Responses to EPA Notice of Data Availability From Ocean Carbon and Biogeochemistry Program

Items in **Blue** are original questions posed by EPA.

OCB RESPONSE

The following comments are compiled from the U.S. Ocean Carbon and Biogeochemistry (OCB) community of scientists (see http://www.us-ocb.org/). The goal of the Ocean Carbon and Biogeochemistry Program is to help coordinate and facilitate U.S. marine biogeochemistry and related ecological research working in conjunction with relevant federal science agencies (e.g, NSF, NASA and NOAA). The OCB is a community of scientists, primarily at academic and research institutions. Much of this information is a summary of the following reviews on the state of ocean acidification research findings, but includes updates from the recent literature.

Dickson AG, Sabine CL, Christian JR (Eds) (2007) Guide to best practices for ocean CO_2 measurements, PICES Special Publication, 3, 191 pp., 2007. (and pH references within) http://cdiac.ornl.gov/oceans/Handbook_2007.html

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Ann Rev Mar Sci 1:169-192

Fabry VJ, Langdon C, Balch WM, Dickson AG, Feely RA, Hales B, Hutchins DA, Kleypas JA, Sabine CL (2009) Present and future impacts of ocean acidification on marine ecosystems and biogeochemical cycles, Report of the Ocean Carbon and Biogeochemistry Scoping Workshop on Ocean Acidification Research (UCSD, Scripps Institution of Oceanography; 9–11 October 2007)

Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006b) Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research. Report of a workshop held 18-20 April, 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the U.S. Geological Survey 88

OCB Subcommittee on Ocean Acidification (2009) Ocean Acidification - Recommended Strategy for a U.S. National Research Program, Ocean Carbon and Biogeochemistry Program, 14 pp.

It should be noted that major recommendations in these reviews are to improve and increase monitoring of ocean acidification, including in the coastal zone, and to increase research on its impacts on marine organisms and ecosystems.

We have addressed the EPA questions in the order that they were proposed in the NODA.

1. EPA is soliciting technical information on measurement of ocean acidification in marine coastal waters, including:

   a. **Technological advances in rapid, continuous, or remote measurement of pH;**

Since the beginning of the industrial revolution the release of carbon dioxide (CO_2) from our industrial and agricultural activities has resulted in an increase in atmospheric CO_2 concentrations from approximately 280 to 387 parts per million (ppm). The atmospheric concentration of CO_2 is now higher than experienced on Earth for at least the last 800,000 years, and is expected to continue to rise at an increasing rate, leading to significant temperature increases in the atmosphere and the ocean surface in the coming decades. During this time, the ocean has absorbed more than 450 billion tons of carbon dioxide from the atmosphere, nearly one-third of anthropogenic carbon emissions. This absorption has benefited humankind by significantly reducing greenhouse gas levels in the atmosphere, thereby partly minimizing global warming. However, when the anthropogenic CO_2 is absorbed by seawater, chemical changes occur that increase the CO_2 partial pressure (pCO_2) and reduce both seawater pH and the concentration of carbonate ion in a process commonly referred to as ocean acidification (Caldeira and Wickett, 2003; Feely et al., 2004; Orr et al., 2005).

As a result, the pH of ocean surface waters has already decreased by about 0.1 pH units, which is equivalent to an overall increase in the hydrogen ion concentration in surface waters of approximately 30%. This increase in hydrogen ions reduces the concentration of carbonate ions which are the building blocks needed by many marine organisms, such as corals, calcifying plankton and shellfish, to produce their skeletons and shells. This loss of carbonate ions produce lower saturation levels for the carbonate minerals, aragonite and calcite, which are the primary mineral phases of the shells and skeletons of many marine plants and animals. Furthermore, in coastal regions ocean acidification can be amplified by other natural and human-induced changes to hasten localized declines in pH and carbonate mineral saturation states (Doney et al., 2007; Feely et al., 2008; Salisbury et al., 2008).

Application of a pH criterion alone will be inadequate to monitor ocean acidification and its ecological impacts. Experimental studies have demonstrated that it is the saturation state rather than the pH or pCO_2 that controls the response of many calcifying organisms (Langdon et al., 2000). Therefore, the OCB community recommends that in addition to pH, additional criteria such as carbonate ion concentration ($[CO_3^{2-}]$) and saturation state (Ω) be considered.

Although pH in seawater has been measured for many decades, a reliable long-term trend of ocean water pH cannot be established due to data quality issues, in particular the lack of strict and stable calibration procedures and standards. Moreover, seawater pH is very sensitive to temperature, and temperature is not always recorded or measured at sufficient accuracy to constrain the pH measurement. Therefore in order to reconstruct long-term pH trends, pH values (or $[H^+]$ activity or concentration) are computed using chemical models with measured values of dissolved inorganic carbon (DIC or C_T), CO_2 partial pressure (pCO_2), and/or total alkalinity (TA or A_T) in seawater.

With major analytical advances over the last two decades, the four so-called inorganic carbon system parameters can be measured with very high accuracy and precision aboard oceanographic research vessels. Of the four parameters, pCO_2 and pH can be measured remotely on moorings or other platforms using autonomous sensors. The analytical procedures for making CO_2 parameter measurements are documented in great detail in the "Guide to Best Practices for Ocean CO_2 Measurements" (Dickson et al., 2007). For ocean acidification research at least two, and preferably three of the four carbon parameters should be measured at each of the sampling depths to ensure internal consistency of the data sets. Accuracy and precision can be obtained from replicate measurement of certified standards (Table 1).

Table 1. Present status (2007) of certified reference materials for the quality control of oceanic carbon dioxide measurements (modified after Dickson et al., 2007).

Analytical Measurement	**Desired Accuracy**[a] **and Certification**	**Methodology**	**References**
C_T Total dissolved inorganic carbon	± 1 µmol kg^{-1} certification: since 1991	Coulometric: All carbonate species are converted to CO_2 gas by acidification of the seawater sample. The evolved CO_2 gas is carried into the titration cell of the coulometer, where it reacts with a colorimetric reagent. Absorption of CO_2 stimulates the hydrolytic production of hydroxide ions (OH-), which stoichiometrically titrates the colorimetric acid. CO_2 is thus measured by integrating the total coulometric OH- production required to achieve full titration. precision and accuracy: ± 0.05% in laboratory setting Methods based on the detection of the liberated CO_2 by an infrared detector are also widely used with a precision and accuracy of ± 0.1%.	Johnson et al. 1985, 1987
A_T Total alkalinity	± 2 µmol kg^{-1} certification: since 1996[b]	Potentiometric titration: A measured amount of acid titrant is added to the sample to reduce the pH to less than 3.55, CO_2-free air is bubbled through the acidified sample to facilitate removal of the evolved CO_2 gas, followed by titration to a pH of less than 3.0. The titration curve is fit to a derivation of the seawater alkalinity equation using a non-linear least squares approach that takes into account the various acid-base equilibria in solution during the titration. The precision and accuracy is ± 0.1% in laboratory setting.	Dickson et al. 2003
pH	± 0.002 certification[c]	spectrophotometric: A pH indicator calibrated on the total hydrogen ion concentration scale. Absorbance ratios are measured at 578 and 434 nm against a reference solution of pure seawater, and absorbance at a non-absorbing wavelength is used to correct for small absorbance variations attributable to manipulations of the 10-cm cylindrical	Clayton and Byrne, 1993

		cuvette. precision and accuracy: 0.002 pH units under field conditions	
$p(CO_2)$[d]	± 2 µatm certification[d]	A known amount of seawater is isolated in a closed system containing a small known volume of air (containing a known initial amount of carbon dioxide) and maintained at a constant, known temperature and pressure. Once the water and air are in equilibrium a sample of the air is analyzed for carbon dioxide content using a gas chromatograph or infrared CO_2 detector which is capable of integrating a constant volume of CO_2	Chipman et al., 1990 Wanninkhof and Thoning, 1993

[a] Adapted considerations outlined in the report of SCOR Working Group 75 (SCOR 1985). They reflect the desire to measure changes in the CO_2 content of sea water that allow the increases due to the burning of fossil fuels to be observed.

[b] Representative samples of earlier batches were also certified for alkalinity at that time.

[c] The pH of a reference material can be calculated form the measurements of total dissolved inorganic carbon and total alkalinity. Also, buffer solutions based on TRIS in synthetic sea water can be certified for pH, but as yet this is not done regularly.

[d] CO_2 in air reference materials are presently available through a variety of sources. However, it is desirable to use a reference material in a seawater medium for a discrete $p(CO_2)$ measurement. Although the thermodynamics of the sea water system suggest that, since the CRMs are certified stable for C_T, A_T, and pH, they should be stable for $p(CO_2)$ a reliable technique for independently determining $p(CO_2)$ to allow proper certification has not yet been developed.

