**Response to Reviewer 3**

We sincerely appreciate the reviewer's careful evaluation of our manuscript and the constructive comments and suggestions. These comments were very helpful in improving the clarity and presentation of the manuscript. We have revised the manuscript accordingly, particularly with regard to the presentation of uncertainty and figure readability. Our detailed responses are provided below.

**Major comments (in excess of those already identified by R1 and R2).**

**Comment 1:** *I am confused on the way uncertainty is reported. Moreover, I think Figure 2 would be more readable (and perhaps demonstrate the authors point more clearly) if all points were plotted on the same graph, with error bars corresponding to differences between individually-measured fish (uncertainty propagated). Why are individual specimens reported independently, rather than grouped by gender and tissue?*

**Reply:** Thank you for this helpful comment. We agree that presenting the data on a single plot improves readability and facilitates comparison among samples. Accordingly, Figure 2 has been revised so that all data points are plotted on the same graph. The primary purpose of Figure 2 is to illustrate tissue-specific differences in isotope ratios within individual fish (e.g., how much higher the liver values are relative to other tissues in the same specimen). For this reason, we present data for each individual specimen separately, with error bars representing analytical uncertainty (2 S.E. of a single analysis consisting of 60 measurement cycles).

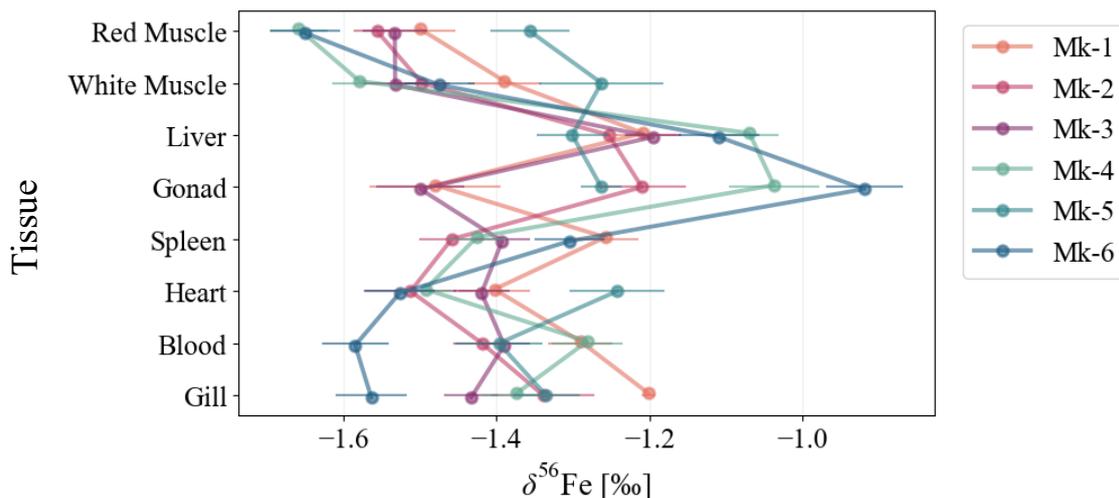**Revision: Figure 2 has been modified as follows:**



**Figure 2: Iron stable isotope ratio in tissues of the six *Scomber japonicus* individuals (Mk1-3: females, Mk4-6: males). Error bars represent 2 S.E. during a single measurement (60 analytical**

**cycles).**

*Figure readability*

**Comment 2:** *Fig 2. Please retain the y axis labels on the bottom half of the plot. Please label all x axes.*

**Reply:** Thank you for the suggestion. We have revised Figure 2 with a single plot as noted above.

**Comment 3:** *Fig. 3. These plots are very valuable but difficult to interpret because there is a lot going on. Could you clarify what the "the gray lines represent $1^{st}$ deviation of the row spectra" means? Is this the difference between the sample and standard? I would recommend plotting the gray lines in a separate plot. Can you please clarify what the red lines are in the caption? (I assume it is a smoothed fit?)*

*Across all plots and throughout, I'd visually emphasize that Mk-1 through Mk-3 are all female, and Mk-4 through Mk-6 are all males. This would assist the reader in interpreting the plots more easily.*

*Rather than referring to your standards as abbreviations, I would suggest using the structure they are meant to represent. For example, changing "Bb-metHb" to "blood methemoglobin." Then, state in the methods that the blood methemoglobin standard is from bovine blood.*

**Reply:** We thank the reviewer for the helpful suggestions regarding Figure 3. The gray lines represent the first derivative of the normalized XANES spectra, which is used to emphasize the gradient and thereby facilitate comparison of subtle shifts in peak positions among spectra. This is not the difference between the sample and the standards, and it cannot be plotted separately from the spectra without losing its intended function. To improve clarity, we have lightened the color of these lines so that they are easier to distinguish.

The red dashed lines indicate the results of linear combination fitting (LCF) using the four standard spectra. We have revised the figure caption to provide more detailed explanations of the lines and symbols, including the purpose of the first derivative and the meaning of the red dashed lines.

