Hydrobiologia (2010) 654:1–5
DOI 10.1007/s10750-010-0361-1

OPINION PAPER

The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks

Comment on: stable isotope dynamics in elasmobranch fishes

Nigel E. Hussey · M. Aaron MacNeil · Aaron T. Fisk

Received: 30 March 2010 / Revised: 7 June 2010 / Accepted: 19 June 2010 / Published online: 19 July 2010

Abstract Stable isotopes of nitrogen and carbon (δ^{15}N and δ^{13}C) provide an important tool to examine diet, trophic position and movement/migration of both aquatic and terrestrial animals. Over the past 10 years, there have been repeated calls to tighten up basic assumptions when applying stable isotopes, one of the most important being the application of accurate, species-specific diet-tissue discrimination factors (DTDFs). Taxa- or species-specific DTDFs are required for (i) predicting dietary sources to a consumer using stable isotope mixing models and (ii) for estimating trophic position relative to primary consumers or known base species. Logan & Lutcavage (2010) recently presented data on stable isotope dynamics in elasmobranch fishes and concluded that DTDFs for teleost fish were suitable for elasmobranch fish, endorsing the generally applied value of 3.4‰. When considering (i) a recent study which found that DTDFs were lower for large sharks than teleost fish (Hussey et al., 2010) and (ii) that the Logan and Lutcavage study did not experimentally address the issue of DTDFs, we would argue that this conclusion is misleading. We demonstrate this point by estimating the proportion of prey items of a captive shark with a known diet history by modelling the δ^{15}N values of the shark and its prey. The often repeated implication of inaccurate DTDFs is clear, with model results highly variable depending on the selected DTDF. In addition, model results for the standard teleost DTDF of 3.4‰ provided erroneous estimates of prey consumption. The suggestion that DTDFs for teleost fish are suitable for elasmobranchs may mislead investigators to choose DTDFs which are likely not applicable to their study species. Caution is therefore warranted in advocating this approach. Continued experimental work to examine stable isotopes in sharks is required and recommendations are made.

Keywords Stable isotopes · Elasmobranch · Shark · Diet-tissue discrimination factor · Fractionation · Mixing model · Trophic position

Handling editor: I. Nagelkerken

N. E. Hussey (✉)
School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey LL59 5AB, Wales, UK
e-mail: Nigel.hussey@bangor.ac.uk

Present Address:
N. E. Hussey
Great Lakes Institute of Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada

M. A. MacNeil
Australian Institute of Marine Science, PMB 3 Townsville MC, Townsville, QLD 4810, Australia

A. T. Fisk
Great Lakes Institute of Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada

Recently, Logan & Lutcavage (2010) undertook a controlled experiment to examine nitrogen and carbon stable isotope (δ^{15}N and δ^{13}C) tissue turnover rates for liver, whole blood and white muscle in juvenile sandbar sharks (*Carcharhinus plumbeus*). In addition, the authors examined the possible impact of urea on δ^{15}N values in coastal skates (*Leucoraja* spp.) and the spiny dogfish (*Squalus acanthias*). The latter experiment was based on previous work by Fisk et al. (2002) on greenland sharks (*Somniosius microcephalus*) that found discrepancies between contaminants and δ^{15}N for estimating trophic position. Fisk et al. (2002) suggested that δ^{15}N values may be low as a result of urea retention in elasmobranchs.

The results of the two main questions posed by Logan and Lutcavage (2010) present important data for interpreting stable isotope data in sharks;

(1) Tissue turnover rates varied with tissue type and were dependent on tissue-specific metabolic rate. The predicted turnover rates were similar to those previously reported for a freshwater stingray, *Potamotrygon motoro* (MacNeil et al., 2006) providing further confidence in their results.
(2) Urea had minimal effect on δ^{15}N values of blood and white muscle tissue. The authors therefore concluded that urea should not complicate the interpretation of δ^{15}N values for understanding diet and trophic position of elasmobranchs.

Logan & Lutcavage (2010) however, clearly state in their abstract, discussion and conclusion that available diet-tissue discrimination factors (DTDFs) for teleost fish could therefore be applied to elasmobranchs. We would question these statements on the suitability of teleost diet-tissue discrimination factors (DTDFs) for elasmobranchs and argue that variation in DTDFs is a major source of observation error in stable isotope studies, particularly in the marine environment.

As Logan & Lutcavage (2010) point out, previous meta-analyses have shown that teleost δ^{15}N DTDFs are highly variable, ranging from -1 to 5.6‰ (Caut et al., 2009). Moreover, there have been repeated calls in the literature for species-specific DTDFs to enable accurate interpretation of stable isotope data (Gannes et al., 1997; Martinez del Rio et al., 2009; Caut et al., 2009; Wolf et al., 2009). This is of particular importance for determining diet contributions using stable isotope mixing models [e.g. IsoSource (Phillips & Gregg, 2003) and MIXsir (Moore & Semmens, 2008)] and for estimating trophic position. Non-specific DTDFs can easily bias interpretation of diet contribution/trophic position and fail to reflect the uncertainty inherent in their selection (Caut et al., 2008, 2009). Because the Logan & Lutcavage (2010) study did not deal directly with or report DTDFs, we feel it is inappropriate for this study to state that teleost DTDFs are suitable for elasmobranchs.