Direct Measurement of pH

The pH in seawater, or the H^+ ion activity or $[H^+]$, has been measured for many years using various electrodes, most commonly a glass-calomel electrode pair. However, these data cannot be used to document a long-term trend because of the lack of electrode accuracy. The major problems include: a) A lack of stable electrodes with sufficient sensitivity (pH~0.001) that are suitable for long-term measurements in seawater; b) A standard hydrogen electrode cannot be used for seawater samples, since the hydrogen gas passed through the sample purges CO_2, thus altering the pH. This means that the pH values obtained by electrode systems cannot be directly related to the thermodynamic standard state of hydrogen; c) pH electrodes may be calibrated using standard solutions (e. g. the TRIS-buffer). However, eliminating systematic errors arising from junction and asymmetry potentials are difficult; d) errors are often introduced due to the exchange of CO_2 gas with the air in a cell's headspace during measurement; e) difficulties in determining errors due to failures in maintaining the samples and electrodes at the same temperature; f) there are four pH scales and the pH scales used for measurements (such as the NBS and TRIS-buffer scales) are often not defined in data reports. These pH scales are not interchangeable and thus the pH scale should always be noted.

Advances in measuring pH spectrophotometrically (Clayton and Byrne, 1993; Wang et al., 2007) make this the current preferred method of pH analyses as the issues with electrodes are circumvented (see a, c, and d above). Measurements can be made with high precision (±0.0005), and accuracy is only limited by errors associated with maintaining and measuring temperature, accurate knowledge of the indicator thermodynamic and spectroscopic constants, and accounting for addition of indicator. Long-term accurate pH_T measurements can now be made on moorings using autonomous spectrophotometric-based pH sensors (Seidel et al., 2008). However, the range of spectrophotometric measurements is tied to the range of the buffers used, which for seawater spans about 0.4 pH units. For inland and estuarine waters with wider pH ranges, other indicator buffers must be sought and cross-calibrated with the TRIS buffers.

Indirect Methods for the Determination of pH

Alternative methods can also be used for computing pH (or $[H^+]$ activity or concentration) in seawater. Strengths and weaknesses of commonly used methods are discussed below.

a) DIC-TA method:

$[H^+]$ may be computed using a chemical model for seawater with DIC (the total concentration of CO_2 species dissolved in seawater = $[CO_2(aq)]+[H_2CO_3] + [HCO_3^-] + [CO_3^=]$), TA (titration or total alkalinity, or A_T), temperature and salinity data. When the concentrations of nutrient salts (i.e. phosphate and silicate) are high, these concentrations are also needed. DIC and TA may be measured with sufficiently high precision and long-term stability.

Strengths of this method: i) Both DIC and TA have been measured widely in the surface and deep ocean (Feely et al., 2004); ii) these properties are used for global ocean circulation-biogeochemistry transport models; iii) change in TA is a direct measure for the precipitation/dissolution of $CaCO_3$ (Feely et al., 2008); and iv) the $[CO_3^{2-}]$ and hence the degree of saturation for $CaCO_3$ may be computed.

Weaknesses of this method: i) While standards for DIC have been available since the 1990s, the standards for TA only become available in the late 1990s; ii) whereas the thermodynamic reference for DIC is tied to the CO_2 gas standard, that for TA is tied to acid concentrations; iii) seasonal measurements of DIC and TA are highly limited; and iv) to compute $[H^+]$, the contributions of boric, phosphoric, silicic and organic acids must be removed from TA. While the concentration of boric acid is believed to be proportional to salinity and not biologically reactive, the concentrations of phosphoric and silicic acids vary in space and time due to biological activity. Although their concentrations are nearly zero in temperate surface ocean waters, the concentrations of dissolved organic compounds (including organic acids) are high and variable in these waters. Chemical models that are commonly used for the calculation of $[H^+]$ include ionic dissociations of "inorganic" acids, but do not include the effects of organic acids. The variability of $[H^+]$ due to organic acids is ignored (Millero et al., 2005).

Because of the complications of TA, the resulting $[H^+]$ values may be less precise and subject to more systematic errors than the DIC-pCO_2 method, which is discussed below. Based on the accuracy limits listed in Table 1 (1 $\mu mol\ kg^{-1}$ for DIC and 2 $\mu mol\ kg^{-1}$ for TA), the pH in surface waters (SST= 20°C, SSS = 35 PSU, TA=2300 $\mu mol\ kg^{-1}$, DIC= 2030 $\mu mol\ kg^{-1}$) can be calculated with a precision of 0.004 and the aragonite saturation state to 0.02.

b) DIC-pCO_2 method:

$[H^+]$ may be computed using a chemical model for seawater with DIC, pCO_2, temperature and salinity data. DIC and pCO_2 can be measured with sufficiently high precision and long-term stability (e. g. Olafsson et al., 2009).

Strengths of this method: i) An extensive seasonal and interannual database for surface water pCO_2 is available (*e.g*., Takahashi et al., 2008; Takahashi et al., 2009); ii) DIC has been measured widely in the surface ocean, although not as extensively as pCO_2; iii) the calibration standards for DIC and pCO_2 are tied to a common thermodynamic standard state of CO_2 gas; iv) unlike the DIC-TA pair, $[H^+]$ may be computed using a chemical model without knowing the concentrations of boric, phosphoric, silicic, and organic acids; and v) pCO_2 in surface water has been measured over the past four decades using the WMO air-CO_2 standard mixtures for the study of the CO_2 flux across the sea surface. The calculated pH values are twice as precise compared to the DIC-TA method. Again, using the accuracy limits listed in Table 1 (1 µmol kg^{-1} for DIC and 2 µatm for pCO_2), the pH in surface waters (SST= 20 C, SSS = 35, pCO2=2300 µmol kg^{-1}, DIC= 2030 µmol kg^{-1}) can be calculated with a precision of 0.002 and the aragonite saturation state to 0.01. That is, the pH can be calculated to similar precision as it can be measured.

Weaknesses of this method: i) While seasonal variability of DIC is large, its seasonal measurements are limited; ii) the pCO_2 data for deep waters are limited; and iii) since pCO_2 is a quantity unrelated to mass (like temperature), it is not useful for mass transport studies as TA and DIC are.

The $[H^+]$ values obtained by the DIC-pCO_2 and DIC-TA methods depend on the pH scale used for dissociation constants. However, the advantage of the pCO_2-DIC method is that it requires only the solubility of CO_2 gas in seawater and the first and second dissociation constants for carbonic acids. In contrast, the TA-DC method requires not only the dissociation constants for carbonic but also those for boric, phosphoric, and silicic acids, and, as stated above, organic acids are often neglected.

c) TA-pCO_2 method:

$[H^+]$ values computed using this pair are subject to the strengths and weaknesses described above for TA and pCO_2. However, unique advantages of this method are that i) the seasonal variability of TA appears to be small and may be parameterized with sufficient precision as a function of salinity and nitrate concentration, and ii) extensive seasonal and interannual pCO_2 observations are available. The precision of the calculated pH using TA-pCO_2 is ~25% better than that of the DIC-pCO_2 method, as pH is not as sensitive to variations in TA as it is to variations in pCO_2 or DIC.

d) pH-DIC and pH-TA methods:

Recent advancement of pH measurements via colorimetric methods has yielded high precision data (Byrne et al., 1998) that are comparable to, or better than the precision of pCO_2 measurements. These spectrophotometric measurements are relatively cheap and easy to perform but require appropriate standards, indicator solutions, and strict protocols to obtain the precision and accuracy necessary to discern multi-year trends caused by uptake of anthropogenic CO_2. However, since these high quality measurements did not commence in the field until the late 1990s, data are very limited and insufficient to determine a long-term trend or characterize geographical variability for seawater pH.

The pH value computed by the DIC-pCO_2 and DIC-TA methods depends on the pH scale used for the determination of the dissociation constants of carbonic, boric and other acids. The pH values measured by colorimetric methods have been found to be consistent with those computed from the DIC-pCO_2 or DIC-TA during cruises when three or more inorganic carbon system parameters are measured (Byrne et al. 1999).

Conclusions

a) Application of a pH criterion alone will be inadequate to monitor ocean acidification and its impacts on coastal marine ecosystems. It is therefore recommended that additional criteria such as carbonate ion concentration and saturation state be considered.

b) Direct measurements of pH using spectrophotometric indicators are the most reliable and straightforward method for quantifying the changes in [H+] due to ocean acidification. Thus far, however, these measurements have limited spatial and temporal coverage.

c) Full characterization of the seawater inorganic chemistry system and ocean acidification requires simultaneous measurement of temperature, salinity and at least two of the four CO_2 system parameters (pH, DIC, TA and pCO_2). The pH scale used must also be reported.

b. Long-term empirical pH data and carbon chemistry measurements, especially those that may demonstrate ocean acidification;

EPA is encouraged to seek out long-term coastal States' and Federal datasets (e.g., USGS, NOAA) on water quality or any monitoring studies that have included pH data collected in coastal State waters. These datasets often have been collected as ancillary data for long-term water quality monitoring (*e.g.*, in conjunction with *E. coli* monitoring), but may be available for other monitoring studies as well. Technically, pH data collected within coastal environments in the U.S. for the past 4-5 decades may not be optimal in resolution and accuracy; however, they might provide EPA with information on long-term trends and seasonal and annual variability of pH that the coastal environments are likely to demonstrate, provided consistent calibrations were performed through time (see technical information above regarding pH measurements). Rigorous statistical evaluation of the data must be applied and metadata must be scrutinized. Many of the historical pH datasets lack sufficient detail on protocol and accuracy to be useful to estimate long terms trends.

