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7.07 Use of Stable Isotopes to Understand Food Webs and Ecosystem Functioning in Estuaries

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Abstract

Stable isotopes have been extensively used to trace element cycles and their incorporation into food webs. This chapter provides a brief introduction to the principles of using stable isotopes as natural or deliberate tracers in estuarine systems, with a focus on (1) assessing the origin and cycling of organic and inorganic elements (mainly carbon and nitrogen), (2) defining estuarine food webs, (3) assessing animal movement or migration, and (4) interpreting stable isotope records in biogenic carbonates as proxies of (past) environmental conditions.

7.07.1   Introduction

Estuaries are highly dynamic and complex ecosystems. The inputs, exchange, and processing of organic matter and nutrient sources in these systems are similarly complex, but play a key role in our understanding of ecosystem functioning as a whole, and the connectivity of estuaries with both terrestrial (or riverine) and coastal marine habitats.

Stable isotopes offer exciting possibilities as natural tracers of the origin and transformations of key biological elements. It is, therefore, not surprising that ecologists and biogeochemists have been actively employing stable isotope approaches in estuarine systems since the 1970s, when the facilities for isotope analyses became increasingly widespread. A large number of still widely cited key papers demonstrating the potential of stable isotopes in studying carbon flow in ecosystems focused on estuarine systems (e.g., Haines, 1976; Thayer et al., 1978; Peterson et al., 1985), and were, without doubt, highly influential in generating interest in the more widespread application of these techniques. These were the 'early days' of stable isotope studies in ecology, and the possibilities must have appeared endless. However, the pitfalls of stable isotope techniques in complex systems, such as estuaries, also became rapidly evident. With often a wide range of possible sources of carbon, nitrogen, or sulfur (the three main elements typically considered), overlapping signatures in the end members, and high spatial and temporal variability in source stable isotope signatures, it soon became evident that stable isotopes alone are not always the miracle solution. The use of complementary proxies or tracers (e.g., biomarkers, ^{14}C, and so on) and the development of appropriate mixing models were, therefore, important steps forward, and are still areas of continued development and improvements.

A few decades after the initial stable isotope surveys in estuarine systems, we are armed with a much more extensive array of advanced analytical techniques: with relative ease, isotope signatures can now be measured for more elements, in smaller samples, and in a wider range of substrates (from inorganic nitrogen sources to dissolved organic carbon (DOC), and from bulk sediments or organisms to specific biomarker compounds). The possibilities to use and further develop these approaches appear endless. There are obviously limitations to what we can hope to gain from deciphering stable isotope signatures in estuarine systems, but there is still much to learn, and isotopes will no doubt remain important tools in studying and unraveling the functioning of estuaries.

This chapter provides an overview of the potential of stable isotope tools in studying estuarine systems, as well as their limitations. The scope of this topic is extremely broad, and we therefore focus on a number of key topics, which are most frequently addressed, that is, (1) delineation of sources of organic or inorganic nutrients (C, N, and S), (2) stable isotopes as tracers in microbial and higher food webs, (3) isotopes as tracers of animal movement, and (4) the use of stable isotopes in natural archives of past environmental conditions.

7.07.2   Identification of Carbon and Nitrogen Sources in Estuaries

7.07.2.1   Isotope Signatures of Potential Organic Matter Sources in Estuaries

The utility of using stable isotopes as natural tracers of the origin of organic matter is based on the potential differences in stable isotope signatures between various sources, and the fact that these signatures are preserved relatively well (or modified within acceptable limits) during mineralization, transport, and burial. Variations in the carbon stable isotope composition of terrestrial plants were first noted in the 1950s, and two decades later, it was generally recognized that $\delta^{13}C$ signatures could be used to distinguish among photosynthetic pathway types (e.g., O'Leary, 1981), with C_4 plants showing typical $\delta^{13}C$ values $\sim-12‰$, and C_3 plants generally approximately $-24‰$ to $-30‰$ (note that δ values are expressed in percentage according to their definition as: $\delta^{13}C = 1000 * (X_{sample} - X_{standard})/X_{standard}$ where $X = {}^{13}C/{}^{12}C$).

