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Incorporating stable isotopes into a multidisciplinary framework to improve data inference and their conservation and management application[§]

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Through its ability to address complex ecological questions and the possibility of analysing large sample sizes to understand population-level processes, the use of stable isotope analysis (δ^{13}C and δ^{15}N) has grown rapidly in recent years. Importantly, it is now becoming an accepted tool to derive data for conservation and management planning at the species, community and ecosystem levels. With this acceptance, however, the stable isotope research community faces new challenges to ensure that data are interpreted and presented effectively to maximise their potential for guiding management. We present a case study on stable isotope trends in the vertebrae of white sharks *Carcharodon carcharias* to show how multiple plausible explanations could be provided to explain the observed isotopic patterns, a point that is likely ubiquitous among isotope studies in ecology. Based on this, we promote that integrating stable isotope data in a multidisciplinary framework will generate the most reliable data for conservationists and resource managers. If this is not possible, we suggest that the isotope community should be more accepting of presenting multiple possible explanations for trends observed in data, rather than focusing on a single interpretation that could potentially misguide management.

Keywords: carbon isotopes, individualisation, maternal influence, nitrogen isotopes, physiological effects, spatial variation

Introduction

Stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) provide powerful chemical tracers to examine diverse questions in ecology, including: (i) ecological characteristics of individual organisms, including diet and resource use (DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984; Peterson and Fry 1987); (ii) niche width and niche overlap (Heithaus et al. 2013; Kiszka et al. 2015); (iii) individual specialism (Hückstädt et al. 2012; Rosenblatt et al. 2015); (iv) movement and migration (Best and Schell 1996; Hobson 1999; Phillips et al. 2009); and (v) the length and structure of, and interactions of organisms within, food webs (Hobson and Welch 1992; Hussey et al. 2014), to name but a few. These tools are now being applied in the study of all types of species from bacteria (Boschker and Middelburg 2002) to the largest terrestrial and aquatic predators (Hilderbrand et al. 1996; Herman et al. 2005), at individual, community, and ecosystem levels, and have particular value for studies on species that are obscure (Navarro et al. 2014), of high commercial value (Estrada et al. 2005; Johnson and Schindler 2009), and/or are critically endangered (Navarro et al. 2009; Seminoff et al. 2012). Importantly, through exponential growth in the application of stable isotopes over recent years and practical and theoretical advancements (Gannes et al. 1997; Wolf et al. 2009), there has been broad recognition of their relevance for informing management (Rubenstein and Hobson 2004; Ramos et al. 2011; Hussey et al. 2014).

The acceptance of stable isotopes as a valuable tool for use by resource managers is a positive step, but this sets a new bar on the quality of data inference and our confidence as a community in their interpretation. To justify the role of stable isotopes as a tool to inform the future management of marine and terrestrial ecosystems, the stable isotope community, as with all disciplines, must systematically and objectively review its methods and approaches to ensure accurate data interpretation. This is particularly pertinent given the number of assumptions associated with the use of stable isotopes (for example, diet and tissue-specific discrimination factors, variable tissue turnover rates, and physiological effects that vary among individuals and species) (Gannes et al. 1997; Martínez Del Rio et al. 2009; Wolf et al. 2009) and the number of quantitative modelling approaches used (which are dependent on the requirement to select relevant prey items and sources and on knowledge of isotopic baselines) (Phillips and Gregg 2003; Moore and Semmens 2008; Parnell et al. 2010; Phillips et al. 2014). Recently, there has been increased use of stable isotopes in conjunction with other established ecological tools, for example, compound-specific isotope analysis of amino acids (CSIA-AA) (Seminoff et al. 2012; Vander Zanden et al. 2013; Hussey et al. 2015a), fatty acids (Kharlamenko et al. 2001; Couturier et al. 2013), trace elements (Werry et al. 2011; Honda et al. 2012), genetics (Clegg et al. 2003;

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Valenzuela et al. 2009), telemetry (Cunjak et al. 2005; Ceriani et al. 2012; Matich and Heithaus 2013; Carlisle et al. 2014), and measures of organism condition (Hobson et al. 1993; Gannes et al. 1998), to name but a few. This is leading to a multidisciplinary approach that inherently will yield more precise conclusions compared to the use of stable isotopes as a stand-alone tool.

