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***Interactive comment on “Anthropogenic stressors and eutrophication processes as recorded by stable isotopes compositions in coral skeletons” by O. Levy et al.***

**Anonymous Referee #1**

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Anthropogenic stressors and eutrophication processes as recorded by stable isotopes compositions in coral skeletons by Levy, et al.

**GENERAL COMMENTS:**

This paper addresses a serious problem in the Red Sea and elsewhere in the world, aquaculture in open ocean pens. This is a serious issue given the increasing global demand for seafood and the need for aquaculture to meet that demand. The authors' goal of detailing a geochemical tool for assessing the impacts of fish farming on coral reefs, in both the past and present is critically important for our understanding of how these operations impact the surrounding environment, and how we must manage these new

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industries to mitigate habitat degradation. The study consisted of two components. The first was a transplant experiment, where small colonies of a hard coral were explanted to an area near fish pens, and a distant, pristine location. The colonies were monitored, stained to assess growth, and later the growth bands were sampled for stable isotope analysis of both C and O in the carbonate. The second part of the study examined three coral cores. Two cores were sampled in the northern tip of Eilat, and one core was sampled at a distant location to the south. Annual bands of the cores were analyzed in similar fashion. The authors found that O isotope ratios tracked SST, and did not vary between sites in both the transplant experiment and coring study. C isotopes in corals transplanted to the fish pen area and the core taken closest to the pen apparently exhibited an enrichment, whereas corals from other sites were unchanged through time. The authors conclude that carbon isotope values of coral skeletons (indirectly) record a signal of intensive fish farming and the resulting “eutrophication” of surrounding waters, though these effects may occur at small spatial scale ( $\sim 1$  km).

While the problem and question are important and of broad interest to marine biogeochemists and coral reef conservationists and managers, at this time I feel that the manuscript is critically flawed and not ready for publication.

#### SPECIFIC COMMENTS:

Firstly, the manuscript is unclear throughout, which is driven by a lack of editing prior to submission. Poor word choice and grammar, redundancies, and mislabeling really impair the readability of the paper. I mention this first because it may be the case that this paper can be saved with some rigorous re-working which may diffuse some my following concerns.

Second, the manuscript lacks a clear hypothesis and mechanism for the proposed relationship between fish farming and skeletal isotope ratios. As the conclusions are completely reliant on the authors’ observed increase of  $d^{13}C$  values my review will focus on these results.

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My understanding is that hard corals acquire the vast majority of skeletal C from seawater. I would like the authors to explain how fish farming would affect the carbon isotope ratio of seawater DIC? In the introduction there is no discussion of the sources of C used by corals, natural sources in this environment, or how these sources would be affected by mariculture. I am left to assume that the authors contend that carbon from fish food and excrement, and/or increased heterotrophy on plankton, increased by farm nutrients is being recorded in the coral skeleton? This reasoning depends on a heterotrophic pathway for C into the coral skeleton, which is an idea that has some support in the literature. Grottoli (2000, 2002) reported increasing  $\delta^{13}\text{C}$  as a result of intensive feeding on brine shrimp. However, in Grottoli (2000), corals exposed to reduced amounts of natural zooplankton also had increasing skeletal  $\delta^{13}\text{C}$ . More recently, Reynaud et al (2002) showed that corals treated with 12 weeks of controlled feeding were no different than unfed controls with respect to skeletal  $\delta^{13}\text{C}$ . Swart et al (2005) may also have some evidence for heterotrophic  $\text{CO}_2$  incorporation into the skeleton, through the indirect analysis of respired  $\text{CO}_2$ . Even so, any contribution to heterotrophy would still be quite small relative to DIC. Given the varied influence of heterotrophy on skeletal  $\delta^{13}\text{C}$ , the authors need to clearly detail the hypothesis and the reasoning behind it in the introduction. The indirect role of N, from phytoplankton and/or fish farm waste I think is a good place to begin, with reference to the discussion in Grottoli (2000). All of this information should be considered in the interpretation of the results in the Discussion section, which is currently very sparse and offers little debate over the alternative hypotheses and future directions for this work.

My perfunctory perusal of the recent literature on C isotopes in coral carbonates has pointed at light as a strong driver of  $\delta^{13}\text{C}$  (Omata et al. 2008, 2005; Rodrigues & Grottoli 2006). Indeed, Levy et al. rightly address light as a confounding variable, and rule out its influence by controlling for depth, and alluding to consistent monthly light measurements across the sites. Given the strong potential for light to influence the interpretation of their results, the authors should report these data. Along these lines, the caption of Figure 1 says “coral colonies transplanted from the navy base to under

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the fish cages. . .”. If the transplants were under the fish cages, how could light not be reduced? This needs to be clearly addressed.

Third, the details of the statistical analyses are completely lacking. If the data were interpolated with software, what was the variation in the raw values? How were the data interpolated aside from using a tool described elsewhere? How was ANOVA used to analyze the d18O and d13C time-series data? Was this done with the individual data, or did the authors use something like ANCOVA to compare the trends? Did they use raw or interpolated data for statistical analyses? How are t-tests used to determine “marked alteration in the pattern and amplitude of the annual d13C relative to “normal” growth period prior to transplantation”? Were they one-tailed or two-tailed tests? To me, the trends in Figure 4 look noisy and very similar. I would like to see a clear explanation of how you analyzed the data to conclude that core A was different from B and C.

