Food Control

# Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species

--Manuscript Draft--

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| **Manuscript Number:** | FOODCONT-D-25-00683R1 |
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| **Abstract:** | In this study, 47 Chinese fish balls (FBs) sold on e-commerce platforms were collected and the ingredient of animal origin were identified using 16S rRNA metabarcoding. The sequencing results were compared to the FBs declared composition (DC) - obtained by combining the trade name and the ingredient list - to assess mislabelling. FBs were considered mislabelled if an ingredient not reported in the DC was detected molecularly, or if an ingredient in the DC was not detected. Excluding 14 FBs that only listed the generic term "fish," the DC mostly included eel (Anguilliformes), shark (Elasmobranch), and freshwater species. The presence of pork or chicken was also declared in several FBs. Overall, 65.9% of FBs (n=31) were considered mislabelled. The following cases were highlighted (the same FB could be included in more than one case, simultaneously): i) 21 FBs containing undeclared pork, chicken, or beef; ii) 9 FBs containing additional fish species which were not declared; iii) 7 FBs not containing the fish reported in the DC.. Threatened fish species were also detected. These mislabelling cases highlight the challenges consumers face in making ethical, religious, and sustainable choices. A more thorough evaluation of the quantitative potential of metabarcoding, as well as the possibility to combine this technique with quantitative methods (e. g. qPCR) should be considered for monitoring the surimi production chain. |
| **Response to Reviewers:** | |

Dear Editor,

please find enclosed the manuscript entitled "**Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species**" to be considered for publication. A brief summary of the attached manuscript is given to introduce the topic.

Seafood supply chain has expanded to the point where a given fish is potentially harvested in one country, processed in another, and consumed in yet another. Therefore, seafood is among the commodities at high risk of mislabelling. Besides deceiving consumers and cause reputational damages to companies, seafood mislabelling may lead to human health risks due to presence of toxic species or the omission of ingredients potentially causing allergies (e. g. crustaceans or molluscs). In addition, mislabelling is suspected of being an important enabler of illegal, unreported, and unregulated (IUU) fisheries.

Surimi, a highly processed seafood product, is particularly vulnerable to mislabeling. Both the inclusion of squid and cuttlefish, which could pose a health risk to consumers allergic to molluscs and the presence of vulnerable species was identified.

Next Generation Sequencing technologies (NGS), high-throughput methods able to simultaneously sequence all the DNA molecules contained in a sample, are the most suitable for the authentication of complex food matrices, including surimi and surimi base products such as fishcakes and fishballs.

In this study, 47 Chinese fish balls (FBs) sold on e-commerce platforms were collected and authenticated using 16S rRNA metabarcoding. The molecular results were compared to the declared composition (DC) - obtained by combining the trade name and the ingredient list - to assess mislabelling. FBs were considered mislabelled if an ingredient not reported in the DC was detected molecularly, or if an ingredient in the DC was not detected. Excluding 14 FBs that only listed the generic term "fish," the DC mostly included eel (Anguilliformes), shark (Elasmobranch), and freshwater species. The presence of pork or chicken was also declared in a number of FBs. Overall, 65.9% of FBs (n=31) were considered mislabelled. The following cases were highlighted (the same FB could be included in more than one case, simultaneously): 1) 21 FBs containing undeclared pork, chicken, or cattle; 2) 9 FBs containing additional fish species not declared; 2) 7 FBs not containing the fish reported in the DC. The mislabelling rate may be even higher, as in some FBs the proportion of the primary ingredient did not align with its listed order. Threatened fish species were also detected. Overall, the 16S rRNA metabarcoding applied in this study allowed to detect a high mislabelling rate in FBs, even

potentially underestimated considering the not-quantitative nature of the method, which limits the ability to determine whether the quantity of the declared ingredients align with their order in the ingredients list. For the first time, the undeclared presence of pork, chicken, and cattle was unveiled in surimi-based products. These mislabelling cases highlight the challenges consumers face in making ethical, religious, and sustainable choices. Therefore, it is strongly recommended to continue implementing monitoring techniques for surimi production lines. Additionally, a more thorough evaluation of the quantitative potential of metabarcoding should be considered.

**Ms. Ref. No.**: FOODCONT-D-25-00683R1

**Title**: Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species

Dear Prof. Editor

Thank you for your kind consideration of our manuscript for publication in ***Food Control*** as a Research Article.

Please find enclosed the revised manuscript entitled "*Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species*" along with our responses to the reviewers' comments. We found the reviewers' feedback highly valuable and constructive, and we have made the necessary amendments to the manuscript to address their suggestions. We hope this revised version meets their expectations.

Please forward all correspondence regarding this manuscript to:

Prof. Andrea Armani,

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Best regards,

Andrea Armani

## Response to Reviewers' comments

## Reviewer #1:

**General comment:** *The manuscript presents a study on the authentication of 47 Chinese fish balls using 16S rRNA metabarcoding, revealing significant mislabeling issues and the presence of undeclared mammalian and avian species. The findings have important implications for food safety, consumer protection, and sustainability. However, several issues need to be addressed to improve the clarity, rigor, and impact of the study.*

**Response:** We thank the reviewer for their valuable comments. In the revised manuscript, we have addressed the issues raised by improving the clarity, rigor, and impact of the study. We believe these revisions significantly improve the manuscript, and we appreciate the reviewer's insightful feedback.

**Major Comments:**

**Comments 1:** *The specific DNA extraction kit (Line 146-153) is not specified.*

**Response 1:** Thank you, the used kit has been reported (**Lines 146-152**).

**Comments 2:** *The primer pair used (16S rRNA) is stated to have broad taxonomic coverage, but no validation data or references are provided to support its effectiveness for detecting mammalian and avian species.*

**Response 2:** Thank you for this consideration. Indeed, this aspect was addressed very marginally in the original version. In this regard, we have added a section in the text where we report the taxonomic coverage of the primers used based on the number of species tested both in the study by Sarri et al. (2014), who projected the primers, and in other studies that used the same primers. We also included two studies that applied them in a metabarcoding approach for the authentication of meat and fish products (**Lines 326-330**). Additionally, we have provided a new table (**Table SM-1**) detailing the species amplified by these primers based on the literature. We hope this information can support the selection of the primers we used for the analysis.

**Comments 3:** *The study acknowledges that metabarcoding is not quantitative, yet the conclusions (e.g., Line 41 "the primary ingredient did not align with its listed order") imply quantitative inferences. This could be misleading and should be clarified.*

**Response 3:** This sentence, reported in the abstract, has been modified to make it less misleading. Additionally, the aspect related to the quantitative interpretation of the results (and mislabelling) has been clarified further in the text (**Lines 416-439**).

**Comments 4:** *A more thorough discussion of the limitations of metabarcoding in quantifying species proportions is needed. Consider including a statement on the potential for PCR bias and its impact on the interpretation of results.*

**Response 4:** This aspect has been further detailed in the manuscript (**Lines 416-439**).

**Comments 5:** *The manuscript should discuss whether the use of threatened species (e.g., sharks listed on the IUCN Red List) in surimi products complies with CITES regulations or Chinese national laws.*

**Response 5:** Thank you very much for your valuable suggestion. We have carefully examined the fish species identified in FBs, particularly those categorized as endangered, vulnerable, or near-threatened by the IUCN, and compared them against List of State Key Protected Wild Animals in China and relevant CITES appendices. We found that incorporating shortfin mako (*Isurus oxyrinchus*) into FB production not only poses potential threats to ecological sustainability but also risks violating both Chinese national and international (CITES) regulations, thus exposing manufacturers to potential legal prosecution. We have incorporated this discussion into the revised manuscript. Again, we sincerely appreciate your insightful recommendation. **(Please see Lines 523-533)**

**Comments 6:** *The Fisher's Exact Test used to analyze the relationship between labeling and price categories may lack statistical power due to the small sample size. Consider using alternative statistical methods or clearly stating the limitations of the current analysis.*

**Response 6:** Fisher's Exact Test (for 2x2 tables) is particularly useful for small sample sizes, especially when the expected frequencies in any cell of a contingency table are less than 5. In these situations, the Chi-squared test may not provide accurate results, and Fisher’s Exact Test offers a more reliable alternative by calculating the exact probability of obtaining the observed distribution of values. This aspect can be found in a number of statistical manual, including Agresti (2002) (this reference has been added in the paper).

**Comments 7:** *Figure 3 (Line 756) could be improved by using more informative visualizations, such as boxplots, to better represent the data distribution.*

**Response 7:** Good suggestion. We have incorporated boxplots into **Figure 3** to better illustrate the distribution of sample prices, enhancing the clarity and informativeness of our visual presentation.

**Comments 8:** *The sustainability implications of using threatened species in surimi production are briefly mentioned but not explored in depth. A more detailed discussion on how this practice impacts marine conservation efforts and potential regulatory measures would strengthen the manuscript.*

**Response 8:** Thank you for your valuable suggestion. We have added further discussion to this section to more effectively address how the use of threatened species in surimi production impacts marine conservation efforts and to explore potential regulatory measures in greater detail.

**Minor Comments:**

**Comments 9:** *There are minor grammatical errors in the manuscript. For example, in the Abstract (Line 39), the numbering of mislabeling cases is repeated (2) 9 FBs....A thorough language review is recommended.*

*Some sentences are overly complex and could be simplified for better readability.*

**Response 9:** The abstract has been amended. The language has been revised throughout the entire manuscript.

**Comments 10:** *Some references are inconsistently formatted (e.g., Reference 67-Zhang et al., 2024 appears twice with different page numbers, Refernce 62-the name of the journal does not have italics).*

*Please ensure all references follow the formatting guidelines.*

**Response 10:** We thank the reviewer for pointing out the issues. We have checked the format of all references and revised them one by one.

## Reviewer #2:

**General comment:** *Zhang et al. purchased 47 Chinese fish balls (FBs) from an e-commerce platform and identified them using 16S rRNA whole-genome sequencing technology. The molecular detection results were compared with the declared ingredients (DC) to assess the*

*labeling errors. The study is highly relevant, addressing food mislabelling issues, particularly in the context of Chinese fish balls. It explores a timely topic using advanced molecular techniques, which could significantly contribute to food authenticity verification and regulatory practices. However, the manuscript needs thoroughly major revised before publication is possible. The following comments should be clarified and included in the revised manuscript.*

**Comments 1:** *The experimental part is rather lengthy: from line 135 to line 179 (from "2.1. Sampling" to "2.3. Library preparation and sequencing"), the description is overly detailed, especially regarding the steps on how to extract DNA from the samples. It is suggested to simplify these steps or present them as an appendix.*

**Response 1:** This part was simplified according to the reviewer's suggestion (**Lines 178-189**). In particular, in section 2.3 ("library preparation and sequencing"), the description of libraries preparation has been deferred to the relevant Illumina protocol that was used. In contrast, the section related to DNA extraction has not been simplified, as we believe the information provided is all necessary. Additionally, another reviewer asked us to include more information regarding the extraction kit.

**Comments 2:** *There are many cases of improper transitions or excessive paragraphs in the text, such as lines 185-186, 218-219, 234-235,249-250, 300-301.*

**Response 2:** The entire manuscript has been carefully revised accordingly.

**Comments 3:** *The introduction part of the text is rather lengthy, which prevents readers from quickly getting to the main point. Please refine the language and logic.*

**Response 3:** We thank the reviewer for their suggestion. However, in our opinion, an introduction of only three pages cannot be considered long, and it is necessary to clearly describe the main topics of interest for the study and detail the aim. Following another of the reviewer's suggestion, we have also streamlined the initial section regarding the labelling policies (**Lines 63-74**, and **119-125**).

**Comments 4:** *Confusing usage of terms: Lines 60 to 70 ("implementation of labelling policy...") mention some international standards and food labelling policies, but the wording is not precise enough. For instance, the part about "Codex Alimentarius Commission" could provide*

*a more specific explanation of its relationship with food labelling and its actual impact, avoiding overly abstract descriptions.*

**Response 4:** Thank you for this right observation. The sentence has been modified to avoid misunderstandings (**Lines 62-72**).

**Comments 5:** *Insufficient discussion on primer selection: Lines 293 to 300 ("PCR generates bias related to primer-template mismatches...") mention the issue of primer selection and bias in PCR, but do not further discuss how to optimize primer design, especially in the case of multi-species mixtures. This part could further explore how to reduce these biases through primer optimization. Furthermore, it is mentioned that deviations may occur in PCR, such as mismatches between primers and templates. However, this part does not elaborate on how to conduct a pre-assessment of primer efficiency. It is suggested to add an experimental step to verify the amplification efficiency of primers in various species, in order to reduce false negative and false positive results.*

**Response 5:** Thank you for this consideration. Also based on reviewer 1 comments, we have indeed observed that this aspect had been addressed very marginally. In this regard, we have added a section in the test where we report the taxonomic coverage of the primers used based on the number of species tested both in the study by Sarri et al. (2014) and in other studies that used the same primers, including two studies that applied them in metabarcoding for the authentication of meat and fish products (**Lines 326-332**). Additionally, we have provided a new table (**Table SM-1**) detailing the species amplified by these primers based on the literature. Finally, in the conclusion section, it was emphasized that this method, at present, has limitations related to the reliability of quantitative results and should therefore be used as a screening method and/or combined with quantitative methods such as qPCR.

**Comments 6:** *Lines 208 to 265 ("The mislabeling rate may be even higher...") mention that the mislabeling rate might be even higher, but there is a lack of further substantive analysis.*

**Response 6:** This sentence has been modified in the text (**Lines 247-259**).

**Comments 7:** *More discussion on the potential impacts of mislabeling on consumers' health, morality and the environment, especially when it comes to endangered species, can be included in the discussion section. In-depth analysis of its effects on the ecosystem can also be provided.*

**Response 7:** Thank you for your valuable suggestion. We have added further discussion to this section to more effectively address issues for consumers' health and morality, and how the use of threatened species in surimi production impacts marine conservation efforts and to explore potential regulatory measures in greater detail.

**Comments 8:** *Although the qualitative characteristics of metabarcoding technology were mentioned, no suggestions were made in the discussion section regarding how to transition from qualitative analysis to quantitative analysis. For instance, it was not considered to combine the data with other quantitative methods, or to explore how to quantify the proportions of different components in the samples in subsequent studies.*

**Response 8:** Thank you, this aspect was detailed in the conclusion section.

**Comments 9:** *The entire research relies on metabarcoding technology, which is a qualitative method and does not conduct quantitative analysis on the relative abundance of components. Although the article mentions an error rate of 65.9%, this data might be underestimated because the changes in species proportions and quantities have not been taken into account. The authors can consider adopting quantitative methods (such as through primer efficiency or experimental mixture analysis) to provide a more comprehensive analysis of misidentification. I suggest adding a further assessment of the quantitative potential of metabarcoding in the data discussion section and exploring how to combine it with existing quantitative techniques (such as qPCR) in the experimental design details.*

**Response 9:** Thank you, this aspect was detailed in the conclusion section

**Comments 10:** *Although the 47 samples have certain representativeness for the research, the sample size is relatively small and may not fully reflect the diversity of fish balls in the Chinese market. Especially if future research aims to be extended to a broader market or food category, the expansion of the sample size becomes particularly important. I suggest increasing the sample size by including samples from different regions, brands or time periods to enhance the general applicability and reliability of the research results.*

**Response 10:** Thank you for the suggestion. based on the literature, we do not believe that 47 samples are too few for a metabarcoding study. However, we will take it into consideration for future studies.