Here, we promote the integration of isotopes within a multidisciplinary context based on a case study of a large, threatened fish, the white shark *Carcharodon carcharias*. We define multidisciplinary as research in which each discipline (or subdiscipline) makes an individual contribution to the overall research findings. Through ontogenetic stable isotope profiles derived from the juvenile portion of white shark vertebrae, we demonstrate that there are several equally viable explanations for the observed isotopic trends. Each interpretation, however, would differentially impact the species risk designation and management planning actions. The use of techniques from multiple subdisciplines within biology (e.g. genetics, telemetry, trace elements) coincident with these stable isotope data would increase our interpretive ability and hence more effectively inform management of this life stage. We recommend, where possible, that incorporating stable isotope data into a multidisciplinary framework will markedly improve their application for conservation and management planning.

Case study: vertebral stable isotope profiles (δ^{15}N and δ^{13}C) of white sharks off southern Africa

Stable isotope profiles (δ^{15}N and δ^{13}C) were derived from 58 white shark vertebrae sampled from animals incidentally caught in bather protection nets off KwaZulu-Natal, on the east coast of South Africa. In brief, dried vertebrae were sectioned and sample material was drilled from the vertebrae every 1–2 mm along the centre of the corpus calcareum. Individual age bands were not analysed and data are presented as sequential sampling throughout ontogeny. This was based on the assumption that similar-sized individuals will have similar band thicknesses and therefore the integration time per sequential sampling point will not differ significantly among individuals. The juvenile portion was defined as samples starting after the angle change (birth) to approximately 200 cm precaudal length (PCL). A linear regression between PCL and vertebral radius found that 200 cm PCL corresponded to approximately 17 mm across the vertebral section from the focus. It has been shown that white shark vertebral samples do not require decalcification prior to isotope analysis; therefore no pretreatment was undertaken (Christiansen et al. 2014a).

The juvenile vertebral isotope profiles (both δ^{15}N and δ^{13}C) of these 58 white sharks showed markedly distinct trends across time and individuals (Figure 1). Values of δ^{13}C and δ^{15}N were highly variable among individuals immediately after birth, ranging from −10.9‰ to −16.5‰ and 12.1‰ to 17.1‰, respectively (Figure 1b, e). Each individual, however, showed consistent isotope values across consecutive sampling points until all individual isotope profiles converged at ~200 cm PCL (Figure 1). The consistency and convergence of δ^{13}C values was more striking than that of δ^{15}N. These trends, both inter-individual differences in δ^{13}C and δ^{15}N values between juveniles and consistency in isotope values through time for each individual are notable and identify divergent life-history strategies during these early years. This would indicate these are likely management-critical years, as variable life-history strategies within the population would require more complex management. The convergence point in isotope values at ~200 cm PCL would suggest these animals then occur in the same food web, based on δ^{13}C, and feed at a similar trophic level, based on δ^{15}N. There are several plausible interpretations, that may act independently or in combination, to explain the observed inter-individual variation.

Viable explanations for observed white shark stable isotope profiles

Maternal influence

For most live-bearing organisms, newborn animal tissues are formed of their mothers' provisions during gestation; hence their stable isotope values reflect those of their mother (i.e. maternal isotopic interference). Knowledge of this maternal-newborn isotopic relationship can consequently be used to examine retrospectively variation in foraging locations among pregnant females. For example, δ^{13}C and δ^{15}N values measured in the hair of northern elephant seal *Mirounga angustirostris* pups provided evidence that mothers from two breeding colonies have distinct foraging habitats (Aurioles et al. 2006). Conversely, this relationship can confound data interpretation of the stable isotope values of newborn/juvenile animals depending on the level of isotopic discrimination between embryo and mother, the rate of elimination of the mother's isotope value, and the incorporation rate of the juvenile's own diet (Matich et al. 2010; Olin et al. 2011). For placental viviparous shark species (those connected by umbilical cord to mother during *in utero* development), the stable isotope values of near-term and newborn young are typically enriched in both ^{13}C and ^{15}N compared to the mother (McMeans et al. 2009; Vaudo et al. 2010). Over time, these initial high δ^{13}C and δ^{15}N values decline as they equilibrate with those of their own diet (Olin et al. 2011), a result of ontogenetic shifts in the diet and habitat use of many marine species driving mature females to consume a different diet and reside in a different habitat to juveniles (Lowe et al. 1996; Alonso et al. 2002; Hussey et al. 2011).