The majority of historical coastal ocean pH data have been measured using glass electrodes, which suffer from liquid junction potential problems due to salinity variations (Whitfield et al., 1985; Cai et al., 1998). The spectrophotometric method, which is widely used in open ocean research, needs further validation in coastal waters. While there are limited sustained measurements of pH and other inorganic carbon chemistry parameters in the coastal ocean, there are sufficient observations from a variety of sources to obtain a first-order idea of variations and expected increases. The main drivers of pH variability in the coastal ocean are changes in temperature, salinity, currents/upwelling, and biological processes. Often a combination of these processes acts synergistically on pH.

Table 2 provides a rough estimate of expected ranges of variability in pCO_2, pH, and aragonite saturation state compared with annual pH decrease due to anthropogenic CO_2 uptake. Upwelling heavily influences the U.S. West Coast and thus large ranges in pH and pCO_2 have been observed (Feely et al., 2008). These upwelled waters have a decreased buffering capacity due to high DIC values. Many fresh waters have limited buffering capacity as well because of the low alkalinities of these waters. The greatest excursions along the East and Gulf Coasts are attributed to fresh water input and annual temperature changes. Since variations in seasonal temperature and biological productivity are greater in coastal regions, larger excursions in pH, pCO_2, and saturation states are encountered than in the open ocean.

Table 2. Approximate ranges of inorganic carbon system parameters for seawater and freshwater[a].

	SST	SSS	DIC	TA	pCO_2	pH	Ω(Ar)[c]	Annual pH[d] change
Freshwater	0	0	329	300	375	7.591		0.0023
	25	0	329	300	860	7.360		0.0023
Seawater	0	35	2178	2300	280	8.161	1.80	-0.0027
	25	35	2178	2300	808	7.773	2.07	-0.0026
Mississippi	20	0	2468	2500	375	8.290		-0.0022
Eastern Boundary	10	34	2345	2400	1000	7.692	1.02	-0.0008
	20	34	2035	2300	375	8.061	2.97	-0.0019

[a] These values are for illustrative purposes only. For each scenario the SST, SSS, pCO_2 and TA are prescribed to representative values encountered in the environment. The DIC, pH, and aragonite saturation state (Ω(Ar)) are calculated with the functional dependence of dissociation constants with temperature and salinity as described in Dickson and Millero (1987) using the program developed by Lewis and Wallace (1998). DIC and TA have units of μmol kg^{-1} and pCO_2 is μatm.

[b] pH at *in situ* temperature on the seawater scale

[c] Saturation state of aragonite. Values <1 indicate that the mineral phase will dissolve. Saturation state values for fresh water are poorly constrained and therefore not presented.

[d] This is the annual change in pH for surface water assuming the pCO_2 increases by 2 μatm yr^{-1}. The example shows that seasonal and interannual changes in pH due to changes in temperature and inorganic carbon chemistry can be up to two orders of magnitude greater than the annual decrease in pH due to uptake of anthropogenic CO_2.

c. Empirical data to demonstrate spatial and temporal variability of pH in near-coastal waters;

There are few high quality measurements of the spatial variability of pH or carbon system parameters in U.S. coastal waters. Feely et al. (2008) demonstrated that on the west coast of North America, the seasonal upwelling of subsurface waters along the coast brings CO_2-enriched waters onto the shelf and, in some instances, all the way to the surface ocean. These waters had pH variations that ranged from 7.6 to 8.2, depending on the state of local upwelling and primary production is surface waters. It appears that the upwelled water, in addition to its original high level of CO_2 resulting from natural respiration processes in the subsurface layers, also contains anthropogenic CO_2 that was sequestered by the water when last in contact with the atmosphere ~50 years ago. An immediate consequence of this additional CO_2 is that the CO_2 concentrations in these upwelled waters will be greater than they would have been in pre-industrial times. Furthermore, each ensuing year will draw on water that has been exposed to the atmosphere still more recently, resulting in yet higher CO_2 levels. Since these "ocean-acidified" upwelled waters are presently undersaturated with respect to aragonite they already pose a potential threat to many of the calcifying aragonitic species that live along such coasts.

In the East and Gulf coasts of U.S., upwelling of deep water has less influence, whereas river export has a strong influence on pH and carbonate saturation in near-coastal waters. River water typically has a lower pH and carbonate buffer capacity (i.e., TA and DIC) relative to seawater (Cai and Wang, 1998; Cai et al., 2008). In the Southeast shelf and the Middle Atlantic Bight, mid-size rivers originating from hills/mountain areas (i.e., "piedmont rivers") have relatively high TA (0.5-1.1 mM) and pH (~7.0) (Cai and Wang, 1998). Small rivers from coastal plains (i.e., "black water rivers") have high humic content but low TA (<0.4 mM) and pH (<6.0) (Cai and Wang, 1998). Humic substances significantly influence pH and carbonate chemistry in these nearshore waters (Cai et al., 1998). However, the pH in these river, estuarine, and nearshore waters quickly approaches seawater values (~8.1). In sharp contrast, in the Mississippi River, the largest in North America, TA (~2.5 mM) and pH (~8.2) are quite high. During plume mixing, after an initial small decrease due to thermodynamics, the pH often increases to a very high value of 8.4, and is accompanied by very low pCO_2 (100 μatm) and DIC (maximum removal up to 400 μM) due to biological uptake of CO_2 at intermediate salinities (15-28 PSU) (Cai, 2003; Lohrenz and Cai, 2006). Then, pH decreases to the seawater endmember value. A recent discussion of the potential effects of river export on coastal pH and carbonate saturation on a larger scale is available in Salisbury (2008).

In the Great Lakes, biannual (spring and summer) open-lake surveys conducted by the EPA include pH and TA measurements. In Lake Superior, TA (mean = 840 $\mu eq/m^3$) exhibits little variability in the open lake, but is persistently higher at river mouths. The pH varies widely across the Lake (range = 6.7-8.0 at $25^{\circ}C$, 7.2-8.3 at observed temperatures). There is concern about the accuracy of the pH data, particularly given evidence of significant systematic bias in electrode pH measurements in fresh water (mean offset = -0.137 pH units, French et al., 2002). Additionally, significant temperature corrections are needed in the reported data as samples are warmed to $25^{\circ}C$ before pH measurements are made (Atilla et al., 2009). These data do not indicate trends in Lake Superior pH from 1996-2006, but it is likely that if there were trends, they would be masked by the significant uncertainties in the data.

d. Methods to statistically evaluate variability of pH in near-coastal waters;

While there are very few direct time-series measurements of pH in U.S. coastal waters, the approaches for evaluating overall trends are generally the same as for the open ocean pCO_2 data (Bates, 2001; Takahashi et al., 2009). This approach consists of a two-step process of: 1) determining seasonal changes in pCO_2 on the basis of the monthly mean values computed from multi-year composite data. Values for months with no measurement are estimated by a linear interpolation using adjacent mean monthly values. Difference between a monthly mean and the annual mean represents the correction to be applied for deseasonalization of the monthly mean. Assuming that the seasonal variability and hence the deseasonalization corrections remain unchanged with time, the monthly mean values obtained for years outside the multi-year composite period are corrected to obtain deseasonalized values. 2) The deseasonalized multi-year values are corrected for temperature changes and plotted as a function of time to obtain the long-term trend in the data (Takahashi et al., 2009). For open-ocean conditions, the long-term pCO_2 trends generally agree with the atmospheric CO_2 increase. In coastal regions, changes in primary production and/or circulation can cause competing or synergistic effects that can further complicate the deseasonalization and interpretation of the overall trends.

e. Other approaches (e.g., carbon chemistry), methods and indicators that could reflect ocean acidification.

There are a few empirical approaches that have been used for estimating carbon chemistry from hydrographic data sets from field observations. For example, the multi-linear regression (MLR) analysis method has often been used to fill data gaps in data-sparse basins via interpolation and extrapolation. The MLR method takes advantage of statistical correlations between frequently measured properties (e.g., temperature, salinity) and the target property of interest. The MLR model usually takes the following form:

$$\begin{aligned} Y = I &+ [\alpha_1 (X_1 - m_1) + \alpha_2 (X_2 - m_2) + \dots + \alpha_p (X_p - m_p)] + \dots \\ &+ [\beta_1 (X_1 - m_1)^n + \beta_2 (X_2 - m_2)^n + \dots + \beta_p (X_3 - m_3)^n] + \dots \\ &+ [\gamma_1 (X_1 - m_1)(X_2 - m_2) + \gamma_2 (X_1 - m_1)(X_3 - m_3) + \dots] + \varepsilon \qquad (2) \end{aligned}$$

where Y is a dependent variable (in this case the unknown that you wish to extrapolate), X_p is the pth independent variable, and m_p is the mean of the X_p values. I is an intercept and α, β and γ are partial regression coefficients of linear, polynomial and interaction terms, respectively. The final term ε is an unexplained random error. This approach has been used to extrapolate DIC and TA data in surface waters from global data sets into regions where such data are unavailable (Lee et al., 2000; 2006). A similar approach can be used to estimate other carbon system parameters from hydrographic data sets collected from shipboard and moored observations in coastal waters. However, since coastal ecosystems have varying amounts of freshwater inputs with uniquely different alkalinity-salinity relationships, this approach should be validated locally with high-quality carbon chemistry measurements on a seasonal basis.

