Overview of the Revision

We sincerely appreciate the Editor's summary and the reviewers' constructive feedback. We have addressed the three major areas of concern highlighted in the editorial summary as follows:

Editor's Key Points	Reviewer' s Major Comments (MC)	Key Point-by-Point Response
1. Strength of interpretations relative to the dataset	Reviewer 1 (MC4, 5), Reviewer 2 (MC2)	R1-MC4: Softened conclusions to reflect trends rather than definitive results. R1-MC5, R2-MC2: Toned down claims about "consistently" higher liver values;
2. Treatment of individual specimens and sample size	Reviewer 1 (MC1, 3, 4), Reviewer 2 (MC2)	R1-MC1, MC4: Re-included all six individuals (Mk-1 to Mk-6) in the analysis; R1-MC3: Replaced boxplots with scatter plots to show individual variability.
3. Clarity and consistency of data presentation	Reviewer 1 (MC2, 3), Reviewer 2 (MC1, 4) Reviewer 3 (MC 1)	R1-MC2: Resolved all numerical discrepancies by consolidating tables and unifying blood volume calculations; R2-MC1: Clarified the definition of uncertainty; R2-MC4: Added data about isotope ratios in previous studies; R3-C1: Revised Figure 2 to clarify tissue-specific differences among all specimens.

Summary of Major Revisions

We have conducted a revision of the manuscript to address these concerns. In particular, these major comments pointed out by each reviewer have been modified:

- Treatment of Individual Specimens (Response R1-MC1): This is the most critical revision. As suggested, we have re-included all six individuals (Mk-1 to Mk-6) in our analysis and recalculated all results, including values and ranges. This ensures that our conclusions are based on the full dataset without any selective exclusion.
- Clarity and Consistency (Response R1-MC2-1 to 2-5): We have resolved the inconsistencies in tissue weights and iron content reporting by consolidating Tables S1 and S2 into a single Table S1 based on precise analytical balance weights. We also unified the blood volume estimation using a single conversion factor (30 mL/kg) and specific gravity (1.05).
- Scientific Rigor of Interpretations (Response R2-MC2): We have revised our discussion to be more cautious. Instead of claiming the liver $\delta^{56}Fe$ is "consistently higher" than all other tissues, we now compare the liver value against the whole-body average (δ_{WB}), which is an objective

and robust comparison.

- Biological Justification (Response R2-MC3): We added details regarding the M-type ferritin subunit in teleosts to justify the application of human-based iron fractionation models to fish, acknowledging both the conserved mechanisms and specific differences.

Response to Reviewer 1

We sincerely thank the reviewer for their thorough and constructive review of our manuscript. The comments raised were extremely helpful in identifying inconsistencies between the text, tables, and figures, as well as in improving the clarity and rigor of our interpretations.

In response to the reviewer's concerns, we have revised the manuscript to correct all inconsistencies in numerical values and sample ranges, and to ensure full consistency among the main text, figures, and supplementary tables. We have also revised the abstract and conclusions to avoid any potentially misleading emphasis on results derived from the selective exclusion of individuals.

Major Comment1*: My first main concern was the removal of two specimens (Mk-1 and Mk-5), after measurements were done, which produce a misleading narrow distribution. Either there is a strong incentive to remove these beforehand (e.g. sample compromised, biological justification), and it should be explicitly stated, or they should be included in the conclusion even if it adds more uncertainty. Moreover, the values resulting from the exclusion of these two specimens is mentioned as a key result in the abstract.*

Reply: We thank the reviewer for this important comment. We agree that post hoc exclusion of specimens requires clear justification, and that removing individuals without sufficient explanation may give a misleading impression of the results.

For Mk-1 and Mk-5, there was no strong a priori justification for exclusion. Therefore, in the revised manuscript, we have chosen to retain all individuals in the analysis. The relevant sections of the manuscript have been revised accordingly. In addition, we have modified the Abstract to remove statements based on results obtained after excluding these individuals, in order to avoid potential misinterpretation by readers.

In Section 4.1, we now present Δ values calculated using all individuals, based on the assumption of $\delta_{Hm} = -1.66‰$ as the reviewer noted below, and discuss the observed variability as biologically meaningful rather than excluding any specimens.

Revision:

[P.1, line15] 2.04±0.22‰→2.72±3.03‰

[P.9, Line 196-208] has been replaced (Please see P.9)

Major Comment2-1: *Secondly, I have noticed several discrepancies between the text and the values in the tables or the figures:*

Maybe there is some factor that I don't know about that is missing, but when I take the wet weight in table S1 and multiply it by the Fe concentration (µmol/gww) in Table S2, lots of the results are different from the one given in the column "Total Fe (mg)" of tableS2. "Total Fe (mg)" of tableS2. It is especially true for blood Fe content (see tables at the end of this review). The small differences can be explained by approximations of both values but as for the others I couldn't figure out the cause behind

the discrepancies. Maybe it has something to do with the conversion from wet weight to dry weight?

Reply: We appreciate the reviewer for pointing out this, and acknowledge that the explanation regarding the discrepancy between wet weight values in Table S1 and the "Total Fe (mg)" values in Table S2 was insufficient in the original manuscript.

We checked the discrepancy and found that the wet weights reported in Table S1 were measured immediately after dissection using a portable balance and include the weight of the sampling containers. However, the "Total Fe (mg)" values in Table S2 were obtained using precise tissue weights measured later with an analytical balance after subtracting the container weight. For blood samples, the wet weight in Table S1 corresponds to the amount of blood that could be physically collected at the time of dissection. However, this value underestimates the actual blood volume since the blood had partially coagulated and was difficult to collect completely. In the manuscript, total blood iron content was estimated using the blood volume conversion factor from body weight (around 30 mL/kg), rather than the collected mass. As a result, the blood iron values in Table S2 are not expected to match calculations based directly on the wet weights reported in Table S1. To avoid further confusion, we replaced Tables S1 and S2 with a single revised Table S1 based on the precise tissue weights. In addition, we revised Fig. S1 to clarify that the values represent weight proportions relative to the total body weight, rather than the combined weight of the eight tissues.

Revision:

- Tables S1 and S2 were merged into a new Table S1.
- Figure S1: weights of other tissues except the analyzed tissues were included.

Table S1: Weight, Fe concentration and stable isotope ratio in chub mackerel tissues (Blood volume is calculated as 30 mL/kg Body weight, and 1.05 as specific gravity according to Olson, 1992 and Davison, 2011).