For juvenile white sharks, an aplacental viviparous species, both δ^{13}C and δ^{15}N values generally increased with time following birth. If, at birth, the initial values of ^{13}C and ^{15}N were enriched relative to the mother, it would be expected that ^{13}C and ^{15}N would deplete as the isotope values became more representative of the individual's own diet/foraging location (Olin et al. 2011; McMeans et al. 2009; Vaudo et al. 2010). Given that the isotope values of large female white sharks are depleted in ^{13}C, likely related to offshore foraging during gestation (Carlisle et al. 2012; Domeier and Nasby-Lucas 2013; see Figure 1a, sharks >315 cm PCL), it is possible that the observed pattern of isotopic convergence in these juveniles reflects the loss of the maternal isotope value over time. Since vertebrae are metabolically stable (Campana et al. 2002), any maternal

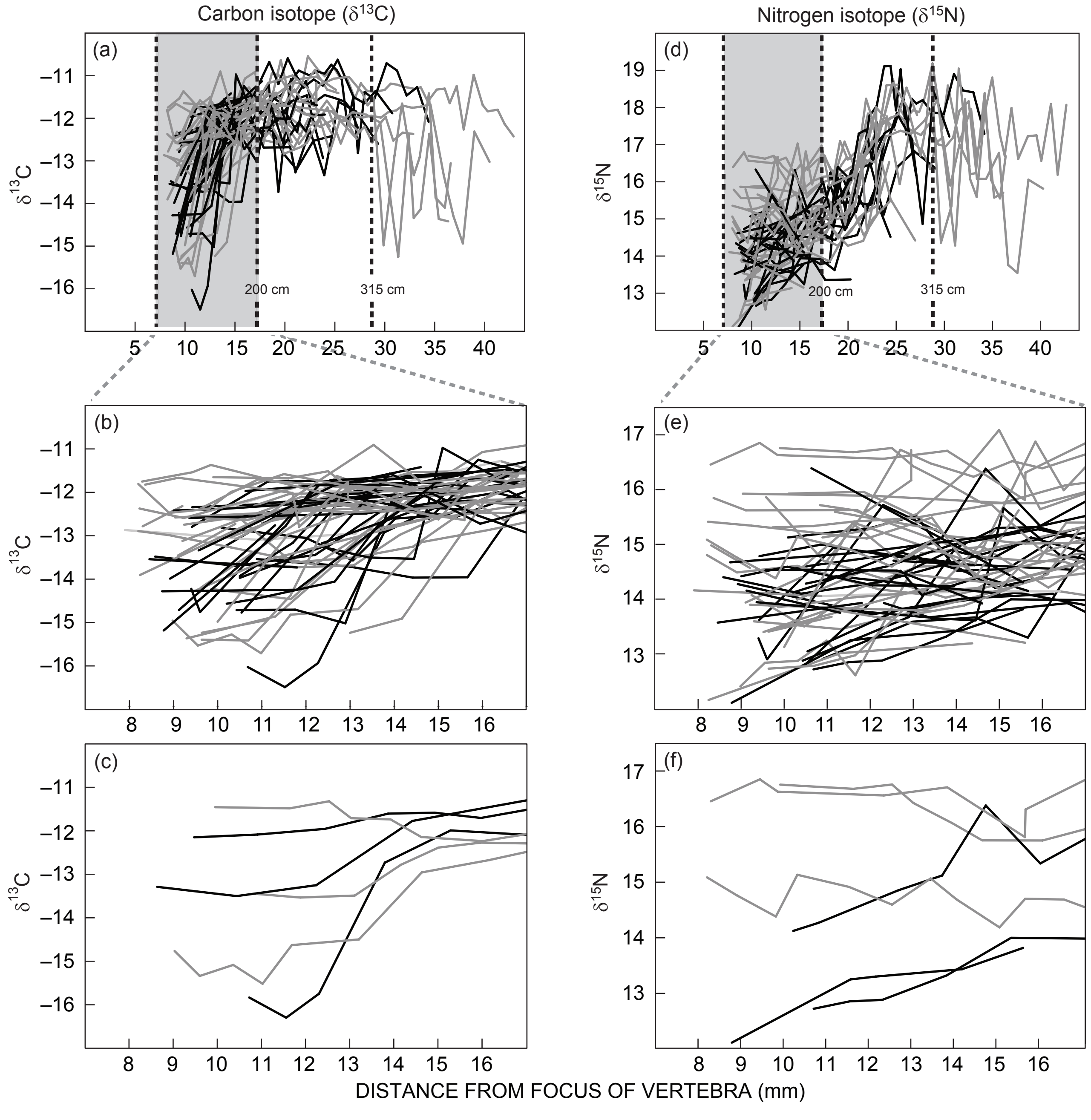

Figure 1: Stable isotope profiles of 58 white sharks from southern Africa. A single line represents each individual sampled approximately every 1–2 mm starting after the angle change (birth); black lines represent males, grey lines represent females. Distance is measured from the centre of the vertebrae (focus). (a) Ontogenetic δ^{13}C values for each individual, dotted lines indicate trend changes in isotope values at approximately 200 cm precaudal length (PCL) and 315 cm PCL; (b) enlarged shaded area from (a), δ^{13}C values for the juvenile portion, less than 17 mm from the focus (~200 cm PCL); (c) δ^{13}C values from six individuals showing consistency in isotope values across sequential sampling points until convergence at approximately 17 mm from the focus; (d) ontogenetic δ^{15}N values for each individual, dotted lines indicate trend changes in isotope values at approximately 200 cm precaudal length (PCL) and 315 cm PCL; (e) enlarged shaded area from (d), δ^{15}N values for the juvenile portion, less than 17 mm from the focus (~200 cm PCL); (f) δ^{15}N values from six individuals showing the relative consistency in isotope values across sequential sampling points until convergence at approximately 17 mm from the focus

