

Stable isotope analysis as a tool for elasmobranch conservation research: a primer for non-specialists

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Abstract. Many elasmobranch species are undergoing population declines on a global scale; however, implementation of effective conservation and management strategies is hindered, to a large extent, by a lack of sufficient data on diet, life history and behaviour. This work is a primer of how stable isotope analysis can be used as a cost-effective, relatively simple tool for examining resource use patterns (e.g. diet, habitat) and generating relevant data in support of science-based elasmobranch conservation and management. Specifically, isotopes can resolve the feeding niches of elasmobranchs, detect ontogenetic trophic shifts and calculate relative diet breadth of different species. Stable isotope analysis can also be employed to investigate the extent of anthropogenic impacts on diet or to infer the source of toxins affecting elasmobranchs. Additionally, this tool can be used to study migration patterns and habitat usage. Depending on the tissue analysed, this technique can also be non-lethal and minimally invasive. The limitations of stable isotope analysis are discussed, and recommendations for future work are presented.

Additional keywords: dietary analysis, ecotoxicology, food web analysis, individual diet specialisation, migration.

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Introduction

Elasmobranchs can play important roles in structuring marine communities (Creel and Christianson 2008; Heithaus *et al.* 2008). However, many species of sharks, skates and rays are experiencing population declines, primarily due to overfishing, by-catch, pollution and habitat degradation (Dulvy *et al.* 2008; Lucifora *et al.* 2011; Gallagher *et al.* 2012). Implementing effective conservation and management strategies is hindered, to a large extent, by a lack of data on diet, life history and behaviour. For example, there is insufficient data on nearly half of all elasmobranchs to support any form of conservation assessment (Heupel and Simpfendorfer 2010). Accordingly, Simpfendorfer *et al.* (2011) identified 26 'research needs' that are considered critical to elasmobranch conservation.

Stable isotope analysis is an increasingly prevalent, cost-effective and potentially non-lethal tool that can be applied to elasmobranch conservation research (see review by Hussey *et al.* 2011). This technique is based on the premise that heavy

isotopes of an element are preferentially retained (e.g. nitrogen isotopes via protein amination/deamination and carbon isotopes via respiration) and that specific ratios of heavy to light isotopes are indicative of specific resource (e.g. diet and habitat) use (Wolf *et al.* 2009). Depending on what resource use is being studied, numerous isotopes can be utilised and answer a variety of ecological and behavioural questions (see Hobson 1999; Davenport and Bax 2002; Jardine and Kidd 2006).

Stable isotope analysis has been used for elasmobranch research for more than two decades and has already helped to increase understanding of their biology and ecology. Additionally, a variety of studies focusing on seabirds, marine mammals, teleosts and marine reptiles have generated innovative applications of stable isotope analysis that have provided data for implementing conservation and management strategies (examples provided below). Many of these stable isotope techniques have yet to be applied to elasmobranchs, providing a promising avenue for generating additional

science-based conservation data for this largely imperiled group of animals.

The work presented here provides a primer for non-specialists interested in utilising stable isotopes to support their research. We describe how stable isotope analysis has been used to generate data for elasmobranchs that fall within the following conservation research needs identified by Simpfendorfer *et al.* (2011): (1) diet and trophic structure, (2) environmental effects on life history, behaviour and spatial ecology, (3) movement and migration and (4) habitat use and preference. We further discuss how novel stable isotope techniques used in studies of other taxa can be applied to elasmobranchs to further address these research needs.

Diet and trophic structure

The USA National Marine Fisheries Service has expressed an interest in creating an ecosystem-based fisheries management plan (Pikitch *et al.* 2004) for several shark species and requested that researchers gather diet and trophic data to facilitate this work (SEDAR 2006). In addition, three of the research needs identified by Simpfendorfer *et al.* (2011) are associated with diet and trophic structure: determining (1) the roles sharks and rays play in aquatic ecosystems, (2) diet of sharks and rays and (3) how these diets change ontogenetically. These research areas are well suited for studies using stable isotope analysis.

