

Responses to Editor comments (Walters et al_R2):

Responses to Editor's General comments 1-7.

1. ***The editor has formatted the manuscript to match the journal's style. Please retain the same.***
Thank you, we have retained the editor's formatting. Regarding the formatting of the Reference section, we used the JoVE style file in Endnote and this produced formatting that was different from what was provided by the JoVE editor.
2. ***Please address all the specific comments marked in the manuscript. We have addressed all specific comments.*** Please see our responses below.
3. ***For the protocol section, please ensure that the steps are discrete action steps, which describe how to perform the action providing all specific details.*** We have revised the protocol section to ensure that each step describes a discrete action.
4. ***Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). Details about the step can be included as a note but notes should be used sparingly and concisely. Further details can be presented in the discussion section.*** We have reviewed each protocol step to ensure that in all appropriate places the imperative tense is used.
5. ***Each step should only contain 2-3 action. Also, we cannot have paragraphs of texts in the protocol section.*** We have revised the protocol section so that no step contains more than 3 actions.
6. ***Once done please highlight 2.75 pages of the protocol including headings and spacings to be used for filming purpose.*** The instructions ask for up to 2.75 pages, not exactly 2.75 pages. We have highlighted ~ 2 pages to be used for filming purposes.
7. ***Before submission, please proofread the manuscript well.*** All authors plus and outside reader have carefully proofread the manuscript. All of the authors and the proofreader are native American English speakers and are well published.

Responses to Specific Editor comments:

1. ***The manuscript needs thorough proofreading. Please proofread the manuscript well for any grammar or spelling issues.***
All of the authors and an additional reviewer have proofread the manuscript carefully and on multiple occasions.
2. ***Please remove the in-text citations from the abstract. Please renumber the references accordingly.***
The in-text citation in the Abstract was removed. Please note that one of the expert reviewers had asked us to add a citation to the Abstract and in response it was added.
3. ***Please remove the redundancy from the protocol and make it crisp.*** Make it "crisp" is vague but we appreciate the follow-up guidance we were given. We have tried our best to make it "crisp" by emulating other papers published in JoVE and following the general guidelines. Again, we would welcome and take under advisement any specific suggestions for making the text "crisper".

The protocol should only contain action steps which direct the reader to do something. Please move the discussion about the protocol to the Discussion. If describing the action, please consider making substeps. So, 1 should be followed by 1.1 which should be followed by 1.1.1. Please ensure you answer the how question, how is this done. Please use imperative tense throughout providing all specific details. The Protocol should be made up almost entirely of discrete action steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step. Please ensure all the steps are written in imperative tense. Please avoid the usage of phrases could be, should be, would be, etc. it should only contain actions of the experiment in a stepwise manner. We have done our best to ensure that each Protocol step describes an action with less than 3 substeps. We have used the imperative tense wherever appropriate. If we have missed any we would welcome if specific instances were identified.

4. ***Reorganized the steps for clarity. Please check.*** The reorganization is fine, thanks.
5. ***Citation?*** We have added a citation (#17, Paffenhöfer and Köster 2011) to support the statement that the algal strains used to maintain doliolid culture have previously been used successfully. Page 4, line 149.
6. ***Do you expose the medium to 12 h light and fark cycle? Please check.*** Reference to light:dark cycle has been removed. This remained from a previous edit and was not removed previously.
7. ***This can be removed.*** The Phytoplankon Culture section has been re-written for improved clarity and “crispness”.
8. ***Reworded for clarity. Please make all the following steps crisper.*** OK, thank you for the specific suggestion. As discussed above, this section of the protocol has been extensively re-written.
9. ***Either make it an action step or convert it to a note. Please do not use the phrases could be, should be etc.*** We have made this an action step. See Step 2.5.4 (page 5, line 180-181).
10. ***How?*** How is described in step 3.1 (page 5, line 188) and a citation is now provided (#15, Ohman et al. 2019).
11. ***These details can be moved to the intro/discussion or can be converted to a note.*** This information has been converted to a note (page 5, lines 190-192).
12. ***How do you estimate chlorophyll a?*** Chlorophyll *a* was estimated by *in situ* fluorescence using a rosette equipped with a chlorophyll fluorometer lowered though the water column. This is indicated as action step 3.2.1 (page 5, lines 197-199).
13. ***What is the link between chlorophyll a and doliolids.*** We have added a Note (page 5, lines 201-203) explaining the link between chlorophyll *a* concentration and doliolids.
14. ***Imperative tense and action steps please.*** We find this section improved and “crisper”.
15. ***???*** We don’t understand what you found confusing in this sentence. Please be more specific with your comments. We hope that the revised section is less confusing.

16. ***Please consider making substeps. We cannot have paragraph of text in the protocol section.*** We have revised step 3.5 by using the imperative tense and moving information about avoiding overcrowding to a new Note referencing Table 3 (page 6, lines 220 – 229).
17. ***Reworded please check.*** Thank you for clarifying this step. The changes are fine.
18. ***Maintain how?*** Sentence is revised to describe specific actions needed to maintain cultures (page 7, lines 268-270).
19. ***Please ensure that the steps of the protocol only contain action. Please consider moving discussion about the steps to the discussion section.*** The action indicated in step 4.4 is reduced to a single concise sentence (page 7, lines 284-285) and the additional information provided as a following note (page 7, lines 287-293)
20. ***This is not a step but discussion instead.*** The action indicated in step 4.5 is reduced to a single concise sentence (page 7, 299-300) with additional information proved as a following note (page 7, lines 302-305).
21. ***Please ensure that you describe the result with respect to your experiment, you performed an experiment, how did it helped you to conclude what you wanted to and how is it in line with the title. e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.*** The Results section includes the synthesis of experiences that resulted in the described protocol and describes “Representative Results” of culture-based experiments to estimate doliolid growth rates. All of the Figures and Tables are referred to in this section although because some of them are presented in the Introduction and Protocol sections they are not presented in the Results section in sequential order. This section was organized to resemble other manuscripts published in JoVE.
22. ***Please include a one liner title for all the figures.*** We have revised the legends for Figures 5&6 to include one liner titles.
23. ***As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:***

In our considered opinion the Discussion section does explicitly focus on these elements. We were not aware of a 3-6 paragraph limit. This limit is not mentioned in the Journals Instructions For Authors. It is our considered opinion that the Discussion section, in its current state, is useful and appropriate. This opinion is echoed by the comments of the expert technical reviewers.

 - a) ***Critical steps within the protocol.*** The critical steps in the protocol are discussed in the 2nd through 4th paragraphs of the discussion (pages 11-12, lines 454-491).
 - b) ***Any modifications and troubleshooting of the technique.*** Possible modification and troubleshooting suggestions are provided in paragraphs 2-4 of the Discussion section in association with the definition of the critical culture steps (see response above).
 - c) ***Any limitations of the technique.*** See page 12, lines 503-310 and the last sentence of the Discussion section.
 - d) ***The significance with respect to existing methods.*** To our knowledge there are no published manuscripts that describe methods for the cultivation of doliolids.

e) *Any future applications of the technique.* Please see the last paragraph of the Discussion section.