Sample ID	Tissue	δ^{56}Fe ± 2 S.E.*	Wet weight [g]	Dry weight [g]	Moisture ratio%	Tissue weight per body weight%	Fe conc. [μg/g w.w.]	Fe conc. [μg/g d.w.]	Total Fe [mg]	Total Fe%
Mk-1	Red Muscle	−1.5 ± 0.05	28.5	13.0	54.5	4.46	144	318	4.12	30.2
Mk-1	White Muscle	−1.39 ± 0.05	333	109	67.1	52.0	4.01	12.2	1.33	9.76
Mk-1	Liver	−1.21 ± 0.04	12.1	2.94	75.6	1.89	68.9	283	0.833	6.1
Mk-1	Gonad	−1.48 ± 0.09	56.4	23.5	58.3	8.81	9.34	22.4	0.527	3.86
Mk-1	Spleen	−1.26 ± 0.04	1.18	0.264	77.5	0.184	721	3210	0.849	6.22
Mk-1	Heart	−1.4 ± 0.05	1.76	0.374	78.8	0.275	121	569	0.213	1.56
Mk-1	Blood	−1.29 ± 0.04	20.2	3.73	81.5	3.15	222	1200	4.48	32.8
Mk-1	Gill	−1.2 ± 0.01	13.5	3.95	70.8	2.11	96.2	330	1.30	9.52
Mk-2	Red Muscle	−1.56 ± 0.03	51.0	24.5	51.9	5.54	93.5	194	4.77	27.5
Mk-2	White Muscle	−1.5 ± 0.04	326	142	56.6	35.4	4.50	10.4	1.47	8.46
Mk-2	Liver	−1.25 ± 0.002	12.0	2.82	76.5	1.30	80.5	343	0.966	5.58
Mk-2	Gonad	−1.21 ± 0.06	81.5	38.6	52.7	8.86	12.3	26.0	1.00	5.8
Mk-2	Spleen	−1.46 ± 0.04	3.76	0.703	81.3	0.409	431	2310	1.62	9.37
Mk-2	Heart	−1.51 ± 0.06	4.30	0.77	82.1	0.468	70.4	394	0.303	1.75
Mk-2	Blood	−1.42 ± 0.04	29.0	5.14	82.3	3.15	196	1110	5.69	32.8
Mk-2	Gill	−1.34 ± 0.07	24.0	6.63	72.3	2.61	63.1	228	1.51	8.73
Mk-3	Red Muscle	−1.53 ± 0.04	33.9	8.35	75.4	5.85	68.9	280	2.34	21.7
Mk-3	White Muscle	−1.53 ± 0.04	182	42.7	76.5	31.3	4.77	20.3	0.867	8.05
Mk-3	Liver	−1.2 ± 0.04	4.57	1.20	73.8	0.788	182	695	0.831	7.71
Mk-3	Gonad	−1.5 ± 0.06	22.3	7.70	65.4	3.84	16.1	46.5	0.359	3.33
Mk-3	Spleen	−1.39 ± 0.04	1.11	0.311	72.0	0.191	891	3180	0.988	9.17
Mk-3	Heart	−1.42 ± 0.04	3.27	0.668	79.6	0.564	262	1280	0.858	7.96
Mk-3	Blood	−1.39 ± 0.03	18.3	2.93	83.9	3.15	174	1090	3.18	29.5
Mk-3	Gill	−1.43 ± 0.04	18.3	4.17	77.3	3.16	73.6	324	1.35	12.5
Mk-4	Red Muscle	−1.66 ± 0.04	72.0	28.0	61.1	7.66	84.7	218	6.09	24.7
Mk-4	White Muscle	−1.58 ± 0.03	374	112	70.1	39.8	3.71	12.4	1.39	5.63
Mk-4	Liver	−1.07 ± 0.04	6.88	1.88	72.7	0.732	234	858	1.61	6.53
Mk-4	Gonad	−1.04 ± 0.06	71.2	16.0	77.6	7.58	10.7	47.6	0.761	3.09
Mk-4	Spleen	−1.43 ± 0.04	2.54	0.648	74.5	0.271	873	3430	2.22	9.01
Mk-4	Heart	−1.49 ± 0.03	2.24	0.523	76.6	0.238	121	517	0.27	1.1
Mk-4	Blood	−1.28 ± 0.05	29.6	6.06	79.5	3.15	349	1700	10.3	41.9
Mk-4	Gill	−1.37 ± 0.04	28.6	7.76	72.8	3.04	69.0	254	1.97	7.99
Mk-5	Red Muscle	−1.36 ± 0.05	36.6	9.83	73.1	5.08	75.0	279	2.75	22.8
Mk-5	White Muscle	−1.26 ± 0.08	326	87.9	73.0	45.3	6.28	23.3	2.05	17
Mk-5	Liver	−1.3 ± 0.05	3.01	0.793	73.7	0.419	162	616	0.488	4.06
Mk-5	Gonad	−1.26 ± 0.03	60.8	12.0	80.3	8.45	5.29	26.8	0.322	2.68
Mk-5	Spleen	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mk-5	Heart	−1.24 ± 0.06	2.54	0.542	78.7	0.353	144	677	0.367	3.05
Mk-5	Blood	−1.4 ± 0.06	22.7	4.5	80.1	3.15	206	1040	4.68	38.9
Mk-5	Gill	−1.34 ± 0.04	21.1	5.22	75.2	2.92	65.1	263	1.37	11.4
Mk-6	Red Muscle	−1.65 ± 0.05	55.4	18.3	66.9	5.54	77.3	233	4.28	17.7
Mk-6	White Muscle	−1.47 ± 0.05	513	150	70.8	51.3	3.85	13.2	1.98	8.19
Mk-6	Liver	−1.11 ± 0.05	7.74	2.02	73.9	0.774	259	993	2.01	8.31
Mk-6	Gonad	−0.92 ± 0.05	61.6	10.9	82.2	6.16	9.17	51.7	0.565	2.34
Mk-6	Spleen	−1.3 ± 0.05	2.92	0.724	75.2	0.292	875	3530	2.56	10.6
Mk-6	Heart	−1.53 ± 0.05	2.29	0.491	78.6	0.229	185	865	0.425	1.76
Mk-6	Blood	−1.58 ± 0.04	31.5	5.85	81.4	3.15	275	1480	8.7	35.9
Mk-6	Gill	−1.56 ± 0.05	38.5	10.5	72.9	3.85	94.8	349	3.65	15.1

- *2 S.E. in δ^{56}Fe represents the standard error during single analysis (60 analytical cycles)

[P.5, line120-121] The hepatosomatic index (HSI) ranged from 1.05–2.14 in females and 0.74–1.26 in males, and the gonadosomatic index (GSI) ranged from 4.26–10.1 in females and 7.25–10.0 in males, consistent with the spawning season in the East China Sea (Shiraishi et al., 2008). Among tissues, white muscle represented the largest biomass fraction (31–52% of total body weight; Fig. S1), followed by gonads (3.8–8.9%), red muscle (4.5–7.7%), gills (2.1–3.9%),

[P.5, line130-132] Mean iron concentrations (± 2 S.D.) were highest in the spleen (3,100 ± 860 μg/g d.w.), followed by blood (1,300 ± 480 μg/g d.w.), heart (720 ± 580 μg/g d.w.), liver (630 ± 510 μg/g d.w.), gills (290 ± 90 μg/g d.w.), red muscle (250 ± 84 μg/g d.w.), gonads (37 ± 24 μg/g d.w.), and white muscle (15 ± 10 μg/g d.w.)

[P.5, line137-144] The total body iron inventory was therefore estimated to be approximately 17–26 mg/kg. Although the liver is the principal iron storage tissue, it accounted for only 4–8% of total body iron. In contrast, blood and red muscle together comprised 51–67% of the total iron burden.

Mean iron isotope compositions (δ^{56}Fe ± 2 S.D.) of each tissue across individuals were as follows: red muscle, −1.54 ± 0.20‰; white muscle, −1.46 ± 0.21‰; liver, −1.19 ± 0.16‰; gonads, −1.23 ± 0.42‰; spleen, −1.37 ± 0.15‰; heart, −1.43 ± 0.19‰; gills, −1.37 ± 0.22‰; and blood, −1.39 ± 0.20‰ (Fig. 2). The net δ^{56}Fe in whole mackerel bodies weighted by tissue Fe contents ranged from −1.50‰ to −1.35‰, with the liver in all individuals showing higher values than the net values.

Major Comment 2-2: *Page 5 line 131 "the total iron content in blood was estimated to be between 4.3 and 9.8mg (Fig. 1B)" these values are incoherent with the ones given in Table S2.*

Reply: As mentioned before, the discrepancy arose from the use of different approaches to estimate blood iron content in the main text and in Table S2. In addition, because reported conversion factors for fish blood volume per body weight vary widely, we initially applied multiple factors to estimate blood iron content, which resulted in inconsistencies among some values.

To resolve this issue, we recalculated all blood iron contents using a unified assumption of a blood volume of 30 mL/kg body weight and a specific gravity of fish blood of 1.05. The values in the text, Figure 1B, and Table S2 have now been updated accordingly and are consistent.

Revision:

[P.5, Line 134–135] Assuming a blood volume of 30 mL/kg and 1.05 as the specific gravity of fish blood (Davison, 2011), the total iron content in blood was estimated to be between 3.2 and 10.3 mg (Table S1).

Major Comment2-3: *Page 5 lines 132-133 again the ranges given are not the ones in Table S2 "gills (1.3-2.0mg)" should be (1.3-3.7 mg), and "liver (0.5-1.6 mg)" should be (0.5-2.0 mg). Or did you remove sample Mk-5 on purpose here?*

Reply: The reviewer is correct. The ranges reported in the main text were due to an error in reading the cell range, which resulted in the omission of the Mk-6 value during data compilation. The ranges for gills and liver have now been corrected in the text to match the values reported in Table S2.

Revision:

[P.5, Line 135-137] Red muscle contributed the next largest Fe pool (2.3–6.1 mg), followed by gills (1.3–3.7 mg), white muscle (0.9–2.0 mg), spleen (0.8–2.6 mg), liver (0.5–2.0 mg), gonads (0.3–1.0 mg), and heart (0.2–0.9 mg).

Major Comment2-4: *Page 7, l.155, section 3.3: Mk-1 and Mk-5 values do not seem to be included, otherwise the ranges given "17-29%" would be "8-31%" as it is the case on page 9 line 175.*

Reply: We apologize for this mistake. The range reported on page 7 (17–29%) was the result of an inadvertent omission of Mk-1 and Mk-5 during data compilation. These samples were not intentionally excluded. The range has now been corrected to 8–31% to ensure consistency with the values reported on page 9 (line 175) and the underlying dataset.

Revision:

[P.7, line 158] whereas ferritin accounted for 8–31% of total Fe in the liver (Fig. 3B).

Major Comment2-5: *Page 7 lines 159-160: it is stated that "females exhibit lower ferritin-bound Fe proportions in the liver, red muscle and gonads than males ($p<0.05$)". However, Figure 3B shows a higher proportion of ferritin (Hs-Ft, red portions) in red muscle and gonads in the female specimens. And in the following text the authors explicitly say that "ferritin represented the predominant Fe form (70-80%)" in ovaries "whereas testes showed highly variable proportions (3-59%)" in agreement with Figure 3B but in contradiction with the previous sentence.*

Reply: We apologize for this oversight. The statement contains an error in the description of sex-specific differences in ferritin-bound Fe proportions. As correctly noted by the reviewer, Figure 3B shows higher ferritin-bound Fe proportions in red muscle and gonads of female specimens, and the subsequent text describing ferritin as the predominant Fe form in ovaries (70–80%) but highly variable in testes (3–59%) is consistent with the figure. We have therefore corrected the sentence to ensure consistency with Figure 3B and the accompanying text. In addition, we deleted p-values in these sentences because they are meaningless given the limited sample size.

