Response to review of:
Effects of increased *p*CO2 and geographic origin on purple sea urchin (*Strongylocentrotus purpuratus*) calcite elemental composition.

M. LaVigne, T.M. Hill, E. Sanford, B. Gaylord, A.D. Russell, E.A. Lenz, J.D. Hosfelt, and M.K. Young

Biogeosciences Discuss., 9,1-34 (2013).

Author response in bold italics:

We would like to thank Maria Byrne for her constructive review of our manuscript. The comments, questions, and suggestions raised in the interactive discussion have greatly improved the manuscript. In this study we examined (1) the geochemical composition of purple sea urchin (Strongylocentrotus purpuratus) skeleton precipitated during both adult and early life history stages; (2) potential differences in geochemical composition among individuals originating from regions spanning a broad latitudinal range encompassing a spectrum of oceanographic regimes; and (3) the impact of ocean acidification on Mg and Sr incorporation into larval and juvenile S. purpuratus skeleton in culture. Both reviewers identified the strengths of the manuscript as being (1) and (2) above, and raised important questions that have strengthened our interpretation of (3) in the revised manuscript.

Below are our point by point responses (in bold italics) to all issues raised in Maria Byrne's review. The manuscript has been revised accordingly.

M. Byrne (Referee)
Received and published: 22 January 2013

The work by LaVigne et al is a very interesting approach to investigate the mineralogy of the sea urchin skeleton along a gradient of increased pCO2 along the coast of California. The title indicates the manuscript it about pCO2 effects – but in fact this work is more a straight forward study of skeletal mineralogy. Thus I suggest a change to the title to better reflect the content, I suggest something like The mineralogy of the skeleton of Sp from populations along the California upwelling/pCO2 gradient and potential effects of increased pCO2 on mineral composition of the adult and juvenile skeleton. The mineralogy in itself is very nice and important data – so it will be better to highlight the strengths of the study – not the marginal data.

The title has been changed to highlight the mineralogy/elemental composition components of the study that both reviewers found to be most compelling and down play the larval culturing results. Updated title:
THE ELEMENTAL COMPOSITION OF PURPLE SEA URCHIN (*STRONGYLOCENTROTUS PURPURATUS)* CALCITE AND POTENTIAL EFFECTS OF *p*CO_2 DURING EARLY LIFE STAGES

The results with respect to the influence of pCO2 are equivocal for two reasons –

1. Strong biological control of mineralization in sea urchins
While the strong biological control on mineralization makes urchins poor <u>recorders</u> of the ambient environment, we would argue that this biological control on mineralization and elemental composition could make urchins more sensitive to ocean acidification. Thus, if biological mineralization pathways are sensitive to ocean acidification and result in changes in skeletal composition, this could impact skeletal solubility. The results of our study, however, suggest that these calcification pathways are somewhat resilient during the adult and juvenile phases, except for larvae originating from the warmest, most buffered site. This evidence for possible geochemical plasticity during early life stages indicates that calcification early in development may be more sensitive to ocean acidification than adults. (A highlight pointed out by reviewer #2).

2. Low sample size of the laboratory studies
We note that the n=2-4 referenced in the review represents 2-4 replicate samples each composed of 20 pooled metamorph skeletons. Thus, each average Mg/Ca and Sr/Ca value is an average of 40-80 individual metamorphs. This does not affect the statistical treatment of the data, and we agree that a greater degree of replication would be best, however, this sample pooling approach was necessary in order to obtain enough material for a single ICP-OES analysis. This point has been noted in the methods of the revised manuscript. Nevertheless, we have edited the discussion of the larval culturing data as noted below:

Thus I suggest that many statements need more balance due to low power of the analyses – for instance p. 17955 – the data do not really 'demonstrate' the role of elevated CO2.
Based on the advice of both reviewers, we have revised the manuscript to highlight the strengths of basic mineralogy component of our study, and down-played several statements regarding the strength of the culturing data (including the sentence suggested above). We have revised the text in several sections of the manuscript to discuss the larval culturing data as "potential or preliminary evidence" for geochemical plasticity in early life stages that we hope will encourage further study (changes made to: Results and Discussion, Conclusion, and Abstract).

Specific comments
What is the carbonate chemistry along the gradient? For instance does the local water vary in Sr.
Our group is involved in recent monitoring efforts to quantify the degree of spatial and temporal variability in carbonate parameters of intertidal ecosystems along the west coast of the U.S. A recent manuscript submitted to a special issue in this journal summarizes the first year of pH data from these locations (Hofmann et al., submitted: Biogeosciences). These data were not available at the time of submission, however, we have included a summary of the pH data in the revised version of the manuscript. In short, the Oregon site experiences the greatest frequency of low pH events as a result of

upwelling, whereas the Southern California site experiences the warmest and most buffered conditions.

