Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators

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Human activities have serious impacts on marine apex predators. Inadequate knowledge of the spatial and trophic ecology of these marine animals ultimately compromises the viability of their populations and impedes our ability to use them as environmental biomonitors. Intrinsic biogeochemical markers, such as stable isotopes, fatty acids, trace elements, and chemical pollutants, are increasingly being used to trace the spatial and trophic ecology of marine top predators. Notable advances include the emergence of the first oceanographic "isoscapes" (isotopic geographic gradients), the advent of compound-specific isotopic analyses, improvements in diet reconstruction through Bayesian statistics, and tissue analysis of tracked animals to ground-truth biogeochemical profiles. However, most researchers still focus on only a few tracers. Moreover, insufficient knowledge of the biogeochemical integration in tissues, fractionation and routing processes, and geographic and temporal variability in baseline levels continue to hamper the resolution and potential of these markers in studying the spatial and feeding ecology of top predators.

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Human-induced changes at sea – through fishery activities, climate change, and release of hazardous contaminants, such as metals and persistent organic pollutants (POPs) – are altering the structure and stability of the marine food web (Halpern *et al.* 2008; Lotze *et al.* 2011). Apical marine predators (eg Figure 1) are particularly sensitive to anthropogenic impacts because of their specific life-history traits, such as long life expectancy, delayed maturity, high rates of adult survival, and relatively low reproductive rates (Heithaus *et al.* 2008). Fisheries for lower-trophic-level species have high economical value but are competing for the main resources of marine predators and can affect the viability of their populations (Cury *et al.* 2011). Moreover, longline fishing and episodic pollutant-associated contamination are responsible for the direct mortality of hundreds of thousands of predatory fish, seabirds, and marine mammals worldwide, and are currently driving many species to unsustainable levels through population declines (Peterson *et al.* 2003; Lewison *et al.* 2004). While this is of major conservation concern, some marine predators can also be used to monitor the health of the marine environment better than other groups of basal-trophic-level organisms. For instance, most of these apical species are exposed to high concentrations of pollutants at the top of trophic webs and many have very large breeding ranges; this allows direct comparisons of pollutant levels among remote areas (Aguilar *et al.* 2002; Hobson *et al.* 2004). These organisms are therefore considered as reliable indicators of the spatiotemporal distribution of marine contaminants (eg Roscales *et al.* 2010). Similarly, those species undergoing long-distance movements may provide unique opportunities to compare pollutant levels between breeding and non-breeding areas, as well as to identify sources of pollution in remote areas. In addition, the spatial dynamics of marine top predators have been reported as an accurate indicator of specific ecosystem-wide changes linked either directly to bycatch associated with hooks and nets or indirectly to climate and overfishing (Worm *et al.* 2005). Thus, marine top predators are among the best candidates to assess the ocean's health and sustainability. However, understanding the underlying drivers and the magnitude of the impacts on the marine environment and its top predators requires more comprehensive insight into the feeding ecology and migration of these organisms.

Trophic and spatial ecology of species has been studied for decades through the use of conventional methodologies, such as dietary analyses or animal banding, but these

In a nutshell:

- Improved knowledge of the spatial distribution and trophic relationships of marine organisms is required to better understand human-induced changes to the marine environment
- Recent advances in the analysis of biogeochemical markers in specific tissues provide powerful tools to effectively trace feeding ecology and migration of marine predators
- Combining multiple tracers and tracking devices in novel ways can aid the conservation and recovery of marine top predators, and provide information about the health and sustainability of marine ecosystems

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approaches have often been found to be unsatisfactory when applied to marine top predators. Dietary analyses underestimate easily digestible prey, require exhaustive monitoring over time to study a population's feeding habits, and are difficult or impossible to undertake when species are not breeding. Similarly, banding programs require a huge banding and searching effort over time (ie several decades) but recovery and resighting rates in the open sea are relatively low, and are biased toward coastal populated areas. Recent advances in electronic tracking devices are helping to fill the current knowledge gap regarding migration patterns of marine predators (Figure 2; Block *et al.* 2011), but tracking studies are usually restricted to a few breeding individuals, often tracked for short periods due to logistical and financial constraints. All of these limitations have led to an increased interest in finding alternative means of investigating the trophic and spatial ecology of marine top predators.