influence would be due to growth from maternally derived nutrients in the whole body of the newborn shark (i.e. muscle tissue). Since there are known gradients in δ^{13}C values between coastal and pelagic waters (France 1995), δ^{13}C values of juveniles would be expected to increase as they begin feeding in coastal food webs. The range of post-birth δ^{13}C values observed in juvenile white sharks may represent differences in maternal habitat (i.e. offshore vs inshore feeding during gestation), rather than actual habitat differences among juveniles.

Individual specialists within a generalist population

It has long been recognised that ecological differences in diet and foraging habitat occur among sex and age classes of species; however, more recently the focus has expanded to include intraspecific niche variation and its potential impact on populations (Bolnick et al. 2002, 2003). Dietary specialisation occurs when individuals within a population use a subset of the total available prey base available to that population. Recognition of this behaviour has generated new insights into the feeding dynamics and interactions across a wide variety of taxonomic groups including gastropods, fishes, birds, reptiles, and mammals (Bolnick et al. 2003). Novel stable isotope approaches have now been developed to quantify inter-individual variation in populations that otherwise would be difficult and labour intensive to study (Newsome et al. 2009; Vander Zanden et al. 2010; Matich et al. 2011). Additionally, analysis of incrementally growing structures (e.g. sea otter vibrissae, turtle scutes, and shark vertebrae) allows for the study of intra-individual changes in isotope values over time (e.g. from months to entire lifetime).

Individual juvenile white sharks showed relatively consistent stable isotope profiles prior to convergence, resulting in the variation in isotope values among individuals likely being much greater than within-individual variation (Figure 1c, f). Under the assumptions of intra-niche variation, this would suggest the potential for individual dietary specialisation among juvenile white sharks. This is supported further by the broad range of teleost and elasmobranch prey identified in the diet of juvenile sharks (Hussey et al. 2012). Previous stable isotope profiles of white shark vertebrae from the North-East Pacific also identified a high degree of dietary specialisation, with isotopic trends similar to those observed in our data, but that study was focused on larger individuals (Kim et al. 2012).