The most common elements used for examining diet and trophic structure in organisms are carbon and nitrogen because they reveal distinct components of a consumer's dietary niche. All stable isotope values are reported in the δ notation, where $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ (the ratio of 'heavy isotope' to 'light isotope') and nitrogen, $\delta^{15}\text{N}$ (the ratio of 'heavy nitrogen' N^{15} to 'light nitrogen' N^{14}). The $\delta^{13}\text{C}$ values vary among different types of primary producers (e.g. C_3 vs C_4 plants, pelagic plankton vs seagrasses), but remain relatively constant as they move from prey to predator, allowing researchers to determine the original source of dietary carbon of a consumer (DeNiro and Epstein 1981; Peterson and Fry 1987). $\delta^{15}\text{N}$ values exhibit stepwise enrichment as they move from prey to predator in a food chain and an organism's trophic position relative to that of others in the same food web can be estimated (DeNiro and Epstein 1978; Peterson and Fry 1987; Post 2002).

As stable isotope analysis relies on isotopes integrating into a consumer's tissues over time, this method can provide long-term estimates of diet that avoid the 'snapshot' bias associated with stomach content analysis; yet stable isotope analysis does not provide the taxonomic resolution that stomach content analysis sometimes generates (Hyslop 1980). Additionally, because many predators have empty stomachs when captured (either as a result of natural feeding behaviour or regurgitation due to capture stress), it is sometimes necessary to capture large numbers of specimens for a stomach content analysis study (Arrington *et al.* 2002). In contrast, all animals have an isotopic signature indicative of long-term average diet regardless of whether or not their stomachs are empty.

Numerous studies have examined the diet and trophic position of elasmobranchs in a food web context using stable isotope analysis, ranging from investigations of dietary niche

overlap in elasmobranch mesopredators (Vaudo and Heithaus 2011), contributions of different primary production sources to communities (Botto *et al.* 2011; Davenport and Bax 2002), community-wide trophic structure analysis (Davenport and Bax 2002; Revill *et al.* 2009; Boyle *et al.* 2012), changes in assemblages over time (Pinnegar *et al.* 2002; Jennings *et al.* 2002) and with other environmental variables (Polunin *et al.* 2001). Many of these studies collected the first stable isotope data for certain species and regions and can therefore serve as important baseline information.

Knowledge of relative diet breadth is important for ecosystem-based management plans. For example, Layman *et al.* (2007) developed summary metrics that allow for comparisons of diet breadth among species. These metrics include $\delta^{15}\text{N}$ range (the difference between the largest/most enriched and smallest/most depleted $\delta^{15}\text{N}$ value within each species), $\delta^{13}\text{C}$ range (the difference between the largest and smallest $\delta^{13}\text{C}$ value within each species) and total occupied niche area (the convex hull area of the polygon represented by all of the $\delta^{13}\text{C}/\delta^{15}\text{N}$ data for each species). Trophic structure within a food web is represented by $\delta^{15}\text{N}$ range, $\delta^{13}\text{C}$ range represents niche diversification at the base of a food web and total occupied niche area represents diet breadth. Other similar measures to estimate diet breadth have been proposed by Turner *et al.* (2010; based on hypothesis-testing framework) and Jackson *et al.* (2011; based on Bayesian approach). Another important factor in determining the dietary niche of organisms is the diet overlap among species and can be estimated by plotting the raw isotopic data from several co-occurring species (or different age-classes of the same species). Diet breadth metrics and diet overlap have been calculated for a community of nearshore elasmobranch mesopredators (Vaudo and Heithaus 2011) and different co-occurring age-classes of sandbar sharks (*Carcharhinus plumbeus*, Shiffman 2011).

Stable isotope values of primary producers can vary drastically in time and space in marine systems (Fourqurean *et al.* 2005, 2007). Hence, raw isotopic data cannot always be used to directly compare among organisms from different locations or times. In these instances, raw isotopic data can be transformed into 'proportional' isotopic data applying isotope values of known source pools (Newsome *et al.* 2007). These ratios can be more ecologically relevant than raw δ values because they can be organismal characteristics (i.e. trophic level), but calculating them accurately requires additional information (i.e. spatially and temporally accurate isotopic baseline values).

