



SDI Review Form 1.6

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	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	The paper provides interesting information on a significant topic for human health. It is well written with a concise style. However, some arguments have to be better addressed. 1. Materials and methods: the number of samples analyzed by HPLC is not clear. Please specified. If it was done on all samples please compare the results with the mouse assay tests. 2. Materials and methods: the phytoplankton analysis is not well described. Uthermol's method is ok but I was wondering if the all phytoplankton community was observed, if only harmful species were identified. If such data are available it can useful to understand which is the contribution of <i>G. catenatum</i> to the phytoplankton or if other harmful algae occurred. 3. Being available both chemical instruments and the mouse assay I would expected the authors to propose an alternative method to the use of mammals. Before reading the entire manuscript I expected to find comparison between HPLC results and mouse assay. The proposed experimental design is, in any case, good and complete. I don't want to jeopardise the paper publication for the use of mice but I strong recommend for future investigations to search for alternative assay (see also part 2 here below). I would suggest for future investigations to compare mouse assay results with those of other methods as suggested by literature. Beyond the ethical issues related to the use of mammals to test toxins, this method was found to be inaccurate and statistically too variable (http://www.fao.org/docrep/007/y5486e/y5486e07.htm). In several country the National regulations indicate the mouse assay as the method to verify toxin occurrence, I do not know which are the rules in Morocco (see point 4), but I think that	1. It was specified in the manuscript. 2. It has primarily targeted <i>G. Catenatum</i> because according to the work already achieved in the same area it is the species that is still responsible for the contamination of the shellfish (Taleb et al., 2001). 3. In Morocco the mouse assay is the method to verify PSP toxins occurrence.



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	researchers should propose better and more reliable methods. 4. In the text a discussion lacks on the National regulations (if any) that indicate when mussels have not to be sold due to toxin concentration. Due to the present findings, was mussel collection interrupted? How much hazardous were the concentrations for human health? In the discussion line 310, it is said that values were always below safety threshold but I would require a major description of mechanism controlling the mussel production in the area. 5. In the discussion session the results of mouse assay were not compared with international literature. Despite the method is not so accurate it is necessary to compare data with the ones from other regions	4. In the National regulations the sanitary threshold is 800 µg STXeq./Kg of meat. If the analysis of shellfish revealed higher than 800 µg STXeq. / Kg of meat, the authority prohibited the exploitation of shellfish in the area.
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<u>Minor</u> REVISION comments	The English is good enough but I would recommend a further check. Line 11 add "light inverted microscope" Line 36 "<i>Alexandrium tamarense</i> (Lebour) Balech" Line 37 correct in "<i>A. catenella</i> (Whedon & Kofoid) Balech" "<i>Gyrodinium catenatum</i> H.W. Graham" After the citation at line 37 please use throughout the text the form "<i>G. catenatum</i>" as at lines 61, 63, 66, 141, 145, 146, 201, 202 and so on. Line 53 delete extra parenthesis Lines 62-63 rephrase "to the appearance of both <i>G. catenatum</i> and the genus <i>Alexandrium</i>" Line 77 "water sampling was collected" In the abstract it is said that samples were collected simultaneously, but in lines 75-78 it is said that tide level was different. Were samples collected in the same days at different hours? Or in close days? Please specify or delete simultaneous at line 8. Fig. 1 add some location name in the upper map...at least Atlantic Ocean, Mediterranean Sea, Morocco... Check spaces where "min" is used at lines 127, 128 Line 130 check point before GCs Lines 133-134 which unit of measure is "_L"? Line 147, 203, 211, 216 correct "L⁻¹" Line 330 "Vale (2008) reported..."	All corrections have been made in the manuscript.
<u>Optional/General</u> comments	Ethical issues : Yes, there are. The mouse bioassay is worldwide used and accepted in the determination of toxin occurrence, although the use of mammals rises several ethical issues and it is recognized to be inaccurate . The research carried out in the present paper has to be accepted but I strongly recommend the author to start the use of alternative methods (pharmacological assays, immunoassays, chemical or separation assays and alternative bioassays) for the future investigation...(see a wide literature on it)	