Revision:

[P.7, line161–162] Sex-related differences were also apparent: females exhibited lower ferritin-bound Fe proportions in the liver, while higher proportions in the red muscle, and gonads than males ~~($p < 0.05$)~~.

Major Comment 3: *Figure 1 (page 6) uses boxplots based on 3 values only. I am not convinced that this is the best statistical analysis one can do on such a small sized sample and especially when comparing groups (p.5 l128-130, section 3.2). Boxplots summarize a distribution and thus need more observations to be robust.*

Reply: We agree with the reviewer that boxplots are not appropriate for such a small sample size (n = 3 per group), as they are intended to summarize distributions and require a larger number of observations to be robust. We have therefore revised Figure 1 by replacing the boxplots with scatter plots showing individual data points. In addition, we have toned down the statistical interpretation in Section 3.2 to avoid overinterpretation of group differences based on a limited sample size.

Correction:

[P.5, line132–133] Male spleens contained significantly higher Fe concentrations than those of females (p = 0.04), and liver and gonads also tended to be higher in males (p = 0.06).

⇒Male spleens tended to have higher Fe concentrations than those of females, and liver and gonads also tended to be higher in males.

[P.6. line 143–144] No significant sex differences were detected, although ovarian δ^{56}Fe tended to be lower than testicular values (p = 0.06).

⇒No sex differences were detected, although ovarian δ^{56}Fe was lower than testicular values.

Figure1 has been changed as follows:

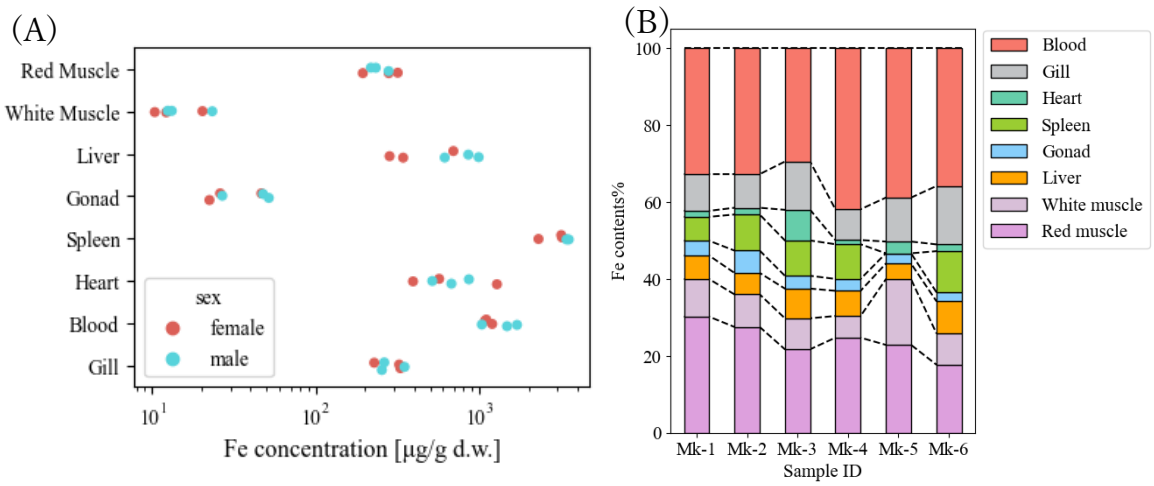

Figure 1: (A) Iron concentration, and (B) Tissue iron burden of the chub mackerels (Mk-1 to Mk-3: female, Mk-4 to Mk-6: male). Total blood contents were assumed to be 30 mL/kg with a specific gravity of 1.05. No uncertainty is reported as multiple sample replicates were not measured. The measurement uncertainty was assumed to be zero.

Major Comment 4: *I would advise the authors to be more cautious with their conclusions as their sample size is small and present some variability.*

Reply: We thank the reviewer for this important comment. We agree that the small sample size and the observed inter-individual variability require cautious interpretation of the results.

We have therefore revised the Conclusions and relevant parts of the Discussion to temper our statements and to emphasize that the observed patterns should be interpreted as preliminary trends rather than definitive conclusions. We now explicitly acknowledge the limitations associated with sample size and biological variability and highlight the need for future studies with larger sample sizes to confirm and extend our findings.

Revision:

[P.1, line15] no ~~significant~~ enrichment of heavy Fe isotope

[P.1, line18] Our results suggested that

[P.13, line294–300]

These results suggest that the high δ^{56}Fe of mackerel primarily reflects dietary iron sources and intestinal uptake rather than internal isotopic fractionation. The relative homogeneity of δ^{56}Fe among major tissues such as muscle and liver compared with the potential isotopic fractionation associated with ferritin formation, suggests that iron isotope pools in fish remain relatively stable. The small variation in δ^{56}Fe among major tissues such as muscle and liver compared with the potential isotopic fractionation associated with ferritin formation, suggests that iron isotope pools in fish remain relatively stable. Although the number of individuals analyzed in this study is limited, these δ^{56}Fe patterns across multiple individuals provide robust constraints on iron isotope behavior in wild marine fish.

Major Comment 5:*The comparison between values seems subjective as the authors state "the δ^{56}Fe values in the liver [..] were consistently higher than those in other tissues", for a maximum difference of 0.35% between the liver and red muscle, and later say that the isotopic offset is "slightly higher but comparable to previous observation" for a difference of 0.46‰ at best. Either both are higher or both comparable in terms of differences. Or are there measurements for other organisms that show even larger isotopic offset? In that case I would understand the "comparable".*

Reply: We agree that the original phrasing may give the impression of a subjective comparison. We have therefore revised the text to harmonize the language used for isotopic differences. Specifically, we replaced "consistently" and "slightly" with a more cautious expression.

Revision:

[P.1, line13-14] In all the specimens, the liver δ^{56}Fe values were ~~consistently~~ higher than the average value ~~those~~ of all ~~other~~ tissues

[P9, Line178-179] The δ^{56}Fe values in the liver of chub mackerel were consistently higher than those in other tissues, indicating depletion of lighter isotopes in this tissue. an enrichment in the heavier Fe isotopes.

⇒All the chub mackerel individuals showed higher δ^{56}Fe values in the liver than δ_{WB} across eight tissues.

Major Comment 6: *p.10 l231-236: it seems the two sentences contradict each other. The part stating "are more likely attributed to variation in intestinal..." excludes the contribution of prey δ^{56}Fe, which is not what you say before and after. Or can you demonstrate why the prey δ^{56}Fe cannot drive the variation in fish δ^{56}Fe?*

Reply: We thank the reviewer for this important comment. We agree that the original wording was overly exclusive and could give the impression that prey δ^{56}Fe does not contribute to the observed variability in fish δ^{56}Fe, which was not our intention.

Our original interpretation was based on the observation that, for sardines and herrings, there were few reported prey candidates exhibiting δ^{56}Fe values as low as those observed in these fish, leading us to suggest that dietary isotopic composition alone was unlikely to fully explain their low δ^{56}Fe values. However, recent observations have identified some of zooplankton taxa exhibiting low δ^{56}Fe values (below −2‰), indicating that prey-derived δ^{56}Fe variability cannot be entirely excluded as a contributing factor. We therefore agree that prey isotopic composition may partially influence fish δ^{56}Fe values, particularly in species with selective feeding habits. Therefore, we have revised the text to clarify that differences in intestinal iron absorption processes are likely a major contributor, but not the sole controlling factor, and that both physiological regulation and prey-derived δ^{56}Fe variability may jointly influence the isotopic composition of marine fish.

Revision:

[P.11 Line250-253] Nevertheless, the δ^{56}Fe differences observed among fish groups such as tuna and mackerel (−1.58‰ to−0.71‰) versus sardine and herring (−2.64‰ to−1.73‰; Hasegawa et al., 2022) are unlikely to be explained solely by isotopic differences in their prey, but are more likely attributed to variation in intestinal iron absorption processes.

⇒The δ^{56}Fe differences observed among fish groups such as tuna and mackerel (−1.58‰ to−0.71‰) versus sardine and herring (−2.64‰ to−1.73‰; Hasegawa et al., 2022) seemed difficult to explain solely by isotopic differences in their prey since all prey species analyzed so far showed higher δ^{56}Fe values than sardine and herring (Hasegawa et al., 2022).

Minor Comments:

[P.2, Line 39] efficient intestinal absorption mechanisms that specifically exist in marine fish

[P.2, line41] However, direct quantification of intestinal iron uptake has been largely restricted

to laboratory animals using enriched isotopes (van den Heuvel et al., 1998, Fiorio et al., 2012), an approach that is not feasible for wild species especially marine fish.

[P.3, Line79] Iron purification was performed used anion-exchange chromatography following Maréchal et al., (1999).