**Comments 11:** *The language of the entire text may need to be polished by native experts to enhance its readability.*

**Response 11:** The language has been revised.

## Reviewer #3:

**General comment:** *The article entitled "Beyond mislabeling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species" investigates the authentication of Chinese fish balls using metabarcoding techniques to identify undeclared species such as mammals and birds. The study reveals a high rate of mislabeling in these products, with the undeclared presence of pork, chicken and cattle, as well as endangered fish species. The study is original and relevant, especially in the context of food fraud and the authentication of marine products. However, major revisions are needed before being published.The structure of the manuscript looks fine, but some relevant information in the text can be important to add or correct (see below).*

**Comments 1:** *Title: However, this is the first time that the findings have been reported in the title.*

**Response 1:** In our experience, several studies do this. Furthermore, we believe that this title is sufficiently engaging to capture the reader's curiosity, and this is also important for the journal.

**Comments 2:** *Introduction: The introduction is well structured, but the importance of the chosen technique is not clear, as it highlights the limitations of the method.*

**Response 2:** Thank you for your suggestion, the potential of the chosen method (especially as screening), despite its limitation respect to the quantitative analysis, has been remarketed throughout the manuscript.

**Comments 3:** *Figure 1 I understood would be enlightening for other culinary cultures, but it's not relevant.*

**Response 3:** Thank you for your suggestion. However, we believe that this figure is interesting considering that it is a little-known product, especially in non-Asian contexts.

**Comments 4:** *Tables: Avoid vertical rules. Place any table notes below the table body. Results and Discussion: The results are clearly presented but could discuss the implications of the*

*findings in more depth, especially in relation to sustainability and consumer health. For example, the detection of endangered species and the presence of undeclared ingredients can have significant impacts on marine conservation. The environmental impact was not mentioned in the discussion, I suggest changing the approach.*

**Response 4:** As for the previous comment, we have added further discussion to this section to more effectively address how the use of threatened species in surimi production impacts marine conservation efforts and to explore potential regulatory measures in greater detail.

**Comments 5:** *The results are not presented in the same order as the materials and methods, or rather, they could combine.*

**Response 5:** Actually, we noted that only the statistical section in M&M does not combine with the results. Statistical sections are often reported at the end of the M&M section in scientific papers, so that we thank the reviewer, but we prefer to maintain the original structure. However, the structure of M&M and R&D was slightly modified to meet the reviewer's request.

**Comments 6:** *It was not clear that the results of the Total DNA extraction and evaluation are presented in the figures 4, 5 and SM-1.*

**Response 6:** These figures and table do not refer to DNA extraction but on sequencing results (taxonomic assignment of the sequences).

**Comments 7:** *Line 364. What is the meaning of sequence abundance?*

**Response 7:** Percentage of sequences assigned to a species respect to the overall sequences in the sample. Clarified in the text.

**Comments 8:** *Figure 4. It would be interesting to quote the Figure during the discussion (as noted in Fig...). As well as Table SM1*

**Response 8:** Done.

**Comments 9:** *Lines 435-437. "Overall, no significant differences (p-value 0.5278) in prices were observed between mislabelled and not mislabelled FBs" in which table can I find the statistical results?*

**Response 9:** We apologize, the real value, although not significant, is actually 0.3262 (modified in the text). This statistical calculation was simply done by tabulating in a 2x2 table

the products considered mislabelled and those not considered mislabeled (rows), and the high or low price range (columns). This information can be extrapolated from the other tables (particularly **Table 1** and **Table 2**) provided, and we did not consider it necessary to include them again. However, below we have provided the tables related to the used data (**Table A**) and the table used for the statistical test (**Table B**).

**Table A. M/N: mislabelled/not mislabelled; L/H: low price/high price (respect to the average price of 17.07 USD/kg)**

| Sample | price USD/kg | M/N | L/H |
|---|---|---|---|
| FB1 | 16.4 | M | L |
| FB2 | 10.9 | M | L |
| FB3 | 26.4 | M | H |
| FB4 | 26.4 | N | H |
| FB5 | 71.1 | N | H |
| FB6 | 46.3 | N | H |
| FB7 | 46.3 | N | H |
| FB8 | 27.3 | N | H |
| FB9 | 19.3 | M | H |
| FB10 | 16.2 | M | L |
| FB11 | 16.2 | M | L |
| FB12 | 13.7 | M | L |
| FB13 | 7.3 | N | L |
| FB14 | 17.6 | M | H |
| FB15 | 13.5 | N | L |
| FB16 | 16.3 | M | L |
| FB17 | 17.6 | M | H |
| FB18 | 17.6 | M | H |
| FB19 | 8.17 | M | L |
| FB20 | 11.6 | M | L |
| FB21 | 4.53 | N | L |
| FB22 | 11.94 | M | L |
| FB23 | 10.41 | N | L |
| FB24 | 27.4 | N | H |
| FB25 | 14.5 | N | L |
| FB26 | 17.8 | M | H |
| FB27 | 22.0 | N | H |
| FB28 | 9.35 | M | L |
| FB29 | 9.35 | M | L |
| FB30 | 9.78 | N | L |
| FB31 | 9.85 | N | L |
| FB32 | 13.72 | N | L |
| FB33 | 13.44 | M | L |
| FB34 | 15.43 | M | L |
| FB35 | 19.01 | M | H |
| FB36 | 19.01 | M | H |
| FB37 | 13.73 | N | L |
| FB38 | 4.57 | M | L |
| FB39 | 4.57 | M | L |
| FB40 | 4.57 | M | L |
| FB41 | 10.99 | M | L |
| FB42 | 20.77 | M | H |

| | | | |
|---|---|---|---|
| FB43 | 11.57 | M | L |
| FB44 | 17.02 | M | L |
| FB45 | 13.77 | M | L |
| FB46 | 19.74 | M | H |
| FB47 | 7.66 | M | L |

**Table B.**

| | **HP** | **LP** |
|---|---|---|
| **M** | 10 | 21 |
| **NM** | 7 | 9 |

**M: mislabelled**
**NM: not mislabelled**
**HP: high price (higher than the average)**
**LP: low price (lower than the average)**

**Comments 10:** *Conclusion: Line 487. it failed to mention possible adulterations under discussion.*

**Response 10:** This sentence has been modified.

**Comments 11:** *To conclude the review, a few general points: Improve the clarity and structure of the article, especially in the Methods section.*

**Response 11:** Done.

**Comments 12:** *Highlight the scientific contributions and originality of the study.*

**Response 12:** Done.

**Comments 13:** *Explore the ethical and sustainability implications in more depth.*

**Response 13:** Done.

**Comments 14:** *Improve tables and figure legends*

**Response 14:** Done.

**Comments 15:** *Standardize references.*

**Response 15:** Done

**Comments 16:** *Correct minor errors and inconsistencies in the text.*

**Response 16:** Done

# Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species

Xia Zhang [a,1], Alice Giusti [b,1], Sihui Li [c], Weide Deng [d], Zhenzhu Sun [d], Yuan Li [d], Hongyuan Peng [d], Jiajie Hu [b], Andrea Armani [b,*], Jing Wen [d,*]

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**Abstract**

In this study, 47 Chinese fish balls (FBs) sold on e-commerce platforms were collected and the ingredient of animal ~~species~~ origin ~~contained~~ were ~~authenticated~~ identified using *16S rRNA* metabarcoding. The ~~molecular~~ sequencing results were compared to the FBs declared composition (DC) - obtained by combining the trade name and the ingredient list - to assess mislabelling. FBs were considered mislabelled if an ingredient not reported in the DC was detected molecularly, or if an ingredient in the DC was not detected. Excluding 14 FBs that only listed the generic term "fish," the DC mostly included eel (Anguilliformes), shark (Elasmobranch), and freshwater species. The presence of pork or chicken was also declared in ~~a number of~~ several FBs. Overall, 65.9% of FBs (n=31) were considered mislabelled. The following cases were highlighted (the same FB could be included in more than one case, simultaneously): i~~1~~) 21 FBs containing undeclared pork, chicken, or ~~cattle~~beef; ~~2~~ii) 9 FBs containing additional fish species which were not declared; ~~2~~iii) 7 FBs not containing the fish reported in the DC. ~~Being metabarcoding not a quantitative analysis, it was not possible to evaluate if the ingredient amount reflects the FB ingredient list. The mislabelling rate may be even higher, as in some FBs the proportion of the primary ingredient did not align with its listed order.~~ Threatened fish species were also detected. These mislabelling cases highlight the challenges consumers face in making ethical, religious, and sustainable choices. ~~Being metabarcoding not a quantitative analysis, it was not possible to evaluate if the ingredient amount reflects the FB ingredient list. Therefore, a~~ A more thorough evaluation of the quantitative potential of metabarcoding, as well as the possibility to combine this technique with quantitative methods (e. g. qPCR) should be considered~~,~~ ~~it is strongly recommended to continue implementing~~ for monitoring ~~techniques for~~ the surimi production ~~lines~~chain. ~~Additionally, a more thorough evaluation of the quantitative potential of metabarcoding should be considered.~~

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**Keywords:** Next Generation Sequencing, DNA, surimi, seafood authentication, fraud, Chinese market, labelling

## 1. Introduction

A food label is one of the most important and direct means of communicating information to the consumer (FAO, 2025; Schifferstein et al., 2021). ~~Implementation of labelling policy have been developed to convey information along the supply chain to the final consumer with the aim of enabling an informed choice~~. At the international level, ~~the Codex Alimentarius Commission provide guidance to on technical and policy issues related to food labelling (FAO, 2025). Specifically,~~ the Codex Committee on Food Labelling has provided by far t~~T~~he Codex General Standard for the Labelling of Prepackaged Foods (Stan, 1985), which serves as the primary Codex tool for conveying food-related information to consumers. According to the standard, pre-packaged food shall not be described or presented on any label or in any labelling in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character in any respect (Stan, 1985). ~~A label, which is the information presented on a pre-packaged food product, is in fact one of the most direct and important ways of communicating information about the food to the consumer (Schifferstein et al., 2021)~~.

This standard has been utilized by countries for ~~harmonization and has also influenced the development of new food labelling policies~~ the implementation of labelling regulations, thus allowing to prevent food sellers from deliberate mislabelling, i. e. the "*false claims or distortion of the information reported on the label*" in many countries (European Commission, 2018). In fact, the complexity of globalized agri-food supply chains which are long, fast-moving, and involve many intermediaries, create opportunities for carrying out mislabelling, that it is now the most common type of food fraud (Yiannaka, 2023). Specifically for seafood, the supply chain has expanded to the point where a given fish is potentially harvested in one country, processed in another, and consumed in yet another. Therefore, seafood is among the commodities at high risk of mislabelling (Silva et al., 2021). Besides deceiving consumers and cause reputational damages to companies, seafood mislabelling may lead to human health risks due to the illicit presence of toxic species or the omission

of ingredients potentially causing allergies (e. g. crustaceans or molluscs) (Giusti et al., 2017a). Finally, mislabelling is suspected of being an important enabler of illegal, unreported, and unregulated (IUU) fisheries (Giusti et al., 2023; Luque & Donlan, 2019; Visciano & Schirone, 2021).

In general, the higher the degree of processing of the seafood, the more mislabelling can be perpetrated, being more difficult to identify ingredients through morphological analysis (Miller et al., 2012). Surimi - a stabilized myofibrillar protein compound obtained from mechanically deboned fish flesh, and derived surimi-based products - in which surimi is often added with non-fish ingredients (cephalopods such as shrimp or cuttlefish, pork, chicken and egg white) and additives - are therefore particularly vulnerable. Surimi-based products are marketed in several combinations of shapes, cooking methods, and ingredients, such as fish cakes, shrimp-based surimi products, fish balls, etc. (Hu et al., 2025; Zhang et al., 2024). It was estimated that over one million ~~MT~~ tonnes of surimi was produced globally in 2020, and the use of tropical fish is especially reported (650,000 MT), followed Alaska pollock (250,000 MT), and freshwater fish (60,000 MT) (Yin & Park, 2023). China is one of the main countries that produce surimi using tropical fish (Leadbitter et al., 2020). Factually, an extremely high number of fish species are reported to be used in surimi production (Giusti et al., 2017a; Giusti et al., 2017b; Hu et al., 2025; ~~X.~~ Zhang et al., 2024).

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Mislabelling in surimi and surimi-based products has been widely reported (Hu et al., 2025; Zhang et al., 2024). The presence of vulnerable species was identified (Galal-Khallaf et al., 2016; Giusti et al., 2017a; Ooi et al., 2021), mainly because the Asian surimi industry frequently depends on species from by-catch fisheries (Leadbitter et al., 2020). The undisclosed inclusion of squid and cuttlefish, which could pose a health risk to consumers allergic to molluscs, was also observed (Chin et al., 2016; Giusti et al., 2017a). These issues further highlight the necessity to proper identify species in surimi and derived products, to better manage overexploited and/or endangered marine resources and safeguard consumers from potential health risk.

The DNA barcoding has been especially used for fish and seafood products authentication (Fernandes et al., 2021; Giusti et al., 2023; Hellberg & Morrissey, 2011; Mitchell & Hellberg, 2016;

Nehal et al., 2021), but its efficiency is limited ~~in~~ for the analysis of multispecies products ~~analysis~~, since only one species (usually the most abundant in the sample) can be identified in a sequencing run. To date, Next Generation Sequencing technologies (NGS), high-throughput methods able to simultaneously sequence all the DNA molecules contained in a sample, are the most suitable for the authentication of complex food matrices, including surimi and ~~SBPs~~ surimi-based products (Giusti et al., 2024). ~~In particular, NGS~~ NGS combined with DNA barcoding, which has been termed as metabarcoding (Fernandes et al., 2021; Taberlet et al., 2012), is among the most promising NGS application for this purpose. ~~However, one significant limitation of this technique is its inability to provide quantitative results regarding the specific proportions of each species present in a sample (Lamb et al., 2019). This can be a critical shortfall when precise quantitative analysis is necessary for compliance with labelling regulations or for detailed supply chain tracking (Deagle et al., 2019; Elbrecht & Leese, 2015). Despite this limitation, NGS remains highly valuable for qualitative assessments, offering a broad overview of species diversity within complex mixtures (Giusti et al., 2024)~~.

Until now, several studies have applied metabarcoding to the authentication of surimi and derived products (Giusti et al., 2017a; Hu et al., 2025; Noh et al., 2021; Piredda et al., 2022; Zhang et al., 2024). In a recent study, we proved that metabarcoding was more effective in authenticating surimi-based products such as Chinese Fish cakes (Zhang et al., 2024) than DNA barcoding. However, in that context, the products were all authenticated by standard DNA barcoding, and an exiguous number of products (n=9) was also tested ~~with~~ using metabarcoding, unveiling the presence of undeclared species. Therefore, in the present study, a more extensive survey aimed at authenticating surimi-based products sold on Chinese e-commerce was presented. In this case, Chinese fish balls (鱼丸) were collected, which are similar in composition to fish cakes being made from surimi, salt, and culinary binder such as tapioca flour, corn or potato starch, and sometimes added with non-fish ingredients of animal origin such as pork and chicken.