In addition, we have updated the labels of the standard spectra to reflect the structure they represent (e.g., "blood methemoglobin" instead of "Bb-metHb") and add labels to distinguish females (Mk-1 to Mk-3) and males (Mk-4 to Mk-6).
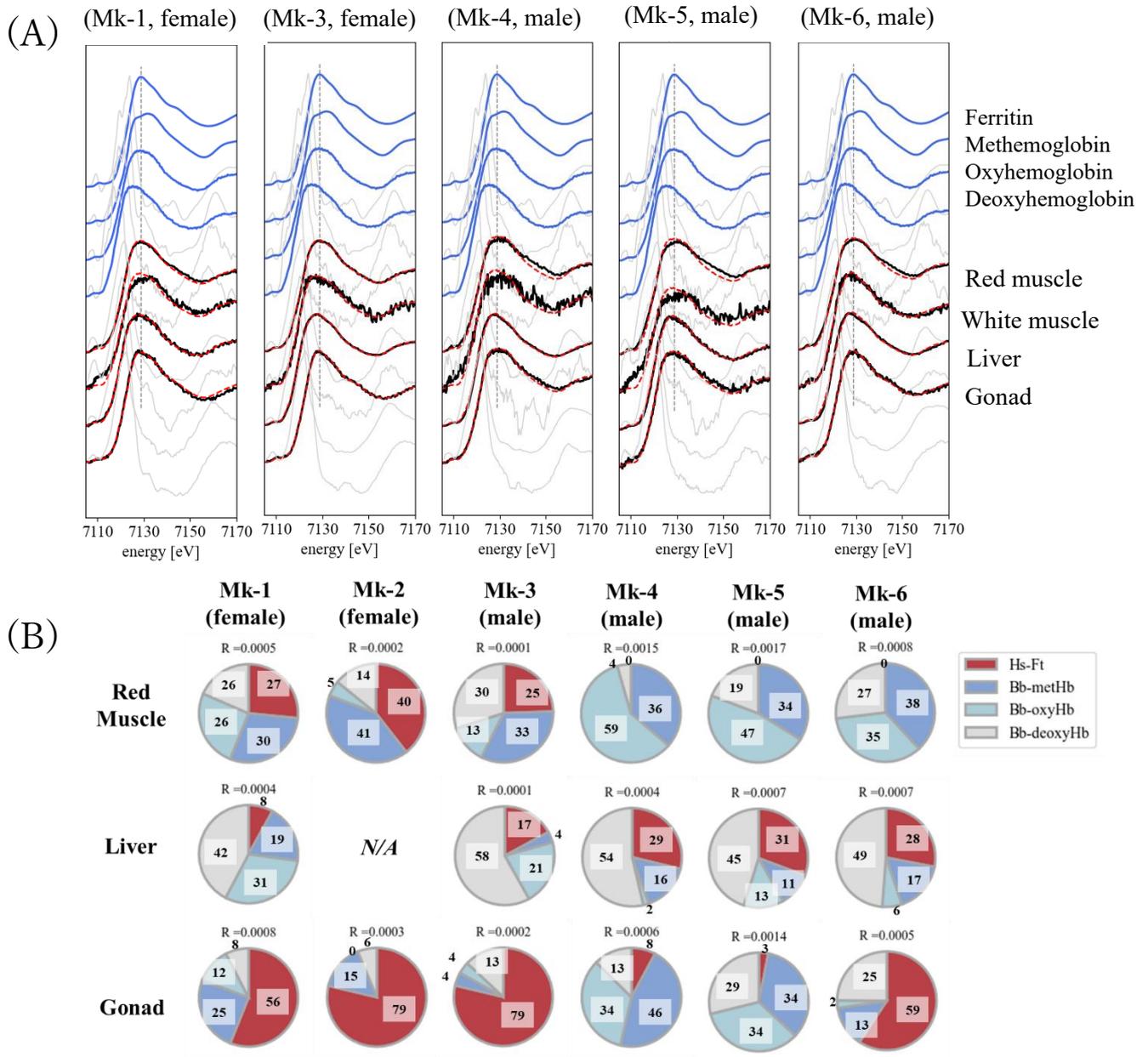
**Revision:**



Figure 3: (A) Fe K-edge XANES spectra of *Scomber japonicus* tissues. **Mk-1 and Mk-3 represent females, and Mk-4 to Mk-6 represent males. The standard spectra were obtained from ferritin from horse spleen and hemoglobin derivatives from bovine blood. Blue lines indicate the spectra of the standard materials, while black lines represent the tissue samples. Red dashed lines show the results of linear combination fitting (LCF) using the four standard spectra. Gray solid lines correspond to the first derivative of the normalized XANES spectra, which highlights the peak top energies, and gray dashed lines mark the peak top energy of ferritin at 7129 eV. (B)** Estimated proportions of each standard materials with linear combination fitting (LCF) of XANES spectra. R value is a goodness-of-fit parameter described in Eq. 4. Each number on the pie charts represents proportions (%) of each component.