Aquatic plants have been found to show widely varying stable isotope signatures (e.g., McMillan et al., 1980; Boon and Bunn, 1994; Hemminga and Mateo, 1996; Boyce et al., 2001). Although the relatively enriched $\delta^{13}C$ values in many aquatic plants have been initially interpreted as an indication for the utilization of the C_4 pathway, it has since been established that, in many cases, the C_3 pathway is operating. C_4 photosynthesis is, however, present in many aquatic species, but it is not associated with the specialized anatomy found in terrestrial C_4 plants (and, evidently, does not offer them the same advantage). In aquatic plants, however, the $\delta^{13}C$ value is not directly indicative of the photosynthetic pathway, and there are several causes for this: (1) the possible ability to take up HCO_3^-, which is isotopically enriched (i.e., has a higher $\delta^{13}C$ value) compared to dissolved CO_2, (2) boundary-layer effects, and (3) the isotopic composition of the dissolved inorganic carbon (DIC) source can vary spatially and seasonally in aquatic ecosystems. Although an in-depth discussion of the wide range of factors that may influence $\delta^{13}C$ signatures of aquatic primary producers in estuarine systems is beyond the scope of this chapter, we describe below the two most important drivers.

7.07.2.1.1   *Variability in substrate (DIC)* $\delta^{13}C$
The isotopic composition of the substrate for photosynthesis (either dissolved CO_2 or HCO_3^-) is an important variable affecting the isotopic composition of aquatic primary producers. Due to the thermodynamic isotope fractionation associated with the equilibrium reaction between CO_2 and HCO_3^-

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$$
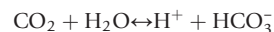
these two possible substrates for photosynthesis have a different isotopic composition, CO_2 (aq.) being depleted in ^{13}C by $\sim9‰$ relative to bicarbonate at $25\,°C$ (Mook et al., 1974). If there is an equilibrium with CO_2 from the atmosphere, $\delta^{13}C$ values for DIC approach $0‰$. Several processes, however, may alter the isotope composition of the DIC pool, for example, autotrophic production in the water column, diffusive efflux of CO_2 to the atmosphere, dissolution of $CaCO_3$, and respiration processes (see Section 7.07.2.5 for a more detailed overview of the effects of these processes). Thus, in seawater with limited

influence from continental organic carbon inputs, the $\delta^{13}C_{DIC}$ shows an overall fairly limited range of values (roughly between –2‰ and +2‰, e.g., Mook and Tan (1991)) although there remains some regional, local, seasonal, and depth-dependent variability. In estuarine and freshwater ecosystems, the effects of respiration are often much more pronounced, as these systems may receive large quantities of organic matter. The respiration of this material, either in the water column or in (intertidal or subtidal) sediments, may result in a significant ^{13}C-depletion of the DIC pool. This often – but not always – results in typical $\delta^{13}C_{DIC}$ patterns along the salinity gradient in estuaries, with lower values in the freshwater stretches (where the respiration of organic matter can be a major source of DIC) and values approaching 0‰ in the marine part of the estuary (Mook and Tan, 1991; Chanton and Lewis, 1999; Hellings et al., 1999). This will be discussed in more detail further on, but the important implication for the identification of organic matter sources is that all local aquatic primary producers in estuaries (whether macroalgae, phytoplankton, microphytobenthos, or seagrasses) will often have strong spatial and/or temporal gradients in their $\delta^{13}C$ signatures, which reflect the gradient in $\delta^{13}C_{DIC}$ (see e.g., Cloern et al., 2002).

7.07.2.1.2 Differences between benthic and pelagic algae

Benthic microalgae have been found to be consistently enriched in ^{13}C relative to their pelagic counterparts by an average of ~7‰ (France, 1995). The mechanism, which is most commonly invoked to explain this, is that the overall fractionation decreases due to boundary-layer effects. The presence of a much larger stagnant boundary layer in benthic or epiphytic algae (up to 7 mm according to MacLeod and Barton, 1998) compared to pelagic algae from the same environment causes diffusion limitation of CO_2, and, consequently, a decrease in the overall fractionation (see France, 1995). This boundary layer will be reduced under conditions of higher water currents, when $\delta^{13}C$ values will decrease. Typical values for intertidal benthic microalgae reported in the literature range between –12‰ and –20‰ (e.g., Couch, 1989; Currin et al., 1995; Newell et al., 1995; Créach et al., 1997; Lee, 2000). This difference in $\delta^{13}C$ values between benthic and pelagic microalgae is consistent enough to be reflected in consumer $\delta^{13}C$ values and has been proposed as a valuable tool to distinguish between benthic and pelagic food sources for coastal animals (France, 1995).