## 2. Materials and Methods

### *2.1. Sampling*.

A total of 47 Chinese fish ball products (FBs) were purchased from Taobao (www.taobao.com), one of the largest business-to-consumer online platforms in China (Figure 1). A convenience, non-probabilistic sampling was conducted, structured to include a proportional number of products per brand according to the e-commerce availability. The FBs were collected to encompass a variety of fish species used in production, as reflected in their trade names. The selection aimed to include both generic and species-specific FBs to ensure comprehensive species identification and authentication analysis. ~~Examples of collected FBs are reported in **Figure 1**.~~

### *2.2. Analysis of trade names, list of ingredients and price*

For each sample, information on the FBs trade name, list of ingredients and price (USD/kg) was retrieved (**Table 1**). Information on the FBs trade names and list of ingredients were combined to obtain the declared composition (DC) of the FBs (with respect to ingredients of animal origin). Fish ingredients and other ingredients of animal origin were discussed separately. In the case of fish ingredients, if only the trade name was reported in the label, the respective scientific name was searched against FishBase (https://fishbase.se/home.htm).

### *2.3. Total DNA extraction and evaluation*.

For each FB, two sample replicates were used for the total DNA extraction, for a total of 94. Two positive control samples (PCs) made of tissue from known species (*Diodon liturosus*/Diodontidae/Tetrodontiformes) and two negative control samples (blank sample with no tissue) (NCs) were included in the analysis. The total DNA extraction was conducted using the TIANamp Marine Animals DNA Kit (TIANGEN, China) according to the manufacturer's instructions. The DNA ~~and~~ evaluation (~~spectrophotometer~~ quali-quantitative analysis) ~~were conducted~~ were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, US). ~~U-1800 spectrophotometer (Hitachi, Japan)~~. ~~according to Zhang et al. (2024).~~ At the end of this phase, total DNA samples of FBs replicates were pooled together, so that the samples returned to be 47.

### *2.4. Library preparation and sequencing.*

The primer pair projected by Sarri et al. (2014) and targeting a fragment ranged from 222 to 252 according to the species ~~region of ~ 200~~ bp of the 16S rRNA was added to Illumina adapter sequences 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG- [forward primer] and 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG- [reverse primer]. For each sample, the following PCR reaction was set: 2.5 µl of 5 ng/µl DNA sample, 5 µl of primer forward, 5 µl of primer reverse, 12.5 µl of 2x KAPA HiFi HotStart ReadyMix (KAPA Biosystems). The following cycling program was used: 95°C for 3'; 35 cycles at 95°C for 30'', 53°C for 30'', 72°C for 15'', and final extension at 72°C for 5'. The two PC and the two NCs were included in the amplifications. The PCR products were checked by gel electrophoresis on a 2% agarose gel. The amplification of fragments of the expected length was assessed by making a comparison with the standard marker SharpMass™ 50-DNA ladder (Euroclone Spa, Milano, Italy). DNA Libraries were prepared according to Illumina® Metabarcoding 16S Metagenomic Sequencing Library Preparation protocol (Illumina, 2013). For libraries purification steps, ~~Then,~~ the NucleoMag® NGS Clean-up and Size Select kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) was used. ~~to purify the amplicons following the manufacturer's instructions. Illumina Dual indices were attached to obtain the final libraries using Illumina® DNA/RNA UD Indexes Tagmentation, following the manufacturer's instructions. The final libraries were further cleaned up using the NucleoMag® NGS Clean-up and Size Select kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) before validation and quantification. For the validation, 1 µl of a 1:50 dilution of each final library was run on an Agilent 4150 TapeStation D1000 ScreenTape assay (Agilent Technologies Inc.) to verify the size. The final libraries were quantified using a fluorometric quantification method (Qubit 4 Fluorometer, Thermo Fisher Scientific, USA). The final DNA concentration was calculated in nM, based on the size of DNA amplicons as determined by Agilent 4150 TapeStation D1000 ScreenTape assay (Agilent Technologies Inc.), and applying the formula reported in Illumina LPG. Finally, aliquots of 5 µl of diluted DNA from each library were mixed for pooling libraries with unique indices~~. ~~Pooled~~ The 47 libraries were pooled, and the pool

was denatured and diluted to 8 pM before Miseq loading. ~~Pooled libraries were~~The pool was loaded using MiSeq Reagent Kit v2 (300- cycles), and the run included a 20% PhiX Control Kit v3 (Illumina) to serve as an internal control.

### *2.5. Bioinformatic analysis, taxonomic assignment and threshold set up.*

Folders containing fastq files with raw reads were processed to generate amplicon sequence variants (ASVs) using DADA2 R package (Callahan et al., 2016). Representative sequences for each ASV were taxonomically assigned through BLAST analysis against GenBank (https://blast.Ncbi.nlm.nih.gov/Blast.cgi) with a minimum percentage identity of 97% and minimum query coverage of 95%. A threshold value was set to filter the final data on FBs composition based on the PCs output. Specifically, the threshold was defined as the first most significant percentage of sequences identified for species (or a higher taxonomic rank) other than the expected one (*D. liturosus*), as performed in a previous study (Giusti et al., 2023). Once filtered, each FBs composition was defined.

### *2.6. Mislabeling assessment and evaluation of environmental impact.*

The FBs compositions obtained by molecular analysis were compared to the DC to evaluate eventual mislabeling. Being metabarcoding a qualitative and not a quantitative method, the FBs were considered mislabeled only when an ingredient (of animal origin) not reported in the DC was molecularly detected or when an ingredient (of animal origin) reported in the DC was not molecularly detected. With respect to fish, a comparison between DC and molecular results to evaluate mislabeling was possible only when this ingredient was reported in a way which allows the association to a taxonomic rank (Class, Order, Family, Genus, Species).

For each fish species molecularly detected in the samples, the IUCN Red List status was checked on the FishBase species-specific page (https://fishbase.se/search.php; accessed on February 2025).

### *2.7. Statistical analyses*

Fisher's Exact Test for 2 x 2 Tables (Agresti, 2002) was used to assess: 1) the association between the DC (whether or not fish ingredient was reported in a way which allows the association to a

taxonomic rank) and price category (high or low, based on whether the price was above or below the average price); 2) The association between the mislabelling evaluation (whether or not the FB was found mislabelled) and the price category. Differences among groups were considered significant for p-values $\leqslant 0.05$. The test was conducted using R software.

## 3. Results and Discussion

### *3.1. Analysis of trade name, list of ingredients and price~~Sampling~~.*

*3.1.1 Fish Ingredients in Surimi-Based Products.* Overall, 44.7% of the FBs (n=21) showed the generic trade name "fish balls", in two cases (FB-35 and FB-46) further detailed as "Freshwater fish balls" and "White fish balls" were reported, respectively. The trade names of the other 55.3% (n=26) of the FBs were distributed as follows: 34.9% (n=9) were labeled as "shark balls", 30.8% (n=8) as "eel balls", 15.4% (n=4) as "mackerel balls", and 3.8% (n=1) as "salmon balls". Additionally, 15.4% of these FBs (n=4) were identified with specific trade names associated with certain species: "Leopard coral grouper balls" for *Plectropomus leopardus*, "Brown-marbled grouper balls" for *Epinephelus fuscoguttatus*, "Black carp balls" for *Mylopharyngodon piceus*, and "Greater lizardfish balls" for *Saurida tumbil* (**Table 1**), which can be found at respective pages on FishBase (https://fishbase.se/search.php; accessed on February 2025). The ingredient list was reported in 44 FBs (95.7%), with 3 FBs (FB-10, FB-43, FB-45; trade name: "shark balls") lacking this information, which is instead mandatory according to China National Food Safety Standard GB 7718-2011. The listed primary ingredient matched with the trade name in 84.1% of the FBs (37 of 44), 13 of which reported as second ingredient in the list other fishery products: 8 of them (showing trade names of "eel balls", "mackerel balls", "shark balls", "salmon balls", "Brown-marbled grouper balls" and "Leopard coral grouper balls") listed generic fish, 4 of them (trade names "shark balls", "mackerel balls" and "fish balls") listed eel (with generic fish as third listed ingredient), and 1 of them (trade name "shark balls") listed Golden threadfin bream, which can be associated to the species *Nemipterus virgatus*. In 7 FBs the trade names did not match with the primary ingredient: in 4 FBs with the generic trade name "fish balls", the ingredient list provided more detailed information, such as eel

(FB-18), eel/shark (FB-17), Grass carp (associated with *Ctenopharyngodon idella*)/Bighead carp associated with *Hypophthalmichthys nobilis*) (FB-26), or marine fish (FB-25), thus excluding the presence of freshwater species; also, 2 FBs with the trade names "white fish balls" (FB-46) and "eel balls" (FB-39) reported *Nemipterus sp.* and the generic fish, respectively, in the ingredient lists (**Table 1**). The DC ~~as regards~~ referring to fish ingredients ~~(obtained by combining information of the trade names and the ingredient lists)~~ are reported in **Figure 2.** The incorporation of multiple fish species in the production of fish balls may serve several purposes. On the one hand, it can enhance the overall flavor profile of surimi-based products or provide a market opportunity for underutilized species; on the other hand, it is also plausible that such blending is intended to partially substitute the primary species—emphasized in the product's trade name—with lower-cost alternatives, thereby reducing production expenses~~.~~ (Hu et al., 2025).

The overall FBs average price was 17.07 USD/kg. The FBs reporting the presence of grouper species showed the highest average price (58.7 USD/kg; range 46.3-71.1 USD/kg), followed by those declaring salmon FB (1 sample, 46.3 USD/kg) (**Figure 3**). The lowest average price (11.78 USD/kg; range 4.57-27.4 USD/kg) was instead associated with FBs reporting the presence of generic fish ~~(~~as reported in **Figure 3**~~)~~. However, no significative association (*p-value* 0.1926) between the DC declaration, and the price category (high and low) was observed, suggesting that FB labelling does not influence its price classification.

Based on DC, and except for 14 FBs for which only the generic "fish" term was available, the presence of eel and shark was reported (alone or combined) in the highest number of samples (14 FBs and 10 FBs, respectively). According to FishBase, eels include species belonging to the Order Anguilliformes, and sharks include species belonging to the sub-class Elasmobranch. The use of eel and shark in surimi-based products like fish balls highlights evolving seafood consumption trends and diversification. Eels are prized in various cultures for their unique taste and adaptability to surimi, enhancing market appeal (Wang et al., 2022). Conversely, sharks are incorporated into surimi in specific markets where they are traditionally favored, potentially increasing product value for

premium markets. The integration of shark meat, often seen as a by-product, into surimi may contribute to sustainable fishing practices by utilizing the whole animal, although it raises conservation concerns due to overfishing (Kittiphattanabawon et al., 2012; Lee et al., 2024). Particularly, the interaction between the direct demand for shark-derived products such as meat, liver oil, cartilage, and fins, and incidental capture, propels targeted shark fisheries and retains accidentally caught sharks as valuable secondary catches, escalating marine ecological risks. It is reported that globally, less than 4% of shark catches are managed sustainably (Zhou et al., 2021). The presence of freshwater species was reported in 5 FBs. Compared to marine fish, freshwater fish offer the advantages of stable supply and relatively lower prices, making them a primary raw material for the production of pre-processed fish products like surimi, especially in Asian countries (Zhang et al., 2023). Additionally, the presence of mackerels, which according to FishBase include species belonging to the Order Scombriformes, was reported in 4 FBs. This is an interesting finding, indicating that mackerel, despite being a dark-fleshed fish, can substitute for traditional, white-fleshed fish surimi bases, such as cod, in the production of abundant and cost-effective surimi products, making it a sustainable option (Somjid et al., 2021). Finally, *Nemipterus sp.* (2 FBs), grouper (2 FBs), lizardfish (1 FB) and salmon (1FB) were reported. Although threadfin bream (*Nemipterus* sp.), and lizardfish (*Saurida* spp.) are reported as the principal white fish employed in Asia for the manufacturing of surimi (Jaziri et al., 2023), these taxa were found to be scarcely represented in FBs. This further confirm that dark muscle fish have garnered increasing interest as a potential replacement raw material for the manufacture of surimi owing to the restricted white muscle fish supplies and the excessive harvesting of white fish (Panpipat et al., 2023; Thongkam et al., 2023).

*3.1.2 ~~Non-Fish Animal~~ Ingredients of animal origin (different from fish) in Surimi-Based Products.*

Most of the FBs (n=39/44; 88.6%) reported the presence of ~~other~~ ingredients of animal origin other than fish in the ingredient lists; in detail, 23 FBs listed pork, 5 FBs listed chicken (reported as chicken, chicken essence, or egg), 9 FBs listed both pork and chicken, 1 FB listed pork and duck egg, and 1 FB listed pork and shrimp. Ingredients of animal origin different from fish, especially pork and

chicken are especially widely reported as used in surimi-based products preparation (Hu et al., 2025; Zhang et al., 2024). ~~Ingredients commonly used in other types of surimi, such as cephalopods (cuttlefish, squids) (Giusti et al., 2017a; Giusti et al., 2017b; Zhang et al., 2024) and shrimp (Hu et al., 2025), do not appear to be used in the production of FBs, at least respect to what extrapolated by the DC~~. Also, no appreciable differences were observed respect to the declared presence of ingredients of animal origin other than fish.

~~*3.1.3. FBs prices evaluation analsyis*. The overall FBs average price was 17.07 USD/kg., and no significative association (*p-value* 0.1926) between the DC declaration (whether or not fish ingredient was reported in a way which allows the association to a taxonomic rank), and the price category (high and low) was observed, suggesting that FB labelling does not influence its price classification. However, t~~The ~~price difference seems to be determined by the type of fishery product, with the FBs reporting the presence of grouper species showed the highest average price (58.7 USD/kg; range 46.3-71.1 USD/kg), followed by those declaringe salmon FB (1 sample, 46.3 USD/kg) (**Figure 3**). The lowest average price (11.78 USD/kg; range 4.57-27.4 USD/kg) was instead associated with FBs reportinged the presence of generic fish (**Figure 3**).. To evaluate this aspect statistically, the Chi-square test would have been deemed appropriate; however, many of the expected frequencies (i.e., the sample number) in the table were below the minimum threshold of 5 required for the Chi-square test to yield reliable results.. Also, no appreciable differences were observed respect to the declared presence of ingredients of animal origin other than fish.~~

### *3.2.~~-~~Library preparation and sequencing output.*

The expected 16S rRNA region was successfully amplified by all the 47 FBs and from the 2 PCs. No amplification bands were instead obtained from NCs. This suggests that the analytical workflow (from total DNA extraction to PCR amplification) was properly conducted, avoiding external contamination and/or cross contamination among samples. Therefore, NCs were not sequenced.

