**Editorial comments:**  
  
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (53550\_R3\_061815.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.   
  
Changes made by the Science Editor:  
  
1. There have been edits made to the manuscript.   
  
Changes to be made by the Author(s):  
  
1. Please upload each Figure individually to your Editorial Manager account as a .png, .pdf, or a .tiff file. Please combine all panels of one figure into a single file.   
  
2. Formatting/Grammar:  
-lines 45-47 – Please correct the grammar in the sentence starting “After hatching, larvae were…” as well as in the sentence after it.  
-Please use consistent units (ie 10 mg/L or 10 mg L−1) I corrected  
-Please correct the grammar in the note under step 2.5.1. I corrected  
-5.2/6.3 – “is described in” instead of “was described at.” OK  
  
3. Additional detail is required:  
-7.9.1 – What standard solutions were used? I added information at 7.9.1.  
-Figures 1 & 2 – Please provide a scale bar for at least one image. I added scale bar  
-Figure 3 – Please define the error.  I added information.  
  
  
**Reviewers' comments:**  
  
**Editor’s Note:**Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please thoroughly address each concern by revising the manuscript or addressing the comment in your rebuttal letter.  
  
**Reviewer #1:**   
*Manuscript Summary:*   
Kataoka and colleagues improved procedures to test the salinity dependence of toxicity for aquatic organisms using fresh water medaka. The authors defined the relationship of the toxicity of the SNCs increasing the deformities of the medaka embryos, obviously increased in conjunction with increasing the concentration of the salinity in the rearing water surrounding the embryos using their new procedures.   
  
*Major Concerns:*  
The content of this manuscript is very interesting, however this reviewer highly recommends that the authors should have somebody who is a native English speaker check the English in their text and who can professionally assist the authors. Some descriptions in the text are a little vague, and the accuracy of their results are compromised by their poor description.   
For example, the use of the term, "egg development", is a little vague. Are you talking about the "embryonic development within the egg"? The phrase "egg development" gives this reviewer the impression that your intention is to say, "oocyte development during the oogenesis", however, my comment does not pertain to the type of description the authors used concerning the phenomena after spawning. After spawning, embryos develop within the egg; however the eggs never develop. Please check the content.   
  
  
Please check the content.   
p. 1 lines 37, 38, & 39  
In this research, different strengths of embryo rearing medium (ERM) (1×, 5×, 10×, 15×, 20×, and 30×) were employed to test the toxicity of silver nanocolloidal particles (SNCs) to medaka eggs.  
What does it mean, "different strengths of embryo rearing medium"? What is "different strength" ? I corrected strength to “concentration”.

p. 2 lines 46 & 47  
The embryos were observed until hatching or 14 days, and then counted hatching rate for 14 days  
Does it mean ".. and then we counted the hatching rate…? There is no subject in the second clause  
  
"counted hatching rate for 14 days"   
Correct the description to "… we counted the hatching rate every day for 14 days".  
  
p. 2 lines 50 & 51  
"The toxicity of the SNCs to medaka eggs obviously increased with increasing salinity."   
  
Correct the description   
The toxicity of the SNCs for medaka embryos obviously increased with the increasing salinity.    
  
p. 2 lines 60 & 61  
"Freshwater environments are more directly affected than saltwater ones by human economic  
and industrial activities."  
  
Change the description:  
"Freshwater environments are more directly affected by human economic and industrial activities than saltwater ones."   
  
p. 2 lines 61 & 62  
"Therefore freshwater environments have been prioritized for testing because they are at high risk from pollution."  
  
Change the description:  
Therefore freshwater environments have been prioritized for testing because they are at higher risk from pollution.   
  
p. 7 lines 267, 268 & 269  
"The effect of salinity on SNC toxicity is very obvious in Figures 1 and 2. Also, we measured phenotypic biomarkers (heart rate, eye size, full body length, and hatch rate) of SNC (10 mg L−1) exposed embryos."   
How obvious? I added information.

Remove "Also"    
"The effect of salinity on SNC toxicity is very obvious in Figures 1 and 2. We measured phenotypic biomarkers (heart rate, eye size, full body length, and hatch rate) of SNC (10 mg L−1) exposed embryos."   
line 270   
  
Correct "In case of heart rate" to "in the case of"  
  
Correct "In case of heart rate in the controls, it was ranged from 29.6….."to "In the case of the heart rates, they ranged from 29.6….. x ERM in the controls."   
  
line 273  
correct "post-hatch larvae" to "larvae" because larvae are not embryos; they have already hatched out.    
  
line 273  
correct "there were no significant difference under control and AgNO3 exposure" to "there were no significant difference between control and AgNO3 exposure"   
What is control here? I compared with the respective 1× ERM solution.  
  
line 275  
Correct "However, the body length was decreased significantly" to "However, the body length decreased significantly"   
  
lines 275 & 276  
Correct "the body length was decreased significantly (P < 0.01) to 4.33, 3.77, and 3.75 mm by  
SNCs exposure" to "the body length decreased significantly (P < 0.01) to 4.33, 3.77, and 3.75 mm due to SNCs exposure"   
  
lines 277 & 278  
"there were no significant difference under control at salinities ranging from 1× to 30× ERM"  
Rewrite this sentence.   
  
line 284  
correct "Decreasing hatch rate indicates the toxic …" to "Decreasing hatching rate indicates the toxic …"   
  
This reviewer realized that the authors need to make a lot of correction in the section of "Discussion," So, this reviewer stopped making corrections here. However, this reviewer highly recommends the authors find a native English speaker, to thoroughly read and correct this part of the article.

The manuscript has been edited carefully by two native-English-speaking professional editors from ELSS, Inc. (elss@elss.co.jp, http://www.elss.co.jp).  
  
*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
  
**Reviewer #2:**   
*Manuscript Summary:*   
N/A  
  
*Major Concerns:*  
It is interesting article using salinity to investgate toxicity. However, this fish species have a very special property on salinity(they could live on fresh water & sea water).  
If the author use other species fish to investigate relationship with salinity and toxicity, I think it maybe difference or couldn't investigate.  
  
Silver nanoparticle could affect on difference ion concentration of medium(water), because silver nanoparticle is a type of ion(Ag+). Differnce concentrion of ion(Na+, Cl-) could be changed the particle size(make AgCl ).  
I coudn't find the result of SEM or TEM to observe the particle size change(before and after). It is very important process in this experiment.

I think image of SEM or TEM need not for this MN, because purpose of this MN is to describe new method of test for the toxicity of chemicals in different salinities by using only a single species of fish.

*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A