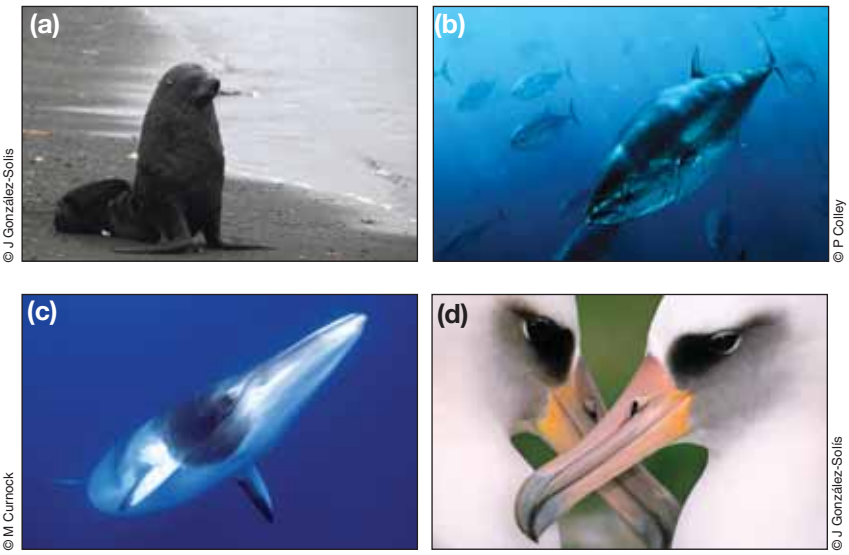

Figure 1. *Uses of intrinsic biogeochemical markers in animal tissues as tracers. (a) The diet, foraging strategies, and migration of several species of seals (including the Antarctic fur seal* Arctocephalus gazella*) are determined by specific isotopic analysis of certain fatty acids in blubber samples or bulk stable-isotope analysis in whiskers (Budge* et al. *2008; Cherel* et al. *2009); (b) natal origin, connectivity, and migration routes, in addition to feeding ecology of different populations of critically endangered tuna species (including the Atlantic bluefin tuna* Thunnus thynnus*), are investigated by analyzing stable-isotope signatures and organochlorine tracers (such as PCBs) in both otoliths and muscle tissue (Ménard* et al. *2007; Popp* et al. *2007; Rooker* et al. *2008; Dickhut* et al. *2009; Olson* et al. *2010); (c) specific feeding patterns and dietary shifts over time can be assessed by determining the elemental and stable-isotope composition of incrementally growing tissues (such as baleen of North Atlantic minke whales* Balaenoptera acutorostrata*; Hobson* et al. *2004); and (d) spatiotemporal assessments of marine pollution can be conducted by analyzing the organochlorine and mercury content of seabirds with wide foraging ranges (such as the Laysan albatross* Phoebastria immutabilis*; Finkelstein* et al. *2006).*

The intrinsic biogeochemical markers

Many chemicals, such as some stable isotopes, trace elements, POPs, or lipids, move through the biotic and abiotic compartments of an ecosystem and can be detected in animal tissues. Several of these chemicals are increasingly used as intrinsic markers, hereafter referred to as tracers, to study the feeding habits or seasonal movements of different organisms (Figure 1; Fisk *et al.* 2002; Budge *et al.* 2008; Rooker *et al.* 2008; Ramos *et al.* 2009a). Although incapable of providing the taxonomic detail achieved by dietary samples or the geographic accuracy of bands and tracking devices, tracers are not hampered by most of the biases and constraints related to these conventional approaches. For example, from the point of view of trophic studies, stable-isotope and fatty-acid analyses of the tissues of predators provide information about the elements and compounds assimilated during digestion, avoiding biases related to prey digestibility. Such analyses can also integrate dietary information from days to years, depending on their biochemical properties and the regeneration rate (ie turnover) of the target tissue (Figure 3; Newsome *et al.* 2007; Caut *et al.* 2009; Williams and Buck 2010). Regarding advantages for spatial ecology, tracer analyses can be conducted extensively on virtually any individual of any species, do not require subsequent animal recovery, and can provide information about movements and past locations (eg breeding or non-breeding areas; Figure 2d) as well as insights into the species' diet in previously occupied areas (Norris *et al.* 2007; Hobson and Norris 2008). However, careful selection of intrinsic markers and target tissues is crucial, given that each tracer and tissue has advantages and limitations, and its interpretation often involves several problematic assumptions (Table 1).

Interest of tracers in trophic ecology

Isotopically speaking, of the 118 known elements, 54 have at least two stable isotopes (ie their nuclides do not decay with time), but only those elements related to the biosphere (plants, animals), the hydrosphere (water), and the atmosphere (gaseous) – ie carbon (C), nitrogen (N), sulfur (S), hydrogen (H), and oxygen (O) – are relevant to ecological research. Although the isotopes of elements have exceedingly similar chemical properties, the slight difference in their mass results in slight differences in reaction kinetics and bond energies, and in the end, this produces differences in isotope abundances in the final product depending on the initial substrate. This process is known as isotopic fractionation and – together with other diverse metabolic phenomena, such as routing processes – it explains why we are not exactly what we eat – that is, why the isotopic composition of a consumer's tissue (final

