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Proof of concept study for the use of biochemical techniques to estimate source ocean of SBT from tissue samples.

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1 Abstract

A developing area of research is the use of biochemical analyses of the flesh of large marine predators, specifically stable isotope analysis (SIA) and fatty acid analysis (FAA), to understand their trophic ecology, population structure and movement. In this proof of concept study we examined their potential utility for distinguishing tissues samples of SBT obtained from two sources: i) wild caught versus purse-seined and fattened (farmed), and ii) different ocean environments (Southern Tasmania, Great Australian Bight, Indonesian spawning grounds). In the case of the farmed fish, we examined inter-annual variation in the tissue of the farmed fish and the small pelagic species they were fed in each season (2013-2015). Unfortunately, avoidable delays in the availability of the restaurant tissue samples meant that only the first part of the study could be completed. The full results will be submitted to the Extended Scientific Committee in 2017. The preliminary results of the first component of the study indicate that farmed SBT and their feed (pilchards) have distinct signatures that can be identified (Compound Specific Inorganic Analysis) and there appears to be inter-annual variation for both these sources. The results for the three sources of wild fish are less definitive. It will require larger sample sizes of known source samples to be analysed before more general conclusions can be made. Notwithstanding this and the small samples sizes available at this time (n=5 per source), there does appear to be the potential to estimate source ocean of SBT using these methods. The samples (n=12) sources from restaurants in Beijing and Shanghai clustered across the four sources (farmed and 3 wild) included in the proof of concept study. In summary, these preliminary results indicate that the techniques appear to have the ability to distinguish between SBT from different regions with CSIA, in particular, able to highlight clear differences between farmed and un-farmed product. Larger samples sizes from the wild sources and further examination of the inter-annual differences are required before more definitive assessment can be made on the utility of the methods and the design requirement for specific applications.

2 Introduction

A developing area of pelagic ecosystem research is the use of biochemical analyses, specifically stable isotope analysis (SIA) and fatty acid analysis (FAA), of the flesh of large predators to understand their biogeography, trophic ecology, and movement behaviour. Stable isotope analysis determines the trophic level of an animal whereas fatty acids can identify actual prey species in a predator's flesh. For SIA, the starting level for each food chain is determined by the stable isotope value of primary producers, which vary regionally in the ocean (Hobson 1999). For FAA, different taxonomic groups from different habitats have unique fatty acid signatures which can be traced in the tissue of predators (Parrish et al. 2015a). As predators move through different habitats, they take on the signature of that area but only after their muscle tissue "turns over" to the new system over a period of time (estimated to be weeks to months; Madigan et al. 2012; Ramos & González-Solís 2012).

Biochemical analyses have been used to identify populations of fish, including tunas, with significantly different isotopic and FA signatures from spatially separated areas (Ramos & González-Solís 2012). Recently, clear spatial differences were detected in SI and FA signatures of albacore tuna caught along the east coast of Australia (Parrish et al., 2015b; Pethybridge et al., in Press). Applied to southern bluefin tuna, these techniques could provide a test to distinguish the source of individual fish (e.g. Australian farms versus wild; or Pacific, Indian Southern Ocean).

Given this, we proposed a proof-of-concept study to investigate whether a) Australian farmed SBT can be distinguished from wild caught SBT and b) whether wild caught fish can be assigned to the ocean of capture, based on the stable isotope and fatty acid signatures of their muscle tissue.

Unfortunately, avoidable delays in the availability of the restaurant tissue samples meant that only the first part of the study could be completed. This report summarises the first component of the study conducted on a subset of the samples to address objectives 1-3. The full results will be submitted to the Extended Scientific Committee in 2017.

3 Objectives

1. Analyse muscle tissue from farmed SBT collected over three years (from CSIRO tissue archive) to determine the variability and stability of the biochemical signatures.
2. Compare the FA signatures in muscle of farmed SBT with feed species used in the tuna farming operations to determine if there is a "farm" signature, which can be linked directly to the prey (feed) species.
3. Analyse muscle tissue from wild-caught SBT and compare the biochemical signatures with farmed fish to determine if clear and consistent differences can be detected.
4. If successful in 1-3, analyse the tissue of 100 SBT from Chinese restaurants to determine the most likely source of these fish.

5. Compare SIA signatures among samples of SBT caught in different oceans of the species range.

4 Methods

4.1 Sample collection

Muscle tissue samples were obtained from three supplier sources.

1. CSIRO's archive: samples were obtained from farmed (2013-2015) and wild SBT.
2. Australia's SBT farm sector: pilchards were obtained from a farm in Port Lincoln in the 2015 season. The fish were from the same batch that were fed to SBT towards then end of the farm season.
3. TRAFFIC: tissue sample were obtained from 12 sashimi-grade tuna from restaurants in China (Shanghai and Beijing) determined to be SBT using genetic species identification (CCSBT-ESC/1609/36 & 37).

Table 1 provides the number of tissue samples selected for analysis by method and source, and Table 2 indicates the number of samples analysed thus far. Table 3 indicates the number of samples analysed

Table 1. Planned number of tissue samples selected for analysis by method for the full study.

METHOD	FARMED SBT	WILD SBT	PILCHARD	SBT - CHINA RESTAURANTS	TOTAL
Fatty acid	80 (20x4yrs)	15 (5x3 areas)	20	12	127
Bulk isotope	80 (20x4yrs)	15 (5x3 areas)		12	107
Compound-specific isotope	20 (5x4yrs)	15 (5x3 areas)		12	47
Total					281

Table 2. Number of tissue samples analysed by method thus far (September 2016) and included in this report.

METHOD	FARMED SBT	WILD SBT	PILCHARD	SBT - CHINA RESTAURANTS	TOTAL
Fatty acid	15 (5x3yrs)	15 (5x3 areas)	20	12	62
Bulk isotope	15 (5x3yrs)	15 (5x3 areas)		12	42
Compound-specific isotope	12 (4x3yrs)	12 (4x3 areas)		12	36
Total					140

Table 3. Source location and year of tissue samples analysed to date. Fatty acid and bulk isotope analysis were undertaken on all samples. Compound specific isotope analysis was only undertaken on SBT from China and on 4 of the 5 samples collected from farms and wild SBT for each location/year (see Table 2). GAB = Great Australian Bight.

YEAR OR SEASON	FARMED SBT - GAB	WILD SBT - GAB	WILD SBT - TASMANIA	WILD SBT- IINDONESIA	PILCHARD - GAB	SBT - CHINA RESTAURANTS
2013	5					
2014	5					
2015	5				20	
2016		5	5			12
2014/15				5		

4.2 Biochemical analyses

Samples were analysed using three methods:

Bulk nitrogen isotopes: This technique is used for investigating trophic position and can give some gross indication of the potential for regional differences, due to different nutrient sources, although it is often difficult to separate differences due to confounding of trophic (food-web structure) and/or nutrient effects.

Fatty acid analysis: This provides dietary (and some trophic) information and therefore has the potential to discriminate regional differences based on biogeographical difference food supply.

Compound specific nitrogen isotopes of amino acids: This method is relatively new but provides more specific trophic estimates than bulk isotopes. It also provides isotopic values for the base of the food chain and, as such, can potentially give better spatial resolution.

All analyses were conducted by the CSIRO Biochemistry Team at the Hobart marine laboratories using quality controlled, calibrated equipment.

5 Results

5.1 Fatty acid analysis

The FAA data were analysed by hierarchical cluster analysis (Figure 1).

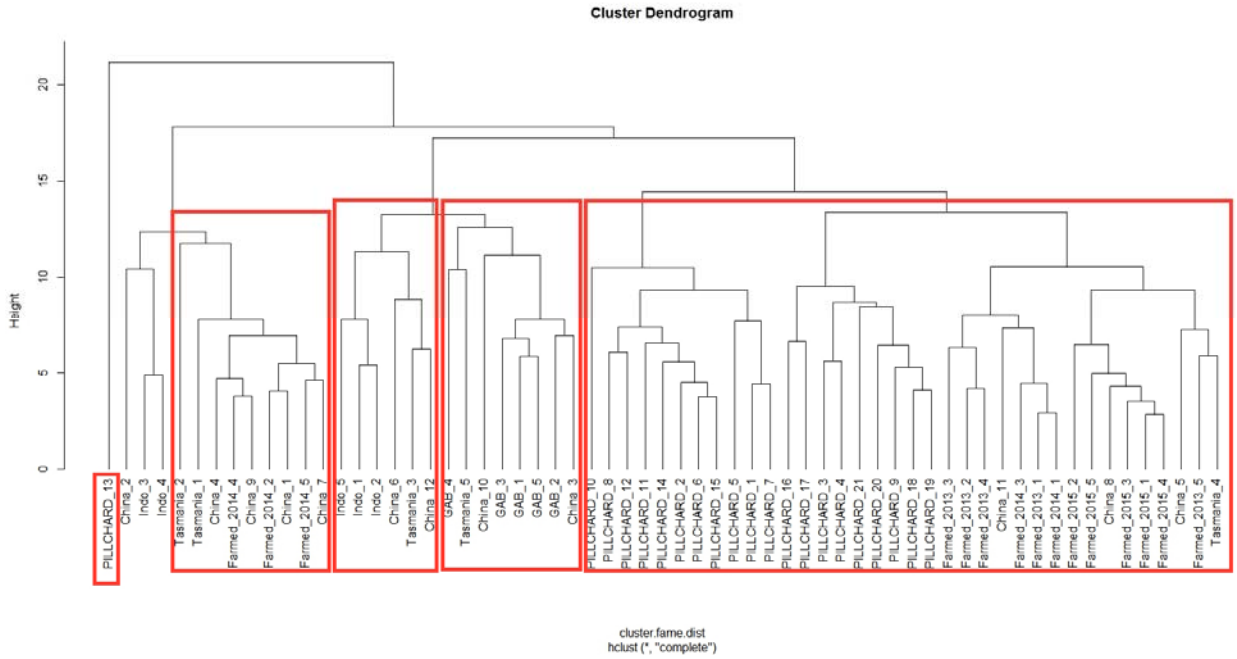

Figure 1: Hierarchical cluster analysis of Fatty Acid Analysis (FAA) for farmed and wild SBT and pilchards used as feed for farmed fish.

The preliminary results for the FAA indicate that the pilchard samples cluster together (with the exception of one individual) and the majority of the farmed SBT also cluster together (with the exception of three 2014 farmed fish associated with the "Tasmanian/China" group. There is no strong evidence of "year" affect in the farmed fish. In the case of the wild source samples, the majority of the wild GAB samples cluster together while other sources are more mixed. The China samples are distributed across sources with no clear pattern.

5.2 Bulk isotope analysis

The results from bulk isotopes for the farmed SBT demonstrate the effect of their narrow sardine diet and the 3-4% difference between the farmed tuna and the sardines is consistent with what would be expected given their respective trophic positions. The low value for farmed fish in 2013 also suggests there may be a difference in diet between 2013 and the other two years of farmed fish (2014-15). There is an apparent difference between the GAB wild sourced samples and the Tasmanian and Indonesian samples, while the China samples cover the broadest range of the SBT samples.

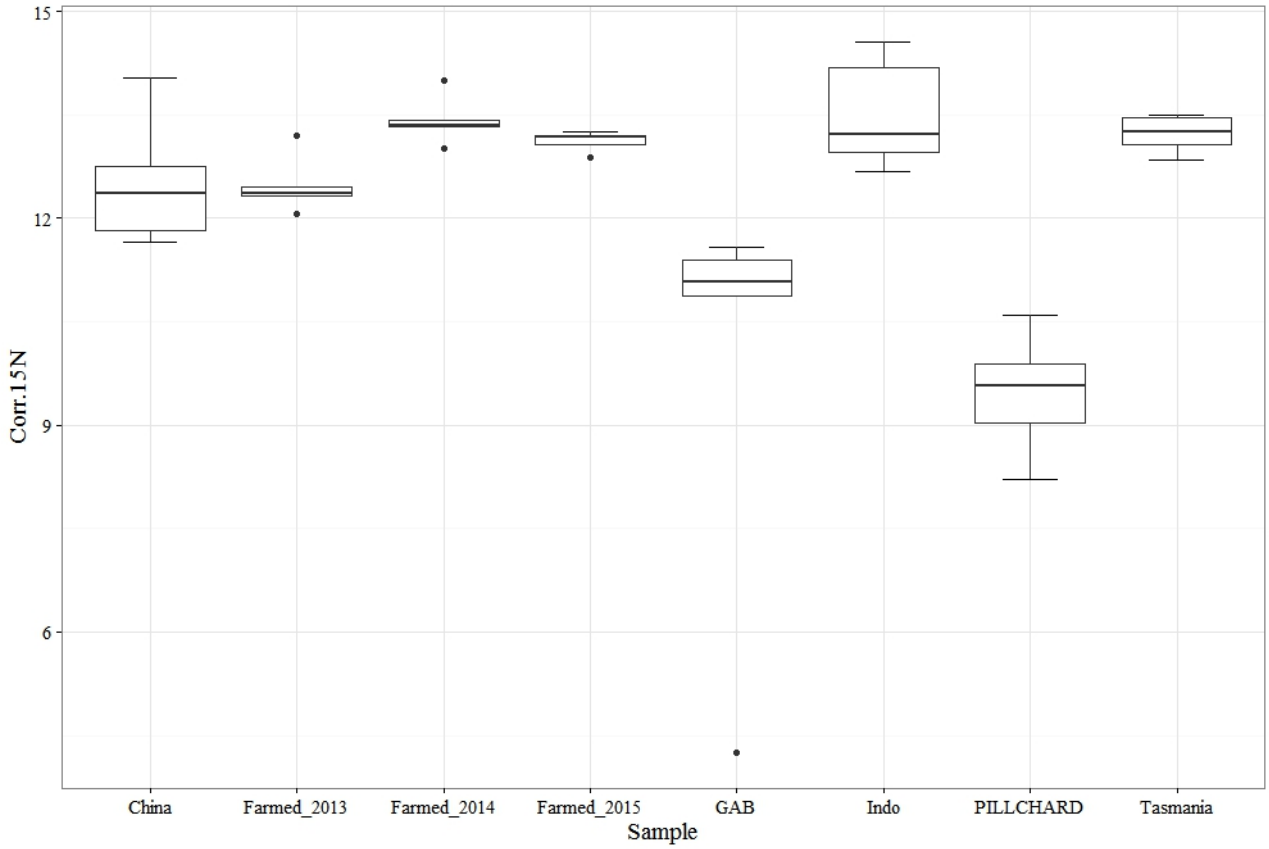

Figure 2: Results of Bulk Isotope Analysis for farmed and wild SBT and pilchards used as feed for farmed fish.

5.3 Compound specific isotope analysis

The Compound Specific Isotope Analysis (CSIA) provides two types of information: i) the trophic position of the animal from which the tissue was sourced in the months before death and ii) the base level of the food chain for the same period.

Trophic Position (TP)

The calculated trophic position shown in Figure 3 suggests there are slight differences between the wild samples with Indonesia being higher than the GAB, which in turn is higher than the Tasmanian samples. The farmed samples had a range of trophic positions with generally higher variation in 2013 and 2015 and markedly lower variation in 2014. The Chinese samples had a calculated TP close to the farmed fish samples from 2014 and GAB samples.

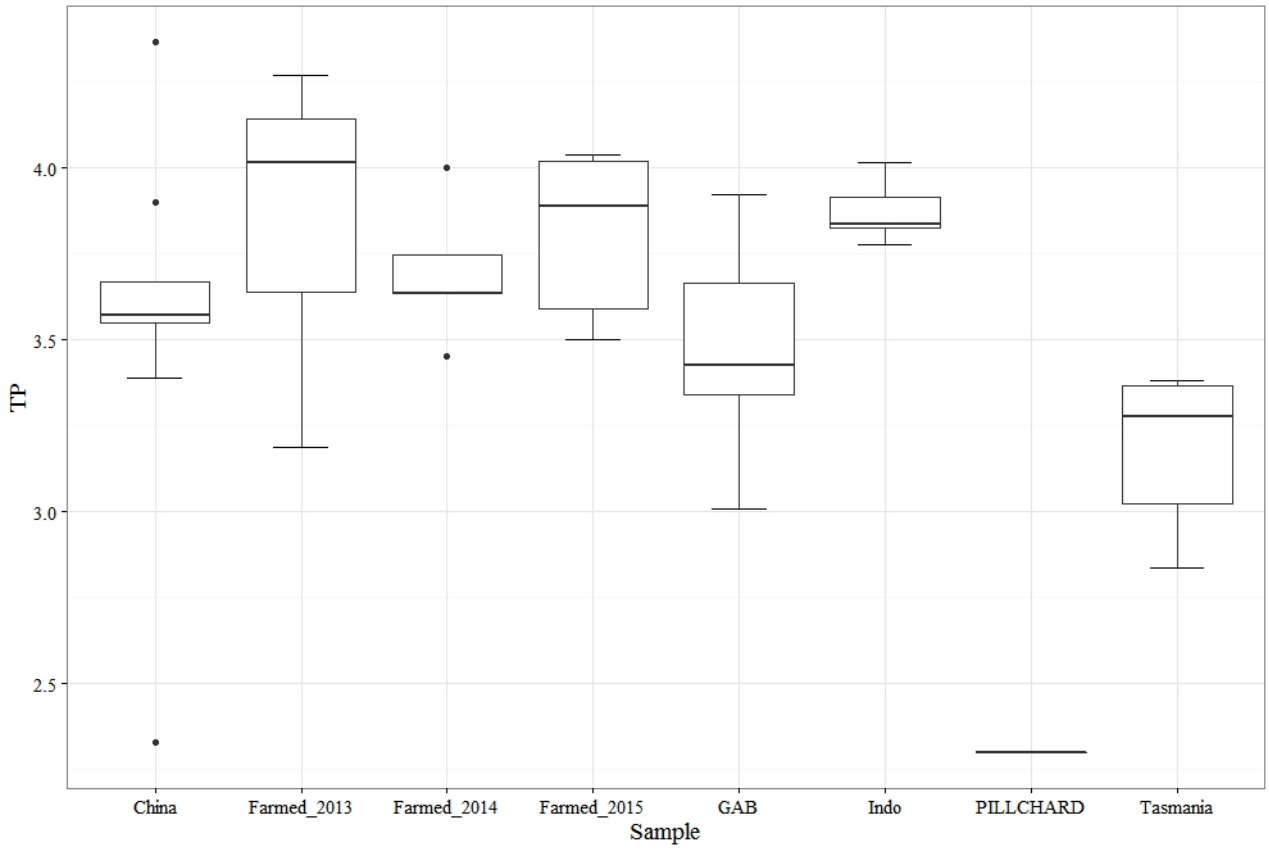

Figure 3: Estimated Trophic Position (TP) from Compound Specific Isotope Analysis (CSIA) for farmed and wild SBT. Note, there were no pilchard samples processed for the CSIA in this component of the project.

Base of food chain (Phe)

The source isotopic information (base of the food chain) is obtained using values for phenylalanine (Phe), which is an essential amino acid and undergoes little trophic fractionation. The initial results in figure 4 suggest there are difference between the wild caught samples with Tasmania being higher than Indonesia, which in turn is higher than the GAB This result is slightly counter intuitive in that you might expect GAB to be higher than Indonesia (values closer to zero are generally associated with more tropical regions) but there is no guarantee that SBT from Indonesia were harvested in that region (given evidence of recent shifts in fleet activities to south of the spawning ground) or were there long enough to assimilate the isotopic signal ($t_{1/2}$ ~ 1-3 months). Anecdotal information based on capture of post-spawn fish (lean and in poor condition) suggests that SBT do not feed, or feed very little, when on the spawning ground. Again, there appears to be differences between the 2014 and 2015 farmed SBT (similar baseline values and close to wild GAB) but 2013 fish are very different. This may reflect a different bait source from a different region being used for the 2013 fish. Again the Chinese samples are quite variable with a mean Phe value close to that of Indonesian sourced samples.

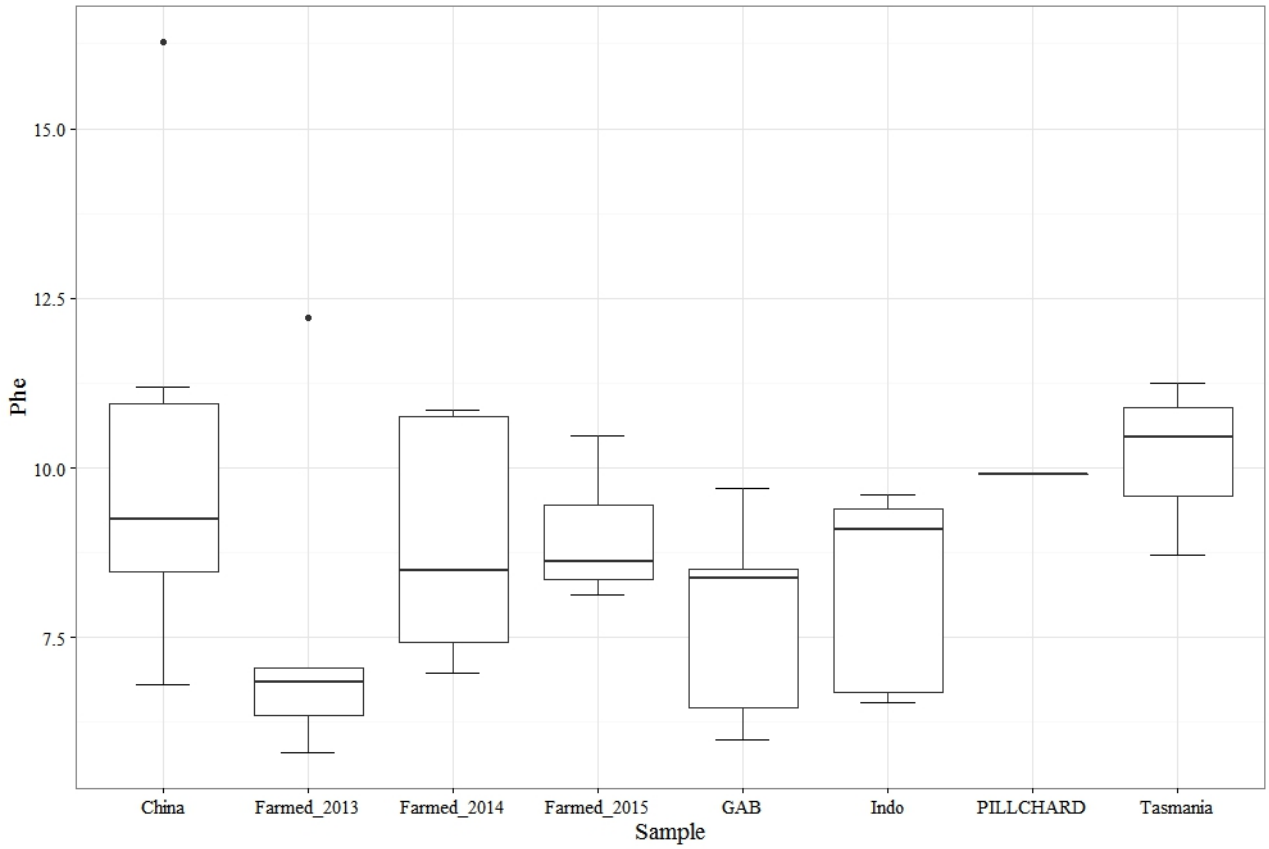

Figure 4: Estimated Phenylalanine (Phe) amino acid from Compound Specific Isotope Analysis (CSIA) for farmed and wild SBT. Note, there were no pilchard samples processed for the CSIA in this component of the project.

All data cluster analysis

As an preliminary attempt to synthesise the the information from the four biochemical techniques, the data from each technique were combined and analysed in a hierarchical a cluster analysis. There are several limiting factors to this analysis (for example the data for the Pilchards is incomplete and the sample sizes for the wild source SBT are small). However, it does provide a useful summary (Figure X5):

- The samples are clustered into 8 major groups separated at the highest level based on the two groups of pilchards and then two groups of largely farmed fish (with China samples).
- Of the farmed fish 2015 (5 of 5 in one cluster) and 2013 (4 of 5 in one cluster) are most distinct, with 2014 being dispersed between the 2013 group and a mixed group of wild sourced samples.
- The wild GAB samples cluster together while Indonesian and Tasmanian fish are more spread possibly reflecting greater variation due to higher rate of migration and or seasonal variation in the food chain in those regions.
- China sourced samples are distributed across the other sample sources.

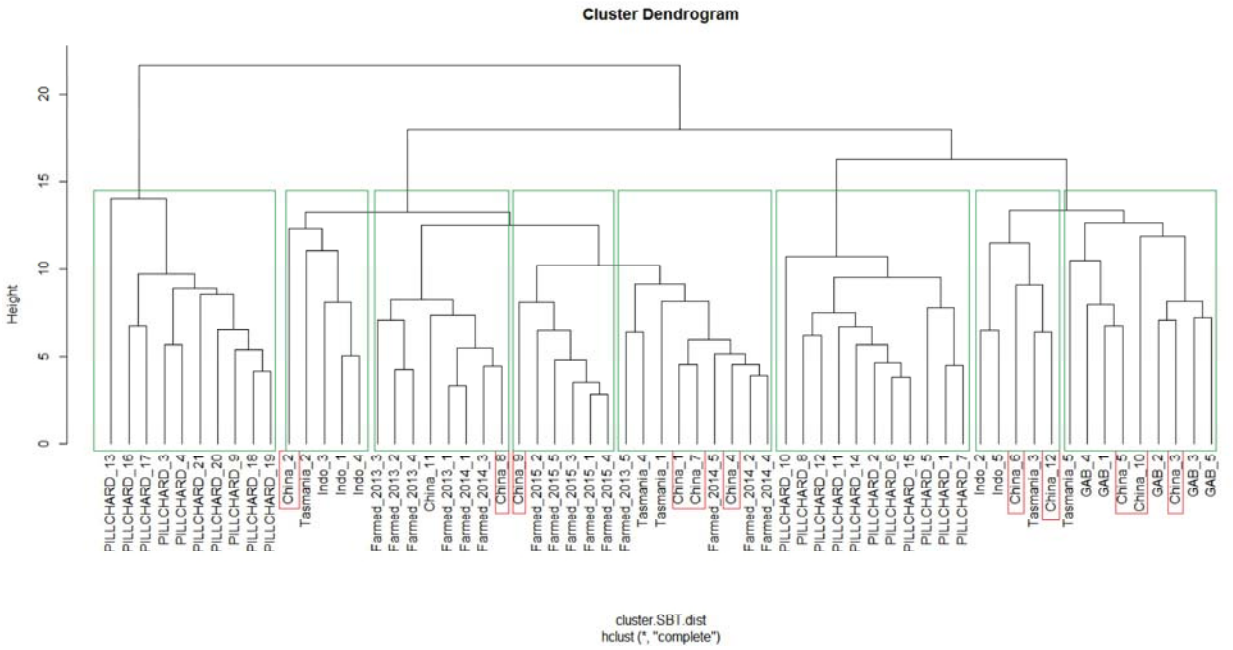

Figure 5: Combined analysis of FAA, BIA and CSIA for farmed and wild SBT and the pilchards used as feed for the farmed SBT. Note, there were no pilchard samples processed for the CSIA in this component of the project.

6 Summary and Conclusions

The aim of this Proof of Concept study is to assess the potential of biochemical techniques to determine the source of tissue samples of SBT, in particular, which ocean/region they were most likely harvested from and whether they are from a wild or farmed SBT. The preliminary results of the first component of the study indicate that farmed SBT and their feed (pilchards) have distinct signatures that can be identified (particular using the Compound Specific Isotope Analysis) and there appears to be inter-annual variation for both these sources. The results for the three sources of wild fish are less definitive. Notwithstanding this and the small samples sizes available at this time (n=5 per source), there does appear to be the potential to estimate source ocean of SBT using these methods. It will require larger sample sizes from known source samples to be analysed before more general conclusions can be made. The samples (n=12) sourced from restaurants in Beijing and Shanghai clustered across the four sources (farmed and 3 wild) included in the proof of concept study thus far. While larger sample sizes are required, the distinct signal for farmed fish suggests there is potential to independently estimate the proportion of farmed product (combined with individual ID from genetic methods to eliminate double counting of individuals) in market surveys using these methods. Larger sample sizes (as per Table 1) will be analysed and presented to the ESC in 2017.

In summary, these preliminary results indicate that the techniques appear to have the ability to distinguish between SBT from different regions with CSIA, in particular, able to highlight clear differences between farmed and un-farmed product. Larger samples sizes from the wild sources and further examination of the inter-annual differences in both farmed and wild samples are required before more definitive assessment can be made on the utility of the methods and the design requirement for specific applications.

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