2008. Recent advances in crustacean genomics. Integrative and Comparative Biology 48:852-868.

Stillman, J. H., and A. Tagmount. 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, Petrolisthes cinctipes. Molecular Ecology 18:4206-4226.

Tagmount, A., M. Wang, E. Lindquist, Y. Tanaka, K. S. Teranishi, M. Wong, S. Sunagawa, and J. H. Stillman. 2010. The porcelain crab transcriptome and PCAD, the porcelain crab microarray and sequence database. PLoS ONE 5:e9327.

Table A2-1. Description of microarrays generated to test RKC samples.

Slide Name	Conditions Tested	Cy3 Sample	Cy5 Sample
153	Reverse Transcriptase specificity	RKC larvae (10µg total RNA) superscript II RT	RKC larvae (10µg total RNA) mmlv RT
154	Preservation method (larvae)	Trizol (10µg total RNA)	RNAlater (10µg total RNA)
157	Preservation method and RNA quantity (adult)	RNAlater (7.5 µg total RNA)	Trizol (3µg total RNA)
158	RKC vs. Porcelain Crab	P. cinctipes larvae	RKC larvae

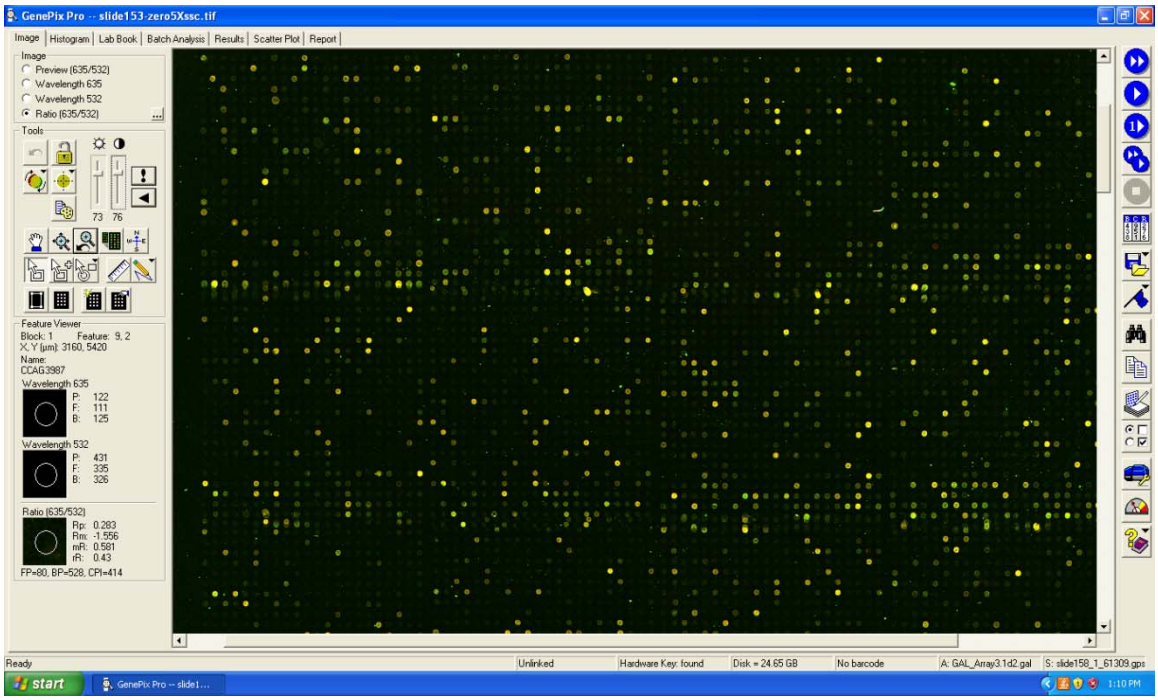


Figure A2-1. Raw microarray image of a portion of slide153.

