NMFS Northeast Fisheries Science Center Ocean Acidification Research 2010-2011

Summary

Ocean acidification research by the Northeast Fisheries Science Center (NEFSC) 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 are working together to ensure that NMFS ocean acidification research provides an integrated research program with publishable scientific results. 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 to assure measurement quality. In FY2010, 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.

Since species-specific physiological response to ocean acidification is unknown for most marine species, research by the NEFSC first centered on the goals of developing and implementing experimental protocols and identifying priority species based on their potential vulnerability to ocean acidification. Our efforts were enhanced by

Focal species groups
• Planktonic Prey
• Fish
• Shellfish

integration of general infrastructure and methodology across 3 regional science centers (NEFSC, NWFSC, and AFSC). A broad research effort is directed toward several taxa including phytoplankton, shellfish and fish. Our fish research will be directed to early life history stages that are more vulnerable to environmental stresses. Commercially important calcareous species (shellfish) are a priority because of their economic value and because these species are likely to suffer direct effects of reduced $CaCO_3$ availability. Planktonic species, representing both calcifying and non-calcifying groups and several diatoms form the basis of most marine productivity, and the effects of increased carbon dioxide on these species will ripple throughout the food web making them a priority. Indeed, suppression of calcifying phytoplankton, coupled with higher carbon availability for remaining phytoplankton, is likely to open niches for proliferation of phytoplankton species that may be relatively rare under current conditions. Thus, identification of taxa that are likely to be "winners" under projected OA scenarios and how these taxa could modify trophic structure has fundamental implications to fishery production.

In FY 2010-2011, analytical ocean acidification laboratories and experimental systems were established at the Milford, CT and Sandy Hook, NJ. Two laboratories were established in order to match expertise resident at each laboratory (e.g., the ability to culture winter flounder and other fish species at the Sandy Hook laboratory and the ability to culture phytoplankton and shellfish at Milford). Experiments were conducted at the Milford laboratory on a phytoplankton monoculture and preliminary work was done on blue crab homeostasis. Following an American

Fisheries Society symposium on Ocean Acidification in September 2011, an investigators meeting was held to discuss challenges and possible solutions to several issues. Monthly teleconferences allow researchers to discuss diverse topics such as laboratory setup, experimental challenges and water chemistry. The information that follows summarizes some of the research efforts taking place in the Northeast Science Center.

Phytoplankton: Experimental Methodology and Development

Laboratory/Science Center/Program: Milford Connecticut Laboratory, NEFSC/NMFS
Principal Investigator(s): Shannon Meseck, Scott Hawley, and Gary Wikfors
Date: 3/1/2011

In 2010, our main goals were: 1) obtain equipment necessary for precise and accurate carbonate chemistry analysis; 2) determine which acceptable methods would work best for altering carbonate chemistry in phytoplankton culture experiments, and 3) design a culturing system that can be used to manipulate carbonate chemistry to four different steady-state pH/CO_2 levels.

There are three acceptable methods described in Riebesell et al. (2010) that can be used for manipulating carbonate chemistry for ocean acidification experiments. These include: 1) bicarbonate addition followed by acidification, 2) CO_2 bubbling, and 3) mixing of high CO_2 water with low CO_2 water. Recommendations regarding the use of these methods with phytoplankton cultures have not yet been established. We tested and compared the bicarbonate addition/acidification method and CO_2 bubbling method for use with phytoplankton cultures. The third method, mixing high and low CO_2 water, was not tested because this method is not easily implemented using aseptic techniques. Total DIC was calculated using a seawater carbonate system program (CO2SYS) using measured pH and an assumed total alkalinity value. The bicarbonate/acid addition method was inadequate in achieving target pH values and initial pH was not maintained with phytoplankton growth. The CO_2 bubbling method produced the target pH values and maintained them after 4 d of phytoplankton growth. Based upon this experiment, the CO_2 bubbling method was determined to be preferable.

Literature Cited:
Riebesell U., Fabry V. J., Hansson L. & Gattuso J.-P. (Eds.), 2010. Guide to best practices for ocean acidification research and data reporting, 260 p. Luxembourg: Publications Office of the European Union.