As widely known, PCR generates bias related to primer–template mismatches and to purely stochastic effects (Piñol et al., 2015). Consequently, in multispecies matrices, some species may increase their relative abundance, others decrease it, while others are not amplified and so remain hidden (Piñol et al., 2015). Therefore, preliminary PCR tests for evaluating primer amplification efficiency are pivotal for the selection of universal primers with a wide species coverage (Giusti et al., 2023). In this way ~~, although metabarcoding is not a quantitative technique,~~ it is possible to ~~limit cases of overestimation and/or underestimation of species present in the samples, as well as~~ minimize the occurrence of false negatives. ~~In this respect, t~~The *16S rRNA* primer pair projected by Sarri et al. (2014) were selected as proved performant in amplifying this region in many ~~in a wind range~~ species of fish, mammal, birds, coleoptera, reptiles, crustaceans, and cephalopods, and they were also used in other studies applying molecular methods to the authentication of foodstuff of animal origin (Stamatis et al., 2015; Wang et al., 2021; Xing et al., 2019; Xing et al., 2020). Particularly, they were successfully used in metabarcoding analysis applied to meat and poultry products (Xing et al., 2019) and fish products (Wang et al., 2021). ~~.~~In a recent systematic review ~~analysing~~ analyzing studies applying metabarcoding to food authentication, they proved to have the highest taxonomic coverage among all the primer pairs used for this purpose (Giusti et al., 2024). Moreover, this primer pair was already used in metabarcoding analysis by Xing et al. (2019). In detail, 126 species were successfully amplified in studies that have used these primers, belonging to fish (43 species), mammals (30 species), coleoptera (22 species), aves (19 species), crustaceans (5 species), reptiles (5 species), and cephalopods (2 species) (detailed in **Table SM-1**). To note, the taxonomic coverage of a primer pair tends to increase progressively as more studies of this nature are conducted, revealing the possibility of amplifying other species with respect to those tested during the primer projecting. For instance, they were proved able in amplifying *D. liturosus,* used as PC in this study. Another aspect that we considered is that they were able to amplify the target region from chicken, listed among the ingredients of FBs. In our previous study (Zhang et al., 2024) we used another primer pair (projected by Chapela et al., 2002) which was not able to amplify avian species (Piredda et al., 2022); however,

in that context, chicken was not listed among the ingredients of the analyzed surimi-based products ~~analysed~~.

After sequencing, an average number of 6,1956.5 sequences for each sample (FBs and PCs) was obtained. The average percentage of sequences maintained after the application of DADA2 R package was 68.0% (range 44.3% - 96.8%).

***3.3. Taxonomic assignment.***

97.81% and 99.03% (average 98.42%) of the sequences obtained from the 2 PCs were assigned to *D. liturosus*, respectively. A low percentage of sequences (1.37% and 0.93%; average 1.15%) assigned to pork (*Sus scrofa*) was also observed in the PCs. Given the high sensitivity characterizing the NGS technologies, the detection of false positives (contaminant DNA) from taxa that was not likely to be present in the sample - that can be introduced at any stage of the metabarcoding process, is frequent (Alberdi et al., 2019; Drake et al., 2022; Giusti et al., 2023; Yang et al., 2021). The certain identification of ~~such~~ false positives is challenging (Drake et al., 2022; Sepulveda et al., 2020). The use of positive controls to define a sequence threshold, below which a detected organism could be deemed a contaminant (false positive), is a widely acknowledged and recommended approach in metabarcoding analysis (Giusti et al., 2024; Piper et al., 2019). A recent systematic review on metabarcoding applied to the authentication of foodstuff of animal origin reported that data were often filtered using thresholds of 1 % or 2 % (Giusti et al., 2024). Based on the PCs outputs, and considering literature data, the threshold value to filter the final data on FBs composition was set to 1%.

The presence of fish species was detected in 97.9% (n=46) of the FBs. Sequences assigned to pork (*Sus scrofa*; *S. celebensis*) and chicken (*G. gallus*) were detected in 91.5% (n=43) and 31.9% (n=15) of the FBs, respectively. Sequences assigned to ~~cattle~~ beef (*Bos taurus*) were also found in 2 FBs (4.3%). Finally, although in percentage below 1%, sequences assigned to sheep (*Ovis aries*) were also detected in 4 FBs (**Table SM-2~~1~~**). Contrariwise, duck (*Anas* spp.), reported in the DC of one FB, was

not found. ~~To note, n~~No sequences assigned to cephalopods or shrimps were detected~~., confirming that these taxa are scarcely or not used in the production of surimi fish balls~~.

With respect to fish, an average number of 5.3 species (or higher taxonomic ranks when sequences were not assigned to the species level) was observed in the 47 FBs, ranging from 1 (FB-27) to 18 (FB-46) (Figure SM-1). This is in line with that reported in our previous study on fish cakes authentication, in which an average number of 5.8 species for each sample were detected by metabarcoding (Zhang et al., 2024).

*Hypophthalmichthys nobilis* (Bighead carp) was the species with the highest prevalence in FBs, being detected in 28 FBs (59.6%), followed by *Muraenesox cinereus* (Daggertooth pike conger), detected in 17 FBs (36.2%). It can be inferred that the high detection rates of the two fish species in FBs may be related to their high production volumes in China. According to the 2024 China Fisheries Statistical Yearbook, the aquaculture output of B~~b~~ighead carp reached 3,349,884 tons, while the catch volume of conger eel amounted to 322,029 tons (BFFA, 2024). Given that these two species are major contributors to China's freshwater aquaculture and marine capture sectors, with low raw material costs, it is possible that producers prefer to utilize them for surimi production, particularly when they can label them as generic "fish". A considerable prevalence (n=9; 19.1%) was observed for *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri*, not identified at the species level as the selected molecular target did not have sufficient inter-species variability in these taxa. Also, *Ilisha elongata* (Elongate ilisha), *Decapterus maruadsi* (Japanese scad), *Pangasianodon hypophthalmus* (Striped catfish), *Trachurus japonicus/T. trachurus/T. declivis* showed considerable prevalences (from 14.9% to 17.0%). Similarly, other major fish species caught in Chinese marine fisheries include the horse mackerel (*Trachurus japonicus*, 253,348 tons), Japanese scad (*Decapterus maruadsi*, 394,026 tons), snapper (Lutjanidae, 133,365 tons), herring (Clupeidae, 8,368 tons), and elongate ilisha (*Ilisha* sp., 59,159 tons) (BFFA, 2024). Moreover, also the Striped catfish, often sold under the name "basa fish" along with *Pangasius bocourti*, was detected. This is a cost-effective Southeast Asian fish used in fish fillets and surimi for its high-quality, boneless white flesh, serving as a cheaper

alternative to cod (Haslawati et al., 2023; Torsabo et al., 2024; Vaishali et al., 2024). All the detected fish species (or higher taxonomic ranks) with the relative number of FBs are reported in **Figure 4**. More details with respect to the percentages of sequences assigned to a species respect to the overall sequences in the sample ~~sequence abundances~~ are provided in **Table SM-2~~1~~**. It should be noted that all the detected fish species are not among those typically used in fraudulent substitutions (Luque, 2019). This is because in surimi production, given the nature of the product, almost any species can be used as a substitute without concerns that the replacement species will closely resemble the original in terms of organoleptic properties

### *3.4. Mislabeling assessment.*

~~Early adopters of metabarcoding hoped it would provide quantitative results, with sequencing reads correlating to biomass in the original sample (Symondson & Harwood, 2014). However, various factors can introduce biases (i.e., primer affinity, PCR conditions or inhibitors, as well as competition among templates) (Deagle et al., 2019; Klapper et al., 2023).~~ However ~~In this study, based on the average percentage of sequences assigned to *D. lituratus* in PCs (98.42%), we could hypothesize that the primary ingredient declared in the FBs should present the highest percentage of sequences assigned to the declared species. If this assumption was reliable, we observed that, only considering FBs in which fish ingredient was reported in a way which allows the association to a taxonomic rank, the primary ingredient did not match with the abundance of assigned sequences in almost 50% (15/31) of the cases.~~ ~~Despite these well known issues~~, Currently, there is ~~still~~ no clear consensus on the quantitative reliability of metabarcoding (Deagle et al. 2019; Lamb et al., 2019~~).~~ Piñol et al. 2019). Metabarcoding can be especially affected by the PCR amplification step using "universal" primers that might instead present different affinities between different species. This results in a final amplified DNA mixture that does not always reflect the original proportion of each species, limiting the quantitative potential of DNA metabarcoding (Bruno et al., 2019; Deagle et al., 2019; Klapper et al., 2023; Lamb et al., 2019). In contrast, metagenomic approaches that do not require PCR amplification (e. g. shotgun sequencing) can successfully estimate abundance, although the costs and

bioinformatic complexity of these methods may be prohibitive in many cases (Ratcliffe et al., 2021). ~~various factors can introduce biases (i.e., primer affinity, PCR conditions or inhibitors, as well as competition among templates) (Deagle et al., 2019; Klapper et al., 2023)~~To date, literature applying metabarcoding to foodstuff authentication (reviewed in Giusti et al., 2024) presented qualitative results. Although the study by Lorusso et al. (2024) recently proposed a semi-quantitative evaluation of the results obtained by metabarcoding applied to processed products (supported by the inclusion of mock communities in the analysis), ~~XX For this reason, a conservative approach in mislabelling assessment was adopted, as in Lorusso et al. (2024). For supporting a semi-quantitative interpretation of the results, which could allow to better assess mislabelling, the parallel analysis of experimental mixtures should be considered (Lorusso et al., 2024). However, based on the average percentage of sequences assigned to *D. litturosus* in PCs (98.42%), we could hypothesize that the primary ingredient declared in the FBs should present the highest percentage of sequences assigned to the declared species. If this assumption was reliable, we observed that, only considering FBs in which fish ingredient was reported in a way which allows the association to a taxonomic rank, the primary ingredient did not match with the abundance of assigned sequences in almost 50% (15/31) of the cases. However, as previously mentioned~~, further targeted studies are needed to evaluate the potential of metabarcoding in quantitative analyses. For this reason, we decided to evaluate mislabeling based only on qualitative results.

By comparing molecular results with DC, it became evident that the percentages of FBs in which pork and chicken were detected were higher (around two/three times) than those in which their presence was declared (**Figure 5**).

Pork and chicken were detected in 10 FBs and 10 FBs, respectively, which not reported them in the DC. These FBs were therefore considered as mislabelled. Sequences of chicken were especially abundant in 7 of these mislabelled FBs (from 30.69% to 75.79%) (**Table SM-2~~1~~**). Sequences of pork were instead less abundant in the mislabelled product (below 15%), except for FB-10 and FB-43 (67.13% and 71.96%), respectively. However, the undeclared use of pork and chicken in FBs

production not only misleads consumers but also raises ethical concerns and dietary restriction-related issues. These concerns are particularly significant for groups with religious dietary restrictions, individuals with specific food allergies, and pescatarians (Giusti et al., 2024; Kirsten et al., 2020). Furthermore, the widespread occurrence of these fraudulent practices highlights systemic problems within seafood supply chains, underscoring the urgent need for stricter regulations.

Interesting to note that in 7 FBs reporting the presence of pork and/or chicken, these species were not detected in the analysis. Probably, in these samples, chicken ingredient was poorly represented in the sample~~, being it detected in high abundance of sequences in other samples~~. However, given that in most of these samples, sequences of pork and chicken in amount below 1% were found (**Table SM-2~~1~~**) (and considering their role as secondary ingredients, e. g. chicken essence), this result was not considered conclusive for identifying these FBs as mislabelled. For the same reason, although the presence of shrimp and duck egg, reported in FB-17 and FB-25, respectively, was not molecularly detected, these FBs were not identified as mislabeled with respect to this aspect. Contrariwise, FB-12 and FB-46, in which ~~cattle~~ beef (*B. taurus*) was not reported in ~~the DC~~DC was detected, were considered as mislabeled (**Table SM-2~~1~~**). To the best of our knowledge, this is the first study detecting undeclared pork, chicken and ~~cattle~~ beef in surimi-based products. The undeclared presence of these ingredients in processed seafood products has implications for consumers' ethical and religious aspects (Lorusso et al., 2024).

With respect to fish, mislabeling was observed in the following cases: In 2 FBs (FB-1, FB-46) reporting the presence of mackerel, no species belonging to the Order Scomberiformes were detected; similarly, no species belonging to the Class Elasmobranch and the Order Anguilliformes were detected in 3 FBs reporting the presence of shark (FB-17, FB-41, FB-42) and eel (FB-42), respectively (this suggests that these ingredients are listed on the label to enhance the appeal of the products, yet they do not actually form part of the ingredients); no *Nemipterus* spp. was detected in the 2 FBs reporting the presence of this genus (FB-11, FB-46); in FB-26, reporting the presence of Grass carp (*C. Idella*)/Bighead carp (*H. nobilis*), the first one was not detected; in FB-34, the declared

Greater lizardfish (*S. tumbil*) was not detected (**Table SM-21**). Finally, in 9 FBs, other fish species in addition to the declared one/s were detected. In FB-12, which DC was “fish, pork and egg”, fish species were not detected at all.

By combining all the findings reported in this section, 65.9% (n=31) of FBs were considered mislabeled (**Table 2**). It should be considered that this percentage might be underestimated, as the mislabelling was based on a qualitative rather than a quantitative assessment. According to the "National Standard of the People's Republic of China GB 7718—2011," ingredients should be listed in decreasing order of the amount added during the manufacturing or processing of the food. ~~In fact, i~~In this respect, in some products it is difficult to rule out the occurrence of deceptive practices, as in the case of FB-4 (not considered mislabelled in this study), which DC was “eel, fish, pork” and sequences assigned to pork were 92.09% of the total in that sample, and only 1.06% were assigned to *M. cinereus* (Anguilliformes, eel). A similar case was highlighted for FB-5, FB-21 and FB-37 (all not considered mislabelled) (**Table SM-21**). In FB-21, in particular, sequences of pork and chicken were found in percentages of 47.68% and 33.20% of the total, respectively, although the primary ingredient in DC was fish. Also, these cases were found in several other FBs already identified as mislabelled for other issues (**Table SM-21**). To note that Lorusso et al. (2024), who interpreted the results in a semi-quantitative manner, considered as correctly labelled only samples in which the proportion of reads of the declared species was at least twice as large as the second most abundant species and constitutes the majority of the bulk. Therefore, although we have decided to not evaluate mislabelling from a quantitative results perspective (number of reads), it would be appropriate to be suspicious in the face of certain results. ~~According to the "National Standard of the People's Republic of China GB 7718—2011," ingredients should be listed in decreasing order of the amount added during the manufacturing or processing of the food. However, ingredients added in amounts not exceeding 2% do not need to be listed in descending order. Further emphasizing that no definitive conclusions can be drawn considering that the method is not quantitative, it is also true that the findings in these samples are highly suspicious and suggest the possibility of mislabelling~~.

The average price of mislabelled FBs was 14.15 USD/Kg. Surprisingly, the more expensive FBs, namely those with DC including grouper (FB-5, FB-6) and salmon (FB-7), were not involved in mislabelling, although in FB-5 the percentage of sequences assigned to pork was considerably higher than *P. leopardus,* as previously described.

Among the most expensive mislabeled FBs (prices higher than 19 USD/Kg), FB-36, FB-42, and FB-46 were those in which voluntary mislabeling for economic gain is highly plausible since the DC "mackerel" (FB-36), "shark/eel" (FB-42), and *Nemipterus sp.* (FB-46) were not molecularly detected, and in their place many other species (sometimes of lower commercial value) were found. Overall, no significant differences (*p-value* 0.32625~~278~~) in prices were observed between mislabelled and not mislabelled FBs.