2. EPA is soliciting technical information on effects of ocean acidification on marine biota, including:

a. Survival, growth, reproduction, and recruitment of reef-building corals and crustose coralline algae;

Our response addresses coral and coralline algae separately:

CORALS

- ***Survival.*** No studies to date have shown that a lowering of seawater pH alone (at least within the range of surface ocean pH values expected for this century) will directly affect coral survival rates. Polyps of two species of corals (*Oculina patagonica* and *Madracis pharencis*) tripled their biomass in waters with pH values of 7.3-7.6, although the skeletons of these corals had completely dissolved leaving the coral polyps with an anemone-like existence (Fine and Tchernov, 2007). Net organic production in coral communities does not seem to increase under elevated CO_2 conditions (Langdon et al., 2003). Indirect effects of ocean acidification on reef corals may threaten their survival more than direct effects. These include:
    - <u>Slower calcification rates.</u> Several studies suggest that coral survival will be indirectly impacted by ocean acidification because of its effects on skeletal growth. In the extreme example above, the vulnerability of "naked" corals to predation and physical damage in a natural reef environment would be greatly increased. It is unlikely that ocean pH will be lowered to levels that will lead to complete decalcification of corals. However, several reviews (Kleypas et al., 2006b; Kleypas and Langdon, 2006) list multiple ways that reduced skeletal growth may impact coral survival rates, because it may: 1) decrease a coral's ability to compete for space, 2) decrease its ability to withstand hydrodynamic and erosional forces, 3) delay its age of sexual maturity, 4) increase the rate of fragmentation and thus in some corals, the rate of asexual propagation, 5) alter the skeletal light-gathering properties for photosynthesis, and 6) alter gamete production and larval development (although see below) or larval recruitment. While many of these hypothesized impacts seem logical (*e.g.,* disappearance of calcifying organisms with proximity to a natural CO_2 vent on a shallow sea floor (Hall-Spencer et al., 2008)), it should be noted that most have not yet been explicitly tested.
    - <u>Increased sensitivity to temperature.</u> In concert with rising ocean temperatures, ocean acidification may have an additional impact on coral survival rates, as it has been shown to be a factor in coral bleaching. The bleaching temperature threshold was lower in corals (*Acropora intermedia* and *Porites lobata*) when pH was decreased to levels expected this century (Anthony et al., 2008).
    - <u>Increased bioerosion rates</u>. Bioerosion rates of coral skeletons by euendolithic algae increases significantly with decreasing pH (Tribollet et al., In press). Low inorganic cementation rates and high bioerosion rates have been

documented in naturally low pH regions such as the Galapagos Islands, but it is not clear whether low pH is the main factor controlling these rates amongst other factors such as temperature and nutrient concentration.

  - Loss of habitat. Coral survival may eventually be impacted if coral reef structures move from a state of construction to destruction, as is predicted to occur this century (Silverman et al., 2009). The habitat loss associated with a shift in the balance of hard ground to sediment ratios, loss of reef structural complexity, and a decrease in habitat diversity may have an impact on survivorship of certain species.

- ***Growth***
  - Decreased skeletal growth in reef-building corals is now a well-documented consequence of ocean acidification (see reviews: Kleypas et al., 2006a; Doney et al., 2009). A wide range of responses has been observed, but on average, a doubling of preindustrial atmospheric CO_2 values results in about a 10-50% decrease in calcification rates of reef-building corals. Langdon and Atkinson (2005) found that in the suite of experimental data on corals and coral communities through 2006, a doubling of atmospheric CO_2 concentration produced two clusters of responses: a 40-83% reduction in calcification or a 1-8% reduction. They attributed this dichotomy to differences in sensitivity across species and/or differences in experimental setup. The response is reversible (calcification will increase if pH is increased). There is no evidence yet that corals can adapt to these changes. It is not yet clear whether calcification rates of non-reef building corals are equally impacted.

  - Most studies have been conducted in the lab, under non-varying conditions, but the results are consistent with recent studies that allowed the control and simulated future pH conditions to vary naturally (Jokiel et al., 2008; Kuffner et al., 2008). This is important because coral reefs waters can naturally exhibit large diurnal swings in pH (*e.g.,* 7.9-8.1 (Bates et al. 2001; Suzuki and Kawahata, 2003)), and it was unclear whether the added suppression of pH by 0.1-0.3 pH units projected for this century would have a significant effect.

- ***Reproduction and Recruitment***

The effects of ocean acidification on coral recruitment encompass responses in gamete production, fertilization rates, planula larvae, larval settlement, and post-settlement growth. There are very few data on any of these aspects. A brief summary of observations are listed below:

  - Jokiel et al. (2008) showed that *Montipora capitata* was able to produce gametes under conditions consistent with a doubling of today's atmospheric CO_2 levels. The same study illustrated that larvae of *Pocillopora damicornis* were able to recruit under the doubled CO_2 conditions.

  - Albright et al. (2008) studied larval settlement and post-settlement growth in *Porites astreoides* cultured under seawater conditions with pCO_2 levels of 380 ppm, 560 ppm, and 720 ppm. The percentage of larval settlement did not differ between treatments, but relative to the 380 ppm treatment, post-settlement growth (skeletal extension rate) was decreased by 50% and 78% in the 560 and 720 ppm treatments, respectively. Albright et al. (2008) also noted that success in coral recruitment, metamorphosis, and growth depends on substrate characteristics, and particularly with microbial biofilms and crustose coralline algae (Heyward and Negri, 1999; Negri et al., 2001; Webster et al., 2004; Williams et al., 2008). Reef-building CCA have been shown to be quite sensitive to ocean acidification (see Kuffner et al., 2008 and Anthony et al., 2008 below). In addition to CCA, the articulated coralline algal genus *Amphiroa* is known to induce settlement of bivalve larvae (Williams et al., 2008), and is also considered sensitive to ocean acidification as evidenced in the overall community response to ocean acidification in the Biosphere 2 coral reef experiments (Langdon et al., 2000). Thus the effects of ocean acidification on coralline algae may affect the recruitment of corals and perhaps other organisms such as bivalves.

CRUSTOSE CORALLINE ALGAE (CCA)

- ***Survival***

CCA are distributed worldwide from the tropics to the polar seas, and are some the deepest living photosynthetic organisms in the ocean. Almost all secrete skeletons of high-Mg calcite, which, depending on the Mg content, is a particularly soluble form of calcium carbonate that is typically more soluble than aragonite. Latitudinal variation in CCA mineralogy and ecology is high, so the response of CCA to ocean acidification is likely to vary considerably within this group. Tropical reef-building CCA, however, appear to show a consistent decrease in both production and calcification in response to ocean acidification.

  - Increased sensitivity to temperature. Similar to the effect of ocean acidification on coral bleaching (described above), the coralline algae *Porolithon onkodes* showed a loss of pigmentation that was increasingly sensitive to warming at decreasing pH (Anthony et al., 2008). A similar effect was observed in the temperate CCA *Lithophyllum cabiochae* (Martin and Gattuso, 2009): temperature-induced mortality in this species was 2-3 times greater when pCO_2 was elevated from 400 ppm to 700 ppm.
  - Field observations. The abundance of coralline algae decreases rapidly with proximity to a shallow submarine CO_2 vent, which suggests that coralline algae could not survive in the low pH waters near the vent (in pH < 7.7, Martin et al., 2008; Hall-Spencer et al., 2008).

- ***Growth***
  - Productivity. One study has examined the effects of pH on daily net production (photosynthesis minus respiration) of the common reef-building algae *P. onkodes* (Anthony et al., 2008). Relative to net production at normal pH (8.0-8.4), net production at pH levels of 7.85-7.95 and 7.6-7.7 was decreased by 50% and 100%, respectively.

  - Slower calcification rates and higher skeletal dissolution rates. CCA may be more sensitive to ocean acidification because of the greater solubility of their high-Mg calcite skeletons, but well controlled studies of the effects of ocean acidification on CCA growth have only recently been conducted. Rhodolith calcification rates decreased 250% (net dissolution) in mesocosms with CO_2 levels elevated by 365 ppm over present day conditions (Jokiel et al., 2008). The surface area of CCA was also reduced from 25 to 4% in the CO_2-enriched conditions (Kuffner et al., 2008). Anthony et al. (2008) showed that calcification rates in *P. onkodes* decreased by 50% when pH was decreased from 8.0-8.4 to 7.85-7.95, and by 130-190% (indicating net dissolution) when pH was decreased further to 7.6-7.7; an increase in temperature had no significant effect. Conversely, Martin and Gattuso (2009) found that calcification rate of the temperate coralline alga *L. cabiochae* increased by 30% under elevated CO_2 alone (700 ppm versus 400 ppm conditions), but decreased by 10% if temperature was also elevated from 22 to 25ºC. The latter study suggested that *L. cabiochae* might have the capacity to acclimate or adapt to higher pCO_2 levels; but also found that net dissolution of *L. cabiochae* skeletons was 2-4 times higher in the 700 ppm versus 400 ppm conditions (Martin and Gattuso, 2009).