Were the progeny of each population exposed to local water?
The culturing experiment was carried out at Bodega Marine Laboratory (BML; Northern CA), and the progeny of all populations were reared in culture using coastal water local to BML that was pre-equilibrated with experimental CO_2 conditions. We have clarified this point in the methods of the revised manuscript.

It would be good to know the elemental composition and saturation states of water from the different sites. I think some of this information is available in the Hofmann monitoring data?
While the carbonate chemistry parameters are now coming available from the Hofmann et al. monitoring network, bottle sampling for elemental analyses are not yet a part of this effort. Although we do not have direct measurements of Sr/Ca and Mg/Ca in seawater samples from the adult urchin collection sites, text and references have been added to the methods section to explain why seawater Sr/Ca and Mg/Ca variability along this latitudinal transect are likely relatively minor. Sr, Mg, and Ca are all conservative ions that generally scale with salinity, with very little variability globally (<0.5%). This suggests that the Mg/Ca and Sr/Ca ratios compared among sites are likely to be relatively constant.

An n=2-4 is very low. In the methods explain what the replication of each measurement was so that we can understand the level of replication for each ANOVA.
Text has been added to the methods to clarify that each sample of 20-pooled metamorphs contained only enough skeletal material for one ICP-OES analysis.

The ANOVA would have had an unbalanced design and this needs to be explained.
Our limited sample size prevents us from randomly dropping replicates to obtain the same degree of reproducibility for all populations in our ANOVA. Thus, we acknowledge that limited sample availability has resulted in an unbalanced design of our ANOVA and small number of samples per population. We have added the following sentence to the Results and Discussion section to clarify this point: "Given the small number of samples from each population and the unbalanced design of our ANOVA, we cautiously interpret these results as providing preliminary evidence that skeletal 'foreign ion' incorporation in the population from Southern California responded differently to elevated CO_2."

On p. 17950 first para – move info to the methods stating which samples were too small for analysis.
Details have been added to the methods section to identify the cultured metamorph samples that were excluded from analysis due to low sample size.

The 5 juveniles that were reared for 5.5 mo – where they put back in control conditions or maintained in the experimental conditions? I presume they were fed – need details.

After the completion of the culturing experiment, the 5.5 month old juveniles were reared in a seawater table using non-prequilibrated seawater. Thus, the juveniles were reared in neither control (equilibrated to 385ppm) nor experimental (1000ppm) conditions. They were fed a mixed diet of several benthic diatom species. This section of the manuscript has been updated to include these details.

I wonder about the citations of the Ries paper – on page 17943 a big change in skeleton mineral content is quoted – but on p. 17954 – the lack of change in the present study is said to be consistent with lack of response to OA by Atlantic urchins. This is a bit confusing.
We included the reference to Ries 2011 in the introduction to emphasize possible effects of CO_2 on echinoderm calcium carbonate. This paper is a key example of the mixed response across taxa with ocean acidification. Ries 2011 is also one of the only experimental studies that has identified a CO_2 impact on echinoderm elemental ratios—for only some taxa. The reference to this work was somewhat vague. This sentence has been edited to clarify that only coralline red algae exhibited a 20% decrease in Mg/Ca, while serpulid worm tubes showed a 16% increase (no urchin taxa demonstrated a change in Mg/Ca).

What did Ries attribute the big change to?
Ries implies that this range of geochemical responses could be a result of species-specific calcification mechanisms (this point is stated in the text). The specific mechanism is difficult to identify, crystal growth rate, however is ruled out by the authors.

Differences in calcification rates is suggested to be a potential rational of the slight difference in the mineral content. Please explain what is meant by rate. Is this growth of the skeleton? For instance knowing from the Yu et al. papers that Sp larvae from the region as this study grown in high CO2 are smaller – does this mean that smaller (but same age) larvae that are growing more slowly have a different skeletal mineralogy?
We have removed much of the discussion of Sr/Ca changes as an indicator of growth rate from the manuscript as per Reviewer 2's comments. Instead, a more speculative tone is taken in the section entitled "Possible controls on Mg/Ca and Sr/Ca in S. purpuratus" Text has been added to clarify that future work will be required to identify whether a relationship exists between skeletal Sr/Ca and skeletal calcification rate before such claims can be made.

p. 17944 – the differences among studies are also due to different methods see Byrne 2012 – Mar Environmental Research
A reference to Byrne 2012 has been added to specify that differences in experimental methods could contribute to different responses to experimental acidification.

What would an 8% increase in Sr mean for the larvae?
In response to both reviewer comments, the text (particularly the Conclusion and Abstract) has been edited to highlight the different geochemical responses observed in the adult vs. early life history stage data. The broader relevance of this study includes

the geochemical resilience of the adult stage calcite in comparison to the possible evidence for geochemical plasticity observed in the Southern California early developmental stages. While further research is required to confirm the causes and specific impacts of geochemical plasticity, these changes in elemental composition could affect the skeletal solubility and may be a result of mechanisms such as changes in calcification rate. However, more work is required to explore these ideas.