Typical ranges of $\delta^{13}C$ for estuarine primary producers are presented in Figure 1. For $\delta^{15}N$, differences between potential organic matter sources are typically not as large as for $\delta^{13}C$, and do not show patterns, which can be generalized. The utility of $\delta^{15}N$ signatures as source indicators in estuarine systems is, therefore, often limited, although it can be a specific tracer to indicate microbial N processing in estuaries (see Section 7.07.2.3) or as a tracer of sewage-derived nitrogen in estuarine food webs (see Section 7.07.4). An important prerequisite to use stable isotope signatures to identify source contributions is that the source signatures should be preserved during partial degradation of organic matter, or, at least, that any changes taking place should be small and/or known. In most short-term degradation experiments, little changes in $\delta^{13}C$ values of litter have been observed (e.g., Zieman et al., 1984; Fenton and Ritz, 1988; Wedin et al., 1995; Fogel and Tuross, 1999; Schweizer et al., 1999), but changes of the order of 1–2‰ have also been reported (e.g., Lehmann et al., 2002; Hill and McQuaid, 2009). In terrestrial systems, the soil organic matter (SOM) pool has been found to be slightly, but consistently, enriched in ^{13}C when compared to the litter of the dominant vegetation, and this enrichment often increases with depth (see Ehleringer et al., 2000). A number of mechanisms have been suggested to induce small (but in some cases, relevant) possible changes in $\delta^{13}C$ of organic matter, that is, (1) differential rates of decomposition for plant components and (2) fractionation during microbial degradation, which are discussed below

Individual plant components can vary substantially in their $\delta^{13}C$ values, according to the specific synthesis pathways. Lignin, for example, is usually depleted by 2–7‰ relative to the bulk plant material (Wedin et al., 1995; Schweizer et al., 1999 and references therein), whereas the cellulose fraction is usually enriched. Thus, the relative nondegradability of lignin has been suggested to result in a depletion in ^{13}C of plant litter during decomposition (e.g., Benner et al. (1991), for salt-marsh ecosystems). Although an appealing hypothesis and although lignin:C ratios obviously do increase during decomposition, the SOM pool is usually enriched rather than depleted in ^{13}C, with the exception of salt-marsh ecosystems (i.e., where Benner and colleagues worked on when they formulated their hypothesis); but the enrichment in the latter case is more likely to be caused by the import of allochthonous material (Middelburg et al., 1997). Wedin et al. (1995) studied changes in bulk $\delta^{13}C$ and lignin $\delta^{13}C$ during 2 years of decomposition for four grass
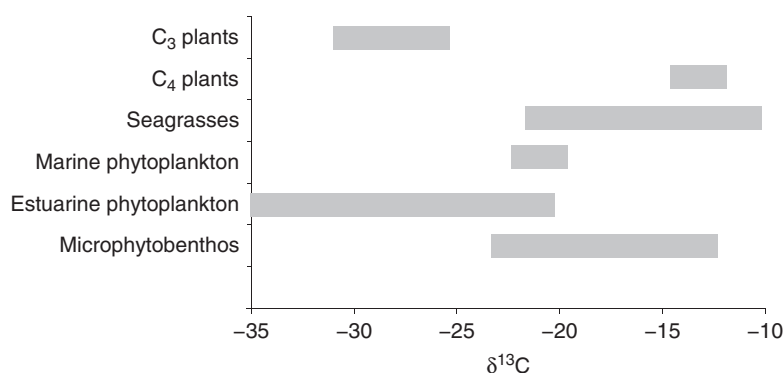

Figure 1 Typical range of carbon-stable isotope signatures for major primary producers and carbon sources in estuarine systems.

species (two C_3 plants and two C_4 plants). Their initial hypothesis that $\delta^{13}C$ values would decrease during decomposition due to the selective preservation of lignin did not appear to hold true: $\delta^{13}C$ values for C_3 and C_4 plants changed little, but in the opposite direction: C_3: from +0.4‰ to +0.7‰; C_4: from −1.0‰ to +1.5‰, and Bouchard et al. (1998) found a decrease in $\delta^{13}C$ values (~1.5‰) of the C_3-salt-marsh grass Spartina. It is, therefore, unlikely that such selective preservation of certain compounds has any consistent significant effects on total SOM $\delta^{13}C$ values.