Phytoplankton: Effects of Ocean Acidification on Phytoplankton Physiology and Nutrition for Fishery-based Food Webs

Laboratory/Science Center/Program: Milford, Connecticut Laboratory, NEFSC/NMFS
Principal Investigator(s): Shannon Meseck and Andrew King
Date: 8/1/2011

Three culture experiments have been conducted thus far to test the effects of varying pH and partial pressure of CO_2 (pCO_2) on the growth rate and physiology of the centric diatom species *Thalassiosira weissflogii,* the pennate diatom *Pseudo-nitzschia delicatissima*, and the prasinophyte *Tetraselmis chui.* In each 2-week experiment, cultures were grown semi-continuously (in logarithmic growth phase) for 7-10 generations to acclimate cells to different pH/pCO_2 levels before physiological measurements were made. The experimental treatments consisted of triplicate culture vessels equilibrated to pH ~8.4, 8.1, 7.8, and 7.2 (equivalent to ~100, ~400, ~1,000, and ~4,000 ppm pCO_2, respectively) by sparging with air/CO_2 mixtures.

Media pHs and total alkalinities were monitored to ensure experimental conditions and for the calculation of seawater pCO_2. After the acclimation phase, we measured specific growth rate using cell counts from flow cytometric (FCM) analysis and collected samples for the analysis of cellular C, N, P, Si, total lipid, carbohydrate, and fatty-acid composition. Supplementary data also were collected during FCM analysis, including autofluorescence and light scatter properties. In addition to those FCM parameters, with a visiting collaborator Dr. Hélène Hégaret (Institut Universitaire Europeen de la Mer, Brest, France), the experiment with *P. delicatissima* also assessed cell viability and total lipid content (using molecular probes and FCM), and maximum quantum yield (photosynthetic efficiency) as measured by fast repetition-rate fluorometery.

Preliminary findings indicate that growth rate of *T. weissflogii* was highest at pH 8.1/400 ppm pCO_2 and lowest at the pH 8.4 and 7.2. *P. delicatissima* growth rate, on the other hand, was depressed under high pH/low pCO_2 (pH 8.4 and 100 ppm pCO_2) and highest in low pH/high pCO_2 treatments. Growth rate data for *T. chui* were unreliable because of cellular adhesion to the culture vessels. Molar C:P and N:P elemental ratios of *P. delicatissima* were found to be elevated in 1,000 and 4,000 ppm pCO_2 treatments. All three species showed pH-related differences in FCM-determined side-scatter profiles (an index of granularity and internal complexity of cells) that will be investigated further. Cellular Si, lipid, carbohydrate, and fatty acid samples are awaiting analysis.

The experiments included cooperation of seven scientists in total, including Andrew King, Shannon Meseck, Jennifer Alix, Mark Dixon, Kelsey Beoff, Hélène Hégaret, and Lisa Milke. These scientists worked on one or more facets each experiment.

Calcium Carbonate Measurements and Experimental Facility

Laboratory/Science Center/Program: James J. Howard Laboratory, New Jersey/NEFSC/NMFS
Principal Investigator(s): Matthew Poach, Daniel Wieczorek
Date: 3/1/2011

The goal of this project was to develop, build, and evaluate facilities to accurately sample and measure calcium carbonate measurement and an experimental system that would provide

seawater with pH, pCO_2, carbonate saturation state, and temperature levels expected under present and future atmospheric conditions. There were two main objectives: 1) establish the capability to accurately sample and measure three carbon chemistry parameters (pH, dissolved inorganic carbon [DIC], alkalinity) and 2) create a prototype experimental system that would expose marine organisms to a range of target pCO_2 and temperature levels

Approach: Work on the establishment of analytical capabilities involved the identification and purchase of instruments to measure three of the four carbon chemistry parameters. The instruments obtained were as follows: 1) a dual-beam spectrophotometer for pH determination using 10 cm spectrophotometric cells; 2) a coulometer for the determination of DIC, and 3) an automated titrator for the determination of alkalinity using the open cell method. Laboratory specific protocols were also developed for sample collection, preservation and analysis. Protocols were based on the standard operating procedures (SOP) outlined in the Guide to Best Practices for Ocean CO_2 Measurements (Dickson et al., 2007). Assessment of analytical proficiency as well as quality control was performed by analysis of certified reference materials (CRM) purchased from the Dickson's Laboratory.

Before the actual creation of a prototype experimental system, work involved the development of an experimental design to produce pCO_2 treatments at target levels. It was determined that the best method for producing target pCO_2 levels was to bubble our incoming seawater with CO_2. Five target pCO_2 levels were then chosen, which include levels recommended by the OA research community as well as higher levels that would help pinpoint biological tipping points. After the design phase, instruments and supplies necessary to implement the experimental design were acquired. Supplies necessary to produce target pCO_2 levels included both pH-stat and mass flow controllers. System design also included water filtration and sterilization elements. Construction of the apparatus that would alter the carbon chemistry of our incoming seawater was started in the summer of 2010.