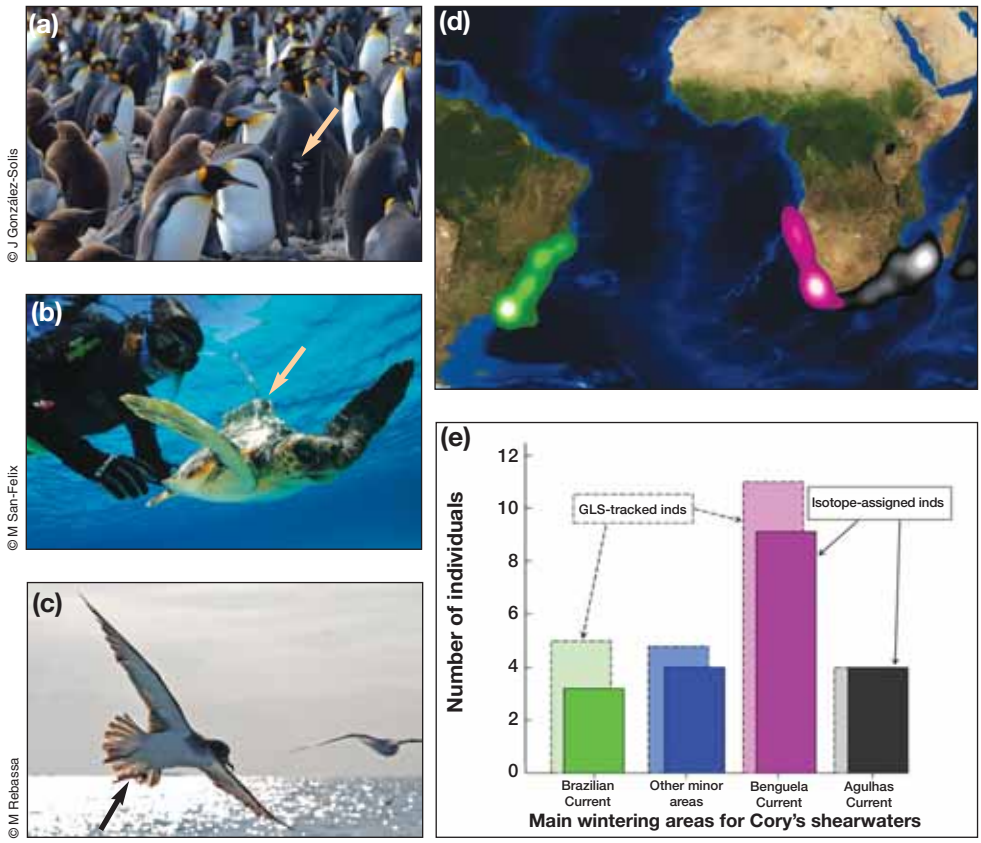

Figure 2. *Many species of marine top predators, such as (a) king penguins* (Aptenodytes patagonicus)*, (b) loggerhead sea turtles* (Caretta caretta)*, and (c) Cory's shearwaters* (Calonectris diomedea)*, can be tracked electronically through the use of Platform Terminal Transmitters, Global Positioning System loggers, or Global Location Sensing (GLS) loggers (arrows). Combining the analysis of different intrinsic biogeochemical markers with electronic tracking of a limited number of individuals offers new ways to reveal foraging areas (Zbinden* et al. *2011), migratory routes, and wintering areas (Ramos* et al. *2009a) of top predators. For instance, when studying the annual migrations of Cory's shearwaters ([d]; 5–90% kernel ranges derived from validated wintering locations), the isotopic composition of feathers molted in winter ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, and $\delta^{18}O$ in the eighth secondary feather) identified the wintering area chosen by each tracked individual: Benguela, Brazilian, and Agulhas currents ([e]; correct cross-validation assignments were 81.8%, 60%, and 100%, respectively; other minor wintering sites included the south-central Atlantic Ocean, Gulf of Guinea, and the Canary Current). The number of individuals tracked with GLS devices is shown in dashed line/light tone bars, whereas the number of individuals estimated through isotopic analyses is represented in solid line/dark tone bars. Further biogeochemical analyses with larger sample sizes should provide stronger evidence and a more meaningful perspective of the spatial ecology of the species of interest. Inds = individuals.*

product; δX_{tissue}) and its diet (initial substrate; δX_{diet}) differ quantitatively (Caut *et al.* 2009). This difference is called isotopic discrimination ($\Delta X_{diet\text{-}tissue}$). Interestingly, this discrimination occurs at each trophic step, summing throughout trophic chains (Figure 4), and thus it can help us to decipher the trophic interactions among organisms (Newsome *et al.* 2007; Boecklen *et al.* 2011). At a local spatial scale, three main stable isotopes (those of C, N, and S) are measured to establish dietary origins and trophic relationships of the species (Table 1). $\delta^{15}N$ in the tissues of consumers reflects dietary protein metabolism, and its values are normally used to infer information about food-web interactions and to show the trophic positions of species (Figure 4; Caut *et al.* 2009). $\delta^{13}C$ is present in all three dietary macromolecules (ie proteins, fats, and carbohydrates), reflecting the various dietary sources of the consumer's tissues. Sulfur in the consumer's tissues is usually only present in S-containing amino acids like cysteine and methionine, and therefore its isotopic analysis exclusively represents protein pathways derived from diet. Typically, $\delta^{13}C$ and $\delta^{34}S$, due to their smaller discrimination as compared with that of $\delta^{15}N$, allow us to trace the different origins of these element inputs into food webs (see WebPanel 1). However, one must be careful about making isotopic comparisons across samples or studies because different factors, such as tissue type, age, body size, consumer's nutritional condition, or dietary quality, contribute to variation in isotopic signatures and the discrimination process (Cherel *et al.* 2005; Forero *et al.* 2005; Caut *et al.* 2009; Boecklen *et al.* 2011).