Multiple nurseries

Carbon stable isotopes have been shown to be effective at discriminating the foraging habitats and migration patterns of animals (France 1995; Hobson 1999). This is based on the premise that carbon stable isotopes in a consumer fractionate conservatively through food webs and thus represent the isotope value of the baseline carbon sources where the animal feeds. In marine systems, pelagic and offshore food webs are readily distinguishable because carbon in pelagic systems is driven by photosynthesis and tends to be depleted in ^{13}C. Large-scale latitudinal gradients in stable isotope values can also occur and have been used to identify the habitat and migration of a range of species (Best and Schell 1996; Cherel and Hobson 2007). Off South Africa, there is a known gradient of δ^{13}C values, such that ^{13}C enriches along the eastern coast from KwaZulu-Natal to the Western Cape (Hill et al. 2006). Moreover, gradients in δ^{13}C values are documented in the tropical western Indian Ocean (Ménard et al. 2007) and across the sub-Antarctic front (Best and Schell 1996; Cherel and Hobson 2007).

The large variation in δ^{13}C values of juvenile white sharks post-birth and their consistency over time prior to convergence could suggest these animals inhabit distinct geographic locations or nurseries. Multiple nurseries could occur in either coastal or pelagic waters, or both, or could be shared across continents. Transoceanic dispersal events have been recorded for white sharks (Blower et al. 2012), indicating that South African females may give birth in Australian waters or that juveniles are capable of transoceanic migrations (Bruce and Bradford 2008). A subadult female white shark was documented making a return migration to Australia from South Africa (Bonfil et al. 2005), although such migration events have yet to be observed in smaller individuals. Alternatively, nurseries could be distributed along the western Indian Ocean coast (i.e. by latitude). The only large, near-term, pregnant female white shark observed in the region was caught off Kenya, supporting this hypothesis (Cliff et al. 2000). Furthermore, in Australia, satellite telemetry data have revealed that juvenile white sharks inhabit two distinct nursery areas, with few individuals migrating between habitats (Bruce and Bradford 2012).

Physiological constraints

The most obvious physiological effect on organismal stable isotope values relates to the effect of fasting. Once an animal fasts, i.e. no longer consumes food, catabolism occurs, leading to preferential excretion of ^{14}N (Hobson et al. 1993). This excretion results in higher δ^{15}N values in a consumer, which confounds quantification of the actual diet of that individual. This relationship is likely ubiquitous across species that fast and/or experience periods of low food availability (i.e. poor condition), because most adapt to reduced food intake through identical metabolic processes. The magnitude of the effect of fasting on stable isotope values, however, is thought to be variable. For organisms that use high levels of protein and maintain low lipid stores or have high energetic requirements (e.g. for long-distance migrations), fasting is thought to result in a greater change in δ^{15}N values (Cherel et al. 2005).

The consequences of fasting effects on a population of a species with highly variable life-history dynamics, such as partial migrations, where some individuals remain resident while others commonly migrate, could lead to large among-individual variation in δ^{15}N values even if individuals feed on a similar diet (with similar δ^{15}N values). There is evidence to support white sharks feeding during offshore migrations (Carlisle et al. 2012); but individuals returning to coastal waters were observed to be in poorer condition, and mixing models indicated a reduced level of foraging in pelagic versus coastal environments (Chapple et al. 2011; Carlisle et al. 2012). The extent of fasting or reduced foraging of white sharks during offshore migrations off southern Africa is unknown, but variable residency and migration dynamics of animals within the population may explain the observed isotopic patterns. Alternatively, highly variable growth may occur during the juvenile life stage, driving physiological variation in the uptake of stable isotopes, for example through variable fractionation. For Atlantic salmon *Salmo salar*, varying growth rates contributed to variation in δ^{15}N values among individuals held under controlled conditions (Trueman et al. 2005). Although δ^{13}C values are reportedly not affected by periods of fasting (Hobson and Clark 1993), there have been mixed results (Cherel et al. 2005; Williams et al. 2007) and little is known regarding these effects in sharks.