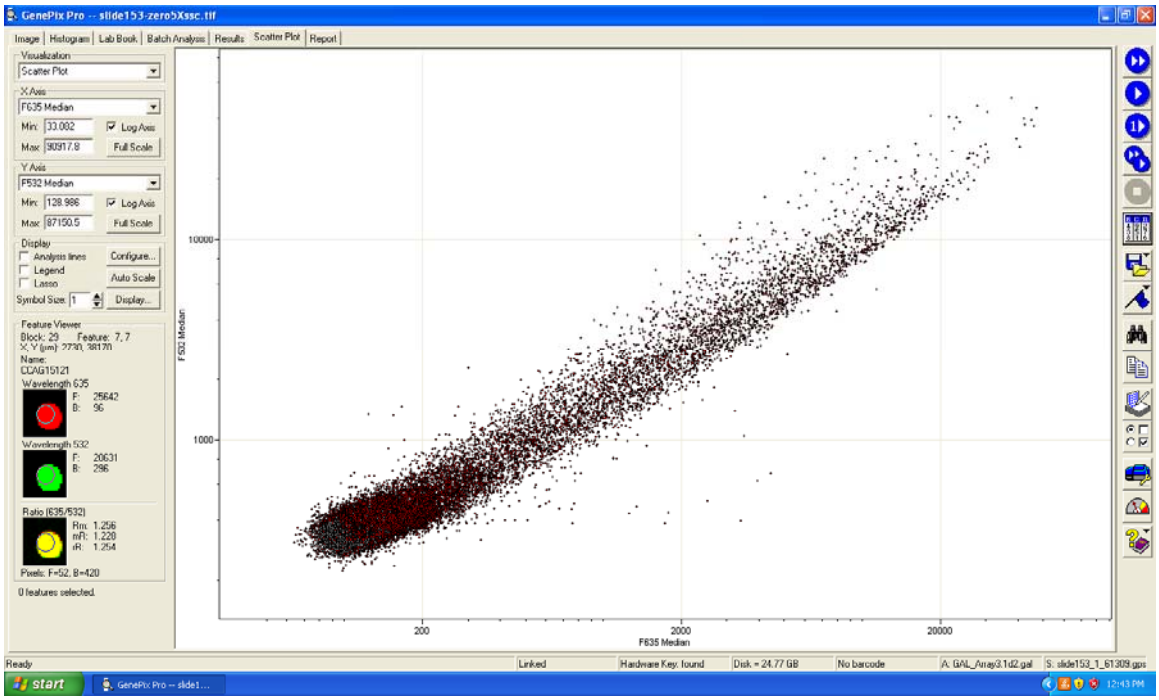


Figure A2-2. Scatterplot of median feature intensities from slide153. Each spot represents one feature on the microarray.

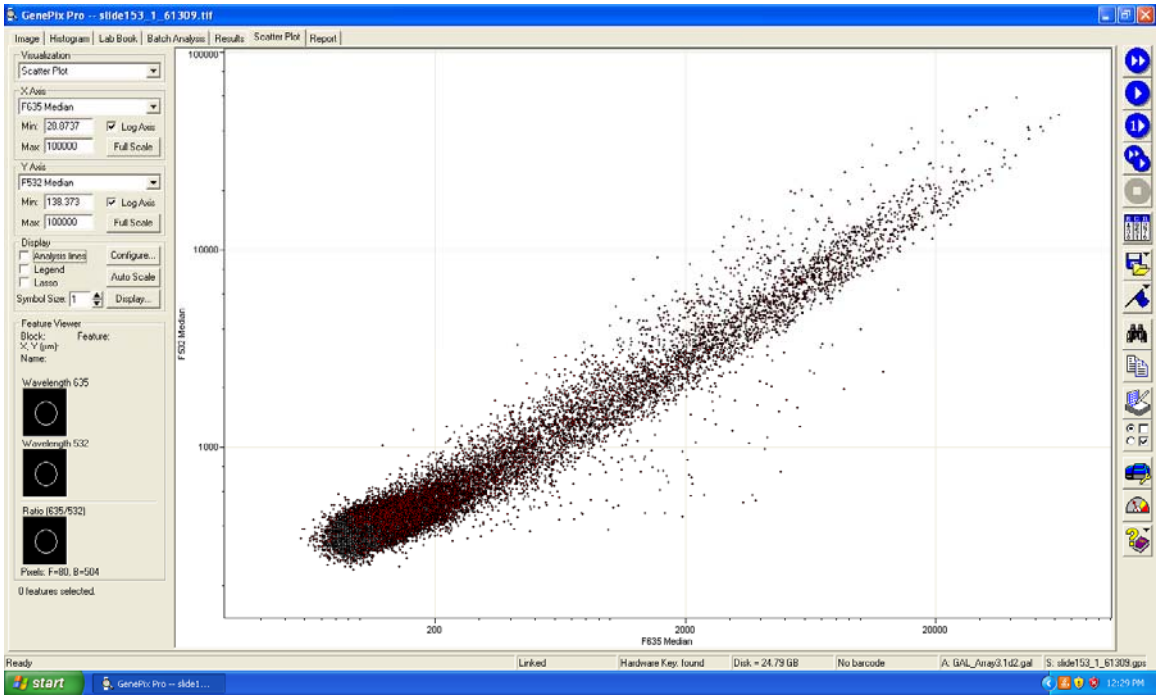


Figure A2-3. Slide 153 washed at a lower stringency than in Figure 2. Some of the low intensity Cy3 (y-axis) samples seem to be present here that are not present in the Cy5 (x-axis) samples in Fig. 2.