Comparing trophic positions of elasmobranchs and co-occurring organisms can determine if they are truly apex predators in their ecosystems, which has implications for both the ecological role of elasmobranchs and the level of fisheries exploitation that can be supported by that species (Cortes 1999). A formula developed by Post (2002) requires the $\delta^{15}\text{N}$ signature for a prey species of known trophic position, the $\delta^{15}\text{N}$ signature for a predator species of unknown trophic position, and the appropriate diet-tissue discrimination factor. For such models to be used appropriately, low trophic level organisms from the same food web must also be sampled in order to develop an isotopic baseline that allows comparisons among different systems and times. This method has been applied to both

shark (Estrada *et al.* 2003) and ray (Sampson *et al.* 2010) species.

As previously noted, the traditional method of determining the diet of elasmobranchs is stomach content analysis, directly examining what prey species the animal has recently consumed (Hyslop 1980). Although a few researchers examine stomach contents utilising non-lethal gastric lavage, stomach content analysis typically involves sacrificing large numbers of the study species (Shurdak and Gruber 1989). In contrast, stable isotope ratios can be used to infer diet by using mixing models that estimate the proportional contribution of various prey items to a predator's diet. These models require sampling both the elasmobranch consumer and an extensive array of other organisms in the food web.

Mixing models can solve a unique solution to scenarios involving n elements and $n + 1$ prey items (e.g. $\delta^{15}\text{N}$ to resolve the contributions of two prey items, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for three prey items). Common models include a linear mixing model called IsoSource (Phillips 2001) and a Bayesian model called MixSIR (Moore and Semmens 2008). Mixing models are often used in non-elasmobranch studies (e.g. Bugoni *et al.* 2010 used IsoSource on seabird populations; Dennard *et al.* 2009 used MixSIR on halibut; *Hippoglossus hippoglossus*). These models are not yet commonly used by elasmobranch researchers, although Vaudo and Heithaus (2011) used MixSIR to determine the relative contribution of phytoplankton-based, algae-based and seagrass-based carbon to the diet of various nearshore elasmobranch mesopredators, Drymon *et al.* (2011) used a mixing model called SIAR to detect regional variation in the diet of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and Boyle *et al.* (2012) used SIAR to estimate the proportional contribution of three major prey groups to the diet of the roughtail skate (*Bathyraja trachura*).

Additionally, other quantitative techniques such as cluster analysis have been used in food web studies that involve elasmobranchs (Davenport and Bax 2002; Revill *et al.* 2009). Although the $n + 1$ prey item limitation can be problematic, Phillips and Gregg (2003) and Phillips *et al.* (2005) have addressed this issue with *a priori* and *a posteriori* aggregation techniques. We encourage the use of mixing models in the future, as the results can be informative for ecosystem-based management and the information these models need (isotopic values for predators and suspected prey species) is often already collected by researchers focusing on isotope analysis.

Some authors have suggested that under an ecosystem-based management model, different age-classes of the same species should be managed as separate species because their diets are so distinct (Lucifora *et al.* 2009; Grubbs 2010). Stable isotope analysis can be used to detect these ontogenetic shifts in diet: different isotopic signatures between different age-classes, or between different tissues within an individual, can indicate a shift in diet if stable isotopes are properly applied (see 'Conclusions and Future Directions' below). Comparing the isotopic signatures of tissues with large differences in turnover rates can allow detection of a change in an individual animal's diet over time. For example, MacNeil *et al.* (2005) took samples from muscle, cartilage and liver tissues from three species of shark and identified diet switches based on different isotopic signatures between the tissues. These types of data can also be useful

in assessing elasmobranch intrinsic vulnerabilities to anthropogenic hazards such as fishing pressure (Gallagher *et al.* 2012).

Measuring the isotopic signature of different subsamples of vertebrae tissue along the vertebrae's radius can also detect diet change within an individual. Estrada *et al.* (2006) analysed the isotopic ratios of several points along the radius of great white shark (*Carcharodon carcharias*) vertebrae and reconstructed the sampled animals' trophic history. The two techniques outlined above require either lethal sampling of sharks or opportunistic sampling of sharks that have been sacrificed for another purpose, which may not always be feasible or desirable (Hammerschlag and Sulikowski 2011). Alternatively, if researchers have access to multiple age-classes of a species, taking one sample each from multiple individuals of discrete life history stages can also detect a diet shift. Shiffman (2011) analysed muscle samples from young-of-year and juvenile sandbar sharks and, after correcting for maternal isotopic influence (see Olin *et al.* 2011), found a significant difference between the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of these age classes.