[P. 4, Line105] following Di Iorio (1981) and Wilson et al. (2013) (delete comma)

[P.5, line 119] curve of Shiraishi et al., (2008)

Comment 7: *p5 5 l.131 "the total iron content in blood was estimated to be between 4.3 and 9.8 mg(Fig. 1B~~A~~)"*

Reply: Thank you for the reviewer's comment. We revised the text because Fig. 1 does not explicitly present numerical values for blood iron content. Since the estimated values are summarized numerically in Table S1, we consider it more appropriate to refer to Table S1 rather than Fig. 1. Accordingly, the sentence was revised as follows:

Revision:

[P.5, line134-135] Assuming a blood volume of 30 mL/kg, the total iron content in blood was estimated to be between 3.2 and 10.3 mg (Table S1).

Comment 8: - *p9 l.188: please check the value "1.99±2.20‰". Using formula 6 and 1.66‰ as delta(hm) (lowest $d^{56}fe$ in red muscle here) I get a mean value of 2.7‰ with all samples and 2.24‰ excluding Mk-1 and Mk-5.*

Reply: We thank the reviewer for carefully checking this calculation. We agree on applying a single δ_{Hm} value of −1.66‰ (the lowest δ^{56}Fe observed in red muscle).

We have recalculated Δ using this definition of δ_{Hm} and revised the text accordingly. When all individuals are included, the estimated Δ values are 4.15 ± 2.84‰ (2 S.D.) for females and 1.76 ± 0.88‰ for males. Because the sample size is limited, we do not interpret these results as evidence for a definitive sex difference. Instead, we have revised the Discussion to emphasize that the higher Δ values observed in females may reflect transient physiological conditions, such as altered iron fluxes during the spawning season, rather than equilibrium isotope fractionation.

Revision:

[P.9, Line196–208] has been replaced as the following sentences.

By assuming that the lowest δ^{56}Fe observed here in red muscle represent δ_{Hm} (−1.66‰), the isotopic offset between ferritin- and heme-bound Fe in the liver ($\Delta = \delta_{Ft} - \delta_{Hm}$) was estimated to be 2.72±3.03‰ (2 S.D.) in all specimens, whereas 4.15 ± 2.84‰ (2 S.D.) in females and 1.76 ± 0.88‰ in males. These estimated values are higher than previous observations in skipjack tuna (*Katsuwonus pelamis*, female, Δ = 1.52‰) and another chub mackerel specimen (Δ = 1.41‰, male, Hasegawa et al., 2023), and, in females, appear higher than the equilibrium isotope fractionation between Fe(II) and

Fe(III) (~2.8‰、Johnson et al., 2002; Welch et al., 2003). The two females exhibited a lower ferritin-bound iron fraction than the three males, while their liver δ^{56}Fe values are comparable to those of the males, resulting in a tendency for their estimated Δ values to be higher. Because of the limited sample size, it cannot be definitively concluded that a sex difference exists. However, one possible explanation is that the release of isotopically lighter Fe from hepatic ferritin following Rayleigh fractionation leads to significant enrichment of heavy Fe in the residual ferritin and results in Δ values larger than those expected under isotopic equilibrium. As the sample consists of mature individuals during the spawning season, it cannot be ruled out that physiological changes relating to spawning may have temporarily altered iron metabolism, particularly in females.

Comment 9: - *p11 l.243-245: "efficient uptake of iron" can be misleading. Fish have low intestinal absorption of Fe, no excretion processes for Fe, which can be toxic, and as you show low Fe storage so it would appear that they limit absorption and intensively recycle absorbed Fe.*

Reply: We thank the reviewer for this important clarification. We agree that the phrase "efficient uptake of iron" was misleading in the context of fish iron physiology. We have therefore revised the text to replace "efficient uptake of iron" with wording that emphasizes efficient retention and recycling of absorbed iron rather than elevated intestinal absorption rates.

Revision:

[P.11, Line263-264]

They may instead have evolved a "recycle ~~absorption~~ dominant" mode of iron homeostasis that relies on intensive recycling of iron to sustain metabolic demands.

Comments about Figures:

Comment 10: - *Fig. 2: The Y-axis legend at the far left of the male plots was attached as well.*

Reply: We thank the reviewer for pointing this out. Following the suggestion of Reviewer 3, we modified the figures into a single combined figure to improve readability.

Comment 11:- *Fig. 3B: The proportions were put around of the pie charts.*

Reply: The proportions have been repositioned in Figure 3B for improved readability.

Comment 12: - *Fig. S5: Point Mk-2 is missing on panel Liver "Total Fe" (0.97 in Table S2)*

Reply: We appreciate the reviewer's comment. We carefully rechecked Figure S5 and found that the data point for Mk-2 in the liver "Total Fe" panel was not plotted because the ferritin proportion for this sample was unavailable. This is due to the fact that we did not obtain enough quality of XANES the liver sample from Mk-2. We have clarified this point in the revised manuscript and Table S2.

Revision:

[P.7, line156] Sentence inserted: We note that the XANES spectra of MK-2 liver was unavailable because the quality of spectrum was not good.

Comment 12: - Fig. S5: Y-axis seems wrong. Gonads "Total Fe" in mg is lower than 1 in Table S2 but you show data going up to 20 on the y-axis.
Reply: We thank the reviewer for pointing this out. The Y-axis scale of the gonads "Total Fe" panel in Figure S5 was incorrect and has been corrected to match the values reported in Table S2.

Comment 13: - Bibliography: "Von Blackenburg" and "Von Heghe" should be at Vs, "Di Iorio" at Ds.
Reply: We appreciate the comment. The bibliography has been corrected and the references are now properly ordered alphabetically.

Response to Reviewer 2

We sincerely thank the reviewer for the careful evaluation of our manuscript and for the constructive comments and suggestions. We are grateful for the time and effort the reviewer devoted to providing these insightful comments, which were very helpful to improve the clarity and quality of the manuscript. In response to the reviewer's suggestion, we carefully revised the manuscript to avoid speculative expression.

Major Comment 1: *I have two main comments. My first comment is I am slightly confused as to what the uncertainty you report in your tables & figures show. If I am understanding correctly, you only measured each tissue from each sample once. So, from sample Mk-1, you measured two splits of Red Muscle: 1) one split for Fe isotopes, which you report with a ± 2 S.E. in Table S2, and 2) Another split for Fe concentrations, which you report with no error for wet weights, dry weights, and calculated total iron in Table S2. What does the uncertainty on your Fe isotope data represent? Is this the analytical uncertainty? Or did you measure multiple Red Muscle tissues from Mk-1, and this is propagated error from those multiple tissue measurements? In addition, as you do not report error on the Fe concentration, I'm assuming that you did not measure multiple samples, and that you are assuming that the measurement uncertainty on this is zero. That is ok if so, just needs to be clarified. You could rectify this by having a brief statement in Section 2.2, like "Each tissue was measured once for both iron isotopes and iron concentrations; tissue replicates (i.e., multiple samples of red muscle) were not performed."*

Reply: Thank you for this important comment. Each sample was measured once for both iron isotope composition and iron concentration, and we did not conduct replicate measurements. Each iron isotope measurement consisted of 60 measurement cycles using MC-ICP-MS. The reported uncertainty for iron isotope ratios (±2 S.E.) represents the internal analytical precision of a single measurement based on these 60 cycles.

Revision:

[P. 3, Line 77-78] add sentence: Iron concentrations were determined from a single aliquot of each tissue sample.

[P. 3, Line 84-85] add sentence: Each tissue sample was measured once for iron isotope composition with 60 analytical cycles.

[SI Table S2] add notation: *2 S.E. in δ^{56}Fe represents the standard deviation during single analysis (60 analytical cycles)

Major Comment 2: *My second comment is about the discussion. Overall, I think the manuscript could be improved by interpreting the results more tightly and to refrain from making statements that perhaps extrapolate beyond the measured dataset.*

For example, your discussion starts with the statement, "The δ^{56}Fe values in the liver of chub mackerel were consistently higher than those in other tissues, indicating an enrichment in the heavier Fe isotopes." However, looking at your Figure 2, this only really appears to be true for Mk-3. The heaviest tissues for Mk-1 appear to be the liver, spleen, blood, and gill (I'm guessing that they are similar because their uncertainties overlap, but it would be helpful if you could calculate P values for significant differences). The heaviest tissues for Mk-2 and Mk-4 are both the Gonad and Liver. For Mk-5, they appear to all be similar, though again I cannot readily tell because there are no P values or other statistics plotted. For Mk-6, it's actually the Gonad that is heaviest. So, the opening statement of your discussion does not really appear to be supported by the evidence.

Reply: Thank you for this helpful comment. We agree that the liver does not always exhibit the highest δ^{56}Fe value among all tissues in every individual. Therefore, we revised the wording in the Discussion to avoid the overstatement. In the revised text, we instead compare liver δ^{56}Fe values with the calculated whole-body δ^{56}Fe values (from Eq.4). We also added the calculated whole-body δ^{56}Fe values for each individual as Table S2.

