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Interactive comment on “Estimation of nutrient contributions from the ocean across a river basin using stable isotope analysis” by K. Nakayama

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A-1 General comments This is an interesting and important paper examining nutrient contributions from the ocean to a river basin using stable isotope analysis. As authors emphasize, estimating the contribution of MDN to the entire river basin is challenging, but necessary to determine the true impact of MDN in riparian ecosystems. However, I'm not convinced about some of the methods, discussions and conclusions of this study. First, the analytical method for escapement of salmon in this study appears to be rough and inadequate, despite the fact that estimation is critical for the calculation of TN transportation. Second, in the abstract the authors state that sea eagles contributed more to MDN transportation for the river basin than bears, but nowhere else in the paper are these results or their implications discussed. Finally, conclusions of this

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study based on stable isotope analysis lack validity. In addition to these general points, this paper includes too many issues to be addressed and is not of sufficient quality to be published in Biogeosciences in its current form. The writing generally lacks clarity as I have elaborated on below in specific comments.

Reply: The manuscript was revised carefully by following your comments. We added more explanation about the methods, and we revised the manuscript thoroughly.

A-2 Abstract Page 5536, lines 5-8: More detailed information about field sampling and data collection should be included here so that readers can understand the paper in its entirety.

Reply: The manuscript was revised by adding more information about field observations.

A-3 Page 5536, lines 12-13: Most salmon-derived nitrogen is excreted as urine rather than as feces (see Hilderbrand et al. 1999). Therefore, evidence from SIA of feces does not support the conclusion. In addition, I can't find any discussion in the main text to support this statement.

Reply: We followed your comment and deleted the sentence pointed out.

A-4 Introduction Page 5536, Line 18: Did the studies cited here investigate the number of SIA studies related to nutrient cycling? If not, please delete them.

Reply: The studies shown in the manuscripts are all related to nutrient cycling or transport.

A-5 Page 5537, Lines 5-6: I think it is redundant to use "suggested" and "may" in this sentence.

Reply: Thank you for your suggestion. "may" was removed from the sentence.

A-6 Page 5537, Line 14: I suggest deleting "of" from this sentence.

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Reply: Thank you for your suggestion. “of” was removed from the sentence.

A-7 Page 5537, Line 25: Do you mean "food web"?

Reply: Sorry about the typo. “food web” is correct.

A-8 Page 5538, Line 2: Please delete the space after the hyphen in "ocean- river".

Reply: Thank you for your suggestion. “-” was removed from the sentence.

A-9 Methods Page 5539, Line 20: Are there any spaces in the apparatus so that most of the salmon can run upstream? If so, please specify the length or rate at which the apparatus prevents salmon run. If not, I think your estimation of the escape rate of salmon (80%) is too high because of biases related to the different observation conditions in each term. Please see below comment (for Page 5543, Lines 12-16) for further details.

Reply: Though we found some spaces in the apparatus and many spawning salmons in upstream sites when we researched, there was no quantitative data and also no past report about such space and escape rate of salmons in our country. The rate 80% is adopted as the highest value in the field. We added this meaning as the word “potential” in the text.

A-10 Page 5539, Lines 22-24: How did you identify individual salmon? Individual identification is quite important for proper estimation of salmon escapement because the average number of salmon that you observed was small (0.49-0.61 h-1). In addition, you should clarify the field conditions that may affect salmon runs (e.g. flow rate, temperature and weather of each observation term) in order to show that each observation was conducted under the same conditions.

Reply: We added more information about the monitoring of salmons and field conditions following comments.

A-11 Page 5540, Lines 8-10: Doesn't the denitrification involve any isotope fractiona-

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tions?

Reply: Thank you for your comments. It is uncertain whether denitrification involves isotope fractionations or not. Therefore, the sentence was revised.

A-12 Page 5540, Lines 16-19: If your analysis included any preliminary sample preparations (e.g. filtration of soil samples or lipid extraction of salmon tissue), please note them here.

Reply: We added the information about sample preparations.

A-13 Page 5540, Line 21: To explain the delta-definition of isotopic values, this equation is suggested to be avoided (See Coplen 2011 below). Coplen (2011) Guidelines and recommended terms for expression of stable-isotope and gas-ratio measurement results. Rapid Commun. Mass Spectrom. 25: 2538-2560.