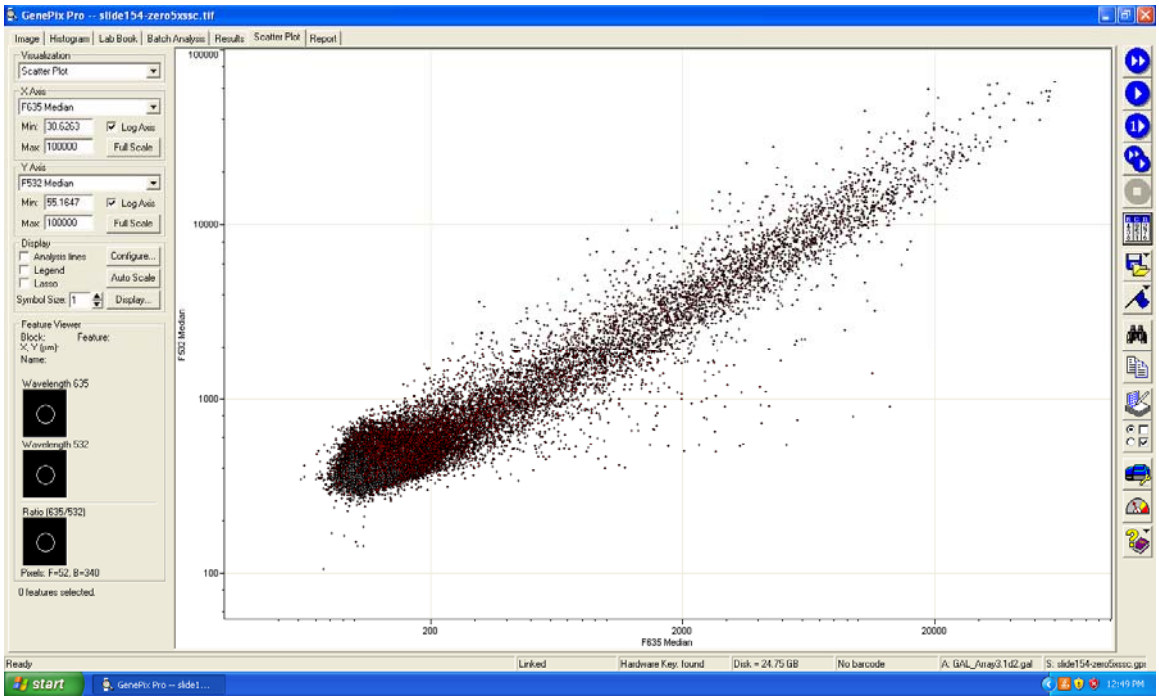


Figure A2-4. Scatterplot of Slide154 showing that both preservation methods work well.