Recent studies have shown the importance of variable resource use among individuals, which may have important ecological and conservation implications (Bolnick *et al.* 2003, 2007, 2011). Individual specialisation, defined as a significantly smaller dietary niche of an individual than the population niche for reasons other than sex, body size, or age class, has been measured in diverse taxa including elasmobranchs (Magurran 1986; Bolnick *et al.* 2003; Matich *et al.* 2011). Stable isotope analysis can be used to detect variable individual feeding behaviour within a population in several ways: (1) examining tissues from many members of a population; (2) utilising different parts of the same metabolically inert tissue deposited at different times (e.g. vertebrae) in individuals; and (3) using multiple tissues with different turnover rates in individuals. When individuals within a population retain the same isotopic signature, one that is different than the signature of the rest of the population, it may indicate individually variable feeding behaviour.

This type of analysis has been used among freshwater teleost fishes such as northern pike (*Esox lucius*, Beaudoin and Prepas 1999) and largemouth bass (*Micropterus salmoides*, Post 2003). Post (2003) used a combination of age and growth analysis and stable isotopes to show that members of a largemouth bass cohort transitioned to a piscivorous diet in different years. Recent isotope work has also suggested that individual specialisation may be occurring among seabirds (e.g. Cherel *et al.* 2006) and marine mammals (e.g. Cherel *et al.* 2007). Additionally, Matich *et al.* (2011) presented evidence that it may be occurring in bull sharks (*Carcharhinus leucas*). Owing to the suite of tissues (and their associated variable turnover times) available to elasmobranch researchers, a greater understanding of individual feeding specialisation is undoubtedly possible. If individual specialisation is found to occur within certain elasmobranch populations, it could alter our models of how the loss of these animals will impact ecosystems (Matich *et al.* 2011). Additionally, variable feeding behaviour may be associated with variable habitat use patterns, which has conservation implications if management plans only incorporate 'typical' behaviour and not the complete range of intrapopulation niche variation (Hammerschlag-Peyer and Layman 2010).

Environmental effects on life history, behaviour and spatial ecology

Anthropogenic disturbance to an organism's diet represents an important changing environmental and ecological condition and thus, might affect the life history, behaviour and spatial ecology of predators such as elasmobranchs. For example, heightened intraspecific competition in response to a steady anthropogenic source of food resulted in acute physiological stress in southern stingrays (Semeniuk *et al.* 2009). Other elasmobranchs are also intentionally fed by SCUBA operators to enhance the viewing experience for tourists (Gallagher and Hammerschlag 2011). A study of Caribbean reef sharks (*Carcharhinus perezii*) in the Bahamas used stable isotope analysis, in combination with acoustic telemetry, to investigate the short-term effects of ecotourism provisioning (Maljković and Côté 2011) on shark diet and behaviour. The study found that the subset of sharks that regularly took the bait (grouper carcasses, *Mycteroperca* spp. and *Epinephelus* spp.) had significantly higher $\delta^{15}\text{N}$ values than the sharks that rarely took bait during feeding dives (Maljković and Côté 2011).

Indirect provisioning via fishery discards can also alter natural trophic pathways for elasmobranchs. Fisheries can introduce food sources via bait, discarded offal (shark livers and viscera) or by-catch. Boyle *et al.* (2012), in a study that included two elasmobranch species, found that nitrogen isotope ratios were lower than expected in large teleost fishes that consumed fishery discards and scavenged more frequently rather than feeding in the benthic food web. Additionally, Bugoni *et al.* (2010) found that seabirds in Southern Brazil that fed upon the fishery offal had similar nitrogen isotope values, but a species that did not consume offal had much lower nitrogen isotope values. Although these studies have used stable isotopes to indicate short-term changes in diet and behaviour of marine organisms via provisioning, more work is needed to fully understand the ecological role of provisioning within fish assemblages and within elasmobranchs.

Anthropogenic disturbances can also come in the form of environmental contaminants. The diet of a fish can represent an integral pathway for tracking the exposure from – and origin of – harmful compounds such as trace heavy metals, organochlorines and other contaminants (see review by Jardine and Kidd 2006). In aquatic systems, compounds can be assimilated into an organism's tissue through ingestion, ventilation (Rawson *et al.* 1995) and even absorption through the skin (Hostynek and Maibach 2006).

