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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_14413
Title of the Manuscript:	Seasonal occurrence of <i>Gymnodinium catenatum</i> and Paralytic shellfish Poisoning in the Mediterranean shore of Morocco
Type of the Article	

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PART 1: Review Comments

	Reviewer's comment	Author's comment <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<u>Compulsory</u> REVISION comments	Summary: This work describes a 3 year sampling study looking at the concentration of the PSP producing dinoflagellate <i>Gymnodinium catenatum</i> and its correlation with the presence of PSP toxins in the mussel species <i>Mytilus galloprovincialis</i> in a commercial growing site in Fnideq on the Mediterranean coast of Morocco. Results showed a correlation between the concentration of <i>G. catenatum</i> and the levels of PSP toxins in mussels. Primary mussel toxicity testing was performed using an in-vivo mouse bioassay. Follow up testing of samples shown to be positive using the mouse assay were analyzed for PSP toxin composition using HPLC with toxin derivatization and fluorescence detection. Although the portion of the study showing the correlation between <i>G. catenatum</i> concentration and mussel toxicity, by mouse assay, is interesting and useful, there are several serious concerns with the follow up toxin composition analysis, especially considering the authors claims of the presence of novel PSP toxin congeners that are not routinely monitored for in most of the shellfish management programs in other parts of the world. These concerns are detailed below: 1. The authors state that they used as standards STX, GTX2/3,	1. These toxins were analysed but their corresponding chromatograms were not shown. This methodology has a complex interpretation and toxin groups can have some degree of overlapping because different toxins produce the same oxidation products. This is why toxin groups were identified. The study did not aim at comparing in detail <i>G. catenatum</i> with mussels, as no Moroccan cultures were available. 2. Not available in culture. A concentrated sample requires collecting several liters of seawater at the appropriate time, or else sensitivity is not achieved - It was from a laboratory culture. 3. This is known from in-house fractionation of extracts with COOH cartridges, as described



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	dcSTX, dcSTX2/3, B1, and C1/2 purchased from the certified reference materials program run by the National Research Council (NRC) in Canada. Additional PSP standards are available from NRC, such as GTX1/4, dcGTX1/4, and NEO. It is not explained by the authors why these toxins were not analyzed for. 2. In figure 3, the authors state that panel A is the toxin profile for a <i>G. catenatum</i> sample from the coast of Portugal. Why didn't the author's test a concentrated water sample from their own study? The cell concentrations were certainly high enough. It is also not stated whether the profile given for the Portuguese sample is from a natural water sample or for a laboratory culture. This could potentially change the ratio of toxins present. 3. Even in the presented toxin profile for <i>G. catenatum</i>, there are several questions. The authors show peaks for C3/4 and B2. How do the authors know that these fluorescent peaks are those compounds since no reference materials are commercially available? The authors have one peak labeled as "GTXs" but do not explain why all of the GTXs would be in one peak for this analysis. Lastly, the authors provide no justification for the identification of the later eluting peaks as GCs-sulfo, GCs-GTX, and GCs-STX, especially without the inclusion of any reference standards for these compounds. If this is detailed in other papers, at least some of that information should be repeated here since that is one of the main novel aspects of the paper. 4. The lower panel for this figure is even more confusing. The authors do not explain why there are separate labels for "dcGTXs" and "C3/4+dcGTXs" as well as "dcSTXs" and "B2+dcSTXs". Also, there are a lot of unlabeled peaks in this chromatogram. Especially considering the lack of standards for many of the labeled peaks in	in Lawrence method (2005), followed by acid hydrolysis. More justifications were done in other papers: Bustillos-Guzman et al., 2011; Gu et al., 2013, now included in this revision. No standards are available. Identification has been done by spectra or wavelength ratios in cases of trace levels. 4. These compounds produce several oxidation products. Explained in text. Naturally fluorescent were not labelled.
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	this figure, I would suggest also running the sample without derivatization. Any fluorescent peaks still present without this step are not PSP related and there are several naturally fluorescent compounds that can be found in shellfish and co-elute with some of the PSP toxins.	
<u>Minor</u> REVISION comments	Additional Comments: I have several additional comments on the paper, detailed below, that could potentially help the authors should they choose to re-work the paper and re-submit. 1. Throughout the paper there are inconsistencies in the use of italics, eg. Lines 37-38 "A. catenella" and "compressum". 2. The references are formatted inconsistently. 3. More detail is required for describing the HPLC procedure used, especially in relation to the derivatizing step. 4. The action level for PSP toxins in shellfish in Morocco is not provided. A line showing this level would be useful in Fig. 2. 5. There are now several widely recognized, and some AOAC approved, HPLC-FLD methods for the detection of PSP toxins, with either pre-column or post-column oxidation. I would recommend the authors use one of these recognized methods as it may fix several of the methodological issues with the paper and strengthen the results and conclusions.	1. Corrected 2. Corrected 3. Improved, but were already described in previous papers. 4. The sanitary threshold adopted by the National regulations is 800 µg STXeq./Kg of meat.
<u>Optional/General</u> comments		