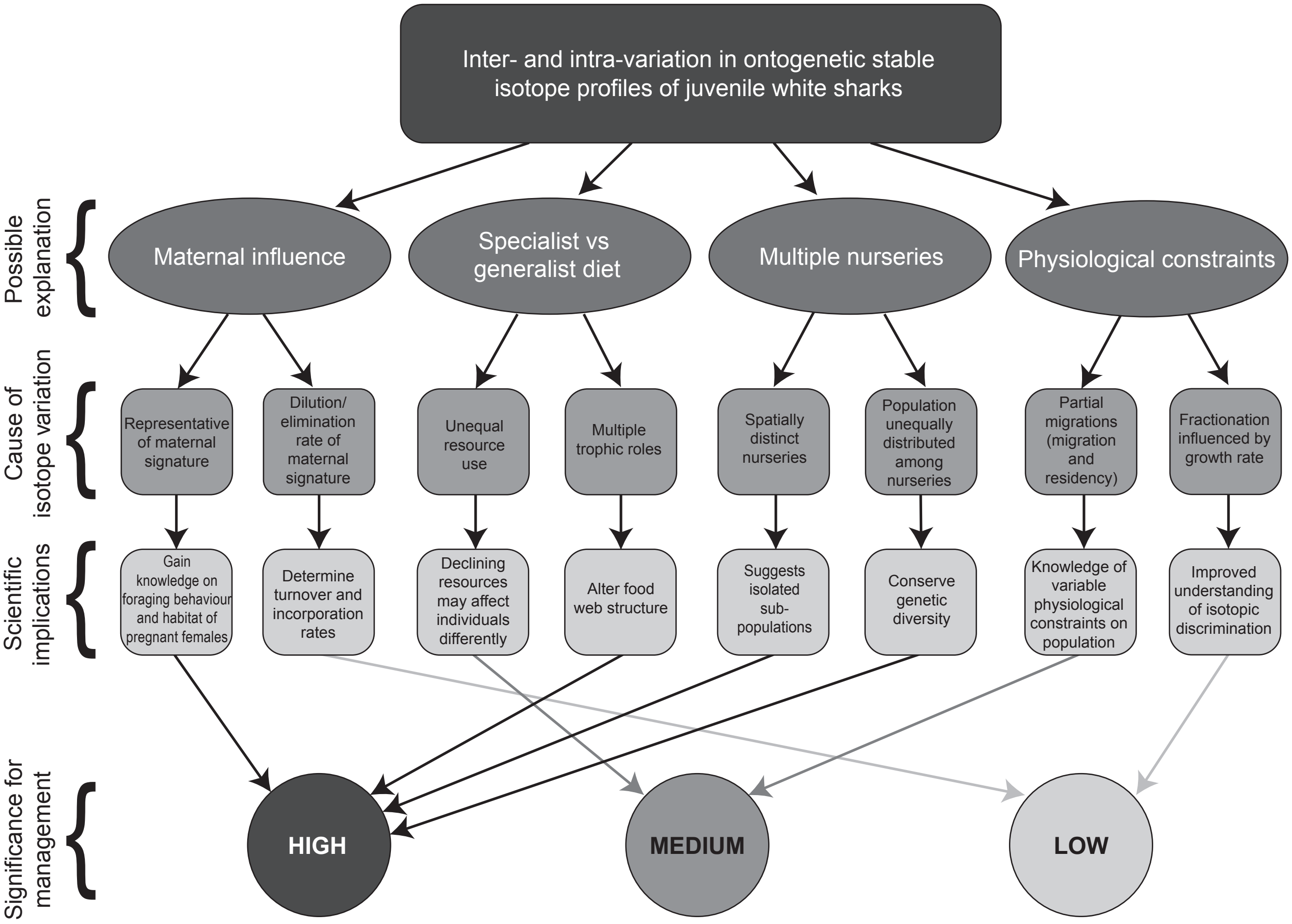

Figure 2: Viable explanations of variation in stable isotope values across the vertebrae of juvenile white sharks from southern Africa and their management implications. Specific causes of each explanation are listed, together with their scientific implications and significance to management (designated as High, Medium or Low impact)