If microbial reactions preferentially use ^{13}C-depleted carbon sources in metabolic reactions associated with litter decomposition, then the residual SOM should become progressively more enriched in ^{13}C. In their review on soil organic carbon dynamics, Ehleringer et al. (2000) pointed out that there is currently no direct, compelling evidence of microbial fractionation during SOM breakdown, and this is confirmed by the in situ experiments of Ekblad et al. (2002). According to Ehleringer et al. (2000), the absence of significant fractionation during mineralization should not be confused with the frequent observation that microbial and fungal carbon is enriched in ^{13}C compared to litter, as not all of the microbial and fungal carbon is likely to be derived from decomposing soil organic matter.

In contrast to the situation for $\delta^{13}C$, where only very small changes have been found to occur in short-term degradation experiments, and where $\delta^{13}C$ shifts over long timescales are fairly consistent in direction, the limited number of studies on $\delta^{15}N$ dynamics during decomposition has resulted in apparently contradictory conclusions. Some authors have reported (large) depletions in ^{15}N during decomposition (e.g., Zieman et al., 1984), whereas others have found significant increases in $\delta^{15}N$ (e.g., Turner et al., 1983; Caraco et al., 1998) – an increase or decrease depending on the oxic or anoxic conditions (Fogel and Tuross, 1999; Lehmann et al., 2002), or no significant changes at all (e.g., Dehairs et al., 2000 and Zieman et al., 1984 for seagrass litter). This apparent contradiction is, however, easily explained: the changes in $\delta^{15}N$ (if any) are not necessarily changes in the isotopic composition of the residual N pool, but are rather the result of nitrogen added by microbial immobilization. Such immobilization ('MAD': microbially added nitrogen; Caraco et al., 1998) has been shown to be significant in the N balance of the decomposing litter (e.g., Caraco et al., 1998), and thus, the magnitude and direction of $\delta^{15}N$ changes will depend on a multitude of factors including the inorganic N substrate used by bacteria or fungi (in most cases NH_4^+), its concentration and isotopic composition, and the degree of fractionation exerted by the heterotrophic community.

7.07.2.2   Conservative Mixing in Estuaries: Mass-Balance and Stable Isotope Considerations

The concept of conservative versus nonconservative mixing and the inclusion of stable isotope signatures in detecting nonconservative mixing in estuaries is an important central concept, which will return regularly in different sections of this chapter, and is therefore introduced separately. Estuaries are known as biogeochemically active zones. Organic matter can be remineralized in the estuarine zone, consumed by fauna, and deposited in intertidal sediments, and new inputs include local primary production or material exported from intertidal areas (mudflats, mangroves, and salt marshes). Similarly, nutrient concentrations can, in some cases, be significantly altered during the water residence time in estuaries, which can lead to both net inputs (release) or sinks (uptake or immobilization) of nutrients. Concentration profiles of elements along the salinity gradient (i.e., with salinity as a conservative tracer for the mixing between freshwater and seawater) have, therefore, been applied frequently to document internal sink or source terms for elements in estuaries (Figure 2(a); see Dittmar et al., 2009), and have also been proposed as a potentially important framework for food-web studies in estuarine systems (Fry, 2002).

A disadvantage of such concentration profiles is that, in some cases, net inputs and outputs of certain elements can be in balance, leading to a 'conservative' concentration profile, which hides possibly intensive processing. Consider, for example, a situation where riverine DOC is continuously degraded during its residence time in an estuary, but where DOC derived from vegetation (e.g., from salt marshes) is also released into the estuary. Here, stable isotope analyses can provide a powerful additional tracer, when sources and sinks have different stable isotope signatures – in this case, relatively ^{13}C-depleted riverine DOC, and ^{13}C-enriched salt marsh DOC.

Conservative mixing in terms of element concentrations along an estuarine salinity gradient can be described as:

$$E_S = \left(\frac{E_M - E_F}{Sal_M - Sal_F} \right) Sal_S + E_F \qquad [1]$$

whereby E is the concentration of the element considered, Sal represents salinity, and the subscripts refer to the marine end member (M), the freshwater end member (F), and the sampling point along the salinity gradient (S).