- ***Reproduction and Recruitment***
  - The response of CCA reproduction to ocean acidification has not been explicitly investigated. However, Kuffner et al. (2008) found that CCA recruitment rates in acidified conditions (365 ppm CO_2 higher than untreated conditions) were reduced by 72%.

b. Anticipated persistence of coral reef communities under future pH scenarios;

Predicting the state of coral reef communities under future pH scenarios is complicated. There is incomplete knowledge of how many organisms will be affected by ocean acidification, and how changes in their ecological functioning will affect the coral reef communities. Question 2a above addresses only the main calcifying organisms on coral reefs, but evidence is increasing that noncalcifying organisms, from seagrasses and macroalgae to fish, also exhibit responses to changes in ocean carbon chemistry (Palacios and Zimmerman 2007; Diaz-Pulido et al., 2008; Munday et al., 2009). Substantially more information exists regarding the effects of ocean acidification on marine calcifiers and on coral reef calcification, than exists on other physiological and biogeochemical responses. The following points therefore focus on the persistence of coral reef communities with respect to calcium carbonate production and its role in reef development. It should be noted, however, that there are considerable gaps in our understanding of how ocean acidification will affect reef communities through changes in microbial processes, nutrient recycling, species competition, species symbioses, diseases, and other reef processes, some of which could strongly affect the ability of coral reef communities to persist with continued increases in ocean acidification. The main points regarding reef persistence under future pH scenarios include:

1) Our knowledge of the response of corals and other reef organisms to ocean acidification is growing but still rudimentary, particularly with respect to understanding community response.
2) Predicting regime shifts in marine ecosystems (*e.g*., the shift from a coral-dominated to an algal-dominated system) is impeded by "our limited knowledge of the drivers, the causative agents for change and the internal dynamics of ecosystems." (deYoung et al., 2008).
3) Atmospheric CO_2 increases are causing not only ocean acidification, but also global warming, sea level rise, and other environmental changes. The combined effects of warming and acidification, for example, are rarely additive; rather, they are synergistic or antagonistic (*e.g.,* Reynaud et al., 2003; Anthony et al., 2008; Martin and Gattuso, 2009).
4) The definition of coral reef communities is complicated by the fact that some coral communities, while dominated by corals, are not building reefs (no net calcium carbonate production). While coral reef structures depend on high rates of calcium carbonate production (mostly corals and coralline algae), it is not clear how much coral communities depend on reef structures (Kleypas et al., 2001).
5) Degradation of reef structures will impact existing coral communities. A loss of structural complexity will decrease habitat diversity that is likely to lead to decreased biodiversity (Gratwicke and Speight, 2005). The loss in biodiversity, particularly in combination with loss of coral reef species through bleaching, disease, and overexploitation, threatens the persistence of coral reef and fish communities in the future (Jones et al., 2004). The future level of reef persistence as a function of pH alone is largely unknown, but the likelihood of fewer coral reefs in the future is high (see below).
6) The threshold where coral reefs will shift from net production/accretion to net dissolution/erosion will vary greatly from reef to reef. This is because a multitude of factors determine the overall calcium carbonate budget and reef-building capacity of a reef, such as carbonate production rates (which are a function of the calcification rates of the organisms and their abundance on reefs), dissolution rates, and carbonate removal rates due to erosion and sediment transport. A recent paper that projected future net carbonate production (production minus dissolution) on reefs and estimated that almost all coral reefs would be in a net state of dissolution by the time atmospheric CO_2 concentrations reached 560 ppm (Silverman et al., 2009).

c. Survival, growth, reproduction, and recruitment of other (non-coral) marine calcifying organisms;

Most calcifying organisms investigated to date show reduced calcification in response to increased pCO_2 and decreased $[CO_3^{-2}]$, calcium carbonate saturation state, and pH (*e.g.,* Gattuso *et al*., 1998; Riebesell *et al*., 2000; Langdon *et al*., 2000; 2003; Fabry et al., 2008). The majority of work has tested warm water corals and coccolithophorid algae (Raven *et al*., 2005; Kleypas *et al*., 2006). Evidence suggests that the calcification rate in corals is controlled by the calcium carbonate saturation state (Gattuso *et al*., 1998; Langdon *et al*., 2000; 2003; Marubini et al, 2001; 2003; Leclercq et al., 2002; Ohde and Hossain, 2004; Langdon and Atkinson, 2005; Schneider and Erez, 2006; Silverman *et al.*, 2007), rather than pH or another parameter of the seawater CO_2 system. Because the $[Ca^{2+}]$ in the ocean today is approximately constant (depending predominantly on salinity), changes in the $[CO_3^{2-}]$ are reflected directly as changes in the calcium carbonate saturation state.

c.1. Planktonic calcifiers

The major planktonic calcium carbonate producers are the coccolithophores (single-celled algae), foraminifera (single-celled protists), and euthecosomatous pteropods (planktonic snails). These three groups of calcifiers account for nearly all the calcium carbonate that sinks from the upper ocean to the deep sea. Planktonic foraminifera and coccolithophores secrete tests or shells made of calcite, whereas pteropods form shells made of aragonite, a type of calcium carbonate that is about 50% more soluble in seawater than calcite (Mucci, 1983).

The calcification response of coccolithophores, foraminifera and pteropods to ocean acidification has been investigated in very few species. Most studies have involved bloom-forming coccolithophores and these species (*Emiliania huxleyi* and *Geophyrocapsa oceanica*) show decreased calcification rates ranging from -25 to 66% when pCO_2 is increased to 560-840 ppmv, respectively in lab and mesocosm experiments. In lab experiments with the coccolithophore *Coccolithus pelagicus*, however, Langer et al. (2006) found that calcification did not change with increased CO_2. Moreover, there is evidence suggesting that at least one coccolithophore species may have the capacity to adapt to changing pCO_2 over long time periods. Experimental manipulations show that *Calcidiscus leptoporus* exhibits highest calcification rates at present-day CO_2 levels, with malformed coccoliths and coccospheres at both lower and higher pCO_2 (Langer et al. (2006). Because no malformed coccoliths were observed in sediments from the Last Glacial Maximum (when pCO_2 levels were about 200 ppmv), the authors concluded that *C. leptoporus* has adapted to present-day CO_2 levels.

Data are limited on response of calcification in shelled pteropods and foraminifera to elevated pCO_2 and decreased calcium carbonate saturation state. Presently, published evidence is available for only two of the ~35 species of planktonic foraminifera and only one of the ~34 euthecosome pteropod species (cf. Fabry et al., 2008; Guinotte and Fabry, 2008). While these data suggest both groups reduce calcification in response to ocean acidification, the small number of species tested precludes the identification of general trends. Species-specific responses are likely and it is possible that the calcification rates of some species may not be sensitive to elevated pCO_2, as has been found in coccolithophores (Riebesell *et al.*, 2000; Langer *et al.*, 2006).

In lab experiments with the planktonic foraminifera *Orbulina universa* and *Globigerinoides sacculifer,* shell mass decreased in response to reduced carbonate ion concentration and calcite saturation state, even though the seawater was supersaturated with respect to calcite (Spero *et al.*, 1997; Bijma *et al.*, 1999, 2002). When grown in seawater chemistry equivalent to pCO_2 of 560 and 740 ppmv, shell mass in these species declined by 4-8% and 6-14%, respectively, compared to the preindustrial pCO_2 value. A positive correlation between foraminiferal shell mass and ambient [CO_3^{2-}] is observed in the sedimentary record as a response to known glacial-interglacial changes in atmospheric CO_2 of the past 50,000 years (Barker and Elderfield, 2002).

As $p$$CO_2$ rises and the calcium carbonate saturation state of the surface ocean decreases, euthecosomatous pteropods and foraminifera may secrete under-calcified or thinner structures (Bijma *et al.*, 1999). This is supported by the observed decrease in calcification in planktonic foraminifera in the modern Southern Ocean, as reported by Moy et al. (2009).

c.2 Benthic invertebrates

At least nine multicellular invertebrate phyla have benthic representatives with calcium carbonate skeletal hard parts (Lowenstam and Weiner, 1989). These taxa secrete calcium carbonate in the form or aragonite, calcite, high-magnesium calcite (>5 mole % $MgCO_3$), amorphous calcium carbonate, or a mixture of these calcium carbonate phases. Many benthic calcifying fauna are prominent in nearshore communities and are economically and ecologically important.

In response to an elevated $p$$CO_2$ level projected to occur under the IS92a emissions scenario (~740 ppmv in 2100), calcification rates in the mussel, *Mytilus edulis*, and the Pacific oyster, *Crassostrea gigas*, decreased by 25 and 10%, respectively (Gazeau *et al.*, 2007). When grown for over 6 months in seawater bubbled with air containing 560 ppm CO_2, a decrease in shell growth was observed in the edible snail *Strombus luhuanus* and a reduction in wet weight was reported in both this snail and two species of sea urchins (Shirayama and Thorton, 2005).

Early calcifying stages of benthic molluscs and echinoids show a strong response to increased seawater $p$$CO_2$ and decreased pH, carbonate ion concentration and calcium carbonate saturation state. In the sea urchins *Hemicentrotus pulcherrimum* and *Echinodetra mathaei*, fertilization success, developmental rates, and larval size all decreased with increasing CO_2 concentration (Kurihara and Shirayama, 2004). Abnormal biomineralization of the highly soluble high-magnesium calcium carbonate spicules in urchin larvae was also observed. Green *et al.* (2004) found that newly settled juveniles of the hard shell clam, *Mercenaria mercenaria,* showed substantial shell dissolution and increased mortality when they were introduced to surface sediments that were undersaturated with respect to aragonite ($\Omega_{\mathrm{Arag}} \sim 0.3$), a level that is typical of nearshore, organic-rich surficial sediments. Within two weeks of settlement, the calcium carbonate shells were completely dissolved, leaving only the organic matrix of the shell.