Although less common than stable-isotope analyses, trace elements and several organic pollutants have also been used to trace the dietary preferences of a great variety of marine organisms (Fisk *et al.* 2002). Specifically, some trace elements, such as mercury (Hg), arsenic (As), cadmium (Cd), and selenium (Se), and several POPs, in particular polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), bioaccumulate in lipid-rich tissues and biomagnify (increase in concentration through the food chain), indicating the trophic position of the consumer (Figure 4; Becker *et al.* 2002; Stewart *et al.* 2004; Bentzen *et al.* 2008). However, as with isotopes, many other factors can also influence trace element and pollutant burdens among consumers, for instance branchial ion uptake, ecosystem compartments (mesopelagic versus benthic prey), trophic steps, specific metabolic rates – greater in homeotherms (those organisms that maintain a stable internal body temperature) than in poikilotherms (the internal temperature of

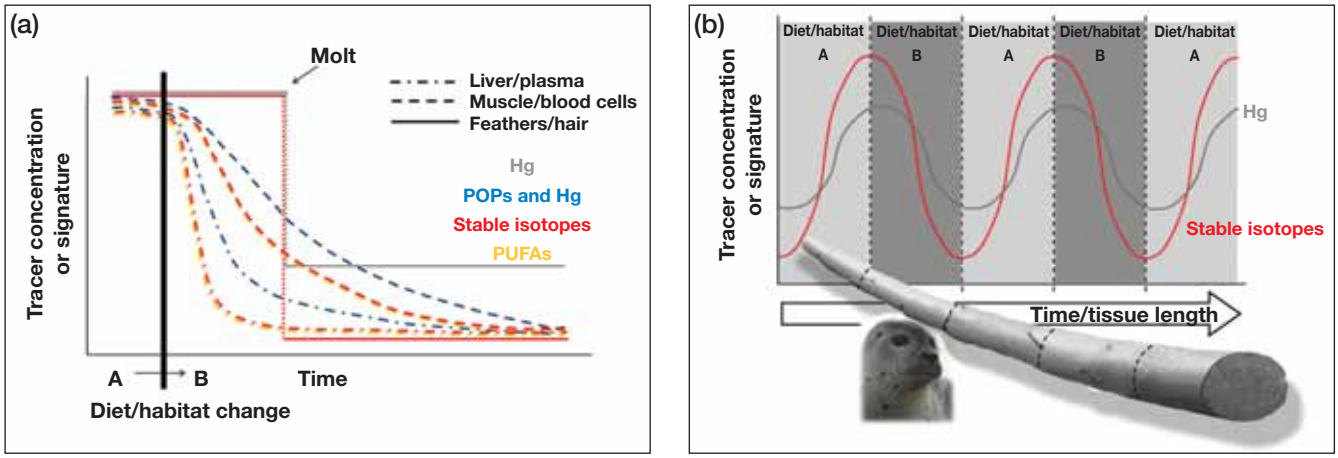

Figure 3. *Tracer dynamics in the tissues of a marine predator that changes either its diet or its habitat (from "A" to "B"); these diets or habitats are biogeochemically distinct. (a) Tissues are grouped according to their turnover rate: liver and plasma (dash-dot lines) can integrate new chemicals from days to a week; muscle and blood cells (dashed lines) can integrate new chemicals from weeks to months; and some keratinous tissues with determinate growth, such as feathers and hair (solid line), can integrate new biogeochemicals from months to years, depending on molting period (dotted lines) (Foglia* et al. *1994; Hobson 1999; Buchheister and Latour 2010). Contrasting dynamics among the tracers can be noted: stable isotopes (in red) and polyunsaturated fatty acids (PUFAs, in yellow) decrease faster than persistent organic pollutants and mercury (POPs and Hg, in blue). For PUFAs, depicted changes only refer to changes in diet. Note that keratinous tissues inherently lack PUFAs and POPs in their chemical structure. (b) Hg (gray line) and stable-isotope (red line) dynamics in calcified tissues (eg teeth, otoliths, bones), chitinous tissues (eg gladius from squid), or keratinous tissues with continuous/indeterminate growth (eg whiskers and claws from seals, scutes from turtles, baleen from whales, claws from seabirds) from a hypothetical marine predator consuming two distinct diets or inhabiting two different biomes throughout its annual cycle.*

which varies considerably with external temperature) – and several other factors, such as age, sex, body size, genetics, reproductive status, and nutritional condition (Aguilar *et al.* 2002; Becker *et al.* 2002; Verreault *et al.* 2009).