Regarding the reviewer's suggestion to calculate P values, we acknowledge that the limited sample size and the absence of replicate measurements constrain the statistical interpretation of the dataset. As also noted by Reviewer 1, statistical analysis would therefore be difficult to justify. Instead, we revised the Discussion to avoid subjective wording and to refrain from interpretations that extrapolate beyond the measured dataset.

Revision:

[P. 9, Line 177-179] The δ^{56}Fe values in the liver of chub mackerel were consistently higher than those in other tissues, indicating an enrichment in the heavier Fe isotopes.

⇒ First, we focused on the liver because it is a central tissue for iron storage in vertebrates and is expected to exhibit iron isotope fractionation through the synthesis of ferritin. All the chub mackerel individuals showed higher δ^{56}Fe values in the liver than whole-body δ^{56}Fe across eight tissues.

Major Comment 3*: Next, the Albarede et al 2011 paper is cited as evidence that ferritin incorporates heavy iron – it should be specified by the authors that this study looked at variation in humans, not fish, and therefore an assumption is made that the same mechanism present in humans is also present in fish. I have no complaints about citing the Albarede paper – there are, I think, few papers on Fe isotopes in fish, but some additional detail is needed to justify this assumption. For example, how similar are human vs. fish ferritin proteins? This is an example where small changes will improve the manuscript from unintentional over-statements.*

Reply: Thank you for this helpful comment. We agree that the study by Albarède et al. (2011) was conducted on human samples, and that applying this interpretation to fish involves an implicit assumption that similar ferritin-related fractionation mechanisms operate in teleosts. To clarify this

point, we have revised the text to state that the interpretation is based on observation from human studies (Albarède et al., 2011). In addition, we added a brief explanation noting that the ferritin structure differs between mammals and teleost fish. Specifically, while mammalian ferritin consists of H and L subunits responsible for iron oxidation and mineralization, respectively, fish possess an M-type subunit with intermediate functional properties. We think that these differences in ferritin subunit may influence iron oxidation efficiency and potentially affect the magnitude of Fe isotope fractionation. Finally, we revised several sentences throughout the Discussion to avoid overinterpretation and to ensure that conclusions remain consistent with the limited sample size and the observed variability among individuals.

Revision:

[P.9, Line 180] Because ferritin preferentially incorporates heavier Fe isotopes during the oxidation in the storage process according to human studies

[P.9, Line 181-184] Insert the following sentences: It should be noted that human ferritin consists of H and L subunits responsible for iron oxidation and mineralization (Plays et al., 2021). While teleost ferritin includes an additional M-type subunit with intermediate functional properties, its effect on iron oxidation and isotope fractionation remains unclear and is not considered further here (Dickey et al., 1987; Andersen et al., 1995).

Major Comment 4: *Section 4.3 was interesting to read, as the authors tackle a question that affects any field that uses the stable isotope composition of organisms – are the isotopic fingerprints a result of differences in source (i.e., diet), or does it reflect isotopic fractionations (i.e., "internal redistribution;" fractionation due to ferritin)? This section would benefit from a figure, where you plot your data vs. the data from other studies that you mention in text (i.e., shipjack tuna, mammals) as well as their prey (i.e., zooplankton, squid, crustaceans). This, I think, would help strengthen the paper as you are adding to a larger body of data that shows an intriguing pattern – that mammals have more depleted Fe isotopes (-3.79 to -1.5‰) vs. fish (i.e., your data set ranges from about -1.6 to -0.8‰, in line with the -1.46 to -0.71‰ values that Hasegawa et al 2022 reports). (Although, it appears that sardine & herring, which you cite as having values of -2.64 to -1.73, are an exception and 'look' like mammals in this framework?) You then argue that since the only potentially fractionating component – ferritin – is a low fraction of total iron, your Fe isotope data likely reflect source instead. You then cite Fe isotope values of various prey to show that they have heavy values (-1.00 to -0.3‰) but note that these are a little too heavy for fish, particularly for fish like sardine and herring which are pretty depleted. So, perhaps this is because more data is needed of their prey (as you note), or maybe fish have a different model of ferritin cycling. For this latter point (starting Line 238), your argument would benefit from some numbers. For example, give the Delta values for humans, mice, and sheep and compare them to your values. Otherwise, this paragraph felt a little too speculative and not*

supported by data.

Reply: Thank you for this helpful suggestion. Following the reviewer's recommendation, we added a Figure 4 that compares the δ^{56}Fe values obtained in this study with previously reported values for marine fish, mammals, and representative prey organisms such as zooplankton and squid. This figure helps place our results within the broader context of Fe isotope variations in marine organisms and highlights the general pattern that some fish species tend to exhibit heavier δ^{56}Fe values than mammals.

As noted by the reviewer, some species such as sardine and herring show relatively low δ^{56}Fe values that overlap with the mammalian range. We have therefore revised the text to acknowledge this exception and discuss the possible influence of dietary sources and physiological processes.

In addition, we included representative Δ values reported for mammals (e.g., humans, mice, and sheep) and compared them with the values estimated in this study. These numerical comparisons have been added to the relevant paragraph in Section 4.3 in order to better constrain the discussion of ferritin-related isotope fractionation and reduce speculative interpretation.

Revision

[P.10, Line 229-236] The range of δ^{56}Fe variation among tissues in chub mackerel was clearly narrower than that reported for mammals. In skipjack tuna, muscular δ^{56}Fe values are consistently high regardless of regional differences (−1.46‰ to −0.71‰; Hasegawa et al., 2022), whereas mammals generally show lower values (−3.79‰ to −1.5‰; Walczyk and von Blanckenburg, 2002; Balter et al., 2013).

⇒The range of δ^{56}Fe variation among tissues in chub mackerel was clearly narrower than that reported for mammals. For example, the differences in δ^{56}Fe values between muscle and liver range from 0.72‰ to 1.71‰ in mice (Balter et al., 2013), 1.64‰ to 2.72‰ in sheep (Balter et al., 2013), and 0.83‰ to 1.75‰ in humans (Walczyk and von Blanckenburg, 2002), whereas the maximum isotopic difference among tissues in the present chub mackerel specimens was 0.73‰. Despite this relatively small internal fractionation, δ^{56}Fe values in marine organisms often show species-specific ranges (Figure 4). For example, muscular δ^{56}Fe values in skipjack tuna are high regardless of regional differences (−1.46‰ to −0.71‰; Hasegawa et al., 2022), whereas mammals generally show lower values (−3.79‰ to −1.5‰; Walczyk and von Blanckenburg, 2002; Balter et al., 2013).

The Following Figure 4 was inserted:

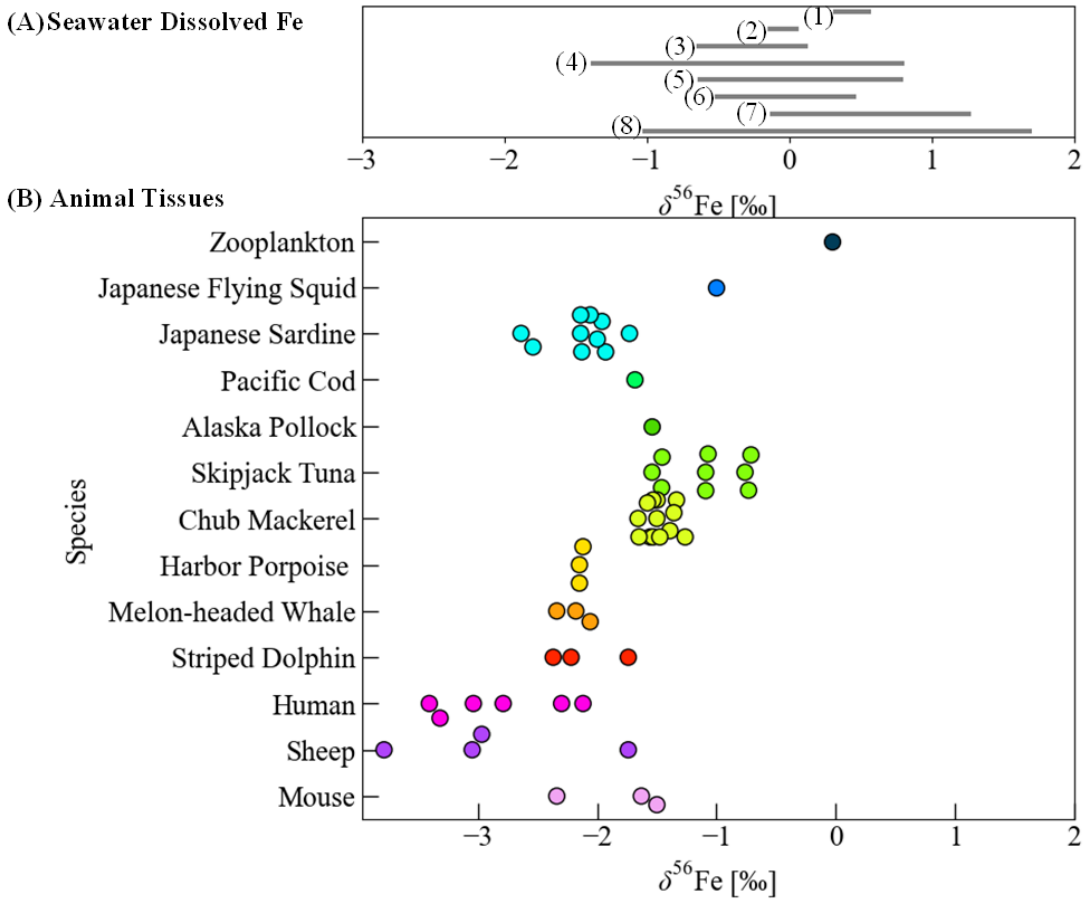

Figure 4. Comparison of δ^{56}Fe values in seawater (upper figure), and marine organisms and mammals (lower figure). Literature data in the upper figure are indicated by reference numbers: seawater from the Pacific Ocean (1–4: Radic et al., 2011; Conway and John, 2015; Pinedo-González et al., 2020; Kurisu et al., 2024), North Atlantic Ocean (5: Conway and John, 2014), and South Atlantic–Southern Ocean (6–8: Abadie et al., 2017; Ellwood et al., 2020; Sieber et al., 2021). The data of chub mackerel includes this study and Hasegawa et al. (2023), while values for zooplankton (whole body), squid (mantle), and mammals (harbor porpoise, melon-headed whale, striped dolphin, human, sheep, and mouse) are compiled from previous studies (Walczyk and von Blanckenburg, 2002; Balter et al., 2013; Hasegawa et al., 2022).

Minor Corrections:

Comment 5*: Lines 180-194: If I am understanding correctly, you are using Equation 6 to calculate the delta value of ferritin (d_{Ft}), and then using that value in your next equation ($D = d_{Ft} – d_{Hm}$) to get D. Could you report in the Supplemental the individual d_{Ft} and Dvalues you get for each fish, rather*

than just reporting the overall D in the main text?

Reply: Thank you for this helpful suggestion. We have now included the calculated δ_{Ft} and Δ ($\delta_{Ft} - \delta_{Hm}$) values for each individual specimen in the Supplemental Information. These values are provided in the newly added Table S2.

Revision:

Table S2, which describes the estimated value of δ_{Ft} (and δ_{Hm}) in each specimen was attached.

Table S2. Whole-body iron isotope ratio (δ_{WB}), and isotope ratios in liver ferritin (δ_{Ft}) and heme (δ_{Hm}) iron compartments in each chub mackerel specimen. The δ_{WB} values were calculated according to Eq.4. The δ_{Hm} value is assumed to be −1.66‰, the lowest value among mackerel samples. The δ_{Ft} values were estimated from Eq.6. The value Δrepresents isotope fractionation between δ_{Ft} and δ_{Hm} (i.e. $\Delta = \delta_{Ft} - \delta_{Hm}$).

Sample ID	δ_{WB}	δ_{Ft}	δ_{Hm}	Δ
Mk-1	−1.36	3.96	−1.66	5.62
Mk-2	−1.44	NA	−1.66	NA
Mk-3	−1.43	1.1	−1.66	2.76
Mk-4	−1.39	0.516	−1.66	2.18
Mk-5	−1.35	-0.513	−1.66	1.15
Mk-6	−1.50	0.281	−1.66	1.94

Whole-body δ^{56}Fe (δ_{WB}) was calculated as:

$$\delta_{WB} = \sum_i (\delta^{56}Fe_i \times T_i) = \sum_i (\delta^{56}Fe_i \times C_i \times M_i) \quad (4)$$

Comment 6: *Line 190: After excluding Mk-5, a D value of 2.04 ± 0.22 was derived for all five samples. It is then stated that this "slightly higher but comparable" to prior studies. Could you also give the uncertainties on the D values from those papers? For example, if the uncertainty for Katsuwomus pelamis was 1.52 ± 0.8, then that would be statistically similar to the value you derive. These additional statistics would enable you to make stronger statements overall.*

Reply: Thank you for this helpful suggestion. The Δ values reported in the previous studies were derived from single individuals and the associated uncertainties were not reported. Therefore, a direct statistical comparison with our value is difficult. We have clarified this point in the revised manuscript and adjusted the wording to avoid implying a strict statistical comparison.

Revision:

[P. 9, Line 198-200] These estimated values are higher than previous observations in skipjack tuna (*Katsuwonus pelamis*, female, Δ = 1.52‰, n = 1; Hasegawa et al., 2023) and another chub mackerel

specimen (Δ = 1.41‰, male, n = 1; Hasegawa et al., 2023)

Comment 7: *Line 221: You write, "As discussed in the previous section, hepatic ferritin iron likely undergoes isotopic fractionation on the order of approximately 1.5–2‰." Do you mean the D value you calculate? If so, how are you getting this 1.5-2‰ range, because your D values are either 1.99 ± 2.20‰ or 2.04 ± 0.22‰, depending on if you include or exclude Mk-5. Are you also including the Delta values from prior studies (K. pelamis = 1.52‰, another chub mackerel = 1.41‰)?*

Reply: Thank you for pointing this out. The fractionation range was intended to reflect both the Δ values obtained in this study and those previously reported for marine fish (e.g., *Katsuwonus pelamis* and chub mackerel). In addition, following the suggestion of Reviewer 1, the average Δ value was recalculated and is now 2.72 ± 3.03‰. To clarify this point, we have revised the sentence to explicitly state the basis of this estimate.

Revision:

[P.11, Line 241] As discussed in the previous section, hepatic ferritin iron likely undergoes isotopic fractionation on the order of approximately 1.5–3‰, based on the Δ values estimated in this study and Hasegawa et al. (2023).

Comment 8: *Line 241: You write, "In contrast to mammals that rely on ferritin-based iron recycling, fish appear to lack a long-term iron storage strategy based on ferritin, despite inhabiting chronically iron-limited environments." Are you making that inference based on your isotopic data alone, or has it been shown separately that fish lack a long-term iron storage strategy based on ferritin?*

Reply: Thank you for this comment. Our intention was not to conclude that fish lack ferritin-based long-term iron storage based solely on the isotopic data presented here. Rather, we intended to suggest that the relatively small inter-tissue δ^{56}Fe variation observed in chub mackerel may indicate a weaker influence of long-term ferritin-based iron storage compared with that reported for mammals. To avoid overinterpretation, we have revised the sentence to clarify that this point represents a possible interpretation of the isotopic patterns and remains to be confirmed by physiological studies.

Revision:

[P.11, Line 261-263] In contrast to mammals that rely on ferritin-based iron recycling, fish appear to lack a long-term iron storage strategy based on ferritin

⇒In contrast to mammals that rely on ferritin-based iron storage and recycling, the isotopic pattern observed in fish may indicate that long-term iron storage plays a less dominant role in iron homeostasis, despite inhabiting chronically iron-limited environments.

Comment 9: *Line 253: I agree that a Rayleigh-type mode would be interesting. Perhaps the authors could take it on in their next study! On that note, I recommend that the authors suggest somewhere in*

the manuscript that biochemical studies on the enzymatic fractionation of Fe isotope incorporation into ferritin or heme from fish is necessary for more detailed interpretations. For example, it would be great if someone could purify those proteins from fish and then perform the experiment in vitro.

Reply: Thank you for this constructive suggestion. We agree that biochemical studies investigating Fe isotope fractionation at the protein or enzymatic level would greatly improve the mechanistic understanding of Fe isotope behavior in fish. We have added a sentence in the Discussion highlighting the importance of future studies that directly analyze isotopic fractionation associated with ferritin and heme formation in fish.

Revision:

[P. 13, Line 287-289] Insert the following sentences:

In the future, applying a Rayleigh-type model may allow quantitative evaluation of the relative contributions of absorption and retention to isotopic fractionation in fish. Combined with XAFS analyses that provide species-level insights into ferritin- and heme-bound iron pools, these approaches could offer a powerful framework for linking biological iron metabolism to marine biogeochemical iron cycling. In addition, further biochemical studies examining Fe isotope fractionation during enzymatic incorporation into iron-binding proteins such as ferritin or heme in fish would help to better constrain the mechanisms controlling Fe isotope distribution in marine organisms.

Comment 10: *Line 260: You write, "Although isotopic fractionation of around 1.5-2‰ occurs in liver ferritin, its effect on whole-body $d^{56}Fe$ is minimal due to the limited ferritin fraction and overall liver iron content." Again, where is this 1.5-2‰ value coming from?*

Reply: Thank you for pointing this out. As noted in our response to Comment 7, the fractionation range refers to the Δ values estimated in this study as well as those reported in previous studies of marine fish. To clarify this point and maintain consistency with the revised discussion, we have modified the sentence accordingly.