Throughout the various phases of both the experimental system creation and laboratory establishment, other NMFS laboratories in Kodiak and Seattle were consulted to promote agency-wide consistency among ocean acidification experiments.

Results: A laboratory with the capabilities to measure seawater carbon chemistry has been established at the James J. Howard Marine Sciences Laboratory. While it is necessary to measure only two carbon parameters to fully characterize the carbon chemistry of seawater, the laboratory has the capability to measure three parameters. Analysis of CRM's indicates that the pH of seawater samples can be measured with a 0.02% error and the DIC can be measured with a 0.2% error. A training program and an SOP have been developed for the safe handling of mercuric chloride, the chemical used to preserve OA samples.

Construction of an experimental system that could produce seawater with five different pCO_2 treatments and five temperature levels was completed. Initial testing of the consistency, precision, and reliability of the CO_2 dosing system has commenced. Biological productivity in the seawater supply lines for the laboratory raises the pCO_2 of the incoming seawater above the level considered ambient by the OA community. At this time, several options to reduce the pCO_2 of the incoming water are being evaluated.

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

Calcium Carbonate Measurements and Experimental Facility

Laboratory/Science Center/Program: James J. Howard Laboratory, New Jersey/NEFSC/NMFS
Principle Investigators: Matthew Poach and Daniel Wieczorek
Date: August 22, 2011

An experimental system to produce 5 different pCO2 treatments was constructed and tested. Protocols for collecting and analyzing samples were also developed. Data from these tests are presently being analyzed for a system report. Preliminary results indicate that stable pCO2 levels could be produced by the system with properly functioning pH electrodes and controllers. Also, the sampling protocols were sufficient to monitor pH and CO2 treatment levels. At this time, a second generation system is being developed to address deficiencies in the performance of the test system (mainly due to poor incoming water quality and occasionally unreliable pH control).

In addition, a modification to the standard sampling and analysis protocols for dissolved inorganic carbon (DIC) was tested. The new method will lead to faster sample analysis and reduce hazardous chemical use and waste generation. Preliminary results of these tests indicate that the new protocol provides DIC measures similar to the standard method but with greater variability. The reason for the variability has been preliminarily identified and adjustments to the new protocols are being made for further comparisons with the standard method.

Resource Finfish Species and Ecosystem Effects

Laboratory/Science Center/Program: James J. Howard Laboratory, New Jersey/NEFSC/NMFS
Principal Investigator(s): Chris Chambers
Date: 3/1/2011

Goals: Understand the impacts of increased CO_2 and associated increased acidity of ocean and estuarine waters on key marine finfish species of the NE USA. The objectives are: 1) to design, quantify, and evaluate these impacts in an experimental context, and 2) to monitor changes in water chemistry at an ecosystem level and use our experimental results to interpret the pattern and consequences of changes in ocean acidity to living marine resources.

Approach: Our focus is on responses expressed in the early life-stages of representative species because recruitment to the adult stage is set during early life and these life-stages are likely to be most sensitive to environmental variations. Further, due to a limited amount of published

information on the response of finfish to high CO_2 levels in their environment, we expect that the responses of finfish may be subtle, interactive, and expressed in a number of different ways,

Specifically, we have 1) identified a number of commercially and ecologically important finfish taxa to be used in our experiments that, collectively, cover a broad subset of ecological and life-history types for our region, 2) assembled an extensive list of sensitive response variables to be used as bio-indicators of responses to high CO_2 levels, and 3) adopted an experimental design (5 × 5 factorial) that has a sufficient range and density of treatment levels of two factors (CO_2 levels and water temperatures) to estimate the functional form of fish responses and to test for possible interactive effects. Our experimental effort is designed to draw the strongest inference possible given the limited prior knowledge by the larger research community regarding ocean acidification (OA) effects.

Results: Initially, we identified candidate resource species to be used in experimentation; developed an experimental design; selected response variable protocols based on ecological relevance and feasibility. In collaboration with Poach and Wieczorek (see previous report), we designed a CO_2 water delivery system and monitoring scheme for that system; identified materials and equipment to be purchased. We constructed a controlled CO_2 water-delivery system that can generate and maintain five discrete levels of pCO_2, constructed a water bath control system for generating and maintaining five discrete levels of water temperature, constructed egg and larval flow-through containers (75 for each life stage) and developed and purchased a salinity mixing system to deliver and maintain pre-set levels of salinities to mimic embayment to coastal shelf environments as required by the candidate finfish species targeted for experimentation.