Traditionally, most ecotoxicology studies on elasmobranchs, including examinations of the concentrations of contaminants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), have relied upon chemical assays of the liver (Gelsleichter *et al.* 2005). Alternatively, researchers can use stable isotopes to elucidate the point of exposure to a toxin (i.e. mercury, PCBs, DDT) at the individual and ecosystem level, elucidating the source of the contaminant (Jardine and Kidd 2006). Several studies have used heavy metals and bioaccumulating contaminants to corroborate stable isotope values in sharks (organochlorides by Fisk *et al.* 2002; cadmium and mercury by Domi *et al.* 2005; mercury by McMeans *et al.* 2010). It is important to note that stable isotope ratios do not

always correlate directly with the concentration of toxins. Some elasmobranchs are top predators and display the associated tendency to bioaccumulate toxins, so elasmobranchs are potentially more threatened by pollution than other marine organisms (Gelsleichter *et al.* 2005). More studies are needed to further understand the degree of threat and stable isotope analysis has already been shown to be an effective technique for studying these processes in other taxa.

Movement and migration

Migration behaviour is related to important ecological processes such as dispersal, predator avoidance, foraging and mating (Nathan *et al.* 2008; Benhamou 2004). To date, elasmobranch migration studies have focussed on expensive, logistically complex methods such as tag and recapture (Kohler and Turner 2001), acoustic telemetry (Sims 2010), or satellite-linked transmitters (Hammerschlag *et al.* 2011). Stable isotopes (primarily $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$ and $\delta^{87}\text{Sr}$) can also be used to track animal migration patterns (Hobson 1999; Layman *et al.* 2012).

These studies are based on the assumption that local food webs differ in their isotopic signatures based on environmental and spatial gradients (Hobson 1999) and these signatures are incorporated into tissues of animals feeding in these food webs (Peterson and Fry 1987). Therefore, the isotopic signature of animals moving between isotopically distinct food webs can indicate the location of previous feeding events, allowing researchers to infer migration patterns (Hobson and Clark 1992). To date, no published studies report tracking of elasmobranch movement patterns using stable isotopes, although Borrell *et al.* (2011) noted that a change in $\delta^{13}\text{C}$ signature between age-classes of whale sharks (*Rhincodon typus*) may indicate ontogenetic changes in habitat usage and Carlisle *et al.* (2012) had similar conclusions for great white sharks.

A variety of isotopes have been used to track the migration of non-elasmobranch animals (see review by Hobson 1999). Isotopes of hydrogen ($\delta^2\text{H}$), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), oxygen ($\delta^{18}\text{O}$) and sulfur ($\delta^{34}\text{S}$) have previously been employed to discriminate animal movements between freshwater and marine food webs (Killingley and Lutcavage 1983; Peterson and Fry 1987; Owens 1988; Zeigler 1989; Marra *et al.* 1998). For example, Harrington *et al.* (1998) found that streams within an agricultural region had food webs with increased $\delta^{15}\text{N}$ compared with streams in a forested area, a finding that allowed them to track movement of Atlantic salmon (*Salmo salar*) between the two systems.

The stable isotope compositions of marine food webs generally had higher values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ compared with freshwater habitats (Peterson and Fry 1987; Ben-David *et al.* 1997; Hobson *et al.* 1997). The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ found in rainfall can differ spatially, which provide insight into large-scale dietary patterns and associated movements among locations (Bowen and Revenaugh 2003; Solomon *et al.* 2009; Layman *et al.* 2012). Further, $\delta^{13}\text{C}$ gradients in food webs can be related to nearshore or benthic linked food webs compared with offshore or pelagic food webs (Dunton *et al.* 1989; France 1995). For example, working with brown shrimp (*Penaeus aztecus*), Fry (1981) found differences in $\delta^{13}\text{C}$ values in shrimp

tissues as they moved between inshore seagrass beds with increased $\delta^{13}\text{C}$ values compared with the more lower values of phytoplankton pelagic food webs. The ratio of sulfur isotopes ($\delta^{34}\text{S}$) varies substantially among primary producers. As such, sulfur isotopes can be used to delineate between benthic-derived food webs compared with pelagic food webs (Fry *et al.* 1981; Peterson and Howarth 1987; Hobson 1999).