Similarly, the fatty-acid signatures of marine species can be traced through several trophic levels up to top predators. Vertebrates are not able to synthesize some fatty acids de novo (eg some long-chain polyunsaturated fatty acids; PUFAs). Prey fatty acids are deposited into consumer adipose tissue with little modification and, in a predictable way, providing insights into the pathways of energy and nutrient transfer (Dalsgaard *et al.* 2003; Iverson 2009; Williams and Buck 2010). As compared to other tracers, the great diversity of these lipid compounds, the suggested absence of geographic variability in baseline levels in some of them, and their unique origin among plants and animals become key points in their applicability (Iverson 2009).

Ultimately, the fundamental theory of each tracer is what defines its ecological interest. For instance, whereas

Table 1. Major traits among intrinsic biogeochemical markers (tracers) used to study the feeding ecology and migration of marine top predators (numbers in square brackets refer to references listed in WebPanel 2)

	Stable isotopes	*Trace elements*	*Lipids*	*Persistent organic pollutants*
Main biogeochemicals	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Hg, Cd, Se, Pb	Fatty acids	DDTs, PCBs
Geographical changes in baseline levels	Yes [1]	Yes [2,3]	Only specific cases [4,5]	Yes [6,7]
Geographic gradients	$\delta^{13}C$ [1,8,9], $\delta^{15}N$ [1,10]	No [3]	No [5]	Possibly [6,7]
Spatial resolution	Relatively low	High [11]	Only specific cases [5]	Low [6,7]
Indicators of trophic level	$\delta^{15}N$ [12]	Hg, Se, Cd [13,14,15]	Only specific cases [4,5]	Some DDTs, PCBs [6,7,16,17]
Potential to reveal specific food sources	$\delta^{13}C$, $\delta^{34}S$ [18,19]	Yes [20]	Yes [4,5]	Low [16,17,21]
Freshwater vs saltwater habitat food sources	$\delta^{13}C$, $\delta^{34}S$ [18,19,22]	Hg [13,20]	Yes [4]	No
Inshore/benthic vs offshore/pelagic food sources	$\delta^{13}C$ [8]	Hg [13]	Yes [4,23]	DDTs, PCBs [16]

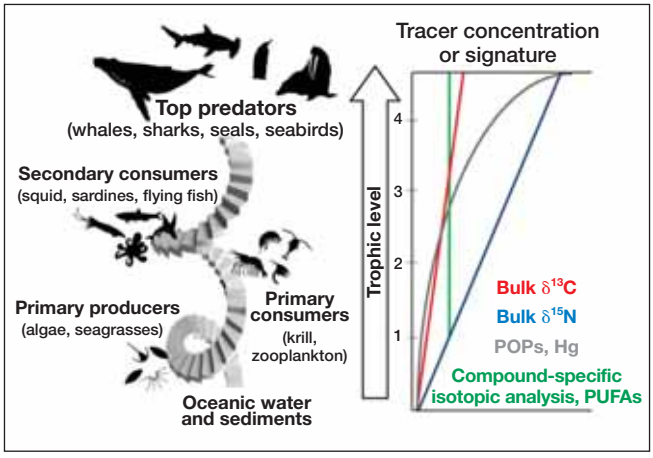

Figure 4. *Tracer dynamics along the marine food chain. Contaminant biomagnification (in gray) increases exponentially with each step of the food chain because contaminants accumulate in tissues faster than they can be metabolized or excreted. In consequence, marine top predators such as whales, seals, sharks, or seabirds are at a greater risk of suffering toxic effects from contaminants than lower-trophic-level organisms. Stable isotopes of N in bulk organic tissues (in blue) increase proportionally 3–4 per mil (‰) with each trophic level, as a result of the animal's metabolism. Isotopic signatures of essential nutrients and PUFA profiles (in green) show little or no change along the marine food web because animals lack the ability to synthesize these compounds.*

stable isotopes typically provide information about the source of proteins (given that samples are usually lipid-extracted prior to isotopic analyses), fatty acids account for the source of lipids. Nevertheless, the different origin and temporal integration of the tracers can offer complementary information as different pieces of the same biogeochemical puzzle. Despite these caveats, studies using tracers have flourished over the past decade to reconstruct the diets of sharks, marine mammals, and seabirds, among others (Hobson *et al.* 2004; Iverson 2009), to characterize trophic relationships among species and individuals (Fisk *et al.* 2002; Budge *et al.* 2008), and to elucidate food-web structuring (Forero *et al.* 2005) as well as the physiological status of the individuals (Cherel *et al.* 2005; Williams and Buck 2010).