***3.4. Possible impacts on sustainability and consumer health.***

Regardless of the cases of mislabelling found in this study, surimi production can sometimes impact the sustainability of ecosystems when threatened or overexploited species are indiscriminately used. These issues have already been widely highlighted in the literature (Detcharoen et al., 2024; Giusti et al., 2017a; Lee et al., 2024; Nugroho et al., 2024). In particular, the depletion of white fish stocks has been extensively documented, with reports indicating that key species used in surimi production, such as Alaska pollock (*Theragra chalcogramma*) and Pacific whiting (*Merluccius productus*), are either overexploited or depleted (Martín-Sánchez et al., 2009). Additionally, in a recent food authentication study of 120 surimi samples from Taiwan, 26 endangered species were identified (Lee et al., 2024).

In our study, it was specifically observed that all FBs with DC including "shark" contained species listed on the IUCN Red List of Threatened Species. In details, *Isurus oxyrinchus* (shortfin mako), detected in FB-3 (as well as in other 5 FBs not reporting "shark in the DC") is considered endangered from 2018 (https://www.fishbase.se/summary/Isurus-oxyrinchus.html; accessed on February 2025). Meanwhile, *Isurus oxyrinchus* has also been included in Appendix II of the Convention on International Trade in Endangered Species (CITES). Similarly, *Rhizoprionodon acutus*/*Scoliodon laticaudus*/*S. macrorhynchos*, detected in FB-10, FB-11, FB-15, FB-43, FB-44, and FB-45, were all

reported in the IUCN as vulnerable or near threatened. However, the shark species identified in FBs in this study were not listed in the List of State Key Protected Wild Animals in China (currently, only *Cetorhinus maximus*, *Carcharodon carcharias*, and *Rhincodon typus* are classified as Level II protected species in China, making their sale and consumption strictly prohibited). Nevertheless, as China is a signatory to CITES, the utilization of *Isurus oxyrinchus* in the production of fish balls (FBs) warrants special attention ~~However, as China is a signatory to CITES, manufacturers of FBs containing *Isurus oxyrinchus* may still face legal prosecution~~ (http://www.moa.gov.cn/govpublic/YYJ/202110/t20211021_6380194.htm).

Overall, the sharks included in the IUCN showed a prevalence of 25.5% in the FBs. This finding highlights an urgent need for stricter enforcement and clearer compliance guidelines regarding the utilization of threatened species in seafood products, as their continued use could significantly undermine marine biodiversity conservation efforts. Indeed, s~~S~~eafood consumption plays a significant role in marine biodiversity conservation, influencing stricter shark fishing regulations and raising awareness about endangered species (Jorgensen et al., 2022; Pacoureau et al., 2021). The high demand for shark fin soup in Asia has historically driven unsustainable fishing practices, leading to international criticism (Zhou et al., 2021). Sharks, which include several species of conservation concern, are now listed under Appendix II of the ~~Convention on International Trade in Endangered Species (~~CITES~~)~~. Although these species are not necessarily endangered, they could face significant threats without tightly regulated trade (Zhang et al., 2021). In response, countries like China have signed the CITES agreement to protect endangered wildlife, and Taiwan implemented measures in 2012, such as mandating whole-shark landings and reducing shark fin dishes in restaurants (China, 2023; Chuang et al., 2016). Despite these efforts, shark exploitation continues, with surimi production emerging as an alternative use for shark-derived products. To address this issue, governments and policymakers should strike a balance between economic interests and ecological sustainability (Koehler & Lowther, 2022). In most cases, the economic gains from the trade of shark fins and meat are marginal and contribute little to national food security (Ferretti et al., 2020). Therefore, as a

responsible global actor, conservation efforts should take precedence over short-term economic benefits in order to curb the broader global drivers behind shark exploitation. The fact that the FBs analysed in our study were labelled with the generic trade name "shark" makes it impossible to determine solely from the label whether the product contains threatened species. Therefore, even when FBs were categorized as not mislabelled based on the fact that the species detected molecularly matched the DC, the observed IUCN status undoubtedly raises a necessary consideration about whether these products are truly compliant with regulations. Besides these shark species, other species detected in the FBs were found as endangered (n=2), near threatened (n=6) or vulnerable (n=5) according to the IUCN Red List. The IUCN status of all detected fish species is reported in **Table SM-3.** These species are typically considered non-target catches during fishing operations (Pham et al., 2023). However, their frequent detection in various seafood products suggests a broader shift in harvesting practices (Hu et al., 2025; Zhang et al., 2021). This indirectly reflects the growing pressure of overfishing on marine fisheries. If high-intensity selective harvesting of target species and bycatch of non-target species continue unchecked, it will lead to declining biomass, disruption of fish community structures, reduced adaptability of aquatic resources to climate change, and alterations in species habitats (Oham et al., 2023). Such trends pose significant challenges to the sustainability and ecological stability of global fisheries. Therefore, improving traceability and transparency within the seafood supply chain should become a regulatory priority.

~~Besides these shark species, other species detected in the FBs were found as endangered (n=2), near threatened (n=6) or vulnerable (n=5) according to the IUCN Red List. The IUCN status of all detected fish species is reported in **Table SM-2**~~.

To note, sequences assigned to pufferfish (*Takifugu* sp.; *Lagocephalus* sp.) were also detected in 10 FBs, although in percentages below 1% (**Table SM-2~~1~~**). Despite, according to the 1% threshold set up in this study, these sequences may derive from contamination at any stage of the production chain, the presence of such species, potentially toxic (Giusti et al., 2019; Yamanoi et al., 2022), should raise awareness regarding potential suboptimal processing practices, which could pose a risk to consumer

health. This aspect, like others related to contamination during production, is undoubtedly an element to consider when aiming to trace the product along the supply chain. It is also important to investigate further the potential presence of irregular channels where at-risk or toxic species are used. Thus, future studies and regulatory oversight should address potential contamination risks and investigate irregular trade channels involving at-risk or toxic species.

## 4. Conclusion

The 16S rRNA metabarcoding applied in this study allowed to detect a high mislabelling rate in FBs~~, even potentially underestimated considering the not-quantitative nature of the method, which limits the ability to determine whether the quantity of the declared ingredients align with their order in the ingredients list~~. For the first time, the undeclared presence of pork, chicken, and cattle was unveiled in surimi-based products~~. Even~~ Some of these ~~in~~ FBs were not considered mislabelled ~~(mislabelling was not evaluated based on~~ due to the quan~~t~~litative nature of our ~~titative~~ method ~~based on the nature of this method~~). However~~,~~ the high number of sequences belonging to these non-fish species raises doubts about the potential for fraudulent adulterations.

Beyond economic damage, ~~this~~ the use of undeclared pork and beef could represent a constraint on consumer choices from both ethical (e. g., pescetarians) and religious (e. g., Muslims) perspectives. In addition, the detection of DNA of fish species reported in the IUCN Red List of Threatened species (particularly sharks) highlights the limited sustainability of these productions. Therefore, while surimi production has the potential to reduce waste along the supply chain ~~through the use of~~using by-products from other processes, inadequate oversight could exacerbate the depletion of already overexploited fish stocks. Consequently, the ongoing implementation of techniques aimed at monitoring these production lines is highly recommended. While research is ongoing to explore the quantitative potential of metabarcoding, the use of quantitative methods such as qPCR, either in parallel or after an initial screening, could provide a more in-depth analysis of surimi adulteration. ~~In this context, while research is in progress to investigate the quantitative potential of metabarcoding~~

~~the use in parallel, or after an initial screening, of quantitative methods such as follow-up qPCR could provide a more in-depth analysis of surimi adulteration.~~

~~For supporting a semi-quantitative interpretation of the results, which could allow to better assess mislabelling, the parallel analysis of experimental mixtures should be considered. However, based on the current limitations regarding the reliability of quantitative results (despite the fact that research is ongoing to investigate them further), it would be appropriate to use it as a screening method and combine it with quantitative methods such as follow-up qPCR. These latter methods, in turn, have limitations related to the number of species detectable in a sample, and therefore, these two methods could complement each other.~~

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## Figures

**Figure 1. Pictures of some fish ball products (FBs) collected and analyzed in this study.**

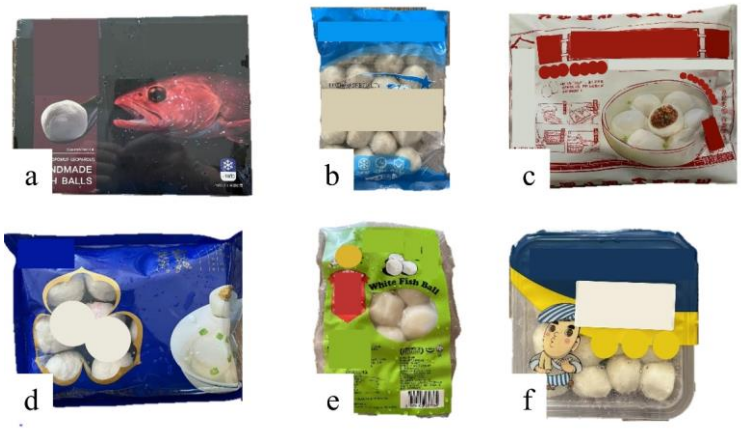



**Figure 2. Information on fish ingredients in DC of the 47 FBs**; FW: Freshwater species

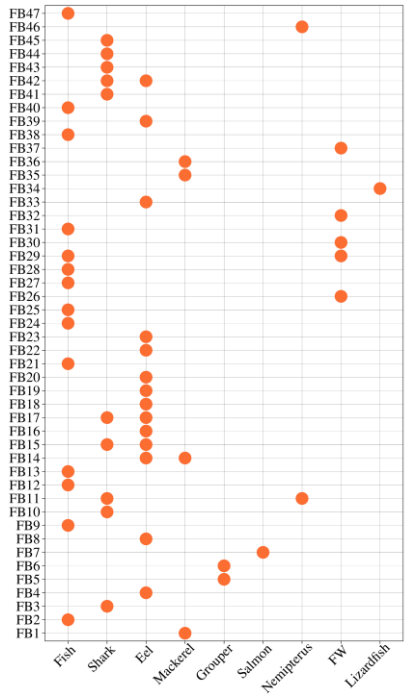
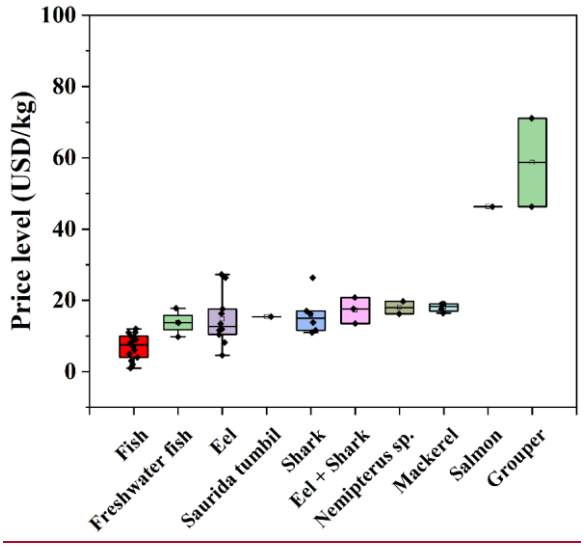


**Figure 3**. Bboxplots illustrating the distribution of **FBs ~~average, lowest and highest~~ prices (USD/kg) divided by DC (only fish taxa).**



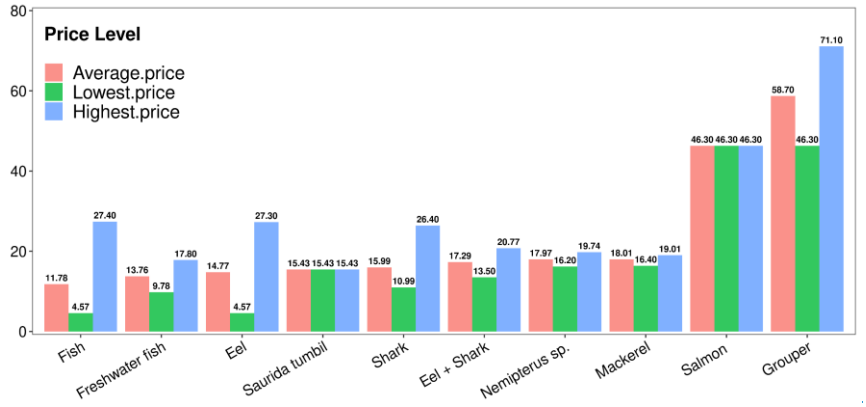



**Figure 4. molecularly detected fish species (or higher taxonomic ranks) with FBs number**.

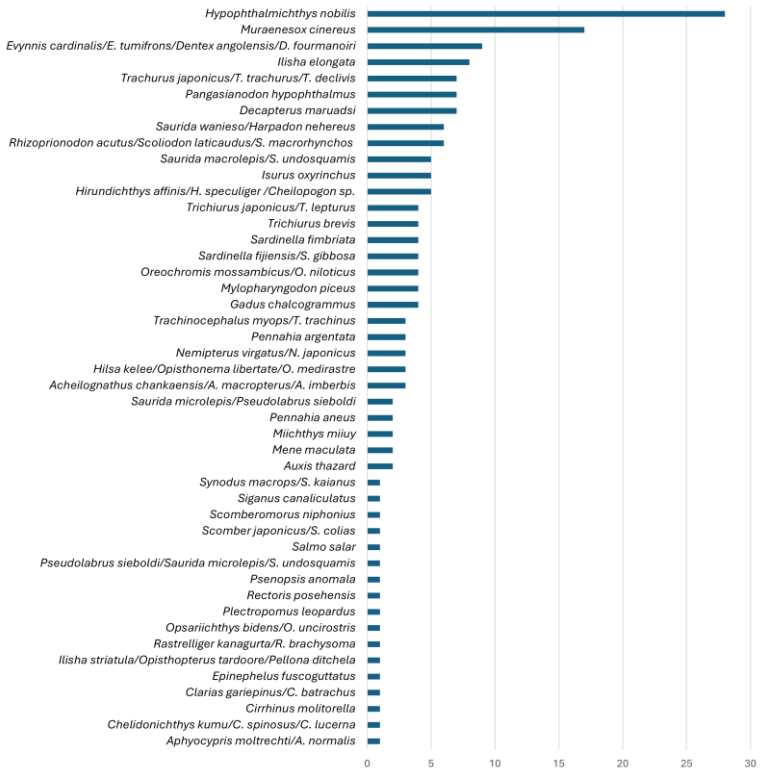
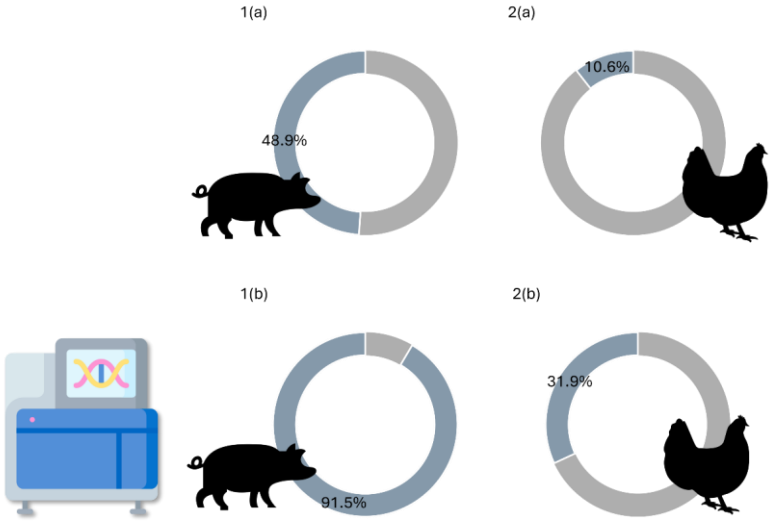