Regarding our target species, we have: 1) established and are maintaining zooplankton (rotifer) cultures necessary as prey for larvae of most of our candidate fish species 2) collected and are now maintaining broodstock of summer flounder, winter flounder, and black sea bass; 3) spawned and are now doing pilot-level rearing of eggs and larvae of black sea bass and summer flounder; 4) are currently spawning winter flounder and maintaining eggs for test rearing and 5) are identifying sources of eggs and permitting requirements for holding sturgeon (Atlantic and shortnose).

Challenges emerged in controlling the stability of our incoming seawater ph. We will resolve this by attempting to degass the experimental system's water with a degassing column. Despite this, we still have the capability to run experiments at 3 different CO_2 levels and 5 temperatures.

Resource Finfish Species and Ecosystem Impacts

Laboratory/Science Center/Program: James J. Howard Laboratory, New Jersey/NEFSC/NMFS
Principle Investigator: Chris Chambers
Date: 8/22/2011

We implemented an experimental evaluation of the concurrent and joint effects of 5 levels of CO_2 and 5 levels of water temperature on the rates of viability, development, and growth of embryos and young larvae of winter flounder (*Pseudopleuronectes americanus*). This initial trial gave us an opportunity to test our system – from treatment delivery, to monitoring water

parameters, to response variable tests of the fish and the general logistics of this new, large-scale experimental system (i.e., 25 treatment combinations x 3 replicates per egg and larval life-stages). We found survival of embryos and young larvae to be more variable in this initial experiment than expected (compared to results from our typical rearing protocol – static water with daily partial water replacement). These and other observations about our first-generation system led us to 1) redesign the water delivery/rearing system in order to provide improved water quality (higher degree of incoming filtration and UV sterilization) and to provide an option for using salinities different from our bay water (~ 24 to 26 ppt from Sandy Hook Bay), 2) modify the rearing components of our system by increasing the size of rearing containers, adding quick-disconnects with cut-off valves, and increase the outflow capacity in this flow-through system, and 3) alter our approach to handling the embryos and larvae, providing higher densities of live feed (zooplankton), and efficiently collecting response variables. We are completing the various components of these changes in our second-generation system and are in the process of testing the efficacy of these modifications. Our next species to be tested in our system is summer flounder (*Paralichthys dentatus*) which spawns in early autumn. We have acquired and continue to supplement the adult summer flounder to be used as parents for this experiment. Lastly, we are concurrently holding adult black sea bass (*Centropristis striata*) whose offspring will be used for experimentation after the summer flounder experiment is completed this autumn.

Marine Species and Ecosystem Modeling

Laboratory/Science Center/Program: Woods Hole Laboratory, Massachusetts/NEFSC/NMFS
Principal Investigator(s): Jon Hare, Mike Fogarty, and Jason Link
Date: 3/1/2011

Goals: Develop and implement modeling approaches to assess the effects of ocean acidification on single-species dynamics and overall ecosystem productivity

Approach: To achieve the above goal, we have established several steps. First is to develop an ocean acidification baseline and then derive reasonable ecosystem specific "scenarios" of ocean acidification conditions in the future. Second is to link the acidification scenarios to primary production and population models to assess the effect of acidification on species and system-level productivity. Third is to link the acidification scenarios to ecosystem models to assess the effect of acidification on ecosystem structure and function.

During year 1 of our project, we have made progress on our first step. Our initial step in addressing the above goal is to develop a baseline of ocean acidification on the northeast U.S. shelf ecosystem and then compare this baseline to current measurements and to climate model projections. We will use data collected during the MARMAP decade to develop the baseline (1977-1987) and use recently collected data during the EcoMon program (2008-2010) to test whether conditions have changed. Global studies indicate a decrease in pH and we will test whether these global patterns hold for the Northeast U.S. shelf ecosystem.