Reply: Thank you. The equation was revised.

A-14 Page 5540, Line 24: It is recommended that ^{13}C values of both organic and inorganic materials should be measured and expressed relative to VPDB (See Coplen et al. 2006 below). Coplen et al. (2006) New Guidelines for $\delta^{13}\text{C}$ Measurements. Anal. Chem. 78: 2439-2441.

Reply: We only conducted SIA by using organic materials. In the future study, we would like to do the analysis by using both the organic and inorganic materials. Thank you for your suggestion.

A-15 Page 5541, Lines 5-6: Which salmon tissue was used for SIA?

Reply: We added the information (chum salmon tissue) in the text.

A-16 Page 5541, Lines 8-11: Your explanation of SSL is unclear. Did you choose SSL from your soil samples collected in each sampling station or did you collect SSL separately from the other soil samples? How many samples of SSL did you collect? Why can you assume that SSL does not include MDN while these are sampled from

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the same region with the other soil samples? Please add a more detailed description of SSL. This is critical to verifying your paper because stable isotope values of SSL are one of the most important sources of information when estimating MDN contribution to the river basin.

Reply: The minimum value was obtained close to the top of the mountain. The elevation is about 1500 m, where MDN is not expected to influence isotope values. Therefore, the following sentence was added in the manuscript.

“SSL was collected close to the top of the mountain, where MDN is not expected to influence isotope values.”

A-17 Page 5541, Lines 12-13: Okay, but please give a more detailed description of the sampling of bamboo grass. Because the sample size of the bamboo grass is small (N=3), you have to discuss why such small sample sizes can represent the isotopic values of the whole bamboo grass target area.

Reply: Three extra samples was added in our results in Fig. 4.

A-18 Page 5541, Lines 16-25: I suggest deleting these sentences since they do not make sense: Predators contribute salmon-derived nutrients to soils directly as excretory substances. Therefore, discussions on the uncertainty of isotopic fractionation of tissues, or comparison with SIA of tissues have nothing to do with your analysis.

Reply: Thank you. The sentences were deleted.

A-19 Page 5541, Lines 22-23: This sentence is not relevant because, in feces, much of the solid matter consists of bacteria and the animals' own tissues such as dead cells and fat, which are included in isotopic fractionation.

Reply: Please see the answer for 18.

A-20 Page 5541, Lines 27-29: What were the isotopic values of soils compared with? Do the soil samples consists of several groups?

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Reply: The sentence was wrong. I am sorry. This sentence was deleted.

A-21 Page 5542, Lines 2-3: Contribution to what? In addition, I do not find the term "subbasin area" elsewhere in this manuscript. Please explain the definition and location of the sub-basin area.

Reply: The explanation about "sub-basin area" was added in the manuscript.

A-22 Results and Discussion Page 5542, Lines 19-21: I can't identify data that support this statement.

Reply: Thank you for your comment. We made the equation (6) by using the field observation results. Therefore, the following sentence was added in the manuscript.

"TPN was modeled by using our field observation results, discharge and TPN as (6)."

A-23 Page 5543, Lines 5-8: Okay, but how did you exclude emission of anthropogenic nitrogen from these estimations?

Reply: This area is registered as the Natural World Heritage. Therefore, there should not be any influence of human activity.

A-24 Page 5543, Lines 12-16: It is likely that the escape rate of salmon calculated here has been overestimated because you did not consider the total number of salmon in each period. Why was the apparatus for catching salmon removed? Isn't it because the spawning season of salmon was over? If so, total salmon abundance should be decreasing from the former- to the latter period, and it results in an overestimation of the escape rate of salmon. In addition, the number of salmon runs should also be influenced by many other factors such as temperature, weather, flow rate, and so on. Therefore, you have to show the evidence that your field observations in each period were conducted in the same conditions. Finally, your estimation of salmon escapement should be made using a sufficient number of observations – not by single observation – because the observed number of salmon passing per hour was quite low (0.49 to 0.61 h⁻¹). From your result, it is unclear that the difference represents anything other

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than sampling variation.

Reply: We think your comments are really reasonable for this issue. Actually we do not know another site to research the escape rate, especially at the similar type of apparatus in Rausu River. Because of all salmon-capturing apparatus in the rivers under the management of fisheries, it is difficult for scientists to remove those apparatus several times, results in deviation from fisheries program in our country. We think that our escape rate should be used as “potential” rate in the field and modified in the text.