**Figure 5. Percentages of FBs reporting the presence of pork (1a) and chicken (2a) and percentages of FBs in which pork (1b) and chicken (2b) were molecularly detected.**



959 **Tables**

960 **Table 1. Fish Ball Products (FBs) collected and analyzed in this study**. Information on the FBs' trade name, list of
961 ingredients and price are reported. *Ingredients of animal origin are highlighted in bold.

| Sample | Trade name | List of ingredients* | price USD/kg |
|---|---|---|---|
| FB1 | Mackerel ball | **Mackerel**, **fish**, sugar, sodium pyrophosphate, sodium tripolyphosphate, water, monosodium, salt, tapioca starch, trehalose, sodium hexametaphosphate | 16.4 |
| FB2 | Fish ball | **Fish**, sugar, sodium pyrophosphate, sodium tripolyphosphate, water, monosodium, salt, tapioca starch, trehalose, sodium hexametaphosphate | 10.9 |
| FB3 | Shark ball | **Shark**, **fish**, sugar, sodium pyrophosphate, sodium tripolyphosphate, **pork**, soy sauce, starch, water, salt, monosodium, sugar | 26.4 |
| FB4 | Eel ball | **Eel**, **fish**, sugar, sodium pyrophosphate, sodium tripolyphosphate, **pork**, soy sauce, starch, water, salt, monosodium, sugar | 26.4 |
| FB5 | Leopard coral grouper ball | **Leopard coral grouper**, **fish**, **pork**, water, starch, soy sauce, salt, sugar, shallot | 71.1 |
| FB6 | Brown-marbled grouper ball | **Brown-marbled grouper**, **fish**, **pork**, water, starch, soy sauce, oil, salt, sugar, shallot | 46.3 |
| FB7 | Salmon ball | **Salmon**, **fish**, **pork**, water, starch, soy sauce, oil, salt, sugar, shallot | 46.3 |
| FB8 | Eel ball | **Eel**, **fish**, **pork**, water, starch, soy sauce, salt, sugar, shallot | 27.3 |
| FB9 | Fish ball | **Fish**, **pork**, water, starch, soy sauce, salt, sugar, shallot | 19.3 |
| FB10 | Shark ball | - | 16.2 |
| FB11 | Shark ball | **Shark**, **golden threadfin bream**, **pork**, starch, garlic, trehalose, salt, water, sugar, shallot, soy sauce, sesame oil, monosodium | 16.2 |
| FB12 | Fish ball | **Fish**, **pork**, water, starch, **egg white**, shallot, soy sauce, salt, sugar, monosodium, soybean oil, sodium pyrophosphate | 13.7 |
| FB13 | Fish ball | **Fish**, **pork**, water, starch, **chicken**, **egg white**, shallot, soy sauce, salt, sugar, monosodium, vegetable oil, sodium pyrophosphate, spice | 7.3 |
| FB14 | Mackerel ball | **Mackerel**, **eel**, **pork**, water, starch, salt, soy sauce, sugar, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 17.6 |
| FB15 | Shark ball | **Shark**, **eel**, **fish**, sugar, **pork**, water, starch, salt, soy sauce, shallot, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 13.5 |
| FB16 | Eel ball | **Eel**, **pork**, water, starch, salt, soy sauce, sugar, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 16.3 |
| FB17 | Fish ball | **Eel**, **shark**, **pork**, **shrimp**, shallot, water, starch, salt, soy sauce, sugar, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 17.6 |
| FB18 | Eel ball | **Eel**, **pork**, shallot, water, starch, salt, soy sauce, sugar, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 17.6 |
| FB19 | Fish ball | **Eel**, **fish**, starch, water, **pork**, soy sauce, salt, sugar, monosodium, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 8.17 |
| FB20 | Eel ball | **Eel**, starch, water, **pork**, soy sauce, salt, sugar, monosodium | 11.6 |
| FB21 | Fish ball | **Fish**, starch, water, **pork**, **chicken**, shallot, soy sauce, sugar, salt, monosodium, pepper, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 4.53 |
| FB22 | Fish ball | **Fish**, **eel**, starch, water, **pork**, shallot, soy sauce, sugar, salt, monosodium, pepper, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 11.94 |
| FB23 | Eel ball | **Eel**, starch, water, **pork**, oil, soy sauce, sugar, salt, pepper, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 10.41 |
| FB24 | Fish ball | **Fish**, water, salt, trehalose, **essence of chicken**, starch, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 27.4 |

| | | | |
|---|---|---|---|
| FB25 | Fish ball | **Marine fish**, water, **duck egg white**, **pork**, starch, salt, monosodium, sugar, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 14.5 |
| FB26 | Fish ball | **Grass carp**, **Bighead carp**, water, **egg**, starch, salt, **lard**, **essence of chicken**, monosodium, ginger, shallot | 17.8 |
| FB27 | Fish ball | **Fish**, **egg white**, **lard**, starch, ginger, shallot, salt, **essence of chicken**, sugar, pepper | 22.0 |
| FB28 | Fish ball | **Fish**, sugar, water, **pork**, **eel**, potato starch, salt, monosodium, soy sauce, shallot, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 9.35 |
| FB29 | Fish ball | **Fish**, sugar, water, **pork**, **eel**, potato starch, salt, monosodium, soy sauce, shallot, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 9.35 |
| FB30 | Freshwater fish ball | **Freshwater fish**, **egg**, ginger, green onion, **pork**, starch, salt, water | 9.78 |
| FB31 | Fish ball | **Fish**, **pork**, **egg**, cornstarch, ginger, shallot, salt, water | 9.85 |
| FB32 | Black carp ball | **Black carp**, **pork**, **egg**, cornstarch, ginger, shallot, salt, water | 13.72 |
| FB33 | Eel ball | **Eel**, **fish**, salt, water, **egg white**, starch, sugar, monosodium, sodium glutamate, sodium hexametaphosphate | 13.44 |
| FB34 | Greater lizardfish ball | **Greater lizardfish**, starch, water, salt, glucose, monosodium | 15.43 |
| FB35 | Mackerel ball | **Mackerel**, water, salt, **egg white**, cornstarch | 19.01 |
| FB36 | Mackerel ball | **Mackerel**, starch, water, salt, glucose, monosodium, spice | 19.01 |
| FB37 | Fish ball | **Freshwater fish**, **pork**, **egg**, potato starch, salt, shallot, ginger, water | 13.73 |
| FB38 | Fish ball | **Fish**, sugar, water, **pork**, **eel**, potato starch, salt, monosodium, soy sauce, shallot, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 4.57 |
| FB39 | Eel ball | **Fish**, sugar, water, **pork**, **eel**, potato starch, salt, monosodium, soy sauce, shallot, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 4.57 |
| FB40 | Fish ball | **Fish**, sugar, water, **pork**, potato starch, salt, monosodium, soy sauce, shallot, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 4.57 |
| FB41 | Shark ball | **Shark**, potato starch, **pork**, condiment, water | 10.99 |
| FB42 | Shark ball | **Shark**, **eel**, **fish**, **pork**, starch, salt, monosodium, water, soy sauce, sugar, **essence of chicken**, sodium pyrophosphate, sodium tripolyphosphate | 20.77 |
| FB43 | Shark ball | - | 11.57 |
| FB44 | Shark ball | **Shark**, **pork**, starch, sugar, soy sauce, water, monosodium, salt, sesame oil | 17.02 |
| FB45 | Shark ball | - | 13.77 |
| FB46 | White fish ball | **Fish (*Nemipterus* spp.)**, water, sugar, **egg white powder**, sodium tripolyphosphate, starch acetate, salt, acetylated distarch adipate, garlic | 19.74 |
| FB47 | Fish ball | **Fish**, sugar, water, **pork**, starch, salt, monosodium, soy sauce, shallot, vegetable oil, sodium pyrophosphate, sodium tripolyphosphate, sodium hexametaphosphate | 7.66 |

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963 **Table 2. FBs considered mislabeled in this study with type of mislabeling**.

| Sample | Information (trade name/ingredient list) | Type of mislabeling |
|---|---|---|
| FB1 | mackerel, fish | Absence of species belonging to Scomberiformes |
| FB2 | Fish | Detection of pork |
| FB3 | shark, fish | Detection of pork |
| FB9 | fish, pork | Detection of chicken |
| FB10 | Shark | Detection of pork |
| FB11 | shark, golden threadfin bream (*Nemipterus virgatus*), pork | Absence of *N. virgatus*; detection of other fish species in addition to shark |
| FB12 | fish, pork, egg | Absence of fish; detection of pork, chicken, cattle |
| FB14 | mackerel, eel, pork | Detection of other fish species in addition to mackerel and eel |
| FB16 | eel, pork | Detection of other fish species in addition to eel |

| | | |
|---|---|---|
| FB17 | eel, shark, shrimp, pork | Absence of species belonging to Elasmobranch |
| FB18 | eel, pork | Detection of chicken; Detection of other fish species in addition to eel |
| FB19 | eel, fish, pork | Detection of other fish species in addition to eel |
| FB20 | eel, pork | Detection of other fish species in addition to eel |
| FB22 | fish, eel, pork | Detection of chicken |
| FB26 | Grass carp (*Ctenopharyngodon idella*), Bighead carp (*Hypophthalmichthys nobilis*), egg, chicken (essence) | Absence of *C. idella* |
| FB28 | fish, pork | Detection of chicken |
| FB29 | fish, pork | Detection of chicken |
| FB33 | eel, fish, egg | Absence of species belonging to Anguilliformes |
| FB34 | Greater lizardfish (*Saurida tumbil*) | Absence of *S. tumbil*; detection of other fish species; detection of pork |
| FB35 | mackerel, egg | Detection of other fish species in addition to mackerel; detection of pork |
| FB36 | Mackerel | Absence of species belonging to Scomberiformes; detection of other fish species; detection of pork |
| FB38 | fish, pork | Detection of chicken |
| FB39 | eel, fish, pork | Absence of species belonging to Anguilliformes; detection of chicken |
| FB40 | fish, pork | Detection of chicken |
| FB41 | shark, pork | Absence of species belonging to Elasmobranch; detection of other fish species |
| FB42 | shark, eel, pork, chicken (essence) | Absence of species belonging to Elasmobranch and Anguilliformes; detection of other fish species |
| FB43 | Shark | Detection of pork; detection of other fish species in addition to shark |
| FB44 | shark, pork | Detection of pork; detection of other fish species in addition to shark |
| FB45 | Shark | Detection of pork |
| FB46 | White fish (*Nemipterus sp.*), egg | Absence of *Nemipterus sp.*; detection of other fish species; detection of pork; detection of cattle |
| FB47 | fish, pork | Detection of chicken |

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- The 16S rRNA metabarcoding allowed to detect a high mislabelling rate in Fishballs
- The undeclared presence of pork, chicken, and cattle was unveiled
- Issues on the sustainability of these productions were highlighted

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**Jing Wen:** Conceptualization; Writing - Review & Editing, Writing - Review & Editing Funding aquisiton

**Declarations of interest: none**

**Beyond mislabelling: Chinese Fish Balls authentication by metabarcoding allows unveiling hidden mammal and avian species**

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## SUPPLEMENTARY MATERIAL (SM)

**Figure SM-1.** Number of fish species (or higher taxonomic ranks) molecularly detected for each FB.

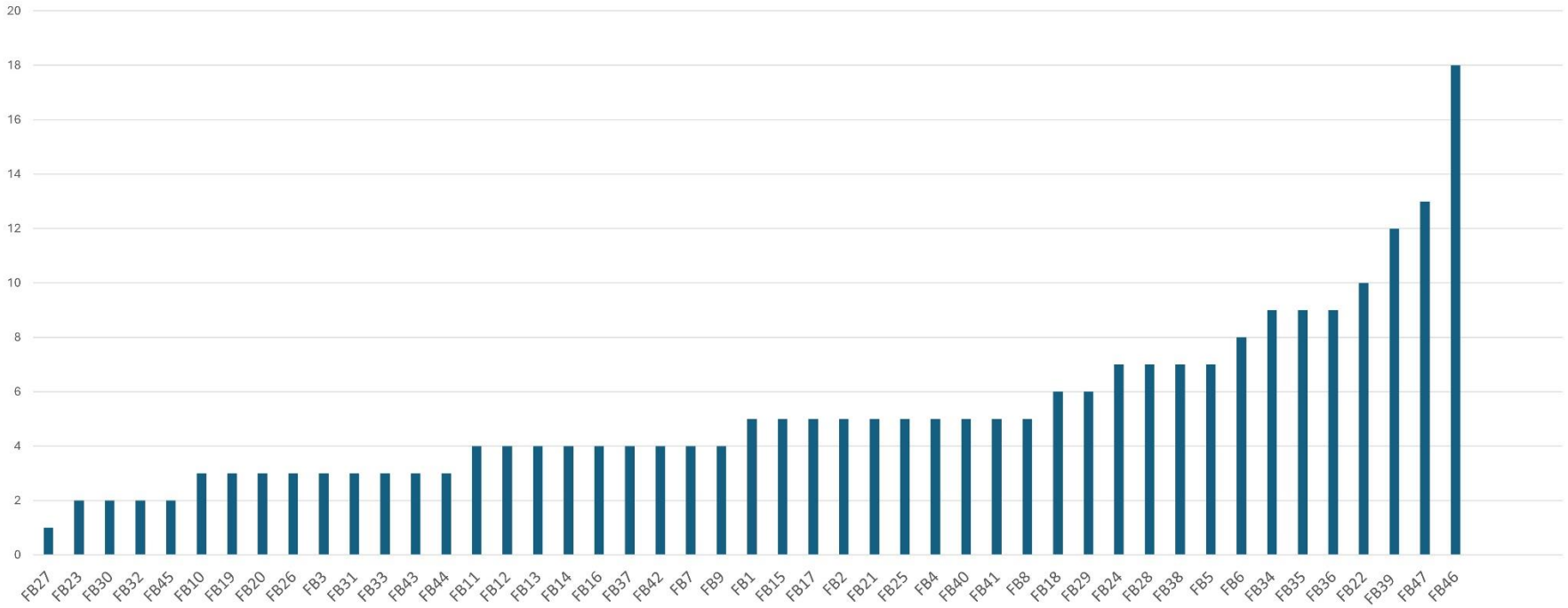


**Table SM-1. Species amplified in the study by Sarri et al. (2014) and other studies.**

| Class | Species |
|---|---|
| Aves | *Alectoris chukar* |

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| Aves | *Alectoris graeca* |
| Aves | *Anas crecca* |
| Aves | *Anas penelope* |
| Aves | *Anas platyrhynchus* [a] |
| Aves | *Anser anser*[a][b][c] |
| Aves | *Columba livia* |
| Aves | *Columba palumbus* |
| Aves | *Coturnix coturnix* |
| Aves | *Coturnix japonica* |
| Aves | *Gallinago gallinago* |
| Aves | *Gallus gallus*[a][b][c][d] |
| Aves | *Meleagris gallopavo*[d] |
| Aves | *Passer montanus* |
| Aves | *Phasianus colchicus* |
| Aves | *Scolopax rusticola* |
| Aves | *Streptopelia turtur* |
| Aves | *Tadorna tadorna* |
| Aves | *Turdus merula* |
| Cephalopods | *Sepia officinalis* |
| Cephalopods | *Loligo vulgaris* |
| Coleoptera | *Aplidia transversa* |
| Coleoptera | *Macraspis tristis* |
| Coleoptera | *Melolontha hippocastani* |
| Coleoptera | *Melolontha melolontha* |
| Coleoptera | *Monochamus sutor* |
| Coleoptera | *Morimus asper* |
| Coleoptera | *Morimus funereus* |
| Coleoptera | *Neodorcadion sp.* |
| Coleoptera | *Niphona grisea* |
| Coleoptera | *Oberea bipunctata* |
| Coleoptera | *Obezema pupillata* |
| Coleoptera | *Oryctes nasicornis* |

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| Coleoptera | *Parmena sp.* |
| Coleoptera | *Pedostrangalia verticalis* |
| Coleoptera | *Philleurus deshave* |
| Coleoptera | *Phytoecia nigricornis* |
| Coleoptera | *Plagionotus arcuatus* |
| Coleoptera | *Rhizotrogus sp.* |
| Coleoptera | *Ruptela maculata* |
| Coleoptera | *Saperda scalaris* |
| Coleoptera | *Scarabaeus sp.* |
| Coleoptera | *Vadonia imitatrix* |
| Crustaceans | *Callinectes sapidus* |
| Crustaceans | *Squilla mantis* |
| Crustaceans | *Astacus astacus* |
| Crustaceans | *Nephrops norvegicus* |
| Crustaceans | *Homarus gammarus* |
| Fish | *Betta splendes* |
| Fish | *Boops boops* |
| Fish | *Carassius auratus* |
| Fish | *Cirrhinus molitorella*[e] |
| Fish | *Coilia mystus*[e] |
| Fish | *Ctenopharyngodon idella*[e] |
| Fish | *Dicentrarchus labrax* |
| Fish | *Engraulis encrasicolus* |
| Fish | *Gadus chalcogrammus*[f] |
| Fish | *Gadus morhua*[f] |
| Fish | *Helicolenus dactylopterus* |
| Fish | *Katsuwonus pelamis*[c] |
| Fish | *Ladigesocypris ghigii* |
| Fish | *Limanda aspera*[d] |
| Fish | *Lophius budegassa* |
| Fish | *Merluccius hubbsi*[d] |
| Fish | *Merluccius merluccius*[d] |

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| Fish | *Micromesistius poutassou* |
| Fish | *Mullus barbatus* |
| Fish | *Mullus surmuletus* |
| Fish | *Oblada melanura* |
| Fish | *Oncorhynchus gorbuscha*[g] |
| Fish | *Oncorhynchus keta*[g] |
| Fish | *Oncorhynchus mykiss*[e] [g] |
| Fish | *Oncorhynchus tshawytscha*[g] |
| Fish | *Oreochromis niloticus*[g] |
| Fish | *Pagellus erythrinus* |
| Fish | *Phycis phycis* |
| Fish | *Prionace glauca* |
| Fish | *Raja miraletus* |
| Fish | *Salmo salar*[a] [b] [c] |
| Fish | *Salmo trutta* |
| Fish | *Sardinella aurita* |
| Fish | *Scomber scombrus* |
| Fish | *Sebastes viviparous* |
| Fish | *Sparus aurata* |
| Fish | *Spicara smaris* |
| Fish | *Tetrapturus angustirostris*[e] |
| Fish | *Thunnus albacares*[e] |
| Fish | *Thunnus tonggoll*[h] |
| Fish | *Trachurus trachurus* |
| Fish | *Trigla lucerna* |
| Fish | *Zeus faber* |
| Mammals | *Bos taurus*[b] [c] [d] |
| Mammals | *Bos grunniens*[e] |
| Mammals | *Bos mutus*[h] |
| Mammals | *Bubalus bubalis*[b] [c] [d] |
| Mammals | *Canis lupus familiaris* |
| Mammals | *Capra hircus*[d] |

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|---|---|
| Mammals | *Capreolus capreolus* |
| Mammals | *Cervus nippon* |
| Mammals | *Equus caballus*[b] [c] |
| Mammals | *Erinaceus europaeus* |
| Mammals | *Felis silvestris* |
| Mammals | *Homo sapiens*[b] |
| Mammals | *Lepus brachyurus* |
| Mammals | *Lepus capensis* |
| Mammals | *Lepus castroviejoi* |
| Mammals | *Lepus europaeus* |
| Mammals | *Lepus granatensis* |
| Mammals | *Lepus mediterraneus* |
| Mammals | *Lepus saxatilis* |
| Mammals | *Lepus timidus* |
| Mammals | *Lepus victoriae* |
| Mammals | *Martes martes* |
| Mammals | *Mus musculus* |
| Mammals | *Mustela nivalis* |
| Mammals | *Oryctolagus cuniculus*[d] |
| Mammals | *Ovis aries*[b] [c] [d] |
| Mammals | *Rupicapra rupicapra* |
| Mammals | *Sus scrofa*[a] [b] [c] [d] |
| Mammals | *Ursus arctos* |
| Mammals | *Vulpes vulpes* |
| Reptiles | *Hemidactylus turcicus* |
| Reptiles | *Hierophis gemonensis* |
| Reptiles | *Lacerta viridis* |
| Reptiles | *Platyceps najadum* |
| Reptiles | *Typhlops vermicularis* |

[a] also amplified by Wang et al. (2021)
[b] also amplified by Xing et al. (2019)
[c] also amplified by Xing et al. (2020)
[d] also amplified by Stamatis et al. (2015)

[e] not tested by Sarri et al. (2014); only amplified by Xing et al. (2020)
[f] not tested by Sarri et al. (2014); only amplified by Stamatis et al. (2015)
[g] not tested by Sarri et al. (2014); only amplified by Wang et al. (2021)
[h] not tested by Sarri et al. (2014); only amplified by Xing et al. (2019)

**Table SM-2~~1~~.** Declared composition (DC) and molecular identification of FBs, with relative sequence abundance (% of the total sequences). Only species (or higher taxonomic ranks) with sequence abundance ≥1% are reported, according to the established filtering threshold.

| Code | DC | Molecular ID | Seq% |
|---|---|---|---|
| FB1[a][e] | mackerel, fish | *Saurida wanieso/Harpadon nehereus* | 63.40 |
| | | *Saurida undosquamis/S. macrolepis* | 14.17 |
| | | *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri* | 11.50 |
| | | *Saurida microlepis/Pseudolabrus sieboldi* | 4.91 |
| | | *Gadus chalcogrammus* | 3.49 |
| FB2[b] | fish | *Saurida wanieso/Harpadon nehereus* | 68.29 |
| | | *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri* | 16.59 |
| | | *Pseudolabrus sieboldi/Saurida microlepis* | 4.29 |
| | | *Gadus chalcogrammus* | 3.98 |
| | | ***Sus scrofa*** | 3.85 |
| FB3[b][c] [e] | shark, fish | *Isurus oxyrinchus* | 90.47 |
| | | *Trichiurus brevis* | 4.14 |
| | | ***Sus scrofa*** | 2.44 |
| FB4 | eel, fish, pork | *Sus scrofa* | 92.09 |
| | | *Isurus oxyrinchus* | 2.57 |
| | | *Ilisha elongata* | 1.43 |
| | | *Hypophthalmichthys nobilis* | 1.23 |
| | | *Muraenesox cinereus* | 1.06 |
| FB5[b] | Leopard coral grouper (*Plectropomus leopardus*), fish, pork | *Sus scrofa* | 41.06 |
| | | *Opsariichthys bidens/O. uncirostris* | 21.89 |
| | | *Muraenesox cinereus* | 11.83 |
| | | *Plectropomus leopardus* | 9.16 |
| | | *Sus celebensis* | 5.87 |
| | | *Pangasianodon hypophthalmus* | 4.78 |
| | | *Trichiurus lepturus/T. japonicus* | 1.72 |
| FB6 | Brown-marbled grouper (*Epinephelus fuscoguttatus*), fish, pork | *Hypophthalmichthys nobilis* | 35.70 |
| | | *Pangasianodon hypophthalmus* | 18.01 |
| | | *Epinephelus fuscoguttatus* | 11.63 |

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|---|---|---|---|
| | | *Sus scrofa* | 9.75 |
| | | *Trichiurus lepturus/T. japonicus* | 5.82 |
| | | *Sus celebensis* | 5.31 |
| | | *Gadus chalcogrammus* | 3.98 |
| | | *Isurus oxyrinchus* | 2.13 |
| FB7 | salmon, fish, pork | *Salmo salar* | 72.29 |
| | | *Hypophthalmichthys nobilis* | 13.07 |
| | | *Ilisha elongata* | 7.30 |
| | | *Sus scrofa* | 3.82 |
| FB8[b][c][d] | eel, fish, pork | *Muraenesox cinereus* | 48.15 |
| | | *Muraenesox bagio/M. cinereus* | 22.11 |
| | | *Sus scrofa* | 18.30 |
| | | *Pangasianodon hypophthalmus* | 3.20 |
| | | *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri* | 2.50 |
| FB9[c] | fish, pork | ***Gallus gallus*** | 75.59 |
| | | *Sus scrofa* | 14.94 |
| | | *Hypophthalmichthys nobilis* | 3.46 |
| | | *Trichiurus japonicus/T. lepturus* | 2.44 |
| FB10 | Shark | ***Sus scrofa*** | 67.13 |
| | | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos/Carcharhinus melanopterus* | 30.18 |
| | | ***Sus celebensis*** | 1.70 |
| FB11[c] | shark, golden threadfin bream (*Nemipterus virgatus*), pork | *Sus scrofa* | 56,82 |
| | | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos/Carcharhinus melanopterus* | 34.32 |
| | | *Sus celebensis* | 5.82 |
| | | ***Pangasianodon hypophthalmus*** | 2.71 |
| FB12[c] | fish, pork, egg | *Sus scrofa* | 89.97 |
| | | *Gallus gallus* | 3.76 |
| | | *Sus celebensis* | 3.15 |
| | | ***Bos taurus*** | 2.07 |
| FB13 | fish, pork, egg, chicken | *Gallus gallus* | 40.98 |
| | | *Sus scrofa* | 38.21 |
| | | *Trichiurus japonicus/T. lepturus* | 16.83 |
| | | *Hypophthalmichthys nobilis* | 1.57 |
| FB14 | mackerel, eel, pork | *Scomberomorus niphonius* | 77.19 |
| | | *Sus scrofa* | 11.71 |

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|---|---|---|---|
| | | *Muraenesox cinereus* | 5.86 |
| | | ***Chelidonichthys kumu/C. spinosus/C. lucerna*** | 2.60 |
| FB15[b] | shark, eel, fish, pork | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos/Carcharhinus melanopterus* | 54.86 |
| | | *Muraenesox cinereus* | 20.09 |
| | | *Ilisha elongata* | 15.32 |
| | | *Sus scrofa* | 7.08 |
| | | *Hypophthalmichthys nobilis* | 1.68 |
| FB16[b] [c] | eel, pork | *Muraenesox cinereus* | 76.68 |
| | | ***Ilisha elongata*** | 10.31 |
| | | *Sus scrofa* | 6.86 |
| | | ***Hypophthalmichthys nobilis*** | 4.36 |
| FB17[c] | eel, shark, shrimp, pork | *Muraenesox cinereus* | 66.71 |
| | | ***Ilisha elongata*** | 13.41 |
| | | *Sus scrofa* | 8.51 |
| | | ***Hypophthalmichthys nobilis*** | 6.92 |
| | | *Sus celebensis* | 3.27 |
| FB18[c] | eel, pork | *Muraenesox cinereus* | 59.21 |
| | | *Sus scrofa* | 23.55 |
| | | ***Acheilognathus chankaensis/A. macropterus/A. imberbis*** | 7.87 |
| | | ***Hypophthalmichthys nobilis*** | 3.50 |
| | | ***Gallus gallus*** | 1.81 |
| | | ***Ilisha striatula/Opisthopterus tardoore/Pellona ditchela*** | 1.14 |
| FB19[c] | eel, fish, pork | *Muraenesox cinereus* | 62.77 |
| | | *Sus scrofa* | 34.22 |
| | | *Hypophthalmichthys nobilis* | 2.01 |
| FB20[b] | eel, pork | *Muraenesox cinereus* | 93.10 |
| | | ***Hypophthalmichthys nobilis*** | 4.34 |
| | | *Sus scrofa* | 1.00 |
| FB21[d] | fish, pork, chicken | *Sus scrofa* | 47.68 |
| | | *Gallus gallus* | 33.20 |
| | | *Hypophthalmichthys nobilis* | 8.73 |
| | | *Trichiurus japonicus/T. lepturus* | 7.71 |
| | | *Miichthys miiuy* | 1.25 |
| FB22 | fish, eel, pork | ***Gallus gallus*** | 41.12 |
| | | *Sus scrofa* | 21.25 |
| | | *Decapterus maruadsi* | 7.35 |

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|---|---|---|---|
| | | *Hypophthalmichthys nobilis* | 6.44 |
| | | *Sardinella fijiensis/S. gibbosa* | 4.45 |
| | | *Muraenesox cinereus* | 4.26 |
| | | *Sardinella fimbriata* | 1.87 |
| | | *Trachurus japonicus/T. trachurus/T. declivis* | 1.38 |
| | | *Auxis thazard* | 1.37 |
| | | *Hirundichthys affinis/H. speculiger/Cheilopogon sp.* | 1.15 |
| FB23[b] [c] [d] | eel, pork | *Muraenesox cinereus* | 79.39 |
| | | *Sus scrofa* | 20.23 |
| FB24[a] [e] | fish, chicken (essence) | *Saurida macrolepis/S. undosquamis* | 73.98 |
| | | *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri* | 9.08 |
| | | *Hypophthalmichthys nobilis* | 2.71 |
| | | *Trachinocephalus trachinus/T. myops* | 1.73 |
| | | *Saurida wanieso/Harpadon nehereus* | 1.44 |
| | | *Synodus macrops/S. kaianus* | 1.40 |
| | | *Decapterus maruadsi* | 1.05 |
| FB25[b] | marine fish, pork, duck egg | *Ilisha elongata* | 43.43 |
| | | *Gadus chalcogrammus* | 28.53 |
| | | *Trachinocephalus trachinus/T. myops* | 20.68 |
| | | *Sus scrofa* | 3.17 |
| | | *Trachinocephalus myops/T. trachinus* | 2.61 |
| FB26[a] [b] [c] | Grass carp (*Ctenopharyngodon idella*), Bighead carp (*Hypophthalmichthys nobilis*), egg, chicken (essence) | *Hypophthalmichthys nobilis* | 79.94 |
| | | ***Aphyocypris moltrechti/A. normalis*** | 10.32 |
| | | ***Mylopharyngodon piceus*** | 9.07 |
| FB27[a] | fish, pork, egg, chicken | *Hypophthalmichthys nobilis* | 99.25 |
| FB28[c] [e] | fish, pork | ***Gallus gallus*** | 62.20 |
| | | *Sus scrofa* | 11.98 |
| | | *Hypophthalmichthys nobilis* | 7.22 |
| | | *Mene maculata* | 4.39 |
| | | *Hirundichthys affinis/H. speculiger/Cheilopogon sp.* | 3.62 |
| | | *Trichiurus brevis* | 1.13 |
| | | *Decapterus maruadsi* | 1.11 |
| FB29 | fish, pork | ***Gallus gallus*** | 70.00 |
| | | *Sus scrofa* | 15.56 |
| | | *Decapterus maruadsi* | 3.41 |

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|---|---|---|---|
| | | *Sardinella fijiensis/S. gibbosa* | 3.11 |
| | | *Hypophthalmichthys nobilis* | 1.55 |
| | | *Hirundichthys affinis/H. speculiger/Cheilopogon sp.* | 1.41 |
| FB30[b] [d] | freshwater fish, pork, egg | *Hypophthalmichthys nobilis* | 87.38 |
| | | *Sus scrofa* | 12.44 |
| FB31[b] | fish, pork, egg | *Hypophthalmichthys nobilis* | 45.42 |
| | | *Sus scrofa* | 27.69 |
| | | *Mylopharyngodon piceus* | 26.32 |
| FB32[b] [c] | black carp (*Mylopharyngodon piceus*), pork, egg | *Mylopharyngodon piceus* | 70.57 |
| | | *Sus scrofa* | 27.91 |
| FB33 | eel, fish, egg | ***Gallus gallus*** | 58.61 |
| | | *Pangasianodon hypophthalmus* | 36.81 |
| | | *Sus scrofa* | 3.88 |
| FB34[b] [c] [e] | Greater lizardfish (*Saurida tumbil*) | ***Trichiurus japonicus/T. lepturus*** | 48.84 |
| | | ***Saurida wanieso/Harpadon nehereus*** | 12.26 |
| | | ***Sus scrofa*** | 5.78 |
| | | ***Pennahia argentata*** | 5.48 |
| | | ***Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri*** | 5.17 |
| | | ***Saurida macrolepis/S. undosquamis*** | 3.98 |
| | | ***Pennahia aneus*** | 3.19 |
| | | ***Muraenesox cinereus*** | 1.77 |
| | | ***Nemipterus virgatus/N. japonicus*** | 1.66 |
| FB35[b] [c] [e] | mackerel, egg | *Trichiurus japonicus/T. lepturus* | 35.70 |
| | | ***Saurida wanieso/Harpadon nehereus*** | 19.88 |
| | | ***Evynnis cardinalis/E. tumifrons/Dentex angolensis/Dentex fourmanoiri*** | 7.83 |
| | | ***Pennahia argentata*** | 7.34 |
| | | ***Nemipterus japonicus/N. virgatus*** | 5.92 |
| | | ***Saurida undosquamis/S. macrolepis*** | 5.02 |
| | | ***Pennahia aneus*** | 3.03 |
| | | ***Sus scrofa*** | 2.02 |
| | | ***Ilisha elongata*** | 1.59 |
| FB36[b] [c] [e] | mackerel | ***Saurida wanieso/Harpadon nehereus*** | 26.35 |
| | | ***Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri*** | 19.40 |

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|---|---|---|---|
| | | ***Oreochromis mossambicus/O. niloticus*** | 16.05 |
| | | ***Saurida macrolepis/S. undosquamis*** | 13.83 |
| | | ***Saurida microlepis/Pseudolabrus sieboldi*** | 5.56 |
| | | ***Nemipterus virgatus/N. japonicus*** | 2.90 |
| | | ***Oreochromis niloticus*** | 2.90 |
| | | ***Hypophthalmichthys nobilis*** | 2.74 |
| | | ***Sus scrofa*** | 1.32 |
| FB37[b] | freshwater fish, pork, egg | *Sus scrofa* | 46.23 |
| | | *Hypophthalmichthys nobilis* | 27.76 |
| | | *Mylopharyngodon piceus* | 24.18 |
| | | *Sus celebensis* | 1.29 |
| FB38 | fish, pork | ***Gallus gallus*** | 60.99 |
| | | *Sus scrofa* | 21.84 |
| | | *Oreochromis mossambicus/O. niloticus* | 6.22 |
| | | *Trachurus japonicus/T. trachurus/T. declivis* | 2.58 |
| | | *Oreochromis niloticus* | 2.46 |
| | | *Clarias gariepinus/C. batrachus* | 1.91 |
| | | *Hypophthalmichthys nobilis* | 1.02 |
| FB39 [c] | eel, fish, pork | *Sus scrofa* | 46.19 |
| | | *Hypophthalmichthys nobilis* | 10.21 |
| | | *Decapterus maruadsi* | 7.51 |
| | | *Hirundichthys affinis/H. speculiger/Cheilopogon sp.* | 6.12 |
| | | *Psenopsis anomala* | 4.50 |
| | | *Sardinella fimbriata* | 2.20 |
| | | *Trachurus japonicus/T. trachurus/T. declivis* | 1.96 |
| | | *Muraenesox cinereus* | 1.74 |
| | | *Sardinella fijiensis/S. gibbosa* | 1.39 |
| | | ***Gallus gallus*** | 1.37 |
| | | *Auxis thazard* | 1.34 |
| | | *Scomber japonicus/S. colias* | 1.32 |
| FB40 | fish, pork | *Sus scrofa* | 76.13 |
| | | ***Gallus gallus*** | 13.29 |
| | | *Hypophthalmichthys nobilis* | 5.74 |
| | | *Sardinella fimbriata* | 2.11 |
| | | *Hilsa kelee/Opisthonema libertate/O. medirastre* | 1.51 |
| FB41[b] [c] [e] | shark, pork | *Sus scrofa* | 39.44 |

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|---|---|---|---|
| | | ***Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri*** | 22.98 |
| | | ***Hypophthalmichthys nobilis*** | 16.57 |
| | | ***Ilisha elongata*** | 12.64 |
| | | ***Pennahia argentata*** | 2.07 |
| FB42[c] | shark, eel, pork, chicken (essence) | *Sus scrofa* | 88.50 |
| | | *Sus celebensis* | 4.36 |
| | | *Gallus gallus* | 2.98 |
| | | ***Hypophthalmichthys nobilis*** | 1.58 |
| FB43[b] [e] | shark | ***Sus scrofa*** | 71.96 |
| | | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos* | 24.49 |
| | | ***Pangasianodon hypophthalmus*** | 2.53 |
| FB44 | shark, pork | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos* | 91.75 |
| | | ***Miichthys miiuy*** | 4.53 |
| | | *Sus scrofa* | 3.13 |
| FB45[b] [c] | shark | *Rhizoprionodon acutus/Scoliodon laticaudus/S. macrorhynchos* | 91.86 |
| | | ***Sus scrofa*** | 7.96 |
| FB46 | White fish (*Nemipterus sp.*), egg | ***Pangasianodon hypophthalmus*** | 15.24 |
| | | ***Sus scrofa*** | 14.40 |
| | | ***Muraenesox cinereus*** | 12.42 |
| | | ***Decapterus maruadsi*** | 8.96 |
| | | ***Sardinella fijiensis/S. gibbosa*** | 5.39 |
| | | ***Rectoris posehensis*** | 5.31 |
| | | ***Trichiurus brevis*** | 4.11 |
| | | ***Siganus canaliculatus*** | 3.37 |
| | | ***Homo sapiens*** | 3.17 |
| | | ***Hypophthalmichthys nobilis*** | 3.02 |
| | | ***Rastrelliger kanagurta/R. brachysoma*** | 2.73 |
| | | ***Sardinella fimbriata*** | 2.63 |
| | | ***Acheilognathus chankaensis/A. macropterus/A. imberbis*** | 2.56 |
| | | ***Bos taurus*** | 1.93 |
| | | ***Isurus oxyrinchus*** | 1.64 |
| | | ***Trachurus japonicus/T. declivis*** | 1.48 |
| | | *Gallus gallus* | 1.38 |
| | | ***Hilsa kelee/Opisthonema libertate/O. medirastre*** | 1.11 |
| FB47[c] [e] | fish, pork | ***Gallus gallus*** | 30.69 |
| | | *Sus scrofa* | 12.37 |
| | | *Trichiurus brevis* | 9.30 |

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|---|---|---|---|
| | | *Hilsa kelee/Opisthonema libertate/O. medirastre* | 8.91 |
| | | *Muraenesox bagio/M. cinereus* | 6.97 |
| | | *Mene maculata* | 5.71 |
| | | *Hypophthalmichthys nobilis* | 3.69 |
| | | *Decapterus maruadsi* | 3.16 |
| | | ***Homo sapiens*** | 2.63 |
| | | *Isurus oxyrinchus* | 2.37 |
| | | *Acheilognathus chankaensis/A. macropterus/A. imberbis* | 2.09 |
| | | *Hirundichthys affinis/H. speculiger /Cheilopogon sp.* | 1.89 |
| | | *Evynnis cardinalis/E. tumifrons/Dentex angolensis/D. fourmanoiri* | 1.02 |

[a] sequences assigned to pork (*Sus sp.*) below 1%; [b] sequences assigned to chicken (*Gallus gallus*) below 1%; [c] sequences assigned to cattle (*Bos sp.*) below 1%; [d] sequences assigned to sheep (*Ovis aries*) below 1%; [e] sequences assigned to pufferfish (*Takifugu sp.; Lagocephalus sp.*) below 1%

**Table SM-32**. IUCN status of fish species molecularly detected in the FBs.

| **Species** | **Common names** | **IUCN Red List Status** |
|---|---|---|
| *Hypophthalmichthys nobilis* | Bighead carp | Data deficient |
| *Muraenesox cinereus* | Daggertooth pike conger | Least Concern |
| *Evynnis cardinalis* | Threadfin porgy | Endangered |
| *Evynnis tumifrons* | Yellowback seabream | Least Concern |
| *Dentex angolensis* | Angolan dentex | Near Threatened |
| *Dentax fourmanoiri* | None | Data deficient |
| *Ilisha elongata* | Elongate ilisha | Least Concern |
| *Trachurus japonicus* | Japanese jack mackerel | Near Threatened |
| *Trachurus trachurus* | Atlantic horse mackerel | Vulnerable |
| *Trachurus declivis* | Greenback horse mackerel | Least Concern |
| *Pangasianodon hypophthalmus* | Striped catfish | Endangered |
| *Decapterus maruadsi* | Japanese scad | Least Concern |
| *Saurida wanieso* | Wanieso lizardfish | Data deficient |
| *Harpadon nehereus* | Bombay-duck | Near Threatened |
| *Rhizoprionodon acutus* | Milk shark | Vulnerable |
| *Scoliodon laticaudus* | Spadenose shark | Near Threatened |
| *Scoliodon macrorhynchos* | Pacific spadenose shark | Near Threatened |
| *Saurida macrolepis* | None | Least Concern |
| *Saurida undosquamis* | Brushtooth lizardfish | Least Concern |
| *Isurus oxyrinchus* | Shortfin mako | Endangered |
| *Hirundichthys affinis* | Fourwing flyingfish | Least Concern |
| *Hirundichthys speculiger* | Mirrorwing flyingfish | Least Concern |
| *Trichiurus japonicus* | None | Not Evaluated |
| *Trichiurus lepturus* | Largehead hairtail | Least Concern |
| *Trichiurus brevis* | Chinese short-tailed hairtail | Not Evaluated |
| *Sardinella fimbriata* | Fringescale sardinella | Least Concern |
| *Sardinella fijiensi* | Fiji sardinella | Least Concern |
| *Sardinella gibbosa* | Goldstripe sardinella | Least Concern |
| *Oreochromis mossambicu* | Mozambique tilapia | Vulnerable |
| *Oreochromis niloticus* | Nile tilapia | Least Concern |
| *Mylopharyngodon piceus* | Black carp | Least Concern |
| *Gadus chalcogrammus* | Alaska pollock | Not Evaluated |

| | | |
|---|---|---|
| *Trachinocephalus myops* | Snakefish | Least Concern |
| *Trachinocephalus trachinus* | None | Least Concern |
| *Pennahia argentata* | Silver croaker | Least Concern |
| *Nemipterus virgatus* | Golden threadfin bream | Vulnerable |
| *Nemipterus japonicus* | Japanese threadfin bream | Least Concern |
| *Hilsa kelee* | Kelee shad | Least Concern |
| *Opisthonema libertate* | Pacific thread herring | Least Concern |
| *Opisthonema medirastre* | Middling thread herring | Least Concern |
| *Acheilognathus chankaensis* | Khanka spiny bitterling | Not Evaluated |
| *Acheilognathus macropterus* | None | Data deficient |
| *Acheilognathus imberbis* | None | Least Concern |
| *Saurida microlepis* | None | Not Evaluated |
| *Pseudolabrus sieboldi* | None | Least Concern |
| *Pennahia aneus* | Donkey croaker | Least Concern |
| *Miichthys miiuy* | Mi-iuy croaker | Data deficient |
| *Mene maculata* | Moonfish | Not Evaluated |
| *Auxis thazard* | Frigate tuna | Least Concern |
| *Synodus macrops* | Triplecross lizardfish | Least Concern |
| *Synodus kaianus* | Gunther's lizardfish | Least Concern |
| *Siganus canaliculatus* | White-spotted spinefoot | Least Concern |
| *Scomberomorus niphonius* | Japanese Spanish mackerel | Near Threatened |
| *Scomber japonicus* | Chub mackerel | Least Concern |
| *Scomber colias* | Atlantic chub mackerel | Least Concern |
| *Salmo salar* | Atlantic salmon | Near Threatened |
| *Psenopsis anomala* | Pacific rudderfish | Least Concern |
| *Rectoris posehensis* | None | Not Evaluated |
| *Plectropomus leopardus* | Leopard coralgrouper | Least Concern |
| *Opsariichthys bidens* | None | Least Concern |
| *Opsariichthys uncirostris* | Three-lips | Not Evaluated |
| *Rastrelliger kanagurta* | Indian mackerel | Least Concern |
| *Rastrelliger brachysoma* | Short mackerel | Vulnerable |
| *Ilisha striatula* | Banded ilisha | Data deficient |
| *Opisthopterus tardoore* | Tardoore | Least Concern |
| *Pellona ditchela* | Indian pellona | Least Concern |

| | | |
|---|---|---|
| *Epinephelus fuscoguttatus* | Brown-marbled grouper | Vulnerable |
| *Clarias gariepinus* | North African catfish | Least Concern |
| *Clarias batrachus* | Philippine catfish | Least Concern |
| *Cirrhinus molitorella* | Mud carp | Near Threatened |
| *Chelidonichthys kumu* | Bluefin gurnard | Least Concern |
| *Chelidonichthys spinosus* | Spiny red gurnard | Not Evaluated |
| *Chelidonichthys lucerna* | Tub gurnard | Least Concern |
| *Aphyocypris moltrechti* | Moltrecht's minnow | Not Evaluated |
| *Aphyocypris normalis* | None | Not Evaluated |