Calcium carbonate skeletal elements are also present in species of other benthic invertebrates such as crustaceans, cnidarians, sponges, bryozoans, annelids, brachiopods, and tunicates. Some of these animals, such as mussels (e.g., Michaelidis *et al.*, 2005), use shell dissolution to support acid-based regulation at high internal $p$$CO_2$.
Apart from warm-water corals, little is known about the effect of elevated ambient $p$$CO_2$ on calcification rates in most of these taxa.

Bibby et al (2007) reported behavioral, metabolic, and morphological responses of the intertidal gastropod *Littorina littorea* to acidified seawater (pH = 6.6). This marine snail produced thicker shells when exposed to predation (crab) cues in control experiments, but this response was disrupted when pH was decreased. The snails also displayed reduced metabolic rates and an increase in avoidance behavior, both of which could have significant ecosystem implications via organism interactions, energy requirements, and predator-prey relationships.

The response of planktonic and benthic calcifying organisms to elevated pCO_2 may not be uniform among species or over time. To date, published research indicates that most non-coral, calcareous organisms show reduced calcification in response to decreased carbonate ion concentrations, however, the limited number of species investigated as well as the small number of studies preclude identification of widespread or general trends. Nearly all studies thus far on the impacts of ocean acidification on non-coral, calcareous organisms have been short-term experiments. Chronic exposure to increased pCO_2 may have complex effects on the growth and reproductive success of calcareous plankton or may induce adaptations that are absent in short-term experiments.

d. Potential changes in community structure and marine trophic links;

d1. Primary productivity

d1a. Phytoplankton and Cyanobacteria

Most species of marine phytoplankton are able to utilize both dissolved CO_2 and HCO_3^- during photosynthesis and, owing to the large pool HCO_3^- in seawater, their growth is generally not thought to be limited by carbon (e.g., Raven, 1997; Tortell et al., 1997; Burkhardt et al., 2001). The relationship between increased CO_2 and primary productivity in the ocean may not be straightforward, however. Evidence from recent laboratory and mesocosm studies suggests that, at least under some conditions, phytoplankton uptake of CO_2 increases under elevated pCO_2 (Hein and Sand-Jensen, 1997; Schippers et al., 2004; Leonardos and Geider, 2005; Riebesell et al., 2007). On the other hand, climate warming increases thermal stratification of the upper ocean, thereby reducing the upwelling of nutrients, and has been linked to observed decreases in phytoplankton biomass and productivity on a global basis (Behrenfeld et al., 2006).

Continued increases in anthropogenic CO_2 may enhance fixation of atmospheric nitrogen and total primary productivity in warm, nutrient-poor tropical and subtropical regions. Nitrogen-fixing cyanobacteria in the genus *Trichodesmium*, which support a large portion of primary productivity in such low nutrient areas of the world's oceans, show increased rates of nitrogen and carbon fixation under elevated pCO_2 (Hutchins et al., 2007; Barcelos e Ramos et al., 2007) . At CO_2 levels of 750 ppmv, *Trichodesmium* increased CO_2 fixation rates by 15-128% relative to present day CO_2 conditions (Hutchins et al., 2007).

d1b. Seagrasses

Seagrasses represent one of the most biologically rich and productive marine ecosystems in the ocean (Guinotte and Fabry, 2008). They create critical nursery grounds for juvenile fishes and important habitat for adult fishes, invertebrates, and mollusks.

Several higher order and endangered species rely on seagrasses for a significant portion of their diet (e.g. dugongs, manatees, and green sea turtles). Seagrass ecosystems are a critical component to maintaining the biological diversity of the oceans and could be one of the few ecosystems that stand to benefit from increasing levels of CO_2 in seawater. Seagrasses are capable of dehydrating HCO_3^- , but many appear to use CO_2(aq) for at least 50% of their carbon requirements used for photosynthesis (Palacios and Zimmerman, 2007). Zimmerman et al. (1997) found that short-term (~45 days) CO_2 (aq) enrichment increased photosynthetic rates and reduced light requirements for eelgrass (*Zostera marina* L) shoots in laboratory experiments. The benefits from CO_2-enriched seawater, however, may be offset by the negative effects of increased temperature on vegetative growth (Ehlers et al 2008).

d2. Secondary productivity and food web impacts

If reduced calcification decreases a calcifying organism's fitness or survivorship, then some calcareous species may undergo shifts in their distributions as the inorganic carbon chemistry of seawater changes. Calcifying species that are CO_2- sensitive could potentially be replaced by non-calcifying species and/or those species not sensitive to elevated pCO_2.

By 2100, surface waters of polar and subpolar regions are projected to become undersaturated with respect to aragonite (Orr et al., 2005). Shelled pteropods are important components of the plankton in high latitude systems, with densities reaching 1000's of individuals m^{-3} (e.g., Bathmann et al., 1991; Pane et al., 2004). If pteropods require seawater that is supersaturated with respect to aragonite, then their habitat would become increasingly limited, first vertically in the water column and then latitudinally, by the shoaling of the aragonite saturation horizon over the next century (Feely et al., 2004; Orr et al., 2005). If high latitude surface waters do become undersaturated with respect to aragonite, pteropods could eventually be eliminated from such regions, with consequences to food web dynamics and other ecosystem processes (Fabry et al., 2008). In the subarctic Pacific, for example, pteropods can be important prey for juvenile pink salmon (*Oncorhynchus gobuscha*). Armstrong et al. (2005) reported that a single species of pteropod (*Limacina helicina*) comprised 15 to 63% by weight of the diet of juvenile pink salmon.

Planktonic ecosystems are complex, nonlinear, and the consequences of ocean acidification on this ecosystem are largely unknown (Guinotte and Fabry, 2008). The interactive effects of elevated pCO_2 and other climate change variables such as temperature could result in substantial changes to species diversity and abundances in many regions. Species interactions may be altered on multiple trophic levels, potentially impacting ecosystem productivity, as well as the cycling of organic carbon and calcium carbonate.

e. Variability of effects in tropical, temperate and polar regions;

Surface ocean pH, carbonate ion concentration and calcium carbonate saturation exhibit large geographical variations from the tropics to the poles under pre-industrial conditions reflecting variations in temperature and seawater chemistry. In cold polar waters, aragonite saturation is lower, and closer to a threshold of 1, the boundary between undersaturated (<1) and oversaturated (>1). It is expected that undersaturated conditions with respect to aragonite will occur in the next 1-2 decades in the Arctic and 2-4 decades in the Antarctic (e.g., Orr et al., 2005; Steinacher et al., 2009). The crossing of this geochemical threshold to undersaturated conditions in polar regions could result in calcium carbonate dissolution unless the organism has some mechanism(s) to prevent dissolution. However, many of the laboratory studies discussed above document biological sensitivity to reduced carbonate ion concentration simply when the extent of the calcium carbonate supersaturation is reduced, but remains well above 1. Therefore, the biological consequences of reduced calcium carbonate saturation state are complex and may differ among particular biological species, communities, and ecosystems. The rate and magnitude of chemical change may be more relevant to biological impacts than simply an absolute value of specific parameters of the inorganic carbon system. More research on field populations is needed to identify areas of particular susceptibility.

f. Estimates of response rates (e.g., rapid, gradual, non-linear) of populations and communities to ocean acidification;

Response rates of populations and communities of marine organisms to ocean acidification have largely been investigated through experimental manipulation work using cultured or collected marine organisms, or natural communities (Langdon and Atkinson, 2005; Hare et al., 2007; Riebesell et al., 2007; Fu et al., 2008). These types of studies typically compare biological responses at a range of CO_2 concentrations chosen on the basis of projected future atmospheric levels. Many investigators have chosen to use present day atmospheric CO_2 concentrations at the time of each study (~360-390 ppm) as a control treatment, and a projected end-of-the-century value of ~750-800 ppm as an acidified treatment. An unstated assumption in these studies is that rates of response in experimental organisms will be more or less linear across the chosen CO_2 interval. For instance, (Feng et al., 2008) found that calcification in coccolithophores decreased by about 50% between 375 and 750 ppm CO_2. Assuming a constant response rate in these organisms, this translates to an annual calcification rate decrease of about 0.4-0.5% at the current atmospheric CO_2 enrichment rate of ~3-4 ppm/year. However, it is clear that response rates of various marine organisms will differ tremendously, and that frequently such responses can be non-linear

Not all biological effects due to ocean acidification will be negative. The growth of the nitrogen fixing cyanobacterium *Trichodesmium* is now known to be stimulated by increasing CO_2 (Hutchins et al., 2007; Ramos et al., 2007). (Hutchins et al. 2007), for example found that nitrogen fixation rates increased by 63% between 380 and 750 ppm CO_2, but then reached a plateau beyond which no further increases occurred even when CO_2 was increased as high as 1500 ppm. This suggests a major biological response of this group to ocean acidification could come over the next century, but that subsequent CO_2 increases could have little additional impact. Primary production has been shown to increase, particularly in concert with warming, in another, non-nitrogen fixing cyanobacteria group, *Synechococcus*, which is one of most abundant photosynthetic organisms on the planet.