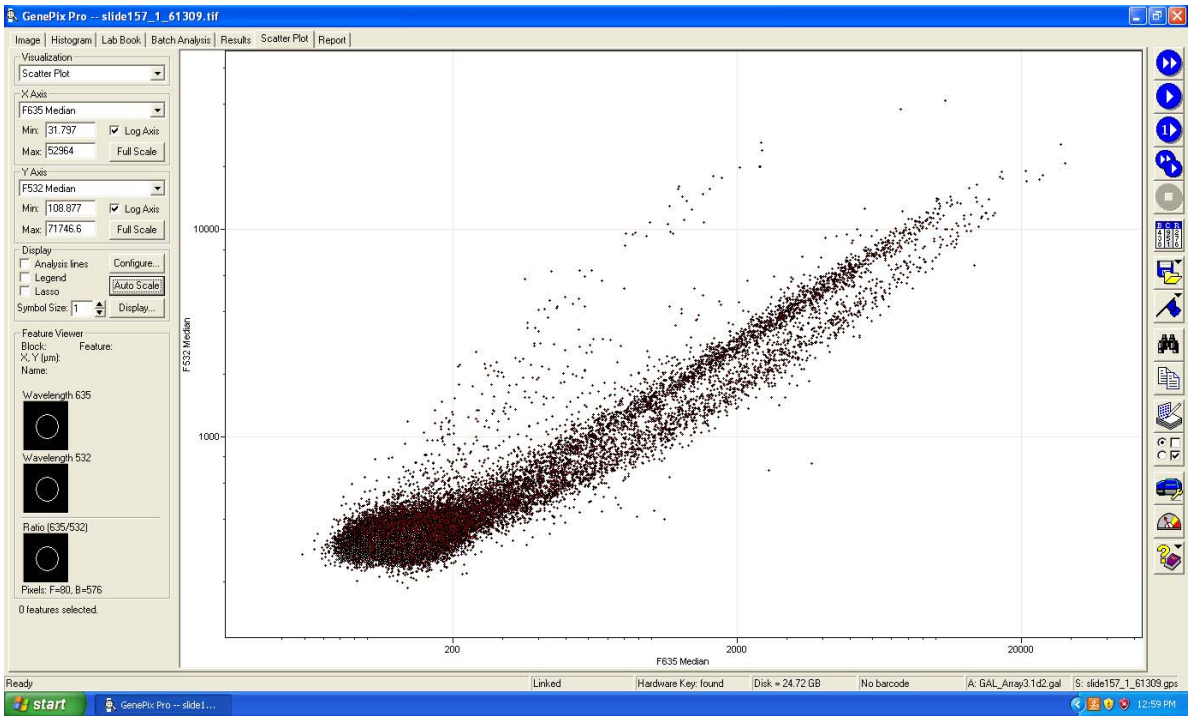


Figure A2-5. Slide 157 analysis of RKC adult tissue preserved with RNA later (y-axis) and Trizol (x-axis).