We can include the stable isotope signatures into this mass–balance equation, which results in the following general conservative mixing equation adapted from Mook and Tan (1991), written out here in terms of carbon-stable isotopes, but applicable to all other elements:

$$\delta^X E_S = \frac{Sal(E_F \delta^X E_F - E_M \delta^X E_M) + Sal_F E_M \delta^X E_M - Sal_M E_F \delta^X E_F}{Sal_s(E_F - E_M) + Sal_F E_M - Sal_M E_F} \qquad [2]$$

where E is the concentration of the element considered, $\delta^X E$ is the stable isotope ratio of the element considered, Sal represents salinity, and the subscripts refer to the marine end member (M), the freshwater end member (F), and the sampling point along the salinity gradient (S).

Conservative mixing curves for isotope ratios typically show a curvilinear shape, which can be either concave or convex, and the shape depends on the end-member concentrations and stable isotope signatures. The mixing curve becomes linear when either the end-member concentrations or the end-member stable isotope signatures are identical.

The inclusion of stable isotope data in evaluating net sinks or sources of elements in estuaries has been applied to a range of substrates, including dissolved inorganic nitrogen (DIN) (Middelburg and Nieuwenhuize, 2001), DIC (Spiker and Schemel, 1979; Coffin and Cifuentes, 1999; Atekwana et al., 2003; Bouillon et al., 2003b, 2007b; Miyajima et al., 2009), and DOC (e.g., Peterson et al., 1994; Cifuentes and Eldridge, 1998; Raymond and Bauer, 2001a).

The stable isotope-based mixing model for conservative mixing in estuaries described above can provide important insights into sources, sinks, and processing of elements, but remains to a certain extent largely qualitative. A number of authors have elaborated on this approach by including other processes into this model, in particular Cifuentes and Eldridge (1998), who proposed a one-dimensional model on DOC cycling in estuaries, and Coffin et al. (1994) and Miyajima et al. (2009), who included the effects of water–air exchange of CO_2 on the distribution of δ^{13}C–DIC along the estuarine gradient. These will be discussed in more detail in the relevant sections below, but provide a first basis for further modifications of such models for other elements or processes.

7.07.2.3   Organic Matter Sources in Estuarine Sediments and Suspended Matter

As outlined in the Section 7.07.2.1, estuaries may receive organic matter from a range of different sources (both imported from outside the estuary, and produced within the estuarine boundaries), and these different sources typically have different, if potentially overlapping, stable isotope signatures. These differences have found applications in (1) studies examining the origin and exchange of organic matter in estuarine sediments, intertidal sediments, and water-column suspended matter, (2) paleo-environmental studies examining historical trends in the origin of sedimentary organic matter, and (3) as baseline information for food-web studies to investigate the relative importance of different organic matter sources or spatial patterns in the food-web base (see section 7.07.4 and 7.07.5).

Intertidal salt marshes have been an important habitat where the use of stable carbon isotope ratios in estuarine systems developed, because salt marshes are typically dominated by either C_4 plants (e.g., *Spartina* spp.) or C_3 plants (e.g., *Typha* spp.; see e.g., Chmura and Aharon, 1995). Early studies in *Spartina* marshes found that, in contrast to the then-accepted belief that these plants were the dominant organic matter source in both intertidal and adjacent habitats, δ^{13}C signatures of sediment organic matter (and fauna inhabiting these marshes) was often strongly ^{13}C-depleted relative to the dominant vegetation (Haines, 1976, 1977). This pattern was attributed either to a large contribution by chemo-autotrophic bacteria (as proposed by Peterson et al., 1980), or to contributions by the local algal production and tidally imported carbon sources such as riverine, terrestrial, or marine organic matter (Sherr, 1982). Sherr (1982) also showed that the importance of *Spartina* as a contributor to the organic matter pools was highest in the intertidal marshes, but much less pronounced in subtidal sediments, and hardly detectable in estuarine suspended matter.

Measurements across a range of C_4-dominated salt marshes later demonstrated that much of the variation in δ^{13}C could be explained by a simple mixing model assuming local *Spartina* production (high δ^{13}C, high %C, and high C/N ratios) and tidally imported carbon sources (lower but variable δ^{13}C, low %C, and low C/N ratios) (Middelburg et al., 1997). Similar conclusions were found (based on the opposite pattern) in intertidal mangrove sediments, where the dominant vegetation uses the C_3 photosynthetic pathway (Bouillon et al., 2003a; see also Bouillon and Boschker, 2006).