In contrast, (Gazeau et al., 2007) showed that calcification of mussels and oysters decreases linearly by 25% and 10%, respectively, as CO_2 is increased up to 740 ppm. If CO_2 is increased above 1800 ppm, however, mussel shells dissolve completely. This finding highlights the importance of understanding potential threshold effects, beyond which responses cease to be linear. Although these thresholds are not yet established for most marine organisms, it is clear that if acidification is allowed to proceed unchecked, ocean pH is likely to reach and exceed such thresholds for numerous economically and ecologically critical species in the relatively near future. Further research is badly needed to establish what these “tipping points” may be for key marine organisms and communities.

g. Adaptability to ocean acidification and broad implications for ecosystem resilience;

Because of inherent temporal limitations in experimental work, the type of organismal response to ocean acidification that we know most about is that of acclimation. Acclimation in this context is the short-term ability of existing individuals and populations to rapidly adjust their physiology to cope with changing pH. While these acclimation responses may yield valuable information to help predict possible longer-term responses, it is important to realize that populations may also respond to long-term pH shifts through true adaptation and evolution. These longer-term processes imply stable, long-term phenotypic shifts that contribute to organismal fitness, or even selection for favorable new variant traits arising through mutation. The potential for marine organisms to adapt and evolve in response to selection by changing pH remains one of the least understood aspects of the ocean acidification field.

Changes in populations mediated by adaptation and evolution usually occur only over many hundreds or thousands of generations, and so typically require extended periods of time. Important clues about how the marine biota may respond to the current anthropogenic acidification event can be found in the paleo-oceanographic literature, since analogous events involving ocean-wide acidification and warming are thought to have occurred in the geological past (Zachos et al., 2005). Sedimentary records of changes in communities of calcareous plankton suggest that marine assemblages likely adapted to some degree to such a natural event, and so suffered only moderate losses of diversity (Bown and Pearson, 2009).

However, even those past ocean acidification events that are considered to be very rapid on geological timescales probably occurred over at least 1000 to 10,000 years (Zachos et al., 2005). In contrast, the current anthropogenic acidification is happening much more quickly, over mere decades or centuries. It is far from clear if or how marine organisms will be able to adapt to pH changes happening at this unprecedented rate. Evolutionary adaptation to acidification also seems much less likely in organisms such as vertebrates and some invertebrates that have long generation times relative to the timescales of ongoing chemical changes in the ocean. Microorganisms, with their short generation times of hours to days, would seem to have the best chance to truly adapt and evolve in response to ocean acidification. Long-term projects using genomic and metagenomic methods are needed to assess whether accelerating anthropogenic acidification is indeed driving microbial evolution.

A way to address these questions experimentally is by maintaining long-term cultures of model marine organisms at altered pH levels. An example of this type of study was presented by Collins and Bell (2004), who examined the long term evolutionary responses of cultured marine phytoplankton to high CO_2 over 1,000 generations. Perhaps not surprisingly, they found that no new specific adaptations to low pH appeared over the course of their selection experiments; novel complex metabolic pathways and capabilities probably take much longer than this to arise through evolution. However, they did find that some cell lines in their experiments lost capabilities such as photosynthetic carbon-concentrating mechanisms (CCMs) that allow them to grow at today's lower CO_2 concentrations. Once the selective pressure to retain these low-CO_2 adaptations was removed through long-term growth at high CO_2, random mutational degradation of CCM genes occurred, rendering these cell lines incapable of growth at lower CO_2 levels. One implication of these findings is that if today's marine organisms are forced to adapt to a higher CO_2 environment, some may lose crucial physiological capabilities that would allow them to survive if and when human remediation efforts are eventually able to return ocean pH to normal pre-industrial values.

Another approach to looking at adaptation is to examine "natural experiments" in which organisms are subjected to low pH, high CO_2 conditions in situations such as when seawater chemistry is influenced by submarine volcanic venting. Since these vents subject local benthic marine organisms to long-term acidification, presumably they can be used as indicators of the capacity for adaptive responses. Hall-Spencer et al. (2008) showed that such naturally acidified communities responded in ways that support some of the conclusions of short-term experiments. That is, acidic conditions reduced the ability of calcifying organisms to survive and build shells, effectively minimizing their contribution to the benthic community. In these acidified sites, their place was taken by the proliferation of non-calcareous, invasive brown algae. Tunnicliffe et al. (2009) found that calcifying mussels were able to grow near highly acidic vents, but they had much thinner shells and would therefore likely be more vulnerable to predation. The results of these natural acidification experiments must be interpreted cautiously for a number of reasons. For instance, other changes to seawater chemistry accompany volcanic venting that are unlikely to occur with anthropogenic ocean acidification, such as sulfide and trace metal enrichment. Nevertheless, these pioneering studies of low pH-adapted marine communities caution us against making the simplistic assumption that most marine organisms can fully compensate for acidification impacts through evolved resistance, if given enough time to adapt.

h. Methods or estimates of the combined and relative importance of ocean acidification in concert with other natural and anthropogenic stressors (e.g., storm damage, pollution, overfishing).

One of the least understood aspects of the ocean acidification problem concerns the effect of ocean acidification when combined with other environmental stressors. The impact of multiple stressors is most acute for sessile organisms such as coral reefs (Anthony et al., 2009; Desalvo et al., 2008; Maynard et al., 2008; Zeevi-Ben-Yosef and Benayahu, 2008). Moreover, if calcification of reef-formers is inhibited by ocean acidification, then the ability of such reefs to withstand the more extreme storm events (expected with global warming scenarios (Huntington, 2006)) would be diminished.

Thus, increased erosion would be expected to result as the reef's ability to rebuild was compromised by ocean acidification. A more intensified water cycle would also result in greater freshwater runoff, which would exacerbate coastal ocean acidification impacts, since freshwater runoff doesn't have anywhere near the buffering capacity of seawater, thus causing even greater pH fluctuations.

Another environmental variable that is expected to be changing due to anthropogenic CO_2 emissions is temperature. Warming can cause acute stress to organisms, such that when combined with lower pH, might yield highly non-linear interactions. The literature on the impacts of thermal stress is enormous, too much to review here. Thermal stress can occur at the cellular level (causing disruption in cell physiology) or the community level (indirect changes in the environment). For example, increases in ocean temperature can affect ocean stratification, which will inhibit the vertical diffusion of nutrients in the sea, leading to nutrient-limited growth (Goes et al., 2004). Gregg et al. (2003) showed reduced global ocean chlorophyll levels corresponding with increases in sea surface temperature, which was hypothesized to be a function of increased stratification. Compounding these impacts with the additional physiological stress imposed by ocean acidification could mean dramatic changes in the ability of specific communities of algae to grow and survive.

Another example of compound effects of multiple stressors is the introduction of toxins to the marine environment along with ocean acidification, for which little work has been done. Much more work has been done on the recovery of lakes from multiple stressors (toxins, acidification, temperature, UVB) (Gunn and Sandoy, 2001; Keller et al., 2003; Keller, 2007). This body of literature has shown that the impact of multiple stressors at the community level is extremely difficult to predict, especially when there is the potential for arrival of exotic species (both competitors and predators) into the ecosystem (Keller, 2007). Optimal approaches for studying the first-order effects of multiple stressors will likely utilize controlled experiments with multifactor analysis of variance (which will also include interaction terms). For whole ecosystem studies, however, such an approach will be much more difficult to design, and reproducibility of the experiments likely more tenuous, due to the stochastic nature of invading species. The time-scales of these multiple stressors could be equally difficult to demonstrate if one stressor has a short-term impact (*e.g.,* temperature) while the other has a longer-term impact (*e.g.*, acidification).

3. EPA is soliciting scientific views on the information presented in the bibliography of this notice.

The study of ocean acidification is a fast moving field and new studies are now being published rapidly on the effects and potential effects of increased pCO_2 in seawater on marine and coastal organisms. It will be important that EPA is familiar with these and with the techniques that are currently being standardized within the scientific community to achieve highest level of accurate and reproducible results in implementing future studies that include collection of pH data.

The bibliography of the notice contains pertinent references to the field of ocean acidification and in general has cited scientifically well-respected, peer-reviewed studies or reviews of studies. We suggest that the Best Practices Guide [Dickson, A. G., Sabine, C. L., and Christian, J. R. (Eds.): Guide to best practices for ocean CO_2 measurements, PICES Special Publication, 3, 191 pp., 2007 (and pH references within) http://cdiac.ornl.gov/oceans/Handbook_2007.html] also be utilized for establishment of technically accurate marine or saltwater pH data.

4. EPA is soliciting information related to EPA's current CWA 304(a) recommended pH criterion for marine waters, including how the criterion could be best expressed, particularly with respect to natural variability.