■ Uses of tracers in spatial ecology

Dietary elements and compounds ultimately derive from soils, geological substrates, and sources of contamination close to or around the area where tissues are formed or maintained, and are integrated in prey tissues and ultimately in their predators. Isotopic signatures – such as those of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ – but also many trace elements and pollutants are known to vary geographically (Figure 5) as a result of differences in nutrient cycling at the base of the food web or different sources of contamination (Dickhut *et al.* 2009; Szép *et al.* 2009; Graham *et al.* 2010). For example, $\delta^{13}C$ signatures typically change at high latitudes, depending on the productivity of the area and the proximity to nearshore or benthic habitats (Cherel and Hobson 2007; Cherel *et al.* 2009), the food-web structuring (Forero *et al.* 2005), or the changes in the diet of predators among different areas (Hobson *et al.* 2004), providing spatially related variability in the biogeochemical composition of the tissues. Given that top predators often move thousands of kilometers over the course of a year and usually show low levels of genetic structuring due to extensive gene flow, different tracers identified in their tissues can be used as geographical markers to identify the regions in which the consumer's tissues were formed. Finally, the tissues provide an overall biogeochemical fingerprint to identify non-breeding areas (Figure 2d), to distinguish between resident and immigrant individuals in a specific area (Herman *et al.* 2005; Ménard *et al.* 2007; Graham *et al.* 2010), to identify interspecific and intersexual segregation in foraging areas (Becker *et al.* 2002; Forero *et al.* 2005), to decipher migratory movements and foraging ecology of marine predators in historical times (Hobson *et al.* 2004; Quillfeldt *et al.* 2010; Rayner *et al.* 2011), or to study natal homing and population mixing (Rooker *et al.* 2008; Dickhut *et al.* 2009).

In this context, the identification of geographic spatial gradients of tracers becomes crucial, because these tracers help us to assign any animal to a specific area with a single capture. In the Atlantic Ocean, preliminary attempts to describe isotopic geographic gradients, also called isoscapes, in $\delta^{13}C$ and $\delta^{15}N$ signatures have been undertaken based on plankton values (Figure 5; Graham *et al.* 2010); however, at the moment, the spatial resolution of such maps in the marine environment remains disappointingly low (ie thousands of kilometers). In the Southern Ocean, a more decisive decrease in $\delta^{13}C$ signatures with latitude has been ground-truthed for several seabird species and can readily be used to assign different top predators to the specific areas where their analyzed tissue was formed (Cherel and Hobson 2007). Similarly, $\delta^{15}N$ isoscapes have also been ground-truthed for tropical Pacific tunas (Olson *et al.* 2010). However, some large oceanic areas, such as the northeast temperate Atlantic, may not exhibit such isotopic gradients (Roscales *et al.* 2011). All the aforementioned studies determined variation in stable-isotope signatures across the ocean by sampling the relevant species and tissues throughout several localities, but this is costly and not always feasible. In this sense, sampling the appropriate tissues from animals tracked with different types of electronic devices is emerging as a powerful tool that can potentially be used to understand the spatial variability of a wide variety of tracers across the oceans (Finkelstein *et al.* 2006; Ramos *et al.* 2009a; Zbinden *et al.* 2011). Despite these substantial advances, there is still much room for improvement, since most studies on the spatial ecology of predators have so far been based on only a few tracers.

In addition to spatial variability, temporal changes in physicochemical and biological composition of primary

producers and consumers are crucial for defining tracer gradients in the oceanic realm. For instance, seasonal fluctuations in stable-isotopic signatures of zooplankton (Hannides *et al.* 2009) are able to be transferred up food chains to top predators, raising concerns about the seasonal stability of biogeochemical values. Temporal variations in the signal of tracers, both at the base of the food web and at the predator level, hamper our ability to draw precise and stable gradients/isoscapes to further track the movement of marine top predators in oceanic ecosystems (Graham *et al.* 2010). This issue can be particularly problematic when contaminants are used as tracers, because their spatiotemporal concentrations depend on discharge from human activities, transport, deposition, persistence, and accumulation in different compartments of the ecosystem (Aguilar *et al.* 2002). The temporal stability of tracers at baseline levels must therefore be considered and incorporated when developing biogeochemical maps and isoscapes for the marine environment.

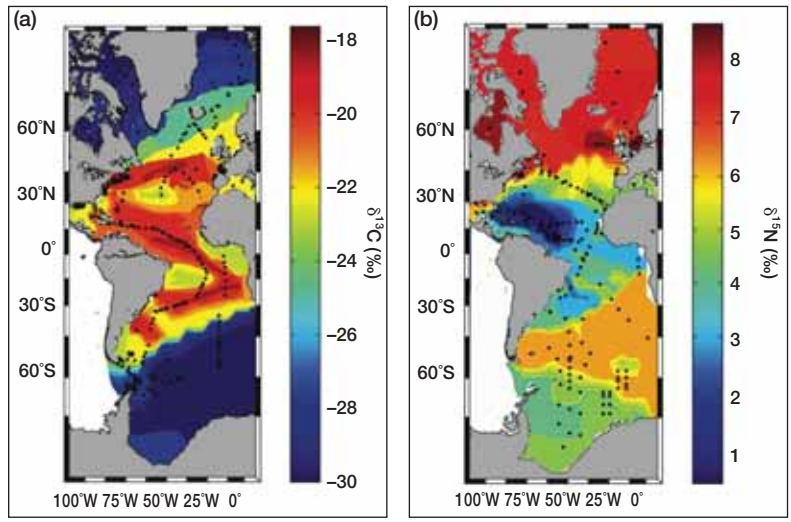

Figure 5. *Isotopic contours (isoscapes) of the Atlantic Ocean from a meta-analysis of published data. Geographic gradients in (a) $\delta^{13}C$ and (b) $\delta^{15}N$ signatures based on plankton samples collected in the Atlantic (sampling locations, between 0–500 m of depth, depicted as black circles; figure from Graham* et al. *2010).*

The relevance of the tissue, integration process, and excretion routes

Several advances in mass spectrometry have led to the possibility of processing smaller quantities of samples in greater numbers, faster, and with improved accuracy and lower costs, thereby providing opportunities to trace ecological processes. However, because many of these tools were originally developed for applications in geochemical systems, there are gaps in our understanding of how these biochemicals become incorporated into biological tissues, the timescales over which they can be used, and the processes that contribute to their distribution, mixing, and turnover within organisms. Each tracer integrates into tissues in a different manner and over different time periods (Figure 3). For instance, while stable-isotope signatures and fatty acids are promptly transferred from the diet to the tissues, trace elements and some pollutants are intentionally mobilized to various tissues and organs with accumulation or excretion purposes, showing a slower and more complex response to changes in diet or habitat (Ramos *et al.* 2009b; Bond 2010). Therefore, when designing any ecological study based on biogeochemical tracers, the selection of the target tracers and tissues becomes a key issue. For stable isotopes and fatty acids, their spatiotemporal integration can range from days to months, depending on the metabolic replacement of proteins and lipids, respectively, of the analyzed tissue. Tissues with high turnover (eg plasma or liver) will integrate isotopic and fatty-acid forms incorporated in the relatively recent past, whereas those tissues that are slowly replaced (eg red blood cells, muscles, or adipose tissue) will integrate over longer time periods (Figure 3a; Foglia *et al.* 1994; Hobson 1999; Buchheister and Latour 2010). Alternatively, tissues that are formed over a specific period but without turnover after their formation – such as eggs, fish scales, calcified tissues (eg teeth, otoliths, bones), or keratinous tissues (eg hairs, whiskers, nails, carapace scutes, baleen, feathers), from a large variety of organisms – are particularly advantageous for ecological research because they remain chemically inert after their formation and can provide information from the same individual over a period of time. It has been largely assumed, then, that isotopic forms of these tissues reflect the composition of the area where they have matured (Hobson and Norris 2008). Thus, specific portions of those keratinous tissues with well-known growth patterns can be sampled at any time of the year to examine feeding habits or geographic locations at concrete periods, regardless of the sampling date (Figure 3b; Hobson *et al.* 2004; Cherel *et al.* 2009; Quillfeldt *et al.* 2010). Pollutant molecules are concentrated in different tissues according to their biochemical properties. For example, organochlorine or organometallic compounds, given their non-polar character, are concentrated in the lipid fraction of the tissue. For such molecules, fatty tissues (eg hypodermic fat) would be the best choice; in the same way, trace metals mainly attached to metallothioneins are concentrated principally in liver, kidney, and muscle, although some metals, such as Hg and lead (Pb), can also be excreted in keratinous or calcified tissues. Although some organs can only be sampled through invasive procedures, many keratinous tissues can be sampled without any detrimental effects; non-destructive biopsy of fat from free-living animals is also feasible in some cases (Owen *et al.* 2010). In addition, recent analytical improvements allow for the analysis of virtually all tracers via minimally invasive procedures, such as blood sampling (Henriksen *et al.* 1998; Roscales *et al.* 2010; Williams and Buck 2010).

The potential of combining tracers in novel ways

As the distribution and integration of tracers in ecosystems and within tissues, respectively, are being clarified, the next aim for ecological research is the simultaneous use of multiple tracers (eg Hebert *et al.* 2009). However, by continuing to rely on more traditional methods, many researchers focus only on part of the entire biogeochemical spectrum. For instance, much attention in trophic and migration studies has focused on both $\delta^{13}C$ and $\delta^{15}N$ signatures, but little research has been conducted on combining those signatures with $\delta^{34}S$, elemental composition, or lipid signatures in study tissues. In spite of this, recent evidence suggests that these relatively unexplored biogeochemicals could be applied to more effectively discriminate among trophic sources (eg Becker *et al.* 2002; Connolly *et al.* 2004; Herman *et al.* 2005), indicate geographical origin (eg Dickhut *et al.* 2009; Roscales *et al.* 2010), and trace migratory movements (eg Szép *et al.* 2009) with more robustness than by relying on $\delta^{13}C$ and $\delta^{15}N$ alone.

Up to now, few ecological studies have successfully combined stable isotopes with trace metals, organochlorine compounds, or fatty acids (eg Herman *et al.* 2005). In some cases, combination with genetic markers and morphological measurements may also provide synergistic effects, such as identifying the origin of predators or assessing ecological divergence among populations (Gómez-Díaz and González-Solís 2007; Foote *et al.* 2009). Recent DNA-based approaches potentially provide more accurate methods for precise diet reconstructions from dietary samples (eg Pompanon *et al.* 2011), but its potential combination with several tracers remains unexplored. Carbon and N signatures have repeatedly been used to identify and quantify the influence of trophic position, food-web structure, and physiology in controlling the accumulation of pollutants in predator tissues (Fisk *et al.* 2002; Stewart *et al.* 2004; Roscales *et al.* 2010). Although these studies have provided important insights into pollutant dynamics and feeding ecology, the independent use of different tracers hampers their overall potential and precision. The simultaneous combination of multiple tracers in common statistical approaches (eg mixing modeling frameworks or in discriminant analyses) has also been largely neglected, despite the potential to optimize the precision of dietary estimations and geographical assignments. However, caution is also needed when combining and interpreting the results of multiple tracers simultaneously, because the functional disparity with which tracers are integrated into tissues (eg for storage, excretion, or rapid consumption) may result in spurious relationships with no biological relevance (Bond 2010).

Conclusions and perspectives

Since the first predictions and calls for appropriate biogeochemical methodologies were conducted a decade ago, there have been substantial improvements in isotopic ecology, and the use of tracers in animal ecology has flourished (Hobson and Norris 2008). Ongoing technological and statistical advances in biogeochemical analysis, such as the use of compound-specific isotopic analyses or the adoption of Bayesian approaches in dietary estimations using mixing models, will likely revolutionize ecological research in the coming years. Yet, in spite of these promising developments, there are still numerous important questions about biogeochemical integration that remain unanswered. For instance, there needs to be a much deeper understanding of the fractionation and routing processes of these tracers in different tissues for their proper interpretation. Several studies have found consistent differences in biogeochemical composition among specific tissues according to their nature and origin. Therefore, the specificity of discrimination factors among different tissues – as well as potential differences in kinetics among biogeochemicals (eg stable isotopes versus contaminants) – must be considered when using these tracers. Moreover, research into several effects, such as the growth state, temperature, O stress, as well as other metabolic factors that influence the incorporation of tracers into tissues, will enhance our ability to use and interpret multiple tracers in an ecological context. Undoubtedly, exhaustive laboratory-based experiments will be required because most of these issues can only be resolved experimentally. Although our call for comparative experiments is not innovative, there is certainly some urgency for conducting them; we therefore echo previous calls for laboratory-based experiments that aim to clarify the biogeochemical integration processes.

Despite evidence that biogeochemical gradients exist in some oceanic water masses, to date, few researchers have analyzed spatial variability of tracers in the marine environment; where such studies have been carried out, they often ignored potential dietary shifts as well as temporal changes in baseline levels. Similarly, trophic studies based on tissue composition consider biogeochemical fractionation throughout the food web only, ignoring geographic variability in the tracers. We emphasize the need to integrate temporal, spatial, and trophic variability when studying both the feeding ecology and migration of marine predators. In this regard, the advent of and continuous improvement in the electronic devices used to track the foraging and migration movements of marine animals will be crucial in teasing apart the different sources of variability among and within tracers. Sampling and analyzing the relevant tissues of tracked animals and matching this to their movements over the course of several years will allow temporal, spatial, and trophic effects on multiple tracers to be distinguished. In addition, the accuracy of dietary information and spatial resolution of isotope-based studies should improve by expanding the spectrum of biogeochemical parameters measured. For instance, by exploiting a greater number of chemical parameters rather than only a few isotopic elements, ecological studies using mineral and pollutant profiles will be

better equipped to assess diets or specific areas used by top predators. Thus, once the different sources of variability are identified and discrimination power is increased, this biogeochemical approach will provide important evidence for assigning specific foraging and non-breeding areas, dietary variability during periods when animals are inaccessible, seasonal interactions between breeding and non-breeding areas, and population connectivity of marine predators.

Such advances in the use of tracers can be applied in many different ways, to resolve specific conservation and management issues in several fields, including fishery management, environmental health, and conservation biology. Results obtained from only a minimally invasive sampling of specific tissues – eg a small amount of keratin, fat, or blood tissue, from just a few animals – can help answer typical management questions related to population dynamics, contamination, or anthropogenic impacts. These questions include, but are not limited to, determining main food sources and trophic relationships, assessing exposure to contaminants, distinguishing resident from migrant animals, identifying population units in species with cryptic population structuring, assigning target or accidental catches to their population of origin, and locating wintering, breeding, or feeding grounds. Resolving these questions not only could improve the conservation and management of predator populations but will be essential in effectively monitoring the sustainability and health of marine ecosystems.

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