Revision:

[P.13, Line 293] Although isotopic fractionation of around 1.5-3‰ occurs in liver ferritin,

Comment 11: *Line 263: You write, "The relative homogeneity of $\delta^{56}Fe$ among major tissues such as muscle and liver suggests that iron isotope pools in fish remain relatively stable, providing new insights into iron transport and isotope systematics within marine food web." When you say this, do you mean that the isotopic fractionation of 1.5-2‰ is larger than the observed range you see in your samples, which is about 0.8‰? (Your data range from about -1.6 to -0.8‰; Figure 2). Stating actual numbers would help make this conclusion stronger. In addition, as far as that 'relative homogeneity' statement goes… you do spend some time pointing out how liver is very isotopically heavy, though see my disagreements above about it being universally isotopically heavy throughout all your samples.*

I'm assuming that your 'relative homogeneity' statement is saying that, despite variations among tissue types, the full range of variation (0.8‰) is smaller than the potential full isotopic fractionation of 1.5-2‰. Is that what you mean?

Reply：We thank the reviewer for this helpful comment. Yes, our intention was to indicate that the observed inter-tissue δ^{56}Fe variability (maximum 0.73‰ among all tissues) is smaller than the potential isotopic fractionation associated with ferritin synthesis (approximately 1.5–3‰ based on Δ values from this and previous studies). We have revised the sentences to clarify this point.

Revision;

[P.13, Line 295-298] The relative homogeneity of δ^{56}Fe among major tissues such as muscle and liver suggests that iron isotope pools in fish remain relatively stable, providing new insights into iron transport and isotope systematics within marine food web.

⇒The maximum δ^{56}Fe range among major tissues was 0.73‰, indicating relatively small tissue isotopic variability. The small variation in δ^{56}Fe among major tissues such as muscle and liver compared with the potential isotopic fractionation associated with ferritin formation, suggests that iron isotope pools in fish remain relatively stable.

Comment 12: Line 287: I appreciate and commend the authors on being forthcoming and transparent about using ChatGPT in improving the English and readability of the manuscript.

Reply: Thank you for your kind comment. We appreciate your positive feedback.

Comments on Figures:

Comment 13: *Figure 1: Could you give some more description in the figure caption? For example, for Panel A, I'm assuming that your boxplot is showing the typical interquartile range (IQR), with whiskers showing 1.5 times the IQR – for example, these are the default parameters when plotting boxplots using pandas in Python. But this should be explicitly specified. In addition, are you plotting or not plotting outliers? Right now, I'm assuming from Panel A that you have no outliers (i.e., no discrete points outside of your boxplots). For Panel B, it would be helpful to restate that percentages were calculated assuming a total blood volume of 30 mL/kg. Also – each proportion is shown with a solid line, but is there some uncertainty on that? If so, you could briefly state, for example, "Uncertainty on calculated iron content (%) is typically ±X% (not shown)." If not (as you do not report uncertainty on your iron concentrations in Table S2), you should state "No uncertainty is reported as multiple sample replicates were not measured, and measurement uncertainty is assumed to be zero."*

Reply: We thank the reviewer for the suggestions. We inserted additional sentences in the caption of Figure 1. We also added a statement noting that uncertainty is not shown because replicate measurements were not performed. In addition, following Reviewer 1's suggestion, the boxplots in

Panel A were replaced with scatter plots in the revised figure.

Revision:

Figure 2: (A) Iron concentration, and (B) Tissue iron burden of the chub mackerels (Mk-1 to Mk-3: female, Mk-4 to Mk-6: male)

⇒ Figure 3: (A) Iron concentration, and (B) Tissue iron burden of the chub mackerels (Mk-1 to Mk-3: female, Mk-4 to Mk-6: male). Total blood contents were assumed to be 30 mL/kg with a specific gravity of 1.05. No uncertainty is reported as multiple sample replicates were not measured. The measurement uncertainty was assumed to be zero.

Comment 14: *Figure 2: Could you provide some additional information in the Figure caption? For example, are the error bars on each discrete point showing a 1 sigma or 2 sigma error? And, per my comments above, I'm assuming that this is analytical uncertainty on a single measurement, not propagated uncertainty over multiple tissue replicates, correct? In addition, you should re-label the y-axis on the lower three panels (i.e., Red Muscle, White Muscle, etc.) for improved readability. And the lower three panels are missing the x-axis label of delta 56 Fe, which should be centered as well.*

Reply: Thank you for this helpful comment. Error bars represent ±2 S.D. internal analytical uncertainty of a single MC-ICP-MS measurement (60 cycles). Because each tissue sample was measured once, the error bars reflect analytical precision rather than propagated uncertainty from multiple tissue replicates. In addition, following the suggestion of Reviewer 3, we modified the figures into a single combined figure to improve readability.

Revision:

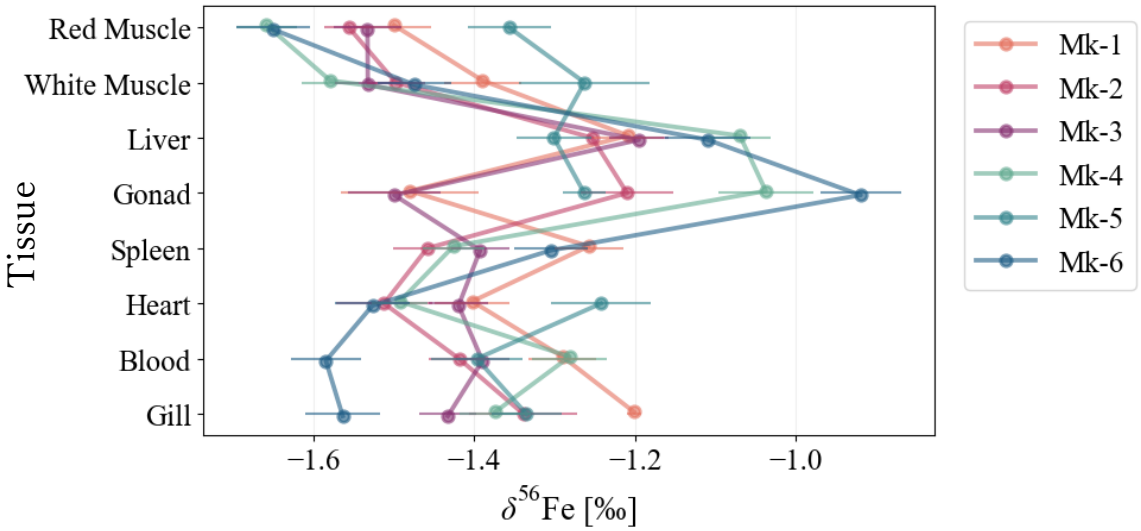

Figure 2: Iron stable isotope ratio in tissues of the six Scomber japonicus individuals (Mk1-3: females, Mk4-6: males). ~~The red line represents average δ^{56}Fe values across the eight tissues. The gray dotted lines represent 2 S.D. values from the average line.~~

⇒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).

Comment 15: *Figure 3: Overall, font size needs to be increased. Panel A is missing some description; it should also say "Black lines represent measured spectra while red lines are reconstructed spectra." And, in Panel B, could you move the percentages on the pie chart to the outside? This would help increase readability.*

Reply: Thank you for your helpful comments. We have revised Figure 3 to improve readability. The font size in the figure has been increased. In Panel A, we added the description "Black lines represent measured spectra while red lines are reconstructed spectra." In Panel B, the percentage labels in the pie chart have been moved outside the chart to improve clarity.

Revision:

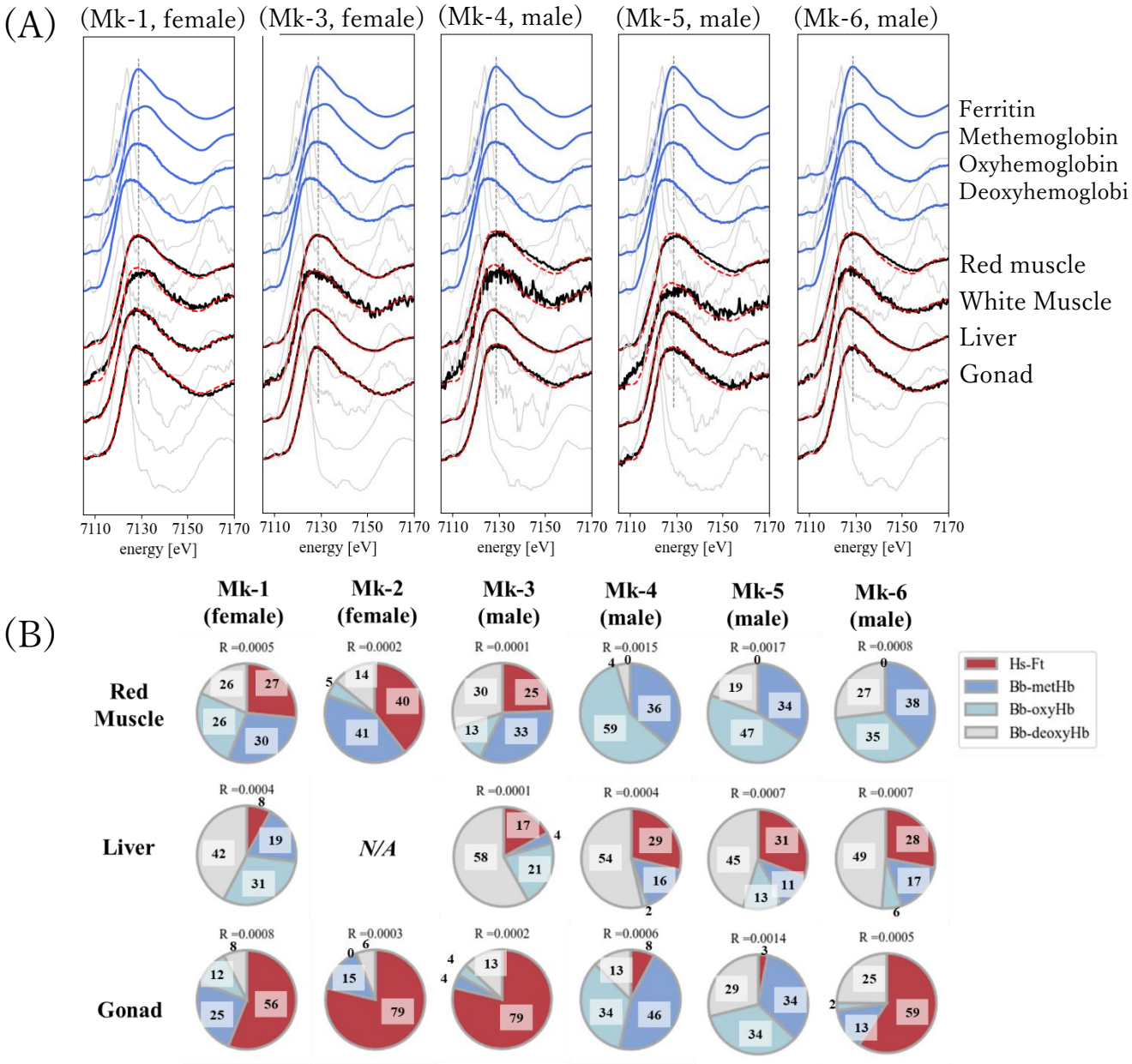

Figure 3: (A) Fe K-edge XANES spectra of *Scomber japonicus* tissues. Mk-1 and Mk-3 represent females, and Mk-4 to Mk-6 represent males. The standard spectra were obtained from ferritin from horse spleen and hemoglobin derivatives from bovine blood. Blue lines indicate the spectra of the standard materials, 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 Hs-Ft 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.

Comment 16: *Overall, I would recommend that you re-format the SI PDF because some of the figures are blurry to the point of being illegible. Figure S1 looks great, but then some (i.e., Fig. S2 and S8) are very blurry. Perhaps some issue with inserting images in the wrong format (i.e., PDF vs. JPG)?*
Reply: Thank you for pointing this out. We have re-formatted the Supporting Information to improve the figure quality.

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:

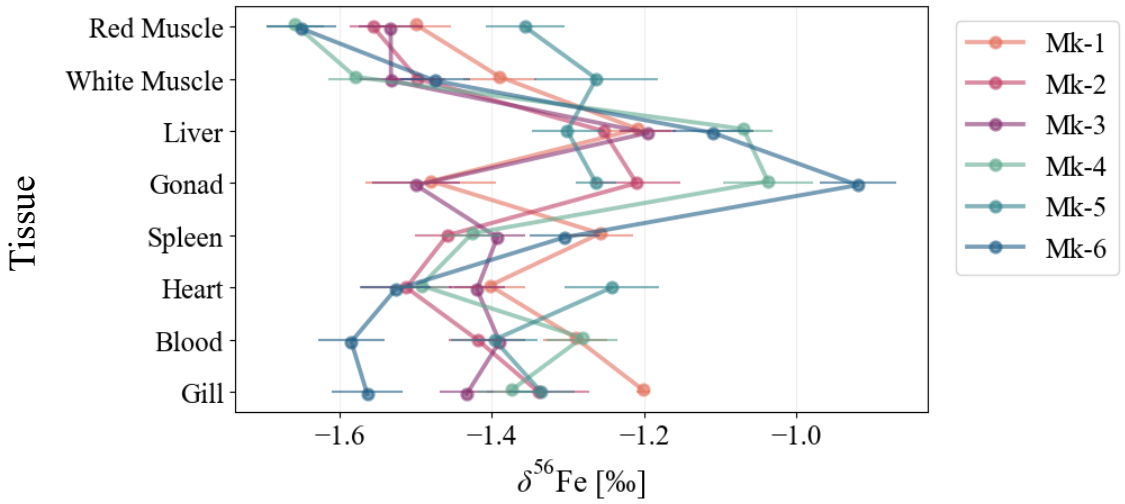

Figure 2: Iron stable isotope ratio in tissues of the six *Scomber japonicus* individuals (Mk1-3: females, Mk4-6: males). Error bars represent 2 S.E. during a single measurement (60 analytical

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 1st 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:

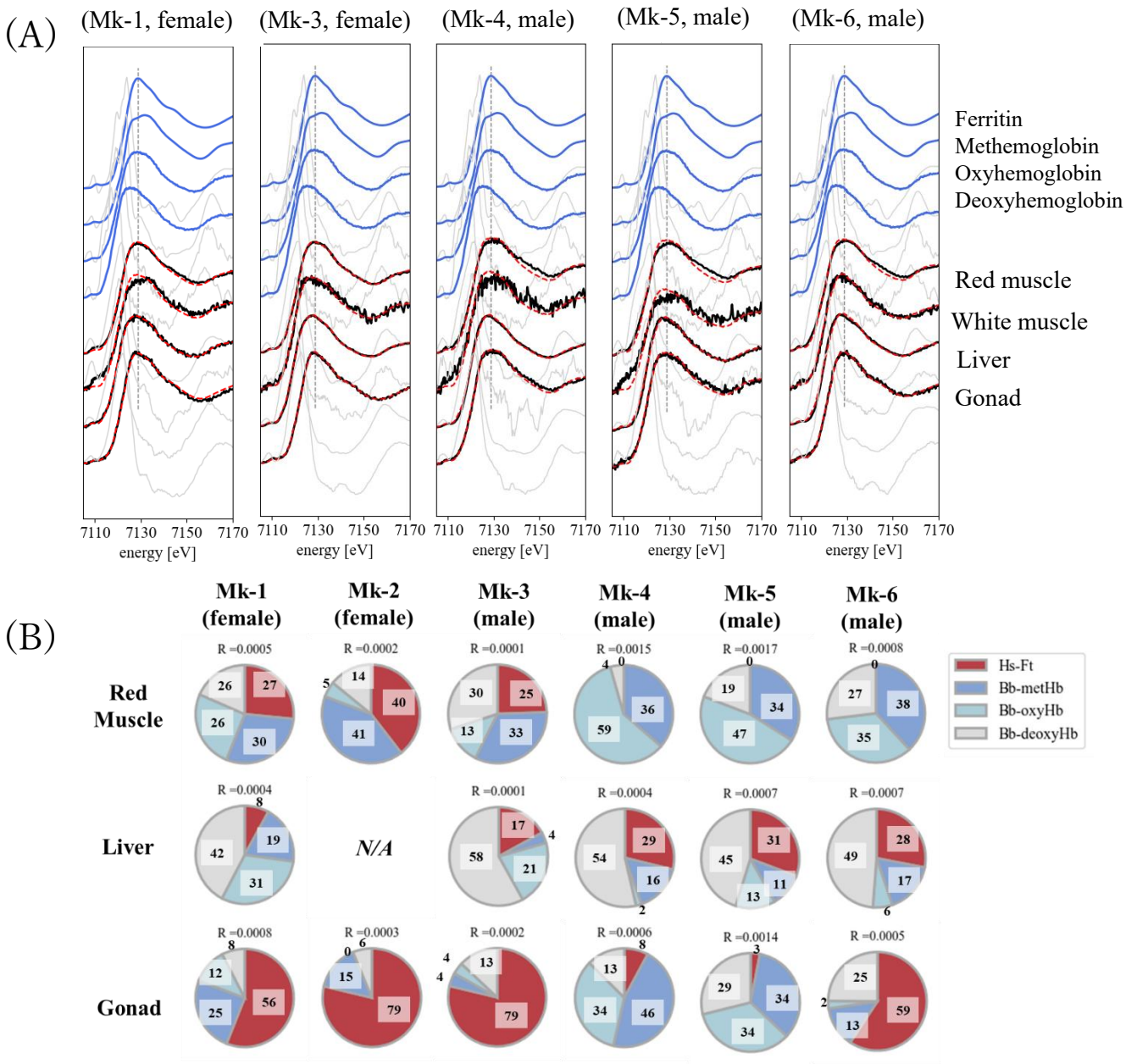

Figure 3: (A) Fe K-edge XANES spectra of *Scomber japonicus* tissues. Mk-1 and Mk-3 represent females, and Mk-4 to Mk-6 represent males. The standard spectra were obtained from ferritin from horse spleen and hemoglobin derivatives from bovine blood. Blue lines indicate the spectra of the standard materials, 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.