In marine fishes, otoliths (calcareous material in the inner ear chambers of teleosts), exhibit growth rings that are deposited in isotopic equilibrium with the surrounding seawater (Nelson *et al.* 1989). Otoliths can be used to identify the isotopic composition of waters previously occupied by the fish. Nelson *et al.* (1989) showed that carbon and oxygen isotopes derived from smelt (*Retropinna retropinna*) otoliths could be used to track the smelt movements among river, lake, estuarine and marine systems.

Given the high variability in habitat use exhibited by elasmobranchs, there are likely to be large differences in isotope signatures of distinct food webs in which they are feeding, making elasmobranchs ideal candidates for isotope migration studies to identify critical habitats (although other confounding factors may influence these results). Although elasmobranchs do not possess otoliths, their vertebrae exhibit growth rings (presumed to be annular) that are deposited in isotopic equilibrium with the surrounding seawater. Thus, akin to teleost otoliths, vertebral samples can be used to identify the isotopic composition of habitats previously occupied by elasmobranchs. However, variable isotopic turnover rates of different elasmobranch species and tissues may complicate this analysis (see 'Limitations of stable isotope analysis' for discussion). Overall, the use of stable isotopes for investigating elasmobranch migration shows promise and coupling isotopes with traditional migration study methods may yield interesting results. Identifying migration pathways will allow for more effective conservation strategies by incorporating spatiotemporal data into management plans (Simpfendorfer *et al.* 2011).

Habitat use and preference

Identifying and characterising critical habitat types (e.g. nursery grounds) linked to important ecological processes such as mating, pupping and foraging are priorities for implementing effective elasmobranch conservation and management strategies (Simpfendorfer *et al.* 2011). Many elasmobranchs are highly mobile and can move among estuarine, coastal and pelagic waters depending on life history stage and time of year. For example, adult bull sharks frequently migrate among inshore and offshore areas (Brunnschweiler *et al.* 2010; Carlson *et al.* 2010; Matich *et al.* 2010).

Given that different tissue types have different isotopic turnover rates (Logan and Lutcavage 2010a), an assay of multiple tissue types (e.g. blood, fin, muscle) can be used to track elasmobranchs' movement patterns on different time scales (i.e. short- vs long-term movements). The use of short-turnover tissues, like blood, contrasted with longer-turnover tissues, like muscle, will identify elasmobranchs not in equilibrium with their current isotopic environment (e.g. new arrivals to that area, following Hobson 1999). Such an approach could have

significant conservation implications for characterising pupping grounds by the identification of newly arrived pregnant females to a localised area.

Limitations of stable isotope analysis

Researchers should note that this technique has inherent limitations that must be considered when interpreting stable isotope data (reviewed by Newsome *et al.* 2007; Layman and Post 2008; Layman *et al.* 2012). Here we briefly discuss how failure to use appropriate diet-tissue discrimination factors, isotopic turnover rates and sample preparation methods can result in inaccurate conclusions, as well as why isotopic routing, maternal contribution bias and spatial/temporal variation in the isotopic signatures of source pools should be considered when conducting a stable isotope study.

An appropriate diet-tissue discrimination factor (DTDF; the change in isotopic signature associated with moving from prey to predator) must be selected to ensure an accurate analysis. Past research has identified a wide range of diet-tissue discrimination factors among different species and among tissues within an individual (Hussey *et al.* 2010a). For example, Hussey *et al.* (2010a) found that nitrogen discrimination factors varied among tissues and were lower than 3.4, a frequently used nitrogen discrimination factor (Peterson and Fry 1987). In contrast, Kim *et al.* (2011) found that the nitrogen discrimination factor for muscle was 3.7. Failure to use accurate DTDFs can lead to inaccurate conclusions (Hussey *et al.* 2010b). Logan and Lutcavage (2010a, 2010b) suggest that when no suitable elasmobranch DTDFs are available, it may be acceptable to use a DTDF from an ecologically similar teleosts (i.e. applying DTDFs from slow-moving benthic flounder to slow-moving benthic skates), but Hussey *et al.* (2010b) disagrees. Hussey *et al.* (2010a, 2010b) and Logan and Lutcavage (2010a, 2010b) recommend future research on DTDFs from ecologically diverse elasmobranchs, including both slow and fast growing species, as well as both generalists and specialists. These DTDFs are particularly important for studies involving mixing models and trophic position. To date, the only DTDFs for elasmobranchs are available from ocellate stingrays (*Potamotrygon motoro*, MacNeil *et al.* 2006), leopard sharks (*Triakis semifasciata*, Kim *et al.* 2011), lemon sharks and sand tiger sharks (*Negaprion brevirostris* and *Carcharias taurus*, Hussey *et al.* 2010a).

