Selection of Peptide Targets for Species-independent Quantitation of Fish Allergens

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Introduction

Detection and quantitation of fish residues in foods for allergen control poses considerable challenges. The diversity of fish used in the food supply necessitates the use of analytical targets that are shared, and are quantitatively similar, in all potential contaminant species. Identifying such targets is challenging. Currently available methods, reliant on ELISA, targeting Parvalbumin, display unacceptable (often 200-fold) variation in performance across fish species rendering quantitation near-impossible. We designed a workflow employing untargeted high-resolution MS with label-free quantitation to identify peptide targets that are present in a broad range of consumed fish, that are similarly abundant, and that are not present in non-fish.

Goals

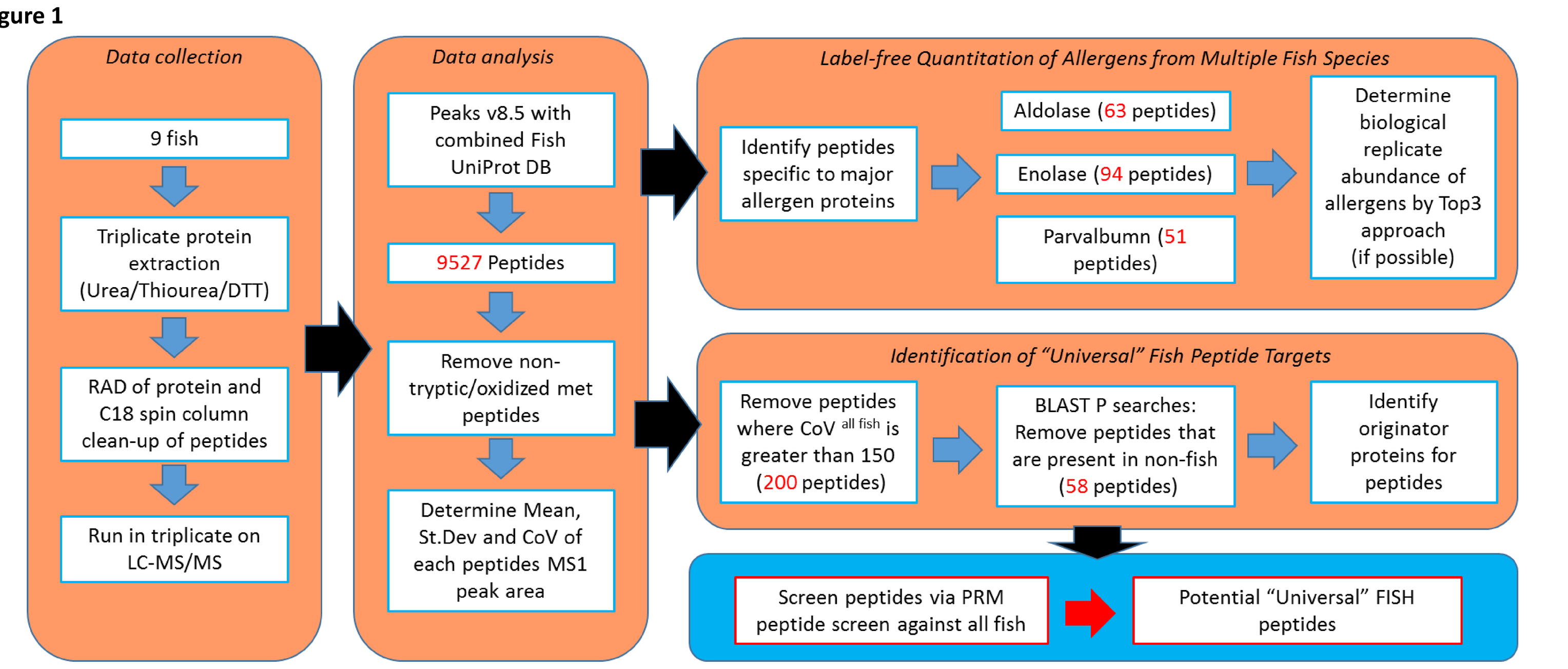
- Determine the suitability of major allergen quantitation for broad-spectrum fish detection.
- Identify peptide targets that are present in a broad range of fish, are similarly abundant, and are not present in non-fish. Use these to develop a method to quantify the major allergens of fish (Parvalbumin, Enolase and Aldolase) across 9 fish species.

Methods

Nine fish species, PCR speciated (cod, pollock, herring, salmon, tuna, skate, tilapia, grouper and halibut) were selected to represent the broad taxonomies of heavily consumed fish. Protein was extracted from 3 replicate samples (0.5 g) of the fillet using a reducing urea/thiourea extraction method (10 mL of 6 M Urea, 2 M Thiourea, 50 mM Tris-HCl, pH 8.6, 50 mM DTT).

Untargeted MS (3 replicate analyses of 3 replicate samples) was performed using a Thermo Scientific™ Q Exactive™ HF mass spectrometer coupled to a Thermo Scientific™ UltiMate™ 3000 nanoRS liquid chromatography (UPLC) system equipped with a Acquity UPLC M-Class Peptide CSH C18 Column 130A, 1.7µm, 75µm x 250mm (Waters Corp). Peptide, protein ID and label-free quantitation was performed using PeaksQ v8.5 with a combined protein database of all sequences available (UniProt) for analyzed fish species. Subsequent analysis of peptide quantitation data was performed using Microsoft Excel. BLAST P searches were performed against the whole NCBI database. **Figure 1** shows an overview of the methodology and peptide selection strategy.

Figure 1

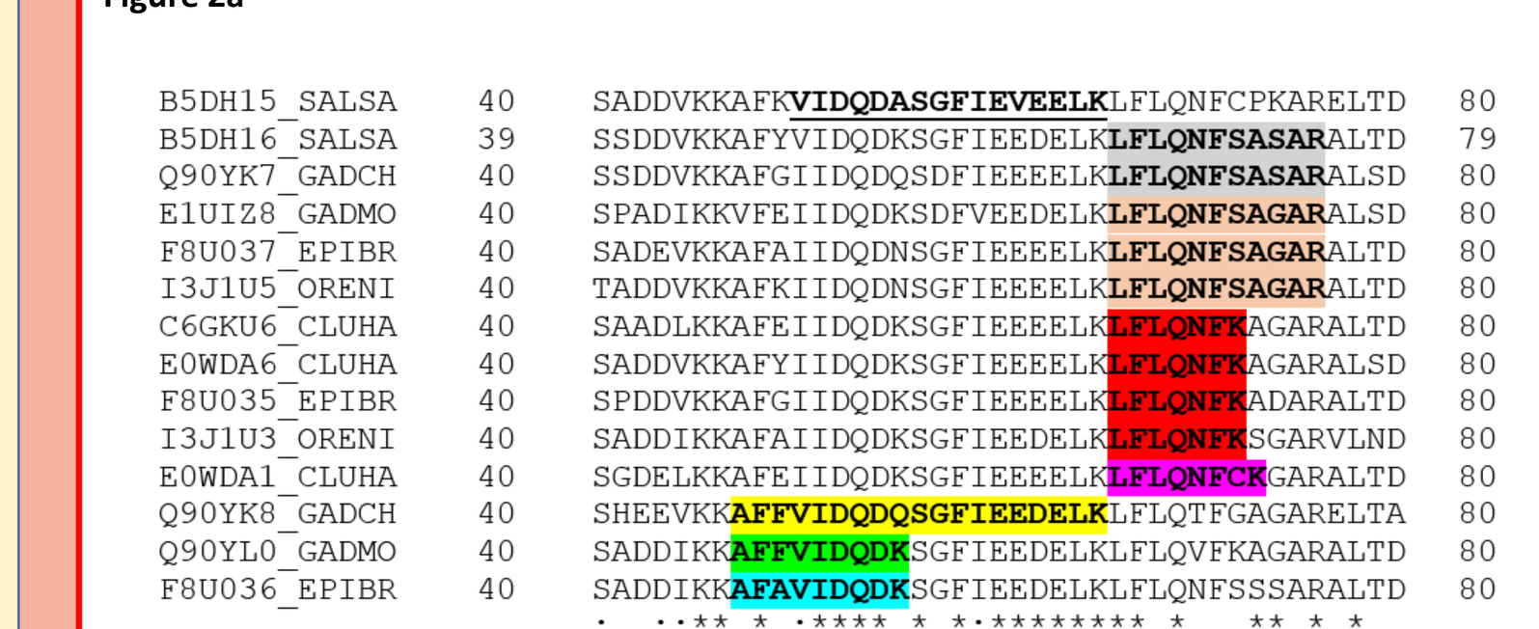


Label-free Quantitation of Allergens from Multiple Fish Species

Parvalbumin

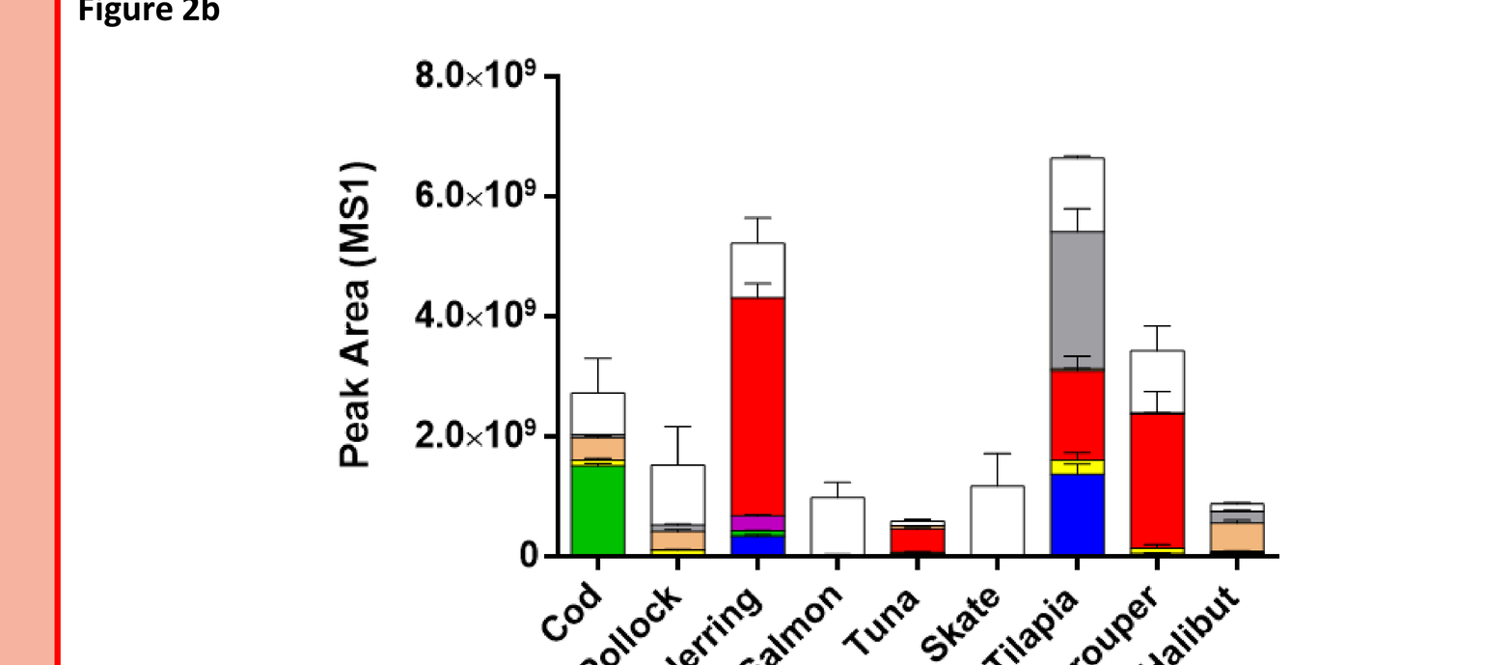
- 51 peptides from 14 Parvalbumin isoforms were detected.
- Label-free quantitation achieved by a single unique peptide to each Parvalbumin isoform family (See alignment in **Figure 2a**).
- Only 4 of the isoform families are present in all fish studied.
- Parvalbumin shows a variable abundance (6-fold difference between Tilapia and Tuna) across the fish species (See **Figure 2b**).
- Parvalbumin abundance in Salmon and skate is due predominantly to one isoform family.
- Tilapia exhibits 4 major Parvalbumin isoform families.

Figure 2a



Alignment of the Parvalbumin isoforms: Performed using Clustal Omega. UniProt accessions identified indicated in alignment. Bold/Highlighted font indicates tryptic peptide used to quantify abundance.

Figure 2b

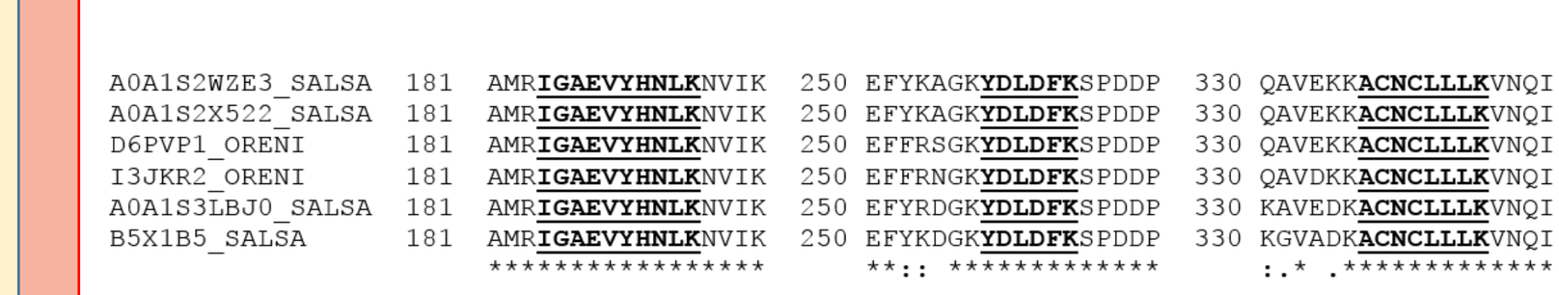


Histogram of Parvalbumin Abundance in 9 Fish from 3 biological replicates: Colored plot represent mean abundance of isoform families as denoted in the key by the tryptic peptide used in quantitation. Standard error of the mean also shown.

Enolase

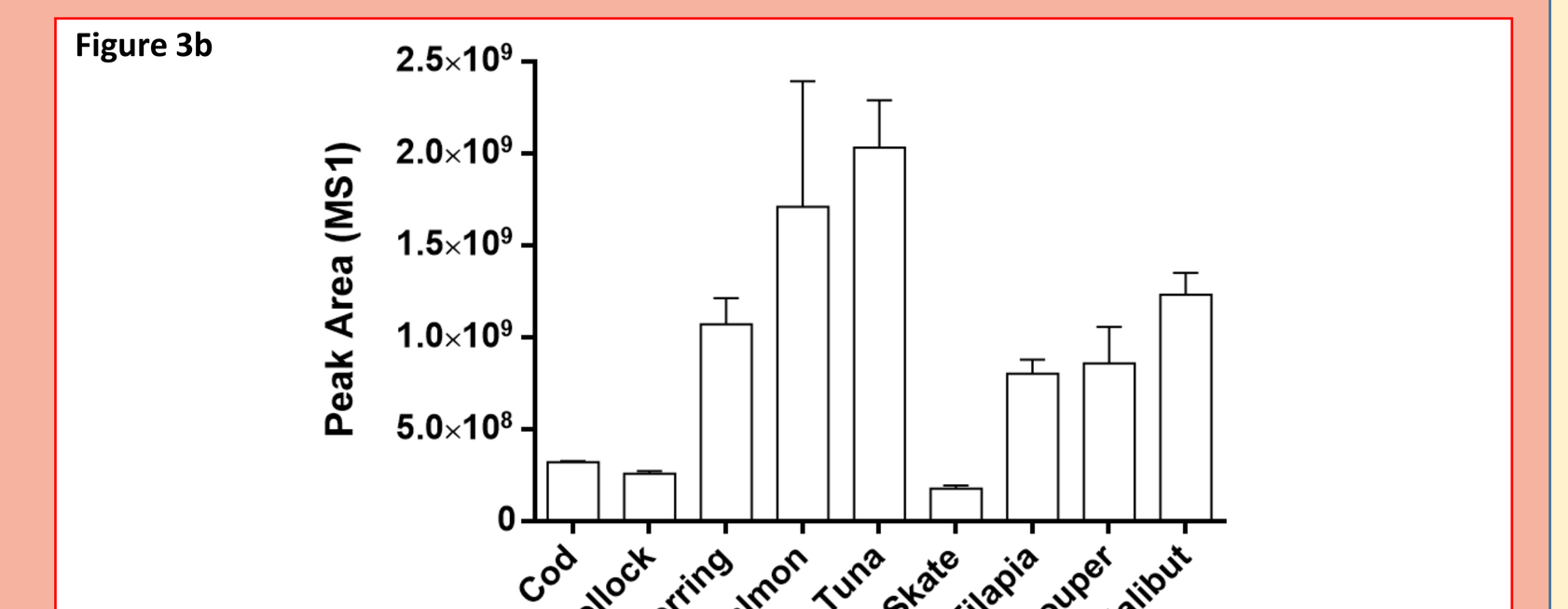
- 94 peptides from 6 Enolase isoforms were detected.
- Label-free quantitation was achieved by a top3 approach (See alignment in **Figure 3a**).
- Enolase shows a highly variable abundance across the fish species (11-fold difference between Tuna and Skate) (See **Figure 3b**).

Figure 3a



Alignment of the Enolase isoforms: Performed using Clustal Omega. UniProt accessions identified indicated in alignment. Bold font indicates tryptic peptides used to quantify abundance.

Figure 3b

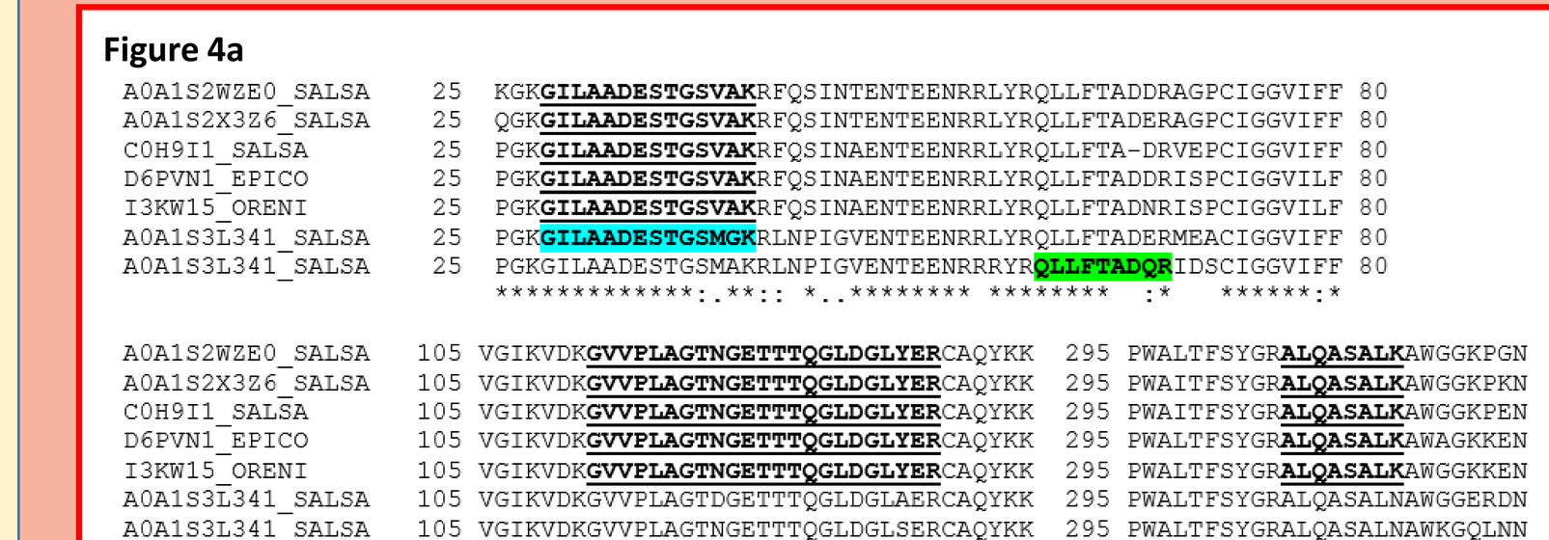


Histogram of Enolase Abundance in 9 Fish from 3 biological replicates: Plot represent mean of enolase as determined by top3. Standard error of the mean also shown.

Aldolase

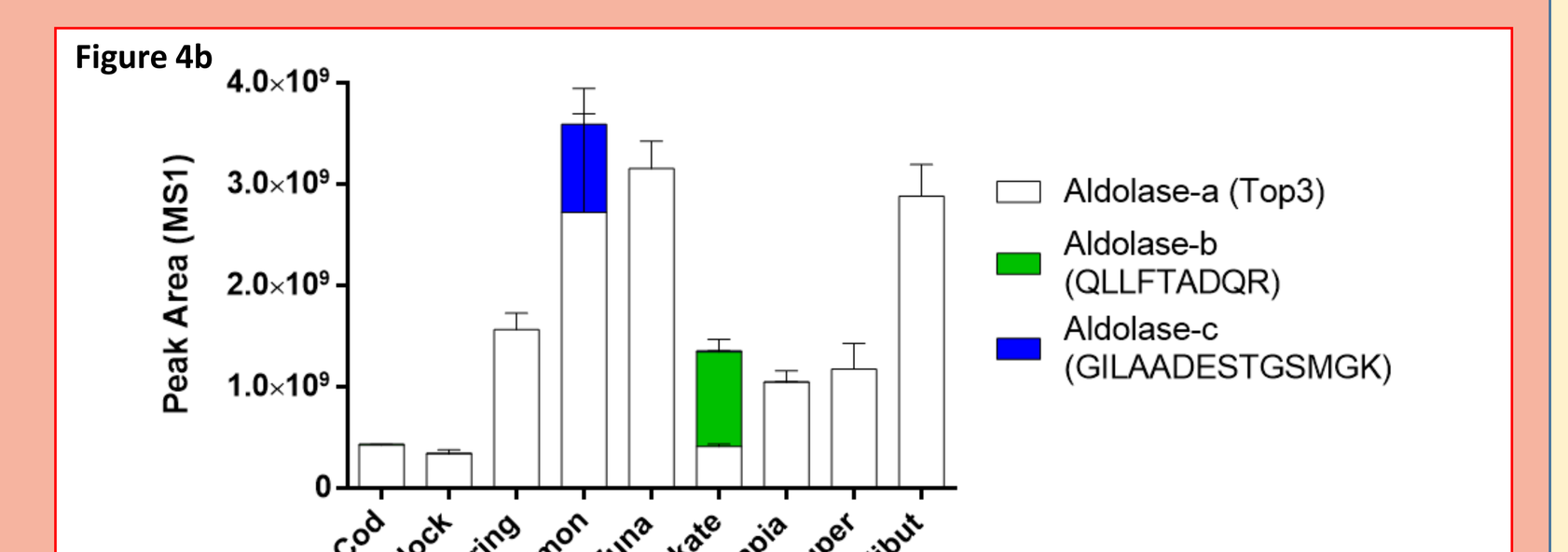
- 63 peptides from 7 Aldolase isoforms were detected.
- Label-free quantitation was achieved by a Top3 approach for the predominant isoform (Aldolase-a) and by a single unique peptide to the other isoforms (b and c) (See alignment in **Figure 4a**).
- Aldolase shows a highly variable abundance across the fish species (10-fold difference between Salmon and Pollock) (**See Figure 4b**).

Figure 4a



Alignment of the Aldolase isoforms: Performed using Clustal Omega. UniProt accessions identified indicated in alignment. Bold/Highlighted font indicates tryptic peptide used to quantify abundance.

Figure 4b

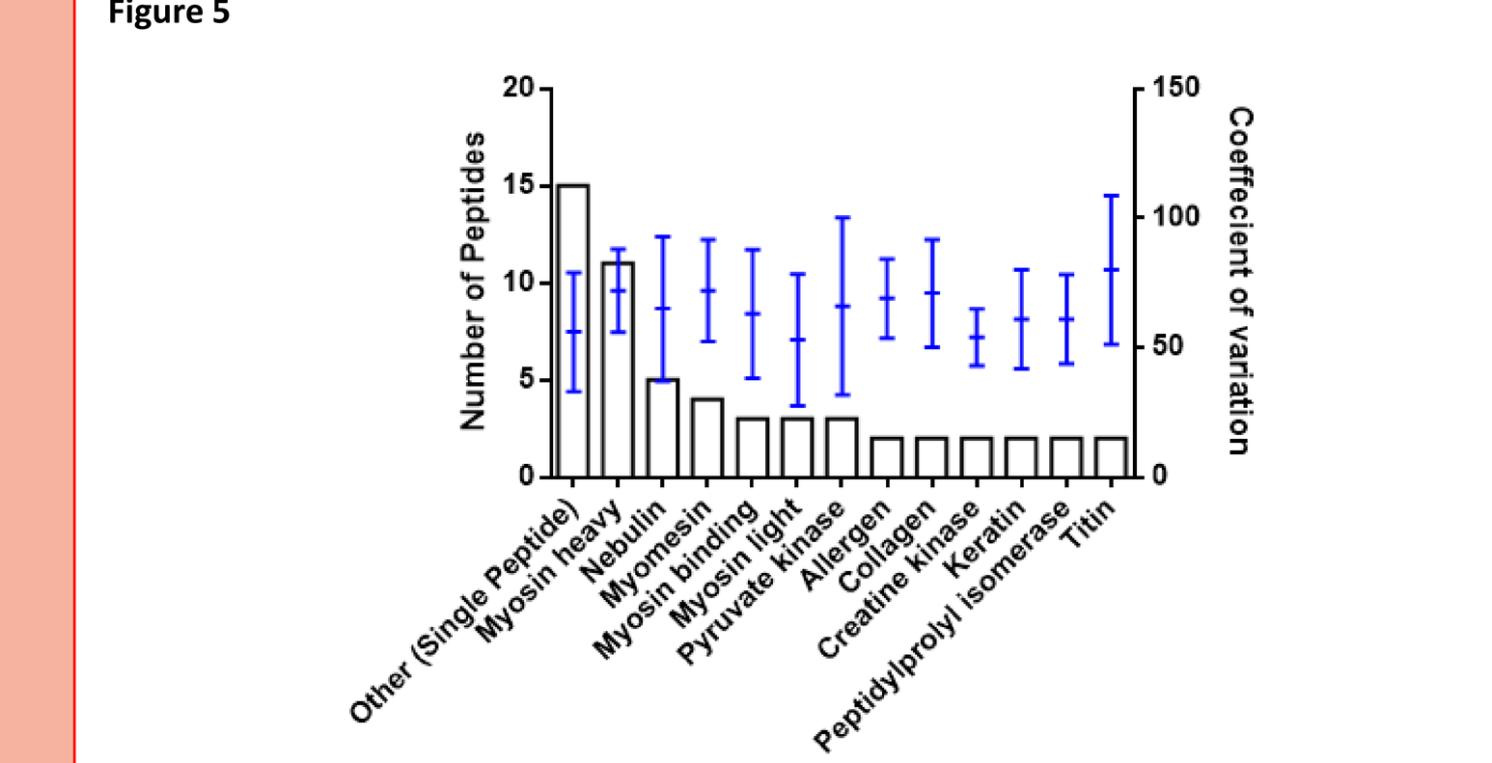


Histogram of Aldolase Abundance in 9 Fish from 3 biological replicates: Colored plot represent mean abundance of isoform families as denoted in the key by the tryptic peptide used in quantitation. Standard error of the mean also shown

Identification of "Universal" Fish Peptide Targets

- 200 peptides met our limitation of <150 CoV $^{\text{all fish}}$.
- After screening these peptides with BLAST P against NCBI, only 25 % of the peptides were shown to be fish-specific.
- The majority of the candidate peptides are, as expected, derived from muscle associated proteins (See **Figure 5**).
- Interestingly, only 1 peptide from the major allergens and 1 peptide from the minor allergens fit our filtration criteria.

Figure 5



Histogram of Candidate "Universal" Fish Peptide Targets: Number of peptides from each family of protein are shown. The mean CoV (across all 9 fish) is also shown as a blue bar. Standard error of the mean also shown

Discussion

- Quantitation of the major allergens of fish reveals high variation of abundance in the allergens.
- A complex Parvalbumin isoform distribution exists in fish.
- Concluding from this work, parvalbumin is likely not an ideal analytical target for fish residue quantitation. This is consistent with the known analytical performance of ELISA methods based on parvalbumin detection.
- Multiple "universal" fish peptide target candidate peptides, derived from non-allergens, have been identified. This pool of candidate peptides comprises 0.5 % of the peptides detected.
- As might be expected, the need to quantify cartilaginous fish (here, skate) complicates the development of a 'universal' fish detection method.

Conclusions and Future Work

Our DDA-dependent strategy of peptide target selection appears to be valid work-flow, and could well be applied to other cross-species studies. However, one limitation to this strategy has been the limited proteome data from some fish. Thus, our quantitation may be biased to the fish species with comprehensive databases (e.g. Salmon and Tilapia). Theses considerations do not impact out target selection strategy though.
Further work will include screening the peptide candidates against each fish, using a PRM methodology and finally in relevant fish spiked food matrices.

Acknowledgements

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