The benefits of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) incorporated into a multidisciplinary approach

While the four above-mentioned explanations are all plausible, the implications of each for the management of the white shark population off southern Africa are highly variable. These management implications are summarised in Figure 2, ranked from low to high priority. In the broader stable isotope literature, it is likely that many studies exist where several equally plausible explanations were possible, but only one was argued. In turn, the selected interpretation may have influenced the perceived conservation status of a species or prospective management actions (Bond and Diamond 2011). Indeed, it is only through multidisciplinary approaches that the initial interpretation of bulk stable isotope data (bulk refers to analysis of whole tissues, as opposed to individual compounds), when used as a stand-alone tool, is being challenged. This certainly does not devalue the application of bulk stable isotopes in ecology; science must advance and build. Importantly though, it reinforces the need for investigators to be cognitive of how they interpret and 'sell' their data and to consider integrating stable isotopes in multidisciplinary frameworks where possible. This is particularly important given the role of science, including stable isotope ecology, in assisting and developing conservation and management actions. Here, we provide a few examples of recently adopted multidisciplinary isotopic frameworks.

Stable isotope analysis provides a single $\delta^{15}N$ and $\delta^{13}C$ value for a consumer, whereas CSIA-AA can provide both a $\delta^{15}N$ and a $\delta^{13}C$ value of the organism (trophic amino acids) and of the baseline of the system (source amino acids) (McClelland and Montoya 2002). This overcomes the limitation of establishing isotopic baselines for the ecosystems an animal inhabits, and is particularly useful for animals that are highly migratory. Through combining these two approaches, it has been possible to determine reliable estimates of trophic position (TP; the level at which an animal feeds in a food web) for two mesopelagic fish families (Myctophidae and Stomiidae) across five global regions (Choy et al. 2012). While bulk $\delta^{15}N$ values produced variable estimates of TP, CSIA-AA revealed that TP was uniform and the observed variation was a result of region-specific water body biogeochemistry (Choy et al. 2012).

Similarly, stable isotope values of herbivorous green turtles *Chelonia mydas* sampled at a nesting ground suggested that individuals fed over several trophic levels, indicating carnivory (Vander Zanden et al. 2013). CSIA-AA revealed that these results were due to variation in baseline seagrass isotope values between foraging grounds; consequently all individuals actually fed at a similar TP (Vander Zanden et al. 2013).

For the above juvenile white shark case study, CSIA-AA could confirm whether variation in stable isotope values is caused by individuals feeding on different prey in the same location, or by individuals inhabiting different regions but feeding on similar prey types. If a combined bulk stable isotope and CSIA-AA approach identified that these animals inhabited different regions, i.e. multiple nursery grounds, this would have important consequences for regional management (Figure 2). While white sharks are protected in South Africa, no legal protective framework exists in other Western Indian Ocean (WIO) countries and sharks are considered to be overexploited by fisheries in the region (Smale 2008). During the newborn/juvenile life stage, white sharks are not easily distinguishable from other species; thus it is possible that they are unknowingly part of fisheries catches. On the contrary, if CSIA-AA revealed juveniles were foraging at different trophic levels in the same location, this would indicate a single nursery for this population, simplifying management efforts.

Telemetry to monitor remotely the movements of animals can also be combined with stable isotope data (Hussey et al. 2015b). To date, telemetry methods are both improving confidence in isotope data interpretation and proposed management actions (Cunjak et al. 2005; Ceriani et al. 2012; Seminoff et al. 2012), but also are revealing dichotomies between movement patterns and previous inferences from stable isotope data. For example, stable isotope profiles of multiple tissues from individual bull sharks *Carcharhinus leucas* inhabiting a riverine/estuarine environment indicated individuals displayed a high degree of dietary specialisation over time (Matich et al. 2011). Recently, through combining stable isotope analysis with telemetry, it was found that these sharks became more generalised on a seasonal basis to take advantage of prey pulses (Matich and Heithaus 2013). Stable isotope analysis of multiple tissues of a highly threatened leatherback turtle *Dermochelys coriacea* population off French Guiana also revealed two distinct foraging groups that agreed with previous satellite tracking data (Caut et al. 2008).