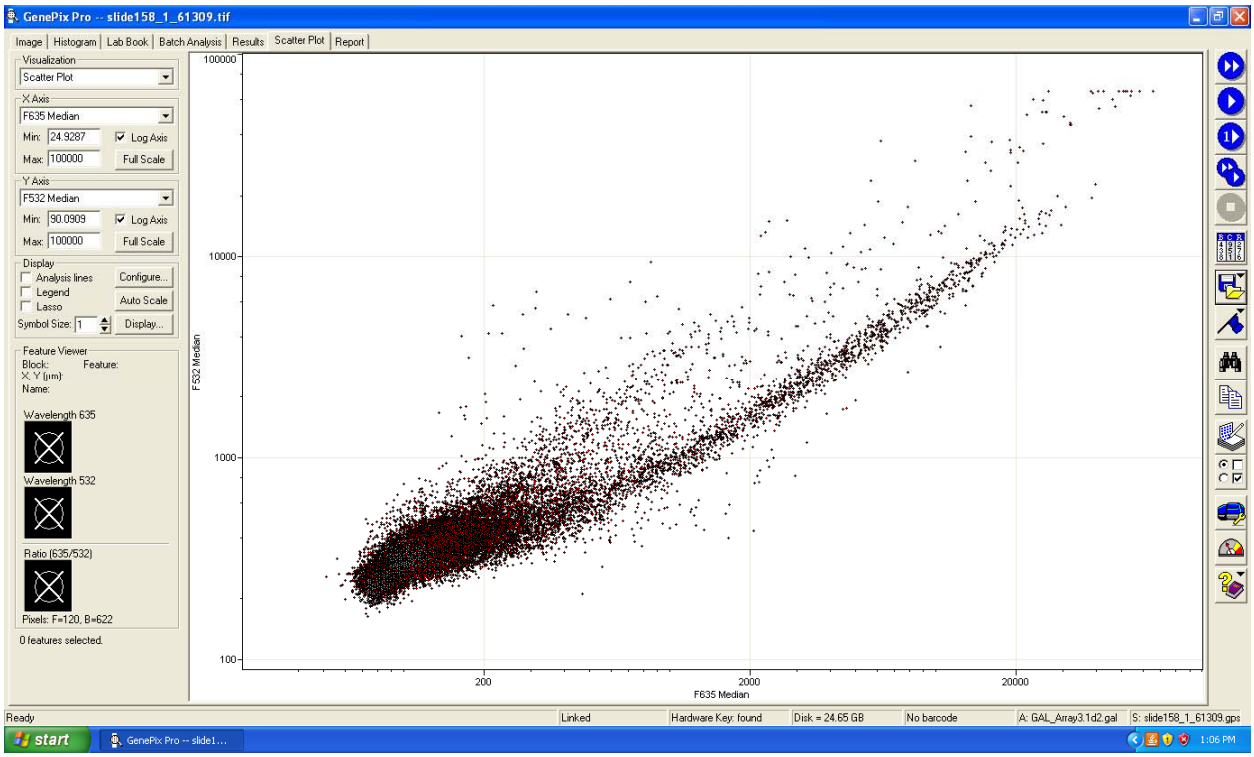


Figure A2-6. Slide 158, comparison of *P. cinctipes* larvae (y axis) and red king crab larvae (x axis). The relatively higher fluorescence is expected in the *P. cinctipes* sample.

Proposal 3: Alaska King Crab Abundance Forecasts

Principal Investigators: Mike Dalton (AFSC) and André Punt (UW)

Project Description:

Problem Statement

Impact assessment in fisheries management usually has biological and economic components. Presently, very little is known about the effects of ocean acidification on fisheries. Bering Sea crab stocks could be sensitive to these effects, and an integrated bioeconomic model would be useful for assessing the potential impacts of this long term global process. This project will develop an integrated bioeconomic model to make long-term (30-50 year) probabilistic projections for Bering Sea king crab fisheries under alternative management and environmental scenarios. The environmental scenarios will involve exogenous forcing from ocean acidification on key life-history parameters (i.e., pre-recruit growth and mortality rates) that will be explicitly represented in the bioeconomic model.

Methodology

The methodology employed will utilize a simple population dynamics model coupled with a reduced-form model of bioeconomic dynamics. Population dynamics will be based on a simplified version of the existing BBRKC stock assessment model. In particular, the simple model has fewer size classes than the full model. It will incorporate potential effects of ocean acidification on pre-recruit survival and growth through physiological response functions that will be calibrated to the experimental results for BBRKC growth and survival. The bioeconomic model will be based on a partial adjustment cost framework that entails adaptive dynamics. The fully coupled bioeconomic model will be used to make forward projections under alternative management and environmental scenarios with respect to explicit model benchmarks, (e.g., MSY), and dynamic comparisons across scenarios (i.e., under scenarios with environmental forcing compared to a baseline without such forcing).

Expected Products:

1) Copies of model code, program input files, and output files with results;
2) Reports that document work on individual tasks listed below;
3) Manuscript for submission to peer-reviewed fisheries or economics journal.

By September 2011, updated estimates of ocean acidification effect on growth and survival values for larvae and juvenile red king crab and the uncertainty of these values will be provided by Bob Foy to Mike Dalton for incorporation into a bioeconomic model of Bristol Bay Red King Crab. By December 2011, these updated estimates will be incorporated into the bioeconomic model and Bristol Bay red king crab abundance will be forecast for experimental treatment levels of ocean pH.

Proposal 4: Calcium Carbonate Measurements

Principal Investigators: Mark Carls, Lawra Vanderhoof, Lawrence Schaufler

Project Description: The purpose of this project is to provide detailed analytical and empirical laboratory support for a variety of research needs across all AFSC laboratories. The principal goals are to 1) operate and maintain the analytical capacity for detailed carbon chemistry measurements to add precision, accuracy, and quality control to routine approximate experimental measures and 2) operate, maintain, and improve a carbon dioxide dosing system for experimental research in collaboration with other investigators. Specific hypotheses, sample sizes, and statistical analyses will be driven by the proposals these measurements support.

Expected Products: Completed sample analyses in support of multiple projects. The analytical capacity and standard operating procedures have been worked out for all major variables, total dissolved inorganic carbon, total alkalinity, pH, salinity, and alkaline metals (Dickson et al., 2007). Specialized centralized processing is required for dissolved inorganic carbon, total alkalinity, and metals analyses due to equipment and support costs and time; thus our group at the Auke Bay Laboratories has agreed to fulfill this role for AFSC groups at Kodiak and Juneau. We expect the chemical data produced by this project will be coupled with biological results from other funded investigations to yield peer reviewed publications.

References:

Dickson AG, Sabine CL, Christian JR. Guide to best practices for ocean CO_2 measurements. Pices Special Publication (2007) 3:1-191.

