Hagfish feeding habits along a depth gradient inferred from stable isotopes

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Supplement 1. Consideration of the mathematical equations used for estimating lipid muscle content from $\Delta\delta^{13}C$ values or C:N ratios for the 3 hagfish species, *Neomyxine biniplicata*, *Eptatretus cirrhatus*, and *Eptatretus* sp. 1

Lipids in muscles are used as energy storage and their state can give an indication of whether specimens have been feeding recently, or have been in a period of food deprivation. Within any species, specimens with high lipid levels have fed recently (within the last month), whereas specimens with low lipid levels have been using their lipid as an energy source (Tocher 2003).

Lipids will have more negative $\delta^{13}C$ values relative to other compounds (DeNiro & Epstein 1977). Consequently, muscle tissues with higher lipid content will have greater negative $\delta^{13}C$ values.

Several equations are available in the literature to calculate the percentage of lipid in different tissues from $\Delta\delta^{13}C$ values or bulk C:N ratios (McConnaughey & McRoy 1979, Kiljunen et al. 2006, Sweeting et al. 2006, Post et al. 2007, Logan et al. 2008). However, it has been suggested that a predictable relationship between bulk C:N ratios with lipid content is highly species- or even population-specific (Fagan et al. 2011). We estimated 2 parameters from our dataset: D (the lipid depletion factor, the isotopic difference between protein and lipids) and I (a constant), following McConnaughey & McRoy 1979), as follows (Eq. 1–3):

$$\delta^{13}C' = \delta^{13}C + D \times \left(I + \frac{3.90}{1 + 287/L} \right) \tag{1}$$

$$L = \frac{93}{1 + (0.246 \times (C:N) - 0.775)^{-1}} \tag{2}$$

$$\delta^{13}C' - \delta^{13}C = D \times \left(I + \frac{362.7}{380 + 287 \times (0.246 \times C:N - 0.775)^{-1}} \right) \tag{3}$$

where D and I were estimated iteratively using non-linear least-squares regression. Using all specimens from the 3 hagfish species, we obtained $D = 7.692$ and $I = -0.093$. Inserting these values into Eq. (1), we obtained proportional lipid content (L, dry weight) in hagfish varying from 38 to 73% for *Eptatretus cirrhatus*, 44 to 91% for *Eptatretus* sp. 1 and 22 to 33% for *Neomyxine biniplicata*. From Eq. (2), we obtained proportional lipid content (L) in hagfish varying from 39 to 78% for *E. cirrhatus*, 43 to 78% for *Eptatretus* sp. 1 and 19 to 49% for *N. biniplicata*.

Using another approach, the arithmetic mass-balance correction method (Alexander et al. 1996, Fry et al. 2003, Hoffman & Sutton 2010), we obtained an average lipid depletion factor of 7.19 ‰ and lipid content of 37 to 85%, for *Eptatretus cirrhatus*, 42 to 85% for *Eptatretus* sp. 1 and 14 to 51% for *Neomyxine biniplicata*. Although the trend is similar, these 2 equations produce quite different results and it is difficult to assess which one would be best to use. Both of these approaches also give very high values for lipid content in hagfish muscles overall which, although not impossible, warrants some caution. By way of comparison, the lipid content of muscle in other hagfishes that have been measured (*E. stoutii*, *Myxine glutinosa* and *E. deani*) has been estimated to be much lower (8, 23 and 34%, respectively) (Kelleher et al. 2001, Drazen et al. 2011). Hagfish do, however, tend to have higher lipid content in their muscles when compared to other deep-sea species. For example, Hoffman & Sutton (2010) investigated lipid correction factors for deep-sea fishes and the highest C:N ratios of untreated muscle was 8.5 for a mesopelagic species (*Lampadena speculigera*), 8.4 for a bathypelagic species (*Sigmops bathyphilum*) and 7.3 for a bathydemersal species (*Hoplostethus atlanticus*). In shallower waters, the highest C:N ratio detected in a series of fish species examined was on average 9.4 (± 9.3 SD) for the yellow-phase freshwater eel *Anguilla anguilla*, while *Salmo salar* had an average C:N ratio of 6.8 ± 2.6 (Kiljunen et al. 2006).

Due to the variation in the results obtained using different methodologies of estimation, and also the discrepancies obtained using these approaches compared with results obtained from previous studies, we did not use estimates of lipid content but instead used $\Delta\delta^{13}C$ values directly as a proxy for the lipid content of hagfish muscles.

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Supplement 2. Rationale for pooling of the hagfish specimens caught at the 2 locations (White Island and Kaikoura) into a single dataset

Our primary objective was to test for potential differences in isotopic signature between hagfish species and also along the depth gradient. The rationale behind pooling data obtained from the 2 locations (White Island and Kaikoura) was to assemble a more robust data set to test these hypotheses. However, it has been shown that isotopic values can change both spatially and temporally, even at relatively small scales (see Deudero et al. 2004). It was therefore necessary to test for the possible effect of location on the isotopic signatures before pooling the data. It is also relevant to note here that the 2 sampling locations were visited in March 2009 and November 2011, respectively. Consequently, any location effect detected could originate from either a temporal effect, a spatial effect, or some combination of the 2 confounding factors.

Although equal numbers of baited pots were deployed at each depth and at each location, not all of these caught hagfish specimens. Thus, the dataset is highly unbalanced (Table 1 in the main article). The effect of location could not be tested for *Neomyxine biniplicata* (having no specimens from White Island) nor for *Eptatretus cirrhatus*, the vast majority of specimens having been caught at White Island. Thus, it was only feasible to test for location effects for *Eptatretus* sp. 1
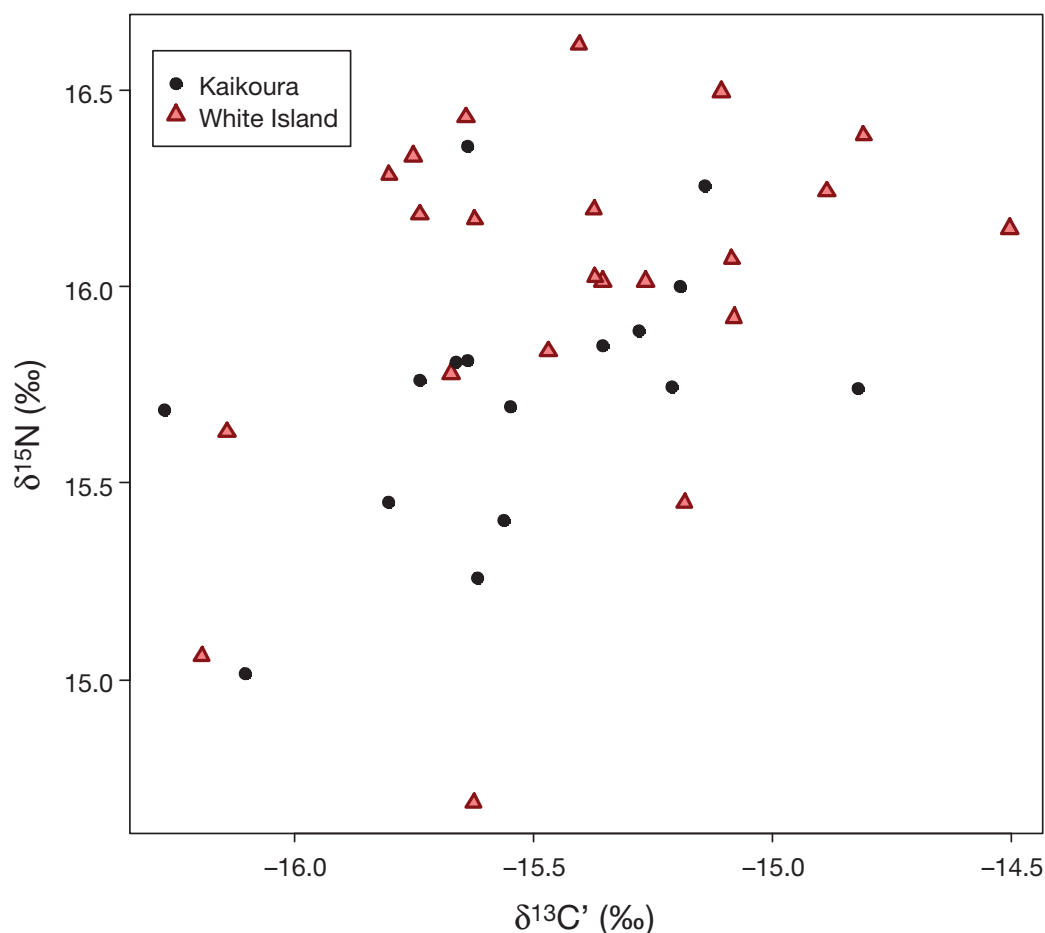
Eptatretus sp. 1

Fig. S1. Origin of the *Eptatretus* sp. 1 stable carbon (δ^{13}C') and nitrogen (δ^{15}N) isotope values

A bivariate scatterplot of δ^{13}C' and δ^{15}N values for this species indicated no obvious effect of location on the stable isotope signatures (Fig. 1). Furthermore, there was no statistically significant effect of location on either δ^{13}C' (ANOVA, $F_{1,37} = 0.911$, p > 0.34) or δ^{15}N values (ANOVA, $F_{1,37} = 3.803$, p > 0.059) for *Eptatretus* sp. 1.

In conclusion, differences found between *Neomyxine biniplicata* and *Eptatretus cirrhatus* could be location-driven, as these 2 different species were indeed caught primarily at 2 different locations. However, there is no evidence that location effects are driving isotopic signature differences for *Eptatretus* sp. 1.

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