For juvenile white sharks, both acoustic and satellite telemetry data could reveal periods of residency, geographical areas of residency, and scales and timing of movements. These data would confirm if the observed isotopic trends relate to the use of multiple nursery grounds, the physiological effect of some individuals undertaking large-scale movements compared to more-resident individuals, and individual and population level feeding ecology characteristics (e.g. specialists vs generalists) (Figure 2). These data would also confirm if, in fact, the variation in stable isotope values relates to the movement and habitats occupied by juveniles or if they were maternally inherited. If the latter were the case, the first isotope data following birth would allow unique insights into the foraging location and trophic position of pregnant female white sharks prior to parturition (Figure 2). For global white shark populations, knowledge of gestation and parturition of females is extremely limited, given that only a few pregnant individuals have been caught and dissected (Francis 1996; Uchida et al. 1996; Christiansen et al. 2014b). The temporal trend of each individual, however, would only allow estimation of the dilution of the maternal isotope values and incorporation rate of the juvenile's diet, with limited management impact (Figure 2).

Combining genetic analysis with stable isotope analysis is also leading to improved insights regarding animal migration patterns and population connectivity. Populations of Wilson's warblers *Wilsonia pusilla* on the North American east and west coasts were determined to be significantly different, based on microsatellite DNA (Clegg et al. 2003). By combining this result with hydrogen stable isotope values, breeding latitude was estimated, allowing for the description of population connectivity between breeding and overwintering sites. For adult female southern right whales *Eubalena australis* sampled at a nursery ground, genetically related individuals had similar $\delta^{15}N$ and $\delta^{13}C$ isotope values, indicating maternally inherited site fidelity to summer feeding grounds (Valenzuela et al. 2009).

White sharks in Australia have been shown to exhibit philopatric behaviour (Pardini et al. 2001; Blower et al. 2012), with sporadic transoceanic dispersal events (Blower et al. 2012). Equally, juvenile sharks have been documented using multiple nursery areas with relatively restricted home ranges (Bruce and Bradford 2012). These behaviours would indicate that if juvenile sharks off southern Africa are using different nursery grounds, then these individuals might be genetically distinct. It would then be expected that these genetically distinct groups of juveniles would have unique isotope values reflecting their foraging locations. For this juvenile white shark case study, correlations between stable isotope values and genetic diversity would provide support for multiple nurseries and confirm philopatric behaviour for this species, with major implications for management (Figure 2).

Conclusion

In modern science, conveying a message to the broader scientific and management community is typically restricted by the word count allowed by the publishing journal. Moreover, when analysing and interpreting large, complex, ecological datasets, common in the data-rich world of today's science, drawing a single explanation or conclusion is difficult and often impossible. Yet, offering multiple interpretations is now viewed as speculative or the result of poor experimental design and has become frowned upon by a majority of scientific journal reviewers. Consequently, researchers are commonly forced into selecting a single explanation to best describe the trends observed in their data. However, ignoring or avoiding alternative explanations of a dataset to avoid negative reviews can not only undermine the overall message, but also hinder the scientific process. Ultimately, this can limit the development of effective management and conservation plans, particularly those that are adaptive. This case study on juvenile white shark vertebral stable isotope profiles revealed that multiple interpretations could be provided that equally explain the observed isotopic trends. One of these could be highlighted preferentially, dependent on the motive of the study or researcher bias, but this could be either to the benefit or detriment of future white shark research and management actions. In this instance, it could be argued that additional research should be carried out prior to publishing data, but in these pressing environmental times – for example, 46.8% of chondricthyan species are currently listed as Data Deficient (Dulvy et al. 2014) – decisions are required based on the best information currently available. We promote the use of stable isotopes incorporated in a multidisciplinary framework to improve our confidence in interpreting data, particularly when outcomes may inform conservation and management actions. If this is not possible, quality stable isotope datasets, with appropriate statistical analyses and potential alternative explanations, should be clearly presented.

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