An appropriate isotopic turnover rate (the amount of time needed for a change in diet to be reflected in a consumer's isotopic signature) must also be used to avoid inaccurate conclusions (Kim *et al.* 2011). For example, $\delta^{15}\text{N}$ turnover rates for ocellate stingrays were calculated to be 265 days for blood and 422 days for muscle (MacNeil *et al.* 2006), but were calculated to be 220 days for blood and 340 days for muscle in sandbar sharks (Logan and Lutcavage 2010a).

Different tissues require different treatments before analysis. Pre-treating the sample by extracting lipids appears to be important for lipid-rich tissues such as liver, but less so for muscle (Hussey *et al.* 2010a; Kim *et al.* 2011). Similarly, elasmobranch muscle has a high urea content, which would influence $\delta^{15}\text{N}$ values if urea is not removed (Hussey *et al.* 2010a; Kim *et al.* 2011).

Isotopes from certain dietary components are routed differently to different tissues, a phenomenon known as isotopic routing, which further complicates analysis because elasmobranchs tend to have diverse diets (Martinez del Rio *et al.* 2009; Hussey *et al.* 2010a). The isotopic signature of a consumer's tissue is representative both of the diet and of the biochemical processes within that consumer (Layman *et al.* 2012).

Maternal contribution bias can affect the interpretation of stable isotopic data from neonate elasmobranchs. Isotopic signatures of embryonic tissue have been found to be enriched in $\delta^{15}\text{N}$ values relative to the mother (McMeans *et al.* 2009; Vaudo *et al.* 2010). The amount of time after birth for a young-of-year animal's isotopic signature to reflect its diet and not that of the mother is a factor of the isotopic turnover rate. Shiffman (2011) found that this process takes ~ 2 months of feeding in the environment for young-of-year sandbar sharks.

Stable isotope analysis may be less useful if source pools do not have distinct isotopic signatures, and δ values can be influenced by variation (spatial and temporal) in the signature of source pools (Newsome *et al.* 2007). Scattering on a $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplot may be the result of variable diet and resource use among individuals of a species, but could also be the result of isotopic difference in the source pools (Matthews and Mazumder 2004). It is important to note that organisms or individuals may have similar isotopic signatures but different ecological niches if source pools have similar isotopic signatures (Layman *et al.* 2012).

Concluding remarks

The limitations of stable isotope analysis are important for researchers to consider. Yet, if applied correctly, stable isotope analysis can be a very powerful tool to examine resource use patterns in elasmobranchs, which can help address selected aspects of the critical conservation research needs identified by Simpfordorfer *et al.* (2011). Stable isotope analysis is easy to incorporate into an existing sampling protocol, as taking small muscle samples, blood samples, or fin clips does not compromise the vitality and survival of elasmobranchs. Additionally, this technique allows researchers to opportunistically sample elasmobranchs caught by fishermen and we encourage this whenever possible (although differential handling of animals before researchers can sample them could potentially impact results). Opportunistically sampling tissues from a large number of individuals at fish markets or tournaments could greatly increase the amount of information known about many species without adding to the anthropogenic impact on those species.

Using stable isotope analysis for elasmobranch migration and ecotoxicology research currently represents an understudied research area when compared with applying this technique to diet studies, but the future is promising. Further studies are needed to compare multiple methods of determining trophic ecology to ground-truth stable isotope analysis results (i.e. stomach content analysis coupled with stable isotope analysis by Boyle *et al.* 2012). Additionally, many analytical techniques and experimental designs focusing on other marine animals could be applied to elasmobranch research. Overall, this

tool can be relatively simple to use, cost-effective and can answer important questions for resource managers in a mostly non-lethal manner. As the technique further develops, so will its application in support of elasmobranch conservation research.

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