Lastly, Fig. 5 strikes me as a nice way to frame the problem. It leaves me wondering, if there is so much input of feed and discharge, and these pollutants carry a large quantity of nitrogen, why then did the authors choose to study C and O isotopes, instead of C/N elemental ratios or d15N? d15N would certainly show an enrichment in the skeletal organic matrix and the living coral tissues as a result of incorporation of organic and inorganic N from fish food and waste, assuming that the fish food is derived from fish, and thus represents a higher trophic level than a coral would occupy (Marion et al 2005; Risk 2009). Measuring d13C seems to be a very indirect metric to address this specific question.

Some other concerns:

- 1) Can the authors be clearer on the species studied? Species specific differences can be an important source of variability (Rodrigues & Grottoli 2006).
- 2) I am also concerned about the design of the coring study. The authors routinely call the three locations, a “gradient”, but I am skeptical. Sites A and B are only ~1

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km removed. Indeed the “gradient” is more east-west than north-south. Also, I am concerned about the sample size of three individual cores. In a previous paper, Levy et al (2006) call for increased sample sizes for coring studies, owing to the observation of high variation among cores sampled from a small atoll. Replicate cores at the “pristine” and fish pen sites would be much more powerful.

3) The authors dismiss anthropogenic nutrients from Eilat as a confounding factor in this study. If sewage treatment effluents do not enter the marine environment it is worth including some supportive evidence either from this work or the literature.

4) Figure 2 confused me. After some time, I realized that the authors might be plotting the data as superimposed on the coral (i.e. a base to tip trend in isotope ratios?). If so please state this in the caption so the reader can quickly orient to the figure. Also, the inset labels (e.g. “FCI”) should be made horizontal. Furthermore, the FC site abbreviations should be included in the text and on the map.

5) If mariculture is a threat to the reefs of Eilat, can you explain why there were no differences in growth rates between the transplants to the fish pens and those taken to the remote site?

6) Include a heading for the methods section. I feel that the method section should include the site information that is now in the introduction. The information presented on the fish pens and Eilat is good.

#### TECHNICAL CORRECTIONS:

Pg 7658 Title & Line 4: “isotopes compositions” should be “isotope compositions”. However, I feel that “isotope values” or “isotope ratios” are more appropriate and should be revised throughout the manuscript.

Line 10: I’m confused by the use of “fractionation” there was no mention of fractionation as driving the variation in  $d^{13}C$ , and there was no variation in  $d^{18}O$ . I don’t think you can say fractionation here when you haven’t mentioned the sources at all.

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Line 13: Be more specific about what “long-term processes” are.

Line 16: Delete first sentence.

Line 20: I’m confused by “different species specific combinations of algal photosynthetic products”. Please revise.

Line 25: “monopolize” seems like an odd word-choice here.

Line 26: two uses of “coral reefs” are redundant.

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Line 12: I suggest, “Stable isotope variations in coral skeletal d18O and d13C reflect. . .”

Line 16: I suggest deleting this paragraph as it is redundant.

Line 18-20: Use of “Although” is causing some confusion. The authors state that usually reefs are affected by one or [more] stressors and Eilat is no different as it has many stressors. Using “although” sets the reader up to think Eilat is different in some way.

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Line 1: delete “the symbiont”

Line 16: “remote” is not a “condition”

Line 23: suggest revising “long-drilled”

Pg 7661

Line 11: “Addition” should be “Additional”

Line 26 & 27: While it is nice to report long-term analytical variation, it is more relevant for this particular study. For example, the long-term variation could mask any substantial drift in a single analytical run. I suggest reporting the precision for the run, indicate what standards were used, and what values you are reporting (standard deviation?).

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Line 4: I now believe that there is a line missing from the page breaks. This is what I see on the printed and electronic version so please check with the editor about this. As it is, there is some confusion about the reference to the location of A and B which needs to be revised.

Line 7: replace “details” with “resolution”

Line 11: replace “does” with “did”

Line 13: please clarify if  $A \neq B = C$ ?

Line 26: please be specific about the “change” you refer to.

References: Please include a heading. Also, I notice that out of the 30 papers cited, only 7 are from the last 5 years.

#### WORKS CITED IN THIS REVIEW:

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Levy, O., Rosenfeld, M., Yam, R., Shemesh, A., 2006. Heterogeneity of coral skeletons isotopic compositions during the 1998 bleaching event. *Limnol Oceanogr* 51(2), 1142-1148.

Marion, G.S., Dunbar, R.B., Mucciarone, D.A., Kremer, J.N., Lansing, J.S., Arthawiguna, A., 2005. Coral skeletal  $\delta^{15}\text{N}$  reveals isotopic traces of an agricultural revolution. *Marine Pollution Bulletin* 50(9), 931-944.

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Interactive comment on *Biogeosciences Discuss.*, 7, 7657, 2010.

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