Population and Ecosystem Modeling of OA on the Northeast U.S. Continental Shelf

Principle Investigator: Jon Hare
Laboratory/Science Center/Program: Narragansett Laboratory, Rhode Island,
Date: 8/18/2011

Status: The initial goal of this project is to develop a climatology of OA parameters on the Northeast U.S. shelf and to evaluate whether there have been changes over the past 40 years. This climatology and change analysis will then be used to inform population and ecosystem models. Multiple shelf-wide surveys of the Northeast U.S. Continental Shelf, from the Gulf of Maine through the Mid-Atlantic Bight, were conducted as part of the NOAA Marine Resources Monitoring, Assessment and Prediction (MARMAP) program. Observations including pH, total alkalinity, temperature, and salinity were collected from the years 1977-1987. These historical data were compared to recent data collected as part of a joint NASA / NOAA project (http://neptune.gsfc.nasa.gov/osb/index.php?section=247) and as part of the NOAA Ocean Acidification Program (Wanninkhof, AOML, East coast observing support). QA/QC has been performed on the historical data; some data is questionable and has been removed from the analyses. A preliminary comparison with recent data has been performed. Additional water sample data (DIC and TAlk) and flow-through pCO_2 data will be added to the comparison before a final analysis is completed. A talk of this work is planned for AGU Ocean Sciences in Feb 2012 and a manuscript is planned. One scientist is involved full-time (a NRC post-doc) and multiple scientists are partially involved in current data collection, advising, and contributing to analyses.

Low pH Effects on an Aquaculture Fish Species

Laboratory/Science Center/Program: Milford, Connecticut Laboratory, NEFSC/NMFS
Principal Investigator(s): Dean M. Perry, Dylan H. Redman and Shannon Meseck
Date: March 1, 2011

Goals: Our goal is to determine the effects of elevated levels of carbon dioxide on otolith condition and growth of juvenile scup, *Stenotomus chrysops* using a flow-through pCO^2 delivery system

Approach: In fish, the otolith is an important sensory organ that senses orientation and acceleration. Fish have evolved with otoliths of a certain size, shape, and mass and any changes due to increased levels of pCO^2 may alter the sensory ability of the fish. Changes in calcification may also affect behavior, including feeding. Further, maintaining otolith growth at increased pCO^2 may have physiological costs in terms of reduced somatic growth so we will measure length and weight, in young-of-the-year scup that have been exposed to increased levels of pCO^2. It was necessary to construct a flow-through seawater pCO^2 delivery system in order to maintain the health and well being of the fish. After consulting with research scientists at the

Woods Hole Oceanographic Institute in April 2010, we designed and started construction on a flow through pCO^2 delivery system with greater seawater flow rates for exposure studies with finfish. The values of pCO^2 that will be used will be 380 ppm (control), a value of 970 ppm, predicted to occur by 2100, and a high value of 2,200 ppm.

Otolith Condition and Growth of Juvenile Scup, Stenotomus chrysops

Laboratory/Science Center/Program: Milford, Connecticut Laboratory, NEFSC/NMFS
Principle Investigator: Dean M. Perry
Date: August 10, 2011

Status of the Research: An OA flow-through treatment system for finfish was constructed during the first 6 months of 2011. The system is operational and the air/CO_2 mixtures entering and exiting the equilibration towers have been tested for pH using both a pH probe and a spectrophotometer. All measurements (pH, pCO_2, and alkalinity) were accurate and precise. We are currently collecting young-of-the-year scup to use in our experiment. The fish will be exposed to 380 ppm pCO_2 (control), a value of 970 ppm pCO_2 and a high value of 1,800 ppm pCO_2. The experiment will run for 6-9 weeks, depending upon the rate of fish growth. After statistical analyses are completed, a manuscript will be prepared for publication in a peer-reviewed scientific journal.

Physiologic Effects on Atlantic Surf Clams

Laboratory/Science Center/Program: Milford, Connecticut Laboratory, NEFSC/NMFS
Principle Investigator(s): Lisa Milke and James Widman
Collaborators: Anne Cohen and Dan McCorkle, Woods Hole Oceanographic Institute (WHOI)
Date: 22 August 2011

Status of the Research: To date, two experiments have been completed and one is in progress. Two short term (~72h) experiments were conducted in mid-July and the beginning of August at the Woods Hole Oceanographic Institute (WHOI) examining the effects of both pCO2 (experiment 1: ambient and 1200; experiment 2: ambient and 2200) and food on early development of surf clam, *Spisula solidissima*, larvae. After ~72 hours, samples were taken for shell growth and weight. Samples are currently being analyzed at WHOI.
A long term (~21 d) long experiment was started August 1st. This is examining the effects of three different pCO_2 concentrations (ambient, 1200 and 2200) upon survival, shell height and shell mass. Samples are being taken every three days to assess these metrics. Mortalities and shell heights are being analyzed at the Milford Lab and animal weights are under analysis at WHOI.