Proposal 5: Growth and Survival of Larval Pollock

Principal Investigators: Thomas Hurst w/ Jeremy Mathis (UAF)

Project Description: The primary goal for FY2011 is to conduct an experiment rearing larval walleye pollock from fertilization through the post-flexion stage at a range of pH conditions. For this experiment, eggs will be collected from the laboratory broodstock and incubated at 4 pH treatments. Each treatment will be represented by at least 3 replicate rearing tanks. After hatch, larvae will be reared to at least 5 weeks post-hatch. Fish will be fed a combination of enriched rotifers and formulated dry food. Tanks will be sampled once per week to determine population growth rates.

We will also make further improvements to the monitoring and control of environmental CO_2 and pH in our fish rearing system. The main issue to be addressed is fluctuation in ambient pH of marine waters off the Oregon Coast, particularly in summer. A second pH regulation system would be added which would prevent periods of naturally low pH from disrupting target pH treatments in experiments.

Pending supplementary funding, research would begin on determining the potential influence of OA-induced changes in lower trophic levels on growth and survival of larval and juvenile walleye pollock. Walleye pollock will be reared with prey containing different fatty acid compositions. Based on existing and newly developed libraries of fatty composition of marine phytoplanktors and zooplanktors from BS/GOA (R. Heintz, unpublished data), we will develop experimental diets representing different primary producer communities. As above, all survival, growth rates, and condition factors will be assessed after 3-12 weeks of rearing. The effects of OA-induced shifts in primary producers on marine fishes will be indicated by significant treatment effects on survival rates, growth rates, or condition factor.

Expected Products:
In FY2011, we will complete an experiment describing the influence of environmental CO_2 and pH on the growth potential of larval walleye pollock. This experiment will be conducted in spring 2011, with data analysis completed by the end of the year. A manuscript describing these results is anticipated in FY2012.

In FY2011, two manuscripts will be developed for publication from experiments completed to date. These manuscripts will also comprise the content of UAF graduate student Elena Fernandez who will complete her thesis in 2011.

Proposal 6: Calcium Carbonate Mineralogy of Alaskan Corals

Principal Investigators: Robert Stone (NOAA, AFSC), Anne Cohen (Woods Hole Oceanographic Institution), John Guinotte (Marine Conservation Biology Institute), Stephen Cairns (Smithsonian Institution), Les Watling (University of Hawaii)

Project Description: 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. In Alaska, many commercial and non-commercial fisheries species are associated with deep corals. Decreases in oceanic pH and resulting decreases in calcium carbonate saturation state could have profound effects on corals dependent on the extraction of calcium carbonate from seawater for skeletal building. Corals will be affected differently depending on their skeletal composition (aragonite vs. calcite), geographical location, and depth. 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. The skeletal composition has been determined for only a few Alaskan coral species and may be composed of aragonite, calcite, high magnesium calcite, or amorphous carbonate hydroxylapatite.

Our next step is to develop standards and use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine the compound ratios of the polymorphous specimens. Adequate funds exist in the FY10 account with WHOI to begin this work in Fall 2010. Funding is sought in FY11 to complete this work and conduct x-ray diffraction analyses to determine the mineralogy of bamboo corals (Family Isididae). The bamboo corals were not amongst those previously included in this project but recent close examination of North Pacific specimens by Dr. Les Watling (University of Hawaii) indicate that they have particularly weak skeletons compared to their counterparts in the North Atlantic where the saturation horizons ware comparatively quite deep. The mineralogy data will form the basis for a risk assessment to be completed in FY11. The mineralogy data will be used in conjunction with species distribution data (depth and geographical) and the present and projected aragonite and calcite saturation horizons in Alaska to predict the effects of ocean acidification on coral resources of the North Pacific Ocean.

Expected Products: The carbonate mineralogy will be determined for all coral species for which samples can be obtained (up to 70 species) and from multiple depth and geographic zones for select corals. This work began in FY10 and will be completed in FY11. The mineralogy data may be submitted independently for publication in FY11 but will form the basis for the risk assessment to be undertaken in 2011.

References

Feely RA, Wanninkof R, Stein J, Sigler MF, Jewett E, Arzayus F, Gledhill DK (NOAA Ocean Acidification Steering Committee). 2010. NOAA Ocean and Great Lakes Acidification Research Plan (Ed. Sutton A). NOAA Special Report, 143 p.

Sigler MF, Foy RF, Short JW, Dalton M, Eisner LB, Hurst TP, Morado JF, Stone RP. 2008. 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. AFSC Proc. Rpt. 2008-07. www.afsc.noaa.gov/Publications/ProcRpt/PR2008-07.pdf