Salt marshes are also highly dynamic ecosystems and can respond to longer-term sea-level changes by expansion (either landward or seaward) and vertical accretion, and, in some regions, show typical zonation patterns, which can be either perpendicular to the coastline (low/middle/high marshes) or along the salinity gradient, with different zones having a different vegetation. Stable isotopes thus have the potential to study past vegetation succession in coastal marshes (e.g., Chmura and Aharon, 1995; Choi et al., 2001). As in most paleo-environmental reconstructions, the use of additional proxies such as *n*-alkanes and/or their stable isotope composition increases the resolution to infer source contributions (e.g., Wang et al., 2003; Tanner et al., 2006).

There are a wealth of studies reporting δ^{13}C and/or δ^{15}N data on suspended organic matter from estuarine systems, and they are commonly combined with other simple parameters such as C:N ratios on suspended matter (e.g., Tan and Strain, 1983; Matson and Brinson, 1990; Fichez et al., 1993; Thornton and McManus, 1994; Cifuentes et al., 1996; Ogawa and Ogura 1997; Prahl et al., 1997; Andrews et al., 1998). Results from such studies are often strongly focused on understanding the behavior or distribution of organic matter within the studied area, which, considering the high diversity of estuaries, their residence times, and the degree of connectivity with adjacent habitats, cannot be discussed here in detail. Particularly relevant, however, is the study by Middelburg and Herman (2007), who compared the distribution as well as elemental and C and N isotopic composition of suspended matter in a range of contrasting European estuaries. In less turbid estuaries (typically with short residence times), δ^{15}N$_{PN}$ (δ^{15}N of particulate nitrogen), distributions are typically uniform, and δ^{13}C$_{POC}$ typically shows conservative mixing between riverine and marine end members (i.e., a strong gradient of more negative values in the upper estuary, gradually increasing to marine signatures with increasing salinity; see Figure 2). Estuaries with higher turbidity (and typically longer residence times) show more extensive processing and mixing of organic matter along the salinity gradient, which is reflected in intermediate salinity enrichments in δ^{15}N (due to microbial N processing, see also Section 7.07.2.6), and δ^{13}C$_{POC}$ signatures in such tide-dominated systems often have a much less pronounced gradient and narrower range (see Figure 2). In estuaries or coastal systems where clear boundaries occur between different habitats, sharp gradients in δ^{13}C$_{POC}$ may occur: dense seagrass beds, for example, act as efficient traps for particulate matter exported by tidal mangrove creeks, leading to an abrupt change in δ^{13}C$_{POC}$ along a salinity gradient across these habitats (Figure 2).