First, it is important to reiterate that a pH criterion alone is insufficient to evaluate effects of ocean acidification on, for instance, marine calcifying organisms. While a decline of the seawater pH may be indicative for adverse ocean acidification effects in some organisms, experimental studies have shown that calcifying organisms such as corals are sensitive to the carbonate ion concentration ($CO_3^{=}$), or the carbonate mineral saturation state (Ω_m) of seawater ('m' stands for the mineral calcite or aragonite; for a discussion of carbonate chemistry parameters, see Section 1 of this response and Zeebe and Wolf-Gladrow, 2001). A seawater carbonate chemistry criterion should therefore include at least two parameters of the CO_2 system. Possible combinations include pH and the carbonate ion concentration (pH-CIC), or pH and the carbonate mineral saturation state (pH-Ω_m). Since Ω_m differs between calcite and aragonite, a pH-CIC criterion may be easier to implement.

If carbonate chemistry changes are to be evaluated for given initial conditions (*e.g.,* if preindustrial TCO_2, TA, temperature, and salinity for a given surface ocean location are known), and changes are solely due to invasion of anthropogenic CO_2, then the pH and $[CO_3^{=}]$ decline are not independent of one another. Nevertheless, for evaluation purposes, two CO_2 system parameters should always be reported based on which water quality criteria may be established (see Zeebe et al., 2008 and Supporting Online Material).

4.1 Shifting pH threshold

It is very likely that pH criteria established based on critical pH thresholds for various species and processes of those species will shift over time in response to changes in other environmental conditions. In particular, as temperatures rises it is likely that this stress will make corals and perhaps other calcifying organisms more sensitive to reduced carbonate ion concentration and pH. As a result the lowest acceptable pH for healthy coral growth would have to be revised upwards as temperature increases. The study by Reynaud et al. (2003) found that at 25°C a reduction in pH(sws) from 8.04 to 7.85 had no significant effect on the rate of calcification of the stony coral *Stylophora pistilata* while at 28°C the same reduction in pH caused a 50% reduction in the rate of calcification. These data indicate the important and strong interaction between temperature and pH that must be taken into account when attempting to establish a safe pH criterion for a species.

4.2 pH threshold process-dependent

It should be recognized that individual species may have more than one process that is sensitive to environmental pH. As a case in point, it is well known that the rate of calcification of adult coral colonies is sensitive to the pH of the seawater through its impact on the aragonite saturation state. On average, a doubling of pCO_2 (560 ppm) results in a 30% reduction in calcification rate (range 10-50% Langdon and Atkinson, 2005). Studies on the effects of pH on the early post-settlement growth of coral larvae show that they can be more sensitive to reduced pH than the calcification of the adult form (Albright et al., 2008), i.e. a 50% reduction for a doubling of pCO_2, equivalent to a pH(sws) of 7.9.

Establishing a pH-CIC criterion for marine waters will have to take into account the latest results of research on pH and saturation state effects on marine organisms. The range of tolerable pH-CIC changes (where "tolerable" means "without substantial detriment to organism fitness") is as yet unknown for many marine organisms. Despite enhanced efforts during the last few years, additional studies (such as long-term experiments) are required to establish the range of tolerable pH-CIC changes for various organisms. However, it is clear at this stage that the response of the various marine organisms to ocean acidification will be inhomogeneous. It is possible that some organisms such as nitrogen-fixing cyanobacteria could benefit from elevated CO_2 (Hutchins et al., 2007). The following represent the types of studies that must be considered when developing recommendations for a pH-CIC criterion.

In laboratory and mesocosm studies, a decrease of 0.2 to 0.3 units in seawater pH (corresponding to a drop of 30-40% in $[CO_3^=]$ in tropical surface waters) slows calcification in many marine organisms, including corals (Kleypas et al., 1999; Langdon et al., 2000), foraminifera (Bijma et al., 1999), and some calcareous plankton (Riebesell et al., 2000; Wolf-Gladrow et al., 1999). Langdon et al. (2000) reported a 37-48% decline in experimental coral calcification, when Ω_{arag} decreased by 23%. Silverman et al. (2009) projected an 80-100% decrease in gross calcification (relative to the preindustrial rate) in 75% of coral reefs around the globe by the time atmospheric CO_2 reaches 750 ppmv. This corresponds to a ~50% reduction in $[CO_3^=]$ in tropical surface waters. At a community $CaCO_3$ dissolution rate of 20%, most reefs would therefore become net dissolvers when atmospheric CO_2 reached 750 ppmv. The projections were worse when a parameterization of future bleaching estimates were included in the calculations (Silverman et al., 2009). These and other studies (see current reference list and references in Raven et al., 2005; Kleypas et al., 2006; WBGU, 2006) should be taken into consideration when establishing a pH-CIC criterion.

Most recently, studies have been published that provide the first indications of field evidence of ocean acidification effects on marine calcifying organisms. For example, De'ath et al. (2009) reported declining coral calcification on the Great Barrier Reef in Australia, though they were unable to attribute the decline to a particular mechanism (acidification, warming, bleaching). Moy et al. (2009) provide evidence for reduced calcification in modern Southern Ocean planktonic foraminifera. In addition to laboratory and mesocosm experiments, these types of field observations, particularly time-series, will be very helpful when establishing pH-CIC guidelines.

Natural Variability

It is important that even if future baseline changes in ocean carbonate chemistry would not exceed the extreme values of the preindustrial/present natural variability, this does not imply that organisms will be able to cope with anthropogenic changes. For example, coral reef communities experience significant diurnal variations in carbonate chemistry (*e.g.,* Frankignoulle et al., 1996), including temporarily low pH and [$CO_3^{=}$] due to reef community metabolism or other causes. Such natural variations are superimposed on a long-term background state or 'baseline'. As anthropogenic CO_2 enters the ocean, the baseline pH and [$CO_3^{=}$] decrease. As a result, calcifying organisms will experience higher average acidity and lower saturation state on a long-term basis and even more extreme minimum values due to the superimposed natural variability. Thus, it is expected that adverse acidification effects on marine organisms as observed in laboratory studies (usually under steady conditions) also apply to the natural environment, where organisms may be exposed to greater variability (this statement only refers to variability, not to other issues such as long-term adaptation). As a corollary, it is also expected that global net ecosystem calcification will decline under decreasing pH and [$CO_3^{=}$], despite potentially large natural variability. This conclusion is supported by the results of recent mesocosm experiments with corals and coralline algae (Kuffner et al., 2008; Jokiel et al., 2008; Andersson et al., 2009).

5. **EPA is soliciting information regarding State and Territorial implementation of the pH criterion related to new information on ocean acidification.** (OCB has decided not to tackle this question).

6. **EPA is soliciting potential strategies for State and Federal water programs to coordinate and enhance Federal data collection efforts, including:**
    a. **Approaches to designated uses for water quality standards that account for different pH regimes (e.g., specific designated uses for areas with organisms that may be more sensitive to significant pH fluctuations such as coral, shellfish, other calcifying organisms) (CFR 131.10, for additional information on designated uses http://www.epa.gov/waterscience/standards/about/uses.htm);**

The EPA should coordinate its water quality monitoring and data synthesis activities with the other Federal agencies, the States, and local municipalities to provide comprehensive long-term data sets of pH and carbon system parameter changes in estuarine and coastal waters. This could be done through the auspices of the Joint Subcommittee on Science and Technology Interagency Working Group on Ocean Acidification (as required by the Federal Ocean Acidification Research and Monitoring Act of 2009). In developing an observational network for ocean acidification we recommend initiating repeat surveys and moored observations to address ocean acidification issues. Time-series stations are urgently needed in coastal regions. Consequently, new moored buoys equipped with carbon system and pH sensors for ocean acidification should be deployed. Bio-optical sensors and optical plankton imaging systems could also be deployed to track possible shifts in abundances of key functional groups. Individual monitoring strategies and physiological response studies for specific ecosystems should be formulated by regional expert teams and coordinated at the national Interagency Working Group level.

b. Scientifically defensible approaches to set and monitor pH criteria.

7. **EPA is soliciting information that may be used to develop guidance and information on ocean acidification pursuant to Clean Water Act Section 304(a)(2) for States and the public. This information may include information on the mechanisms of ocean acidification, methodology development for analysis, and statistical analysis.**

There is great urgency among members of the national and international research communities to plan and conduct viable and compelling experiments that will improve our understanding of the potential biogeochemical and ecological impacts of ocean acidification. An international research workshop on best practices for ocean acidification research took place November 19-21, 2008 in Kiel, Germany. The workshop, which was co-sponsored by the European Project on Ocean Acidification (EPOCA), the International Ocean Carbon Coordination Project (IOCCP), the Ocean Carbon and Biogeochemistry Program (OCB), and the Kiel "Future Ocean" Excellence Cluster, convened approximately 40 scientists from 10 countries to establish an international agreement on best practices for ocean acidification research. The workshop focused on seawater carbonate chemistry, experimental design of perturbation experiments, measurements of CO_2-sensitive processes, and data reporting and usage. The participants have drafted a "Guide to Best Practices in Ocean Acidification Research and Data Reporting" that is currently undergoing open community review.

Building on this effort, OCB is developing a hands-on ocean acidification short course for Fall 2009 that will convene members of the biological and chemical oceanography research communities to gain mutual insights on optimal ocean acidification experimental design. The short course will build on the recommendations from the "Guide to Best Practices in Ocean Acidification Research and Data Reporting", and provide a mechanism for educating scientists on appropriate chemical and biological techniques and protocols related to ocean acidification.

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