Insight

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Literature review: isotope ratios in seafood

Seafish requested Leatherhead to provide information on the current state of knowledge on use of isotope ratios for demonstrating country of origin for prawns / seafood. This included a literature review to establish known information and the potential use, application and limitations of this method. This review relates to stable isotopes, distinct from radioactive isotopes which are unstable.

Stable isotope ratios

Stable isotopes are different forms of the same element which differ in the number of neutrons in the nucleus of the atom. The different numbers of neutrons in the nucleus give the atoms different weights, and this affects the way that the isotopes are distributed in the environment or in plants and animals. The atoms are incorporated into chemical compounds and as these undergo chemical reactions in plants or animals the ratio of the heavier and lighter elements changes depending on those processes. The ratio is also changed as a result of physical processes such as evaporation and condensation of water.

For example, oxygen has the stable isotopes ^{18}O and ^{16}O. The O in water may therefore be either ^{18}O or ^{16}O. Hydrogen also has stable isotopes, ^{1}H and ^{2}H, so water can also contain either of these. There are a number of permutations of the different H and O isotopes in water, leading to water molecules with a range of weights. As water evaporates from the oceans, more of the lighter water molecules evaporate than the heavier ones, resulting in a change in the ratio in the clouds compared to the original ratio in the sea. As the clouds move inland there are further cycles of evaporation, condensation and precipitation and at each stage the ratio changes further, so that the groundwater shows a gradient of isotope ratios from the coast to inland. The isotope ratio in the groundwater has been shown to be correlated to animal products and tissues of animals drinking the water.

Stable isotope ratios in food authenticity

Stable isotope ratios may be determined by Isotope Ratio Mass Spectrometry (IRMS) and are a measure of the relative levels of different isotopes of a particular atom. Depending on the atom selected, the isotope ratios in a material may reflect differences in the isotope ratio in the soil or water where the material originated, or, in the case of animals, may reflect the isotopic makeup of the diet or feed. Different isotope ratios are affected by different factors, and these can be exploited to determine food authenticity related to geographical origin or feed type. This is summarised in Table 1.

Stable isotope ratios have been evaluated for the determination of the geographical origin of a wide range of products including meat, dairy products, coffee, wine and cereal crops.

Table 1: Summary of factors affecting isotope ratio

Isotope ratio	Fractionation due to	Uses
$^{2}H/^{1}H$	Evaporation, condensation, precipitation	Geographical origin
$^{13}C/^{12}C$	Different plant metabolism pathways C3 and C4 plants	Diet (which can be used to indicate geographical origin, also farming method)
$^{15}N/^{14}N$	Changes through the food chain agricultural practice	Diet (which can be used to indicate geographical origin)
$^{18}O/^{16}O$	Evaporation, condensation, precipitation	Geographical origin
$^{34}S/^{32}S$	Bacterial	Geographical (marine)
$^{87}Sr/^{32}Sr$	Age of the rock and Rb/Sr ratio	Underlying geology, geographical

Table based on Kelly *et al.,* 2005

Stable isotope ratios in seafood

Several studies have been carried out looking at the effect of changing diet on the stable isotope ratios in fish. $\delta^{15}N$ was shown to change in the plasma, muscle, heart and liver of cod fed a specific diet, although the rates of change and time taken to achieve a steady state were different in the different tissues. All had reached a steady state after 40 days (Olsen *et al*., 2015).

As discussed above, a number of different tissues may be sampled and the isotope ratios may be different between tissues from the same fish. Fish scales have been used in ecology studies, particularly where rare species are being sampled, in order to avoid sacrificing the fish. Dixon *et al.* (2015) studied different areas of fish scales (adult Atlantic salmon), corresponding to growth at different times and therefore in different environments. Differences were seen in $\delta^{13}C$ values in different growth zones, whilst $\delta^{15}N$ showed no significant differences. Whilst their study was not directed towards authenticity or determination of geographical origin, the authors discuss that the 2nd summer scale material could give an idea of how the fish has been feeding during its most recent summer at sea.

Many of the studies reported have found that selection of the right tissue can be key in using the data to discriminate between fish from different origins. A further confounding factor which may need to be considered, depending on which isotopes and which tissues are being measured, is the post-harvest use of water for freezing or icing, which may have had a different isotope ratio from the original sample (Carter *et al*., 2015).

Use of stable isotopes in authenticity

There have been many reports of the use of stable isotope ratios to authenticate products such as wine, milk, meat, honey (e.g. Drivelos and Georgiou, 2012; Primrose *et al*., 2010) but these are all land-based or originating on land. There are fewer reported studies on the use of stable isotope ratios to demonstrate the authenticity of fish or seafood, although isotope ratios in seafood have been used for other purposes such as to monitor the migratory patterns of salmon or changes in diet of specific groups. Amongst the research which has investigated the use of stable isotopes in seafood authenticity, much of the work has concentrated on being able to distinguish farmed from wild fish, or organic from conventionally farmed

fish. Some work has also been carried out to identify the most reliable tissues for studying isotope ratios in fish, although as this is often directed at minimising the harm to endangered species.

One of the few studies which has looked at isotope ratios specifically to infer geographical origin was by Kim *et al*., (2015) and looked at commercial fish of three species (Mackerel, Yellow Croaker and Pollock) locally sourced from Korea and also imported from a wide geographical area. Stable isotope ratios of carbon and nitrogen were measured and the following observations were made:

- $\delta^{13}C$ values were distinct for each fish species
- $\delta^{13}C$ values from fish within the four sites studies in Korea were very similar
- Mackerel and Yellow Croaker from various sites in Korea showed significant differences in isotopic values ($\delta^{13}C$ and $\delta^{15}N$) from internationally sourced fish of the same species
- Pollock from Japan and Russia varied from each other
- $\delta^{15}N$ values for mackerel and yellow croaker showed slight variations related to geographic origin, but $\delta^{15}N$ values for pollock did not

The authors discuss the sources of the variation of the isotope ratio:

- Different hydrogeomorphic characteristics between the basins are known to affect the C signatures of fish
- Precipitation and temperature variations can affect the stable isotope ratio of organisms
- In the case of C, isotope ratios reflect the isotope ratio of the diet

The authors compared the $\delta^{13}C$ of the fish in their study to reported values of the $\delta^{13}C$ of the particulate organic matter found in the regions where the fish originated, and suggest a good correlation. They suggest that the different $\delta^{13}C$ values are due to the different seawater temperatures, as others have reported a positive correlation between decreasing seawater temperature and lighter $\delta^{13}C$ values of particulate organic matter. The authors also suggest that the relatively small variations in $\delta^{13}C$ within mackerel from four sites in Korea could be due to seasonal and dietary differences.

Overall, Kim *et al*. (2015) concluded that '$\delta^{13}C$ could be used as a single discriminatory variable to distinguish between various geographic origins' and that it was a better tool than $\delta^{15}N$. This is in contrast to work published by Chaguri *et al*. (2015) where the authors concluded that $\delta^{15}N$ was a better indicator. That study also looked at Croaker, this time from Brazil. That study found statistical differences in carbon and nitrogen isotopic ratios between geographic origins (two different locations in Brazil), and seasonal differences in $\delta^{15}N$ in one location; these differences were also put down to differences in diet, with the seasonal variability thought to be due to seasonal changes in feed preference and availability. It was also discussed that isotope ratios in fish are affected by periods of scarcity of food, when the fish use reserves present in the body.

One of the key considerations in using stable isotope ratio, or any other marker for authenticity, is in having sufficient data available covering all variables known to affect the marker. Most of the published studies relating to fish authenticity using stable isotopes have analysed relatively few samples. A study by Thomas *et al*., (2008) did cover several variables (171 samples covering 32 origins within Europe, North America and Tasmania; sampling over all seasons in 2 consecutive years; fish raised by different practices) but this study was aimed at discriminating between wild and farmed fish and not geographic origin. Even then, the

authors concluded that to increase the certainty of identification of fish from an unknown source, another non-isotopic measurement (linoleic acid) should be made alongside the isotope measurement.

The selection of the most appropriate chemometric tools for analysing the data is also critical. Ortea and Gallardo (2015) investigated the use of stable isotope ratio and multi-element analysis to determine production method, origin and species in shrimps. For the stable isotope ratio data they measured $\delta^{13}C$ and $\delta^{15}N$ and noted that of these the $\delta^{13}C$ could be used to discriminate samples originating in aquaculture facilities from wild samples, using the simplest scatter plot analysis of the data. As for the other studies outlined above, differences between values in samples from different farms were put down to differences in the diet used for feeding. More robust discrimination was obtained using multivariate methods to analyse the data; several different techniques were used (PCA, cluster analysis, k-means classification and discriminant analysis). The best performance for discriminating wild from farmed samples was using discriminant analysis on stable isotope ratio values, or on a combination of stable isotope data and element analysis (five elements – As, Cd, Pb, P, S). The authors noted, however, that whilst on an initial sample set they could distinguish wild from farmed samples using only stable isotope data, addition of further samples into the sample set reduced the accuracy of the classification, demonstrating the caution required when drawing conclusions from limited data sets.

Classification of origin using stable isotope ratio in the Ortea and Gallardo study was less accurate than classification of production method; the success rate was higher using elemental analysis data, or a combination of the two. Discriminant analysis was again the method of choice for analysing the data set. Similarly, classification of species was most accurate when the combined stable isotope and elemental data was used, but the authors commented that DNA and proteomics methods might be better for species characterisation, judging their results against other published data. However it was also noted that a larger data set with samples from more diverse geographic origin would be required for a more robust evaluation of the stable isotope / elemental analysis approach for determining species.

As mentioned earlier, few studies on isotope ratios in fish have been published specifically concerned with authenticity issues, but isotope ratios in the study of marine ecology have been used for some time, and the studies can provide clues as to the possibilities or drawbacks of using this technique. A study published by MacKenzie *et al.* (2011) concluded that 'carbon isotope ratios can be used to identify the location of open ocean feeding grounds for any pelagic animals for which tissue archives and matching records of sea temperature are available'. Another study on yellowfin tuna and swordfish (Menard *et al.*, 2007) found that latitude and body length both affected $\delta^{15}N$, the former due to differences at the base of the food web, and the latter due to larger fish being able to access a wider variety of prey in deeper waters. This draws attention to the fact that whilst isotope ratios can be determined accurately using appropriate equipment, the values then require comparison to known values or ranges to determine authenticity. As discussed, many factors such as diet, sea surface temperature and food availability all affect the isotope ratio and therefore databases need to be produced and continually updated taking account of all these factors.

Discussion

Whilst relatively few studies have been published on the use of stable isotope ratios for authentication of fish and seafood, there appears to be increasing interest in the possibility of using this method. Data on use of stable isotope ratios to monitor changes in diet or migration patterns suggests that the technique might have some potential. However the relatively small studies reported so far would need to be repeated to take into account more variables in order to be sure that the suggested correlations still held true. In addition, some studies have shown that isotope ratios need to be used alongside other measures (e.g. elemental analysis) in order to increase the certainty of classification.

If it could be shown, after collection of more data, that correlations could be made between isotope ratio and geographic origin of fish, then the challenge would be to build and maintain databases of values from authentic samples, covering the required range of variables known to affect the ratio

Factsheet reproduced from
Literature review on isotope ratios in seafood,
Leatherhead Food Research,
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Summary of data

Species	Origins	Method/isotopes	Details	Reference
Geographical origin				
Prawns: *P. monodon, Litopenaeus vannamei, Fenneropenaeus indicus, Fenneropenaeus merguiensis, Farfantepenaeus notialis, Pleoticus muelleri, Pandalus borealis*	FAO71, Argentina, North Atlantic, Farms A, B, C, Mozambique, Nigeria, Senegal	IRMS, $\delta^{13}C$, $\delta^{15}N$	Promising data for discrimination of production method and geographical origin. Less accuracy for species determination. Elemental analysis adds to accuracy of methodology.	Ortea and Gallardo, 2015
Croaker: *Micorpogonias furnieri*	Brazil (two sites, North East and South East)	IRMS, $\delta^{13}C$, $\delta^{15}N$	Carbon and nitrogen isotopic ratios statistically different between geographical origins. Some seasonal difference in $\delta^{15}N$ observed	Chaguri *et al*, 2015
Mackerel, yellow croaker and Pollock: *Scomber japonicas, Larimichthys polyactis, Larimichthys crocea, Theragra chalcogramma*	Korea, Australia, China, Norway, Oman, Italy, Japan	Elemental analyser with stable isotope mass spectrometer, $\delta^{13}C$, $\delta^{15}N$	$\delta^{13}C$ could be used to distinguish between geographic origins. $\delta^{15}N$ values from mackerel and yellow croaker but not pollock showed some variation based on geographic origin.	Kim *et al*., 2015
Farming method				
Salmonids (Salmon and trout)	German market; samples originating from organic and conventional aquaculture and wild stocks	$\delta^{13}C$ of lipid fraction, $\delta^{15}N$ of defatted dry matter	Combining $\delta^{13}C$ and $\delta^{15}N$ data allowed differentiation of wild, organic and conventional-farmed salmon, though authors highlight some doubts over whether this would be true for different salmon species than those studied. Trout results showed that isotope ratios reflected the feed, and samples from the same farms obtained at different dates could be different due to different feed, meaning the limits on $\delta^{15}N$ and $\delta^{13}C$ would need to be checked and revised over time.	Molkentin, J *et al*, 2015
Atlantic Cod: *Gadhus morhua*	N/A	IRMS, $\delta^{13}C$, $\delta^{15}N$	Use of stable isotopes to trace diet alterations. Analysis of fatty acids and stable isotopes; fatty acid data concluded to be better tracer than stable isotopes in this study.	Olsen *et al*., 2015

Species	Origins	Method / isotopes	Details	Reference
Atlantic salmon (*Salmo salar* L.)	UK/European fish, various examples	$\delta^{13}C$, $\delta^{15}N$	Discusses that $\delta^{13}C$ is strongly dependent on fractionation of carbon isotopes during photosynthesis by the phytoplankton community. This is affected by dissolved carbonate concentrations, cell growth rate, degree of light and nutrient limitation. Cell growth rates and dissolved carbonate concentrations are related to temperature, and vary with distance and time across ocean basins, hence the carbon isotope composition of fish varies according to feeding location.	MacKenzie *et al.*,2011
Prawns (range)	Australian and 'imported'	^{2}H, ^{18}O, ^{13}C, ^{15}N	Isotope composition of recovered water could not be used to distinguish between prawns of different origin. Meat and chitin were both studied. The authors concluded that $\delta^{2}H$ and $\delta^{13}C$ data for the meat component could be used to distinguish Australian and imported species.	Carter *et al.*, 2015
Salmon fry	Hatchery and wild	Otolith $\delta^{13}C$ and $\delta^{18}O$	Discriminant analysis of data for both isotopes demonstrated highly significant differences between hatchery and wild fry. The authors suggest this might be useful in discriminating the origin of returning adult salmon.	Tomida et al,2014
Atlantic Salmon, *Salmo salar*	Wild and aquaculture origin, Newfoundland	$\delta^{13}C$, $\delta 1^{5}N$	$\delta^{13}C$ and $\delta^{15}N$ in combination used to distinguish between wild and aquaculture origin	Dempson and Power, 2004
Pacific white shrimp (*Litopenaeus vannamei*)	Mexico and Ecuador	$\delta^{13}C$, $\delta^{15}N$	Use of $\delta^{13}C$ and $\delta^{15}N$ to distinguish between wild-caught shrimps and aquaculture shrimps.	Gamboa-Delgado et al., 2014
Cod (Murray)	Australia, different farms with different systems	$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$	$\delta^{13}C$ and $\delta^{15}N$ could be used to link fish to a specific commercial diet; $\delta^{18}O$ could be used to link fish to a specific water source.	Turchini et al,2009
Atlantic salmon (*Salmo salar*)	Europe, North America, Tasmania; wild and farmed	$\delta^{18}O$, $\delta^{15}N$	$\delta^{15}N$ measured on choline, $\delta^{18}O$ measured on total oil could be used to discriminate between wild and farmed fish. Greater certainty in identification of unknowns would be achieved by also measuring linoleic acid.	Thomas et al, 2008

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