The majority of studies comparing the isotopic and elemental signatures of estuarine sediments and those of estuarine suspended matter consistently show significant differences in the characteristics of these organic matter pools (Tan and Strain, 1983; Matson and Brinson, 1990; Ogawa and Ogura, 1997; Middelburg and Nieuwenhuize, 1998). Typically, sediment δ^{13}C signatures are more uniform along the estuarine gradient, and are more ^{13}C-depleted than estuarine particulate organic carbon (POC). Together with the higher C:N ratios they usually record, this suggests that while sedimentary C may contribute to suspended organic matter through resuspension, these two carbon reservoirs should not be considered to be of similar origin.
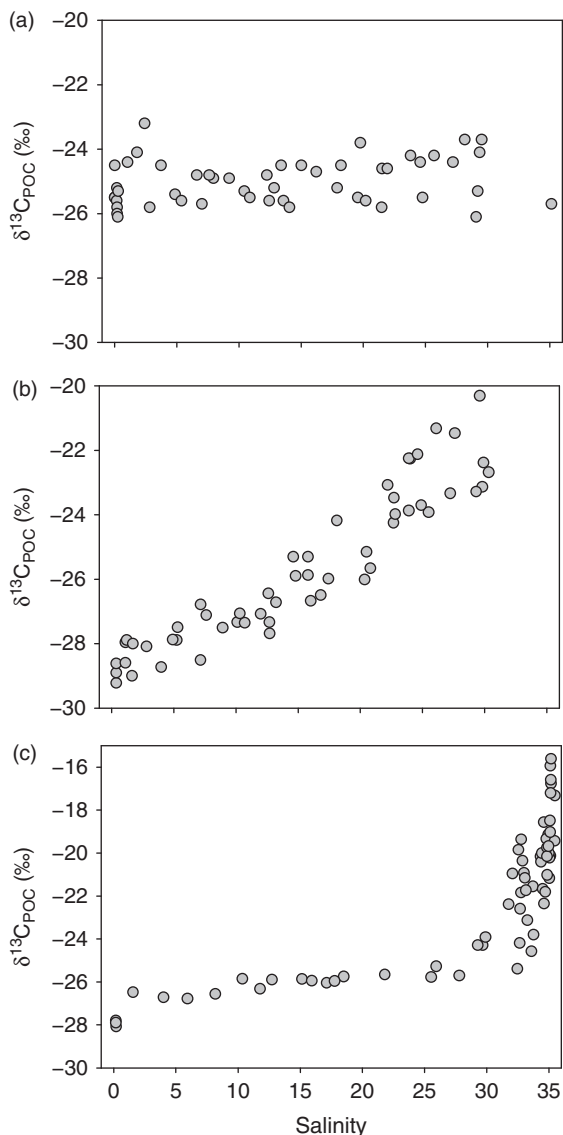
Figure 2 Contrasting examples of the distribution of $\delta^{13}C_{POC}$ along estuarine salinity gradients. Data from (a) the Gironde estuary (France), (b) the Scheldt estuary (Belgium/the Netherlands), and (c) the Kidogoweni, Gazi Bay (Kenya). Note that for (a) and (b), data from different seasons are pooled and note that the y-axis scale for is different in (c). Data from Middelburg and Herman (2007) and Bouillon, S., Middelburg, J.J., Dehairs, F., Borges, A.V., Abril, G., Flindt, M., Ulomi, S., Kristensen, E., 2007c. Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania). Biogeosciences 4, 311–322.

As in many other applications of stable isotopes, it must be stressed that while they are potentially powerful proxies, they cannot always unambiguously resolve source contributions because of a large degree of uncertainty, due to the potential overlap in $\delta^{13}C$ signatures of different sources in estuaries, and often small end-member differences and potential microbial influence on $\delta^{15}N$ signatures of particulate N. Detailed source characterization of organic matter in estuarine systems thus greatly benefits from additional proxies such as C/N ratios (although these are altered significantly during diagenesis), chlorophyll *a* data, or molecular markers such as *n*-alkanes

and/or their isotope composition (e.g., Naraoka and Ishiwatari, 1999; Jaffé et al., 2006), sterols (Canuel et al., 1995; Chikaraishi et al., 2005), lignin composition (Prahl et al., 1997; Dittmar et al., 2001), ^{14}C composition (reviewed by Raymond and Bauer (2001b)), or carbohydrate and amino acid composition (e.g., Loh et al., 2006).

7.07.2.4   Origin of Estuarine Dissolved Organic Matter

DOC is a key component of the overall organic carbon pool passing through estuarine systems (often higher in concentrations than POC). Although the residence time of DOC in estuarine systems is intrinsically shorter than that of particle-associated POC – which may go through deposition and resuspension cycles – DOC can be highly reactive and undergo losses through bacterial remineralization, photo-oxidation, and flocculation. On the other hand, estuarine systems may also have internal inputs of DOC, for example, from phytoplankton production, or release from intertidal systems such as salt marshes and mangroves (Raymond and Hopkinson, 2003; Dittmar et al., 2006), or from subtidal seagrass beds or macrophytes, if present.

Although some estuaries show evidence of conservative behavior of DOC along the salinity gradient (see e.g., Abril et al., 2002), there is now ample evidence that the dynamics of DOC in estuarine systems is often highly complex: some systems show clear evidence of net inputs of DOC (e.g., the Gironde, Ems, and Sado estuaries; the York River estuary, and mangrove-lined tidal creeks; see Abril et al., 2002, Raymond and Bauer, 2001b, and Bouillon et al., 2007c, respectively), while others show a net loss of DOC along the salinity gradient (e.g., the Scheldt and Mekong estuary, see Abril et al., 2002 and Bouillon and Connolly, 2009, respectively). As pointed out in Section 7.07.2.2, concentration profiles can only be interpreted as the 'net' result of both losses and inputs and, thus, even a conservative concentration profile may be the result of balanced inputs and losses, thereby masking active biogeochemical cycling of DOC. Stable (and radiocarbon) isotope data offