Chapter 12
Combining Isoscapes with Tissue-Specific Isotope Records to Recreate the Geographic Histories of Fish

Ernst B. Peebles and David J. Hollander

Abstract After the *Deepwater Horizon* (DWH) oil spill, interest in marine animal movement was heightened by recognition that some individual animals had been cryptically exposed to the oil, and that some of these exposed individuals later moved, introducing oil contamination to geographic areas that were beyond the initial domain of direct oil impact. Forensic methods based on internally recorded stable-isotope records can be used to address the issue of movement by contaminated individuals. Different tissues provide stable-isotope histories that reflect different periods in the individual's history, ranging from just a few recent days in the case of blood plasma to the entire lifetime in the case of eye lenses and otoliths. Isotopic offsets between tissue types (e.g., liver and muscle) within the same individual can be used to measure the relative site fidelities of different individuals. Among individuals that have low site fidelity, geographic movements can be estimated by comparing lifetime isotope trends with background maps of isotope variation (isoscapes). The process of isotope conservation within the vertebrate eye lens is described, and practical application of forensic methods and data interpretation are discussed.

Keywords Isoscapes · Isotope gradients · Site fidelity · Animal migration · Cryptic exposure · Tissue-specific isotope analysis · Eye-lens anatomy · Crystallins · Eye-lens isotopes · Compound-specific isotope analysis

12.1 Introduction

There are many reasons for investigating the geographic movement of animals, and most of these involve resource management. In the case of the *Deepwater Horizon* (DWH) oil spill, interest in marine animal movement was heightened by concerns that some animals would not or could not avoid contaminated waters and sediments,

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and others would become contaminated and then move, introducing the contamination to areas that were beyond the initial domain of direct oil impact.

In cases of direct oiling of animals by crude oil floating at the sea surface, the general location and means of animal contamination are often readily apparent, as oiled animals are often directly observed to be near or within areas contaminated by surface oil. In contrast, the processes behind other more cryptic types of oil exposure are usually difficult or impossible to recognize visually. One cryptic process is body contact with crude oil in the form of its water-accommodated fractions (WAF). WAF includes both dissolved oil components and invisible microdroplets of oil that are not buoyant enough to overcome friction with seawater and thus remain suspended at depth in accordance with Stokes' law. Other forms of cryptic oil exposure include consumption of oil-exposed prey, ingestion of WAF while drinking seawater to maintain osmotic balance, and body contact between benthic animals and oil-contaminated sediments. Detection of cryptic oil exposures requires chemical analysis of soft tissues (e.g., muscle, liver), body fluids (e.g., blood, bile), or hard parts (e.g., otoliths, Granneman et al. 2017).

Once cryptic oil exposure has been detected, the question arises "Where did the exposure to oil take place?" This is a relevant question because there is an important distinction between a fish being exposed to oil elsewhere and then swimming into local waters and a fish being cryptically exposed within local waters. While neither outcome is desirable, the second outcome means there was a source of cryptic oil in local waters, even if no surface oil had been previously detected there [i.e., undetected oil could exist locally as invisible WAF, as sediments containing oil-bound marine snow (MOSSFA), as interstitial sediment porewater WAF, or as contaminated prey moving into local waters]. For example, Murawski et al. (2014) reported oil-related PAHs in red grouper from areas of the West Florida Shelf that had not been exposed to surface oil.

In the likely scenario where there is no artificial tagging program available that can recreate the individual geographic histories of fish that have had known, cryptic, exposure to oil, the researcher is left with forensic approaches for addressing the geographic exposure question. There are several hard and soft tissues that can be used to forensically recreate the lifetime histories of individual fish; these approaches were recently reviewed by Tzadik et al. (2017). One of the most promising forensic approaches involves stable-isotope analysis of various tissues. Different tissues provide stable-isotope histories that reflect different periods in the individual's history, ranging from just a few recent days in the case of blood plasma to the entire lifetime in the case of eye lenses and otoliths. During the course of recreating individual geographic histories, other information obtained from stable isotopes is likely to shed light on the individual's trophic position and basal-resource dependence (i.e., whether the individual ultimately depended on phytoplankton, benthic algae, or a mixture of these as biomass drivers at the base of its food web). All of this new insight has potential utility toward developing a process-based understanding of oil-spill impacts.

Once individual isotopic histories have been obtained, interpretation requires comparison of the individual's stable-isotope values with background values from the seascape around it. Maps of background stable isotopes are referred to as "isoscapes" (Fig. 12.1).

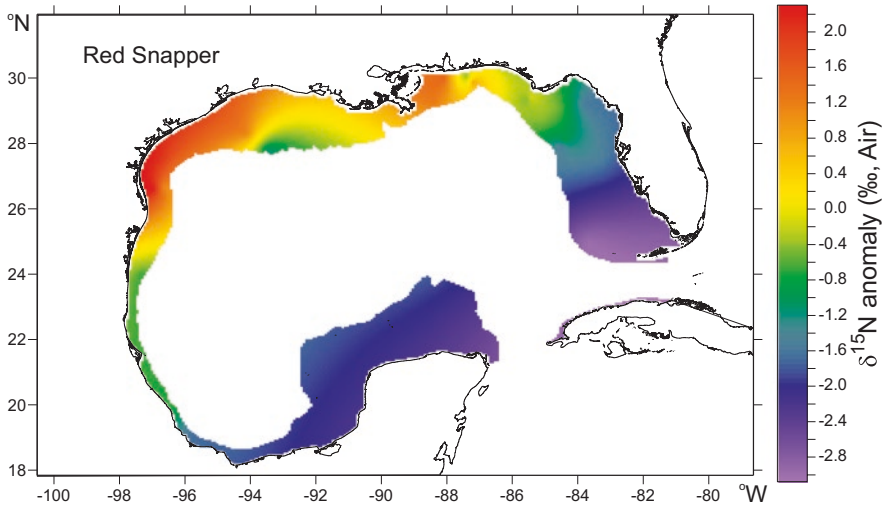


Fig. 12.1 Empirical isoscape (based on kriged data, not a statistical model) of continental shelf $\delta^{15}N$ based on red snapper (*Lutjanus campechanus*) muscle, expressed as differences from the grand mean value. The grand mean was subtracted because correlations between $\delta^{15}N$ and either fish weight or fish length were not significant. This isoscape is derived from 34 locations distributed around the perimeter of the Gulf of Mexico

12.2 Marine Isoscapes

As recently as the 2000s, marine isoscapes were unavailable for most of the world's oceans and coastal waters. The large-scale isoscapes that were produced (e.g., those presented by McMahon et al. 2013) were the products of correlative models that helped interpolate among empirical observations, and these empirical observations were often spaced far apart from each other. Although isoscape models may appear to provide detailed depictions of isoscapes across large areas of the world's oceans, they often do so in areas (such as the Gulf of Mexico (GoM)) where there were very few empirical observations to support the models. Given the lack of detailed, empirical isoscapes, marine ecologists began to create higher resolution isoscapes for their own geographic regions of interest, sometimes including assessments of seasonal and annual variation (e.g., Radabaugh et al. 2013; MacKenzie et al. 2014; Radabaugh and Peebles 2014). These isoscapes have focused on $\delta^{13}C$, $\delta^{15}N$, and to a lesser extent, $\delta^{18}O$, whereas terrestrial isoscapes, which have received far more research attention than marine isoscapes, have focused on $\delta^{2}H$ (deuterium) from precipitation in addition to vegetation-based $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ (Hobson and Wassenaar 2007).

The precipitation-based $\delta^{2}H$ and $\delta^{18}O$ isoscapes used by terrestrial ecologists reflect predictable phase change, Raleigh distillation, and mixing-based offsets that generally coincide with latitude and climate ("meteoric" effect, Smith and Freeman 2006; West et al. 2010; note that $\delta^{2}H$ and $\delta^{18}O$ isoscapes can also be derived from plants). As precipitation-borne isotopes become incorporated into

animal tissues, including hard parts (i.e., bird feathers and mammal teeth and hooves), additional fractionations take place, and the resulting offsets are modeled using "transfer functions" to produce organism-specific isoscapes.

Most of these computations can be automated online by the IsoMAP program (https://isomap.rcac.purdue.edu/isomap/), which assigns geographic-origin probabilities (gridded map files) to each individual animal. The user must calibrate the transfer functions using empirical data specific to his or her study and may post-process IsoMAP's output to accommodate additional information not supported by IsoMAP's algorithms (Bowen et al. 2014). The methods for developing and using marine isoscapes are not as advanced as the methods associated with IsoMAP and terrestrial isoscapes. There is currently no marine equivalent of the IsoMAP online workspace, and IsoMAP offers little direct utility to the marine ecologist other than to provide an example of a successful, automated approach that could be mirrored for application to the marine environment.

Marine $\delta^{13}C$ and $\delta^{15}N$ isoscapes may be based on isotope measurements of primary producers (trophic position 1.0), herbivores (trophic position 2.0), or omnivores and predators (trophic position >2.0). Basing marine isoscapes on herbivores such as bivalves, sponges, tunicates, or sea urchins (Radabaugh et al. 2013) is particularly appealing because herbivores integrate short-term fluctuations in the primary-producer isotopic baseline, and, unlike phytoplankton, many are either stationary (sessile organisms) or slow-moving (sea urchins). Terrestrial ecologists often assume spatial stationarity (i.e., little or no spatial variation in isoscapes over time), whereas marine ecologists generally recognize that this assumption is more problematic in the marine realm, where dynamic processes prevail. In all cases, isoscapes can only be useful if they capture spatial variation, and isoscapes are most useful if the cause of spatial variation is understood.

12.3 Overview of Variation in $\delta^{13}C$ and $\delta^{15}N$ Isoscapes

In coastal ecosystems, prominent causes of variation in baseline $\delta^{13}C_{biomass}$ include freshwater runoff or tidal exchange with slow-moving backwaters (notably swamps and marshes), where DIC (dissolved inorganic carbon) evolving from microbial respiration of primary-producer detritus is more likely to be recycled into new primary production, causing a portion of the DIC molecules to undergo repeated photosynthetic fractionation, dramatically reducing $\delta^{13}C_{biomass}$ (photosynthetic fractionation is strongly negative) and contributing to a trend of progressively more depleted $\delta^{13}C_{DIC}$ (Keough et al. 1998). Low (<−22‰) and highly variable $\delta^{13}C$ values are associated with the DIC recycling process.

$\delta^{13}C_{biomass}$ is also indicative of the light environment, with primary producers from lower-light settings having higher $\delta^{13}C_{biomass}$ values (less photosynthetic fractionation) than those from higher-light settings (higher photosynthetic fractionation); at a global scale, benthic algae have about 5‰ higher $\delta^{13}C_{biomass}$ values than phytoplankton from the same location (Radabaugh et al. 2014 and references cited therein), and this difference gets passed on to higher trophic positions, providing an indication

of individuals' relative dependence on different basal resources (i.e., plankton-based vs. benthos-based primary producers). Benthic algae become unimportant beyond the outer continental shelf due to lack of light reaching the sea floor, and DIC recycling is less likely in the deep sea due to a relative lack of plant detritus and higher likelihood of CO_2 molecules fluxing into the global ocean and atmosphere as a result of turbulent water flows (i.e., thus becoming less available for local recycling). As a result, the $\delta^{13}C_{biomass}$ values of open-ocean phytoplankton tend to be somewhat monotonous over space and time, hovering between −19 to −22‰ (but can be as high as −16‰ and as low as −30‰, Rau et al. 1989) at most lower and middle latitudes (McMahon et al. 2013).

Finally, $\delta^{13}C$ is also strongly influenced by the type of vascular plant that contributes detritus to the food web (C3, C4, and CAM photosynthetic pathways). However, in the coastal ocean, these distinctions are only important at locations that receive vascular plant detritus from wetlands or from riverine inputs and at locations that define the transition between mangroves (C3) and *Spartina*-dominated (C4) coastal biomes.

$\delta^{15}N$ has particular utility in recreating individual trophic and geographic histories (Wallace et al. 2014; Quaeck 2017), as it has been demonstrated that geographic variation in $\delta^{15}N_{biomass}$ often exists as predictable gradients that are potentially useful for reconstructing the geographic histories of individual animals (McMahon et al. 2013; Radabaugh et al. 2013; Radabaugh and Peebles 2014). These $\delta^{15}N_{biomass}$ gradients exist between areas of the coastal ocean where the mass-balanced influences of surface runoff from land (and possibly denitrification, cf. Chang et al. 2002) result in elevated $\delta^{15}N$ and oligotrophic areas where nitrogen fixation is dominant and $\delta^{15}N_{biomass}$ is low (e.g., oceanic gyres and other nitrogen-poor seas). In fishes, individual histories for $\delta^{15}N$ are of particular interest because $\delta^{15}N$ is difficult to obtain from otoliths due to the otoliths' low nitrogen content, and thus the ability to obtain lifetime $\delta^{15}N$ histories for fishes is particularly novel.

12.4 Effects of Scale on Isoscapes

Given that the above causes of isotopic variation may vary at different spatial and temporal scales, it can be expected that the patterns observed within isoscapes may also be sensitive to the effects of scale. For example, at a scale of hundreds of kilometers in the eastern GoM (Fig. 12.1), the general trend in $\delta^{15}N$ is an increase from low values toward the southeast, where nitrogen fixation is more common, to higher values toward the northwest, where large riverine inputs are present (Atchafalaya/Mississippi/Mobile Rivers). Yet at a much smaller scale within this range, at a span of tens of kilometers, this trend is reversed as one moves northward away from the mouth of the Suwannee River, which has elevated $\delta^{15}N$ values (Michael Poniatowski, FWC, pers. comm.). The Suwannee River is a much smaller river than the others, and thus it does not contribute substantially to the larger-scale trend observed at the scale of hundreds of kilometers, yet it nevertheless creates its own localized trend, one that is recognizable at the smaller scale of tens of kilometers.

12.5 Tissue-Specific Isotope Analysis

When conducting stable-isotope studies, the primary reason for considering tissue type is selection of the time scale that is most relevant to the research question being addressed. Any vascularized tissue, even bone and teeth, can be expected to turn over (i.e., to have its mass partially or totally replaced by new tissue over time). Turnover rates are usually described as the length of time required to reach a specified percentage of turnover (e.g., the number of days required to reach 90% turnover). Different vascularized tissues (blood, liver, muscle, bone, etc.) typically have different turnover rates, generally ranging from a few days in the case of blood plasma to about a year in the case of bone (Gaston and Suthers 2004; Buchheister and Latour 2010; Heady and Moore 2013).Turnover rates can vary substantially with variation in the individual's growth and metabolic rates (MacNeil et al. 2006).

In stable-isotope studies of aquatic organisms, muscle has been the most commonly analyzed tissue type. At lower latitudes such as the GoM, isotopes from muscle represent conditions over a duration of several months, more or less, prior to the specimen's collection date. However, small organisms are often analyzed intact due to difficulty isolating enough mass of a given tissue type; this results in an isotope value that reflects the mass balance of the contributions of different tissue types to the overall dry weight of the analyzed sample. Very small organisms such as phytoplankton cells or zooplankton may be composited (i.e., multiple cells or individuals dried, ground to powder, and analyzed together). It should be noted that differences in the types of tissues analyzed can lead to the false perception of isotopic differences between organisms that do not actually exist.

12.6 Site Fidelity Based on Two or More Tissues (Tissue Comparison Method)

The fact that isotopic turnover rates are tissue-specific can be exploited to provide new insights. Given enough time after an isotopic diet shift, isotope values within different tissues will eventually equilibrate with the new diet but will do so at different rates, provided the tissues' turnover rates are different. Thus, if an individual animal moves to a new location that has an isotopically different dietary baseline, then its liver will reflect the new diet faster than its muscle because liver tissue has a faster turnover rate.

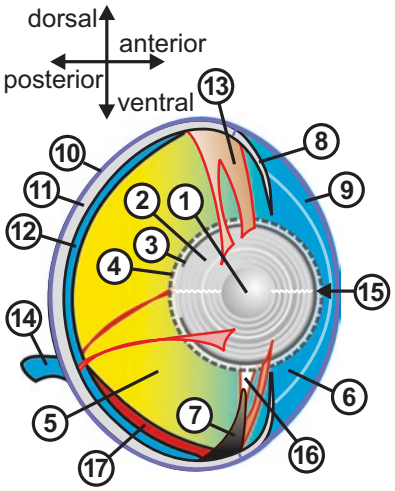
Isotopic offsets between tissue types within the same organism can therefore be used to measure the relative site fidelities of different individuals, species, sexes, or whatever factor the researcher chooses to evaluate. There is a major caveat when doing this, however. One cannot assume that all tissues come to the same equilibrium value after an isotopic diet switch. For example, muscle $\delta^{15}N$ from marine fishes equilibrates at a value that is approximately 1.8‰ higher than liver (Julie Vecchio, USF, pers. comm. of unpublished data). This equilibrium offset is a tissue-

specific constant that must be subtracted from original muscle-liver offsets in order to assign relative site fidelities. After making this correction, the researcher may compare the sign of the offset (i.e., whether it is positive or negative) to determine the likely direction of movement within the local isoscape's isotopic gradients.

Another caveat is that some tissues contain storage molecules. In fishes, lipids (fats and oils) may be stored in various tissues, including muscle, liver, and mesenteries. Oily fishes such as clupeids (herrings and sardines), scombrids (mackerels), and carangids (jacks and scads) store lipids within muscle, and this storage is dynamic, changing with the seasons, prior to spawning, or as feeding conditions change over space and time. This dynamic behavior in lipid storage creates problems with temporal interpretation of isotope results. To avoid this storage problem, it has become common practice to extract lipids from dried, powdered tissues prior to isotope analysis. Lipid extraction, which is achieved using various organic solvents such as dichloromethane (DCM) or a mixture of hexane and acetone, is sometimes automated using an accelerated solvent extractor (ASE). It is important to realize that lipid extraction not only removes storage lipids such as triacylglycerols (fats) but also removes more ubiquitous lipids such as sterols (associated with cell membranes). Lipids are hydrocarbons and thus have very high elemental C:N ratios. As an alternative to lipid extraction, empirical equations have been developed that allow the sample's elemental C:N value (i.e., its relative lipid content) to be used to adjust observed $\delta^{13}C$ to correct for the presence of lipids (Post et al. 2007). Note that $\delta^{15}N_{biomass}$ values are not affected by the presence of lipids, as lipids contain very little nitrogen. In fact, as hydrocarbons, lipids are generally considered to be devoid of nitrogen, yet nitrogen-bearing functional groups can be attached to certain lipid types (fatty acids, specifically). However, the contribution of these functional groups to $\delta^{15}N_{biomass}$ is considered negligible.

12.7 Lifetime Isotope Records from Eye Lenses

Eye-lens layers preserve lifetime isotopic records (Lynnerup et al. 2008; Nielsen et al. 2016; Tzadik et al. 2017). As an individual animal grows, new eye-lens layers (new laminae) form successively at the outside of the lens (Nicol 1989; Greiling and Clark 2012). During the initial part of new-lamina formation, the mass-dominant structural proteins produced within the lamina (crystallins) reflect dietary isotope ratios (Wistow and Piatigorsky 1988). However, final development of the new lamina involves attenuated apoptosis, which destroys organelles and genetic material that are required for new protein synthesis (Shi et al. 2009; Wride 2011). Attenuated apoptosis improves the optical properties of the newly formed lamina by removing these light-scattering materials, but it also halts new protein synthesis and traps existing isotopes that were originally associated with incipient formation of the new lamina. Likewise, formation of the next (outer) lamina incorporates the dietary isotopes of the next time period, and so forth.



1. lens nucleus (primary fiber cells - embryonic)
2. lens cortex (secondary fiber cells)
3. lens epithelium
4. lens capsule
5. vitreous humor
6. aqueous humor
7. retractor lentis muscle
8. iris
9. cornea
10. sclera
11. choroid
12. retina
13. central suspensory ligament
14. optic nerve
15. anterior pole of lens, with suture line (white)
16. retractor lentis tendon
17. falciform process

Fig. 12.2 Anatomical features of the teleost (bony fish) eye. Except in very small specimens, the lens nucleus would be much smaller than depicted

Poor vascularization appears to be an important requirement for isotopic conservation within tissues of any type. In addition to the eye lens, examples of poorly vascularized soft tissues include cartilage, which is avascular, and ligaments and tendons, which contain avascular regions. During embryonic eye development in vertebrates, the hyaloid artery and its associated network of blood vessels (the tunica vasculosa lentis) nourish the newly developing eye lens and its primary fiber cells, but this system of blood vessels soon disintegrates during continued development, leaving the post-embryonic lens without a direct source of vascularization. The retractor lentis muscle (Fig. 12.2) is the most proximal vascular tissue to the post-embryonic lens; it is vascularized via the falciform process or via the retina in species where the falciform process is diminished or absent. However, the retractor lentis muscle is isolated from vascular communication with the eye lens by the poorly vascularized retractor lentis tendon (Fig. 12.2). In the absence of direct contact between the eye lens and vascularized tissues, most isotopic communication with the post-embryonic eye lens likely occurs through the vitreous and aqueous humors, which are clear, colorless fluids that bathe the lens capsule; the highly elastic lens capsule surrounds the lens epithelium and the newly forming laminae that are produced by the lens epithelium. One important function of the lens capsule is to influence molecular communication between humor fluid and the lens epithelium (Danysh and Duncan 2009).

As the result of interest in cataracts and other lens pathologies that affect the human eye, the physiology and biochemistry of mammalian lens maintenance have been the subject of extensive research, and much of this research has concentrated on the movement of proteins within the lens. Such protein movement, if present, could possibly interfere with isotope records by redistributing proteins along the

radial axes of the lens (centripetal diffusion). Protein diffusion during embryonic development of the lens nucleus is suspected of being somewhat isotropic, but there appears to be very little post-embryonic centripetal diffusion of proteins among laminae within the organelle-free zone (i.e., the post-apoptotic zone), which includes the lens nucleus and all post-apoptotic laminae within the lens cortex (Shi et al. 2009, Fig. 12.2). The small amount of centripetal diffusion that does occur within the organelle-free zone results in nominal isotopic deviations equivalent to 0.5–1.0% annual carbon turnover; this turnover appears to be limited to the water-soluble fraction of crystallins and is not of sufficient magnitude to mask the temporal details of the atmospheric bomb pulse recorded by eye-lens isotopes (Stewart et al. 2013). Eye-lens laminae are composed of bundles of secondary fiber cells that derive from the equatorial lens epithelium and elongate from this equatorial point of origin toward the two poles of the lens, at which point the secondary fiber cells meet and interdigitate with fiber cells from other "longitudes" within the epithelium, forming a "suture" that may have taxon-specific morphology. New protein synthesis allows new secondary fiber cells to elongate until they reach the suture near the poles, after which they undergo attenuated apoptosis (Shi et al. 2009; Wride 2011). During this period of fiber-cell elongation from the equatorial epithelium to the poles, proteins are exchanged among elongating cells, but this exchange appears to be limited to lateral (concentric) diffusion among cells of similar age, rather than centripetal diffusion among laminae (Shi et al. 2009).

Collectively, the above circumstances allow eye lenses to be used to recreate lifetime isotopic histories for a variety of organism types, including humans (Kjeldsen et al. 2010; Lynnerup et al. 2010; Stewart et al. 2013), sharks (Nielsen et al. 2016; Quaeck 2017; Quaeck-Davies et al. 2018), bony fishes (Wallace et al. 2014; Quaeck 2017; Quaeck-Davies et al. 2018), and cephalopods (Parry 2003; Hunsicker et al. 2010; Onthanks 2013). Human eye-lens-isotope applications have focused on matching lifetime trends in radiocarbon (^{14}C) to temporal trends in atmospheric radiocarbon that have resulted from nuclear bomb testing in the 1950–1960s (i.e., the "bomb pulse"). This approach was developed as a forensic tool for humans that provides a fairly accurate estimation of birth year (Lynnerup et al. 2010), but the method can also be applied to other long-lived vertebrates such as sharks (Nielsen et al. 2016). Parry (2003) provided an early example of the application of stable isotopes (vs. radiocarbon) to the recreation of individual isotopic histories for two species of squid, and his approach was successfully repeated by other cephalopod biologists (Hunsicker et al. 2010; Onthanks 2013; Meath et al. 2019). In comparison, the application of eye-lens isotopes to bony fishes is relatively recent (Wallace et al. 2014; Quaeck-Davies et al. 2018).

Due to practical limitations on experiments involving long-lived organisms or organisms that are difficult or impossible to keep captive for sufficient periods of time, experimental validation of isotopic conservation within eye lenses has not been widely attempted, with the exception of one recent study by Granneman (2018), who documented an eye-lens isotope shift that followed an isotopic diet switch in a captive bony fish (red drum, *Sciaenops ocellata*). She found that mean

90% assimilation of the new diet was achieved by about 54 days after the $\delta^{15}N$ of the diet had been reduced by 3‰.

The net effect of the above processes is the formation of (1) a conservative organic record within the lens nucleus and elsewhere within the organelle-free zone and (2) non-conservative organic records at the newly forming outermost laminae that become conservative once the outer laminae undergo attenuated apoptosis. Aside from differences in cellular organization (Greiling and Clark 2012), fish eye lenses have the same anatomical, cellular, and compositional features as mammalian eye lenses (Nicol 1989; Wride 2011). Notably, because the eye lens is protein-rich, it provides ready access to useful $\delta^{15}N$ records that are, by comparison, very difficult to obtain from largely inorganic hard parts such as otoliths (Tzadik et al. 2017).

It follows from this history of methods development that there are likely many other taxa to which this general approach can be applied. Cephalopods are unique among invertebrates in having a large eye lens that can be manually delaminated to create isotopic time series, whereas most vertebrate groups possess eye lenses that can be manually delaminated (exceptions being eyeless cave-dwelling/subterranean species). In particular, lower vertebrates (fishes, amphibians, reptiles) are particularly well suited to this approach because they undergo continuous and substantial eye-lens growth during life. In contrast, much of the eye-lens growth in higher vertebrates (birds, mammals) occurs while the organisms are still embryonic, and thus only the outermost lens contains post-embryonic information.

12.8 Practical Solutions for Eye-Lens Analysis

In most cases, fish eye lenses can be readily delaminated using two forceps and a stereomicroscope. Periodically adding small amounts of clean, fresh water facilitates the delamination process (dry method, Wallace et al. 2014), or, alternatively, the lens may be delaminated while immersed in water within a petri dish (wet method, Stewart et al. 2013). Although the wet method results in greater loss of water-soluble material from the lens, experiments with fish eye lenses, wherein the wet method was used for one eye of the individual and the dry method was used for the other eye, did not reveal any statistical difference between the two methods (Julie Vecchio and Amy Wallace, USF, pers. comm. of unpublished data). The wet method tends to be the faster of the two and results in better temporal resolution (more, thinner dissected laminae per lens).

Exceptions to convenient delamination involve very long-lived species, which may have naturally lost the most water-soluble crystallins from a large part of the central eye lens, causing it to become hardened and highly resistant to delamination. In such cases, one solution is to delaminate the lens as much as possible, and then section the remaining hardened core through its center using a slow-speed saw equipped with two or more diamond blades that are separated by appropriately sized

spacers (i.e., the same equipment used to section otoliths). The resulting thin section can then be carefully broken apart using a scalpel under a stereomicroscope, using water as needed (Kurth 2016).

Hardened lens cores also exist in smaller, shorter-lived species, but these tend to represent smaller proportions of the overall lifespan and thus may not require subdivision. Note that the term "core" is defined by practicality rather than anatomy, as the cores typically contain both the embryonic lens nucleus (primary fiber cells) as well as hardened, adjacent laminae (secondary fiber cells). In the event that the core from one eye is of insufficient mass to be analyzed by the available IRMS instrumentation, then cores of similar diameters from both eyes of the same individual can be composited to provide a larger sample, as both left and right eyes contain very similar, if not identical, isotope records (Wallace et al. 2014, Fig. 12.3).

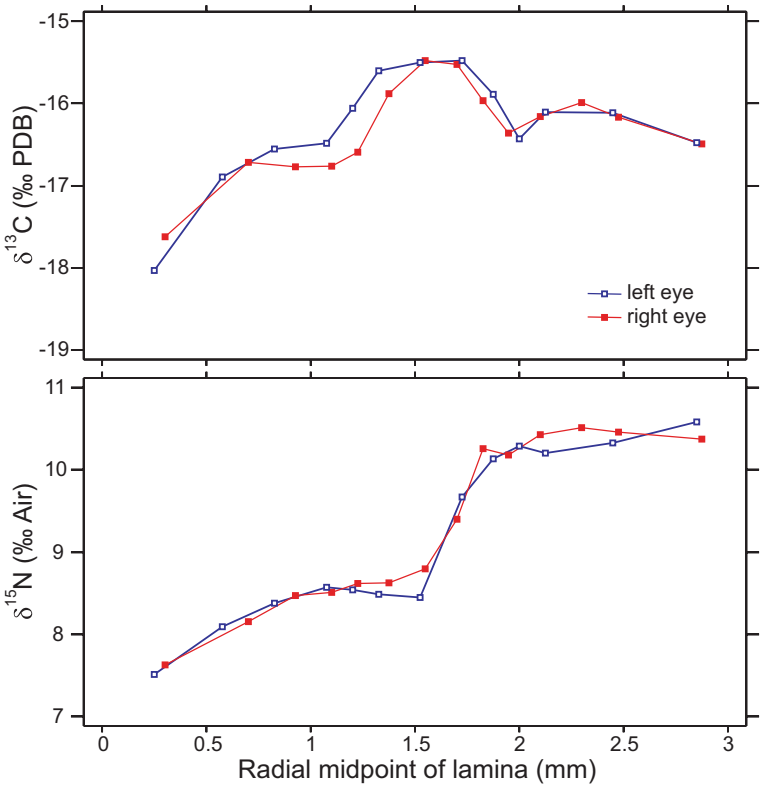


Fig. 12.3 Comparison of lifetime trends in $\delta^{13}C$ and $\delta^{15}N$ in the left and right eye lenses of a single white grunt (*Haemulon plumieri*). Early life is toward the left (from Wallace et al. 2014). In contrast, lifetime isotopic trends in tilefish (*Lopholatilus chamaeleonticeps*), a burrow-inhabiting (stationary) species, increase together in a more-or-less straight line from the lower left to the upper right, reflecting gradual increases in trophic position during life

Successively isolated laminae can be organized on aluminum foil placed on the lab countertop. Isolated laminae tend to dry very quickly at room temperature and do not typically require the use of a drying oven. Powdering is also relatively straightforward, as the dried laminae have a crystalline consistency that is highly friable, and either a mortar and pestle or a dental ball mill will work well to powder the samples.

While the laminated microstructure of the vertebrate eye lens suggests it can be interpreted in the same manner as otolith microstructure (i.e., by recognizing daily, annual, or other growth increments), this is difficult to do in practice. Comparisons with otolith-based ages reveal that individual laminae typically represent a time period of 3 months or less (and approximately 1 month in the case of the squid *Doryteuthis plei*; Meath et al. 2019). However, when an ocular micrometer is used to measure both lamina thickness and lamina position within two lenses from the same individual, it becomes evident that delaminations of the left and right eyes do not result in consistent, corresponding sequences of laminar thicknesses and positions (Wallace et al. 2014). Instead, lamina thickness appears to be influenced by the dexterity of the technician rather than by the locations of inherent, structural interfaces between adjacent laminae. Although inherent interfaces may be visually apparent within lens thin sections, exact separation at these interfaces can be difficult or impossible to achieve during manual delamination, and thus the radial midpoints (mm from lens center) of laminae dissected from the left and right eyes typically do not match (Fig. 12.3). If radial comparisons are to be made between left and right eyes or among eyes from different individuals, then an interpolation method such as cubic splining will be required first.

Given our present inability to directly interpret eye-lens microstructure as a chronometer, the relative positions of the laminae can be used instead to calibrate lifetime events. It is known that the center of the lens represents the embryonic stage and that its outer edge represents the time near collection, and so the relative positions of the laminae between these two points represent different times during life. The lens grows as the fish grows, and the shape of this relationship can be modeled. Specifically, an ocular micrometer can be used to determine laminar midpoints, and these can be converted to fish lengths using regressions that relate total lens diameter to fish length. Special care needs to be taken when interpreting the intercept of these relationships, however, just as special considerations are taken when otolith dimensions are related to fish length (see Campana and Jones 1992). In practice, lens diameter is measured before and after every lamina removal, and radial midpoints are calculated by averaging these pairs of measurements. In bony fishes, the lens diameter is very similar to the pupil diameter (Fig. 12.2), which is fixed (Walls 1942; Jagger 1997; Dahm et al. 2007). Measuring pupil diameters in bony fishes can be a practical alternative to dissecting and directly measuring lens diameters (a minor conversion factor may also be calculated and applied, if desired). At this point in time, the most defendable currency for representing points in life within eye-lens records is estimated fish length rather than estimated fish age.

12.9 Interpretation of Lifetime Isotopic Histories from Eye Lenses

The first consideration to make when interpreting lifetime isotopic records (Fig. 12.3) is to recognize that the synthesis of new eye-lens protein is proportionate to growth in some manner, which means that periods of slow growth or no growth will either not be represented or will be represented poorly. This can include important life-history events such as spawning migrations, wherein a fish could live and grow at an isotopically distinctive location, then make an energetically expensive spawning migration, and then return to feeding at the isotopically distinctive location. In such a scenario, there would be no isotopic record of the spawning migration.

Another consideration is that trends within isotopic records reflect both geographic movement and changing trophic relationships. Furthermore, regarding geographic movement, it can be very difficult to distinguish the movements of a predator from that of its prey, as either or both could result in isotopic shifts. However, different methods can be combined to address this question. For example, if a predator is known to have high site fidelity (e.g., from tagging studies or the tissue-comparison method described above), and the predator is also known to prey upon seasonally migrating coastal pelagic fishes (from diet studies), then it can be argued that the isotopic shift was more likely to originate from prey movement. In some cases, the tissue-comparison method could be applied to both predator and prey, with prey samples being obtained from recently ingested stomach contents or from a separate, dedicated collection effort.

Before addressing geographic movement, however, the issue of changing trophic relationships should be addressed, if at all possible. There are two primary conditions that cause trophic relationships to change at the predator level: changing baselines and changing trophic positions. In the case of $\delta^{15}N$, both of these issues can be addressed directly through isotopic analysis of source and trophic amino acids (compound-specific isotope analysis, CSIA; Chikaraishi et al. 2009; Ellis 2012; Layman et al. 2012). The $\delta^{15}N$ of source amino acids provides the isotopic baseline, after which the isotopic offset between the $\delta^{15}N$ of source and trophic amino acids can be used to calculate trophic position with relative precision. Moreover, it is entirely feasible to apply this CSIA approach to individual eye-lens laminae, thereby allowing the reconstruction of lifetime trends in baseline and trophic position. After accounting for these two effects, any remaining trends will be the result of geographic movement of predator or prey.

Finally, another promising approach would be to reconstruct individual isotopic histories for species that are already well-known (model species). Repeating this for a combination of model species that includes similar and disparate life-history types is likely to be particularly informative. Future work should also include additional efforts to validate eye-lens records under experimental conditions (e.g., Granneman 2018) and should explore novel combinations of approaches that are designed to overcome ambiguities in isotope-based results.

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