A-25 Page 5543, Lines 19-21: Which region is this statement referring to? Is it Shiretoko region or the entire Hokkaido islands? Please clarify your target area here.

Reply: The counts were obtained in the Rausu River of Shiretoko. The sentence was revised.

A-26 Page 5543, Lines 23-24: Same as above comment.

Reply: The values are for Shiretoko. “Shiretoko” was added in the sentence.

A-27 Page 5544, Lines 5-7: Please include the isotopic value of each sample.

Reply: This sentence was deleted.

A-28 Page 5544, Lines 6-7: I’m wondering if the 15N of SOM (about 0.1‰ from fig. 3) was really attributed to the 15N of MDN. According to the other field investigation in Japan (see Wada et al. 1984 below), 15N of SOM in the surface layer of subalpine forest soils ranged from -2.3‰ to +2.8‰ which is almost the same as your data in fig. 2 (-2.5‰ to +2.5‰). Many factors can cause isotope enrichment in 15N values of SOM, such as N₂O reduction, nitrification and denitrification. Therefore, you have to discuss why the 15N value of SOM in this paper is not affected by the isotopic fractionations between litter and SOM. Wada et al. (1984) Natural abundance of 15N in reference to paddy soils implications on soil organic matter with special in Japan: biogeochemical the nitrogen cycle. Geo-chemical Journal, 18: 109-123.

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Reply: Thank you for your comments. To answer your comment, we need to clarify each process in ecological system, which controls 15N of SOM. Since our main target is to understand the budget how much MDN is included in SOM, which is finally transported from inland to the ocean due to runoff process. As shown in a new figure, Fig. 3, particulate nitrogen is associated with suspended sediment strongly. Therefore, Figure 6 was revised by deleting 22.9 %, and the sentences were revised.

A-29 Page 5544, Line 8: Suggest to replace the word "feces" with "droppings".

Reply: Thank you for your comment. "feces" was replaced by "droppings" in the sentence.

A-30 Page 5544, Lines 9-10: Please include citation.

Reply: This sentence was deleted.

A-31 Page 5544, Lines 8-12: So what? This result doesn't indicate that sea eagles contribute more than bears to MDN in this region.

Reply: This sentence was deleted.

A-32 Page 5544, Lines 15-16: Again, the concentration of MDN in feces/droppings is not related to the total amount of MDN that is imported by each predator, because the total amount of MDN depends on the total volume of their feces/droppings.

Reply: This sentence was deleted.

A-33 Page 5544, Lines 19-21: You have to show the isotopic values of soil samples here so that readers can validate the contribution rate of MDN to SOM.

Reply: In Fig. 2, the values are all shown.

A-34 Conclusions Page 5545, Line 27 to Page 5546, Lines 1-2: To which result does this statement refer?

Reply: The value is shown in Lines from 23 to 24 in Page 5543 as follows:

“Therefore, annual TN transported by chum and pink salmon is estimated to be 1542 kg yr⁻¹ and 3216 kg yr⁻¹, respectively.”

A-35 Acknowledgements Suggest to replace "an anonymous reviewer for their..." with "anonymous reviewers for their".

Reply: Thank you. The sentence was revised by following your comment.

A-36 Table 1: As the AE pointed out, all abbreviations used in the table, figures and captions should be defined or explained in each caption. Also, sampling points of bamboo grass should be indicated here.

Reply: Thank you for your comments. The all captions were revised.

A-37 Figures and table Figure 2(a): Please include the location where the field observation to count salmon run was done.

Reply: Thank you. The location of salmon run station was added in Fig. 2.

A-38 Figure 2(b): There should be an error in the legend of 15N value (-2.5‰ to -2.5‰).

Reply: Thank you. The values for the bar were revised.

A-39 Figure 3: What do the points and error bars in this figure mean?

Reply: Sorry. They indicate the standard deviations. The explanation was added in the caption.

A-40 Figure 3: I can't find the isotopic point of SSL in this figure.

Reply: Please see the black circle, which shows the SSL.

A-41 Figure 3: Consider replacing "sea eagle feces" with "sea eagle droppings".

Reply: Thank you. "feces" was replaced by "droppings".

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C2603/2015/bgd-12-C2603-2015-supplement.pdf>

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