More importantly, the suggestion by Logan & Lutcavage (2010) that DTDFs for teleost fish can be applied to elasmobranchs may be inaccurate based on a recent experiment under semi-controlled conditions to examine DTDFs for two large sharks, the sand tiger (*Carcharias taurus*) and the lemon shark (*Negaprion brevirostris*) (Hussey et al., 2010). This study reported a δ^{15}N DTDF for sharks of 2.29‰ ± 0.22 (mean ± SD) based on lipid extracted shark and prey muscle tissue. Although small in sample size ($n = 4$), there was low variation in DTDFs among individual animals (sand tigers: 2.27, 2.15, and 2.14‰; lemon shark: 2.60‰). These data provide an alternative and likely more accurate estimate for elasmobranchs than the standard 3.4‰ (Post, 2002) used by Estrada et al. (2003) or reported meta-analyses values [2.96‰: Vanderklift & Ponsard (2003); ~2.5‰: Caut et al. (2009)].

The effect of an inaccurate DTDF on predicted diet contributions can be illustrated by applying known δ^{15}N data for a consumer (shark) and sources (prey) to an IsoSource model and comparing model predictions against the actual contribution of those sources to the consumer. For example, the Deep Sea World sand tiger from Hussey et al. (2010) had a mean white muscle tissue δ^{15}N value of 14.99‰ ± 0.19. The shark fed on three prey sources, trevally (*Pseudocaranx dentex*; 42.96% of total diet fed over a 1-year period), saithe (*Pollachius virens*; 33.67%), and mackerel (*Scomber scombrus*; 23.37%) with δ^{15}N values for the prey sources being 14.41‰ ± 0.48, 11.97‰ ± 0.74 and 13.13‰ ± 0.16, respectively. IsoSource model results for mean percentage contribution of prey sources using DTDFs ranging from 1.8 to 3.8‰ in 0.1 increments are presented in Fig. 1.

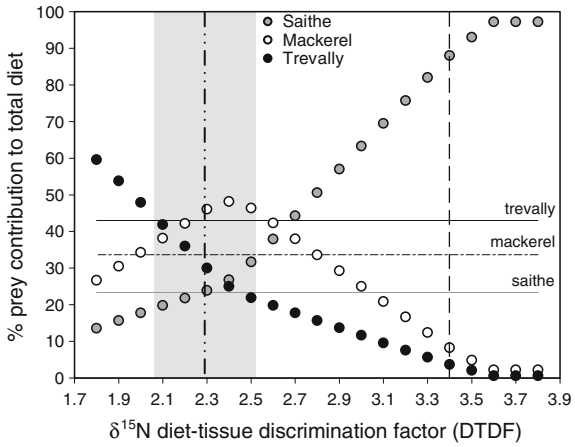

Fig. 1 IsoSource predicted mean prey source contributions to the total diet of the Deep Sea World sand tiger shark (*Carcharias taurus*) using incremental diet-tissue discrimination factors and known δ^{15}N values for the consumer (shark) and source (prey) muscle tissue. Accepting IsoSource calculates a range of values for each source, mean percentage values were selected to highlight the systematic changes in prey contributions as a result of varying DTDF. *Horizontal lines* represent the actual percent contribution of sources to total diet over a 1-year-period prior to sampling. *Dash-dotted vertical line* and *grey box* represent the mean DTDF ± SD (2.29 ± 0.22) derived for large sharks by Hussey et al. (2010); *dashed vertical line* is mean DTDF (3.4 ± 0.98) derived by Post (2002) for consumers in lake systems

There was large variation in model predicted results for the percentage diet contribution of the three prey sources with varying DTDF (Fig. 1). A DTDF ≥2.7 found that saithe was the major dietary component while a DTDF ≤2.1 found that trevally was the most important dietary component. Therefore, if a DTDF of 3.4‰ was used, the model would indicate that the shark was feeding predominantly on saithe (88%), while using the reported value of 2.3‰ (Hussey et al., 2010), estimates were more similar to the actual percentage diet contributions of the three prey sources: mackerel (46.1%), trevally (30.0%) and saithe (23.9%) (Fig. 1). We do accept, however, that this animal was used in the original calculation of the DTDF in Hussey et al. (2010). In addition, on the premise that DTDFs are governed by the δ^{15}N value of diet (Overmyer et al., 2008; Caut et al., 2009), we would expect a DTDF of 2.15‰ for this animal based on the equation provided by Caut et al. (2009) and a total diet δ^{15}N value of 13.27‰ (Hussey et al., 2010). For a DTDF of 2.1‰ (within 1 SD of the mean δ^{15}N DTDF derived by Hussey et al., 2010), the estimated dietary proportions were trevally (42.96%), saithe (38.2%) and mackerel (19.8%), values very near identical to the true diet of this shark (Fig. 1). This provides strong evidence for lower DTDFs for sharks than teleost fish and the generally applied 3.4‰. The often-repeated (Gannes et al., 1997; Ben-David & Schell, 2001; Caut et al., 2008, 2009; Wolf et al., 2009) implication is clear—inaccurate DTDFs can greatly bias diet contribution estimates from mixing models. It is also important to reiterate that DTDFs can be tissue specific (Pinnegar & Polunin, 1999; Hussey et al., 2010).

Furthermore, Logan & Lutcavage (2010) used an exponential decay curve model to calculate δ^{15}N tissue turnover rates. To account for uncertainty, the authors used both a high (3.4‰) and a low (1.5‰) DTDF estimate in the model calculation and concluded that δ^{15}N turnover rate estimates were similar to those reported for a freshwater stingray (MacNeil et al., 2006). Careful examination of their results, however, shows that the low DTDF provided the most comparable results to that of MacNeil et al. (2006) (Table 1), providing further evidence that DDTFs are lower than 3.4‰ in elasmobranchs.

The primary results of the Logan & Lutcavage (2010) study are important for advancing our understanding of stable isotopes in elasmobranchs. Over the past few years, however, there have been repeated calls for the tightening of basic assumptions in stable isotope ecology (Gannes et al., 1997; Caut et al., 2008, 2009; Wolf et al., 2009). It is therefore important that stable isotope ecologists are aware of these issues and move away from generalised assumptions developed from lake ecosystems (Jardine et al., 2006).

As both Hussey et al. (2010) and Logan & Lutcavage (2010) emphasise, further experimental

Table 1 Turnover rate estimates for δ^{15}N in elasmobranch muscle tissue

Study	Species	Δ^{15}N value	Tissue	m ± SE (day^{-1})	Half life (days)	95% Turnover (days)
Logan & Lutcavage (2010)	*C. plumbeus*	High	Muscle	0.009 ± 0.001	78.8 ± 9.0	340.5 ± 38.7
	C. plumbeus	Low	Muscle	0.0007 ± 0.001	105.9 ± 9.5	457.8 ± 41.0
MacNeil et al. (2006)	*P. motoro*		Muscle	0.0041 ± 0.00042	98	422

work is required to examine DTDFs for elasmobranchs. This work could examine the effects of (i) diet [high vs. low quality (Robbins et al., 2005) and high δ^{15}N diet vs. low δ^{15}N diet (Felicetti et al., 2003; Caut et al., 2009)] and; (ii) growth rate [juvenile vs. adult (Trueman et al., 2005)] on DTDFs. In addition, DTDFs for a range of elasmobranch species are required. Future work could examine indicator species for specific life-strategies through aquaria or laboratory studies, for example, large zooplanktivores [whale shark (*Rhincodon typus*); manta ray (*Manta birostris*)], large mobile generalists [sandbar and bull (*Carcharhinus leucas*) shark] and epibenthic less mobile generalist species [leopard shark (*Triakis semifasciata*) and southern stingray (*Dasyatis americana*)].

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Author Biographies

Nigel E. Hussey is a postdoctoral fellow based at the University of Windsor, Canada. His main research is focused on understanding trophic interactions and how these structure marine and freshwater systems. His research employs field techniques, including acoustic and satellite tracking technology and chemical tracers. Dr. Hussey is currently involved in ecosystem-based projects in the Arctic, the Red Sea, and off Southern Africa.

M. Aaron MacNeil is Research Scientist in Marine Biodiversity with the Australian Institute of Marine Science. His research focuses on understanding the role of fishes in marine ecosystems, the effects of disturbance on fish community structure, and the development of sustainable fisheries. Working in a wide range of environments, from tropical to polar regions, his work examines the relative importance of specific ecological and anthropogenic processes through the use of hierarchical statistical models. Currently Dr. MacNeil is working to link social–ecological data to understand how socio-economic forces affect the sustainability of reef resources and to quantify recovery rates of coral communities on the Great Barrier Reef.

Aaron T. Fisk is an Associate Professor and Canada Research Chair in Trophic Ecology at the Great Lakes Institute for Environmental Research, University of Windsor. His research focuses on the mechanisms and processes that structure, and the impact of multiple stressors on, trophic relationships and food web function in aquatic ecosystems. Chemical tracers, including stable isotopes, fatty acids, elements and contaminants, are important tools in his research. Understanding the underlying mechanisms that control the movement of these tracers through food webs have and continue to be a focal point of Dr. Fisk's research program. His research projects include lab and field studies, from tropical streams to higher arctic marine ecosystems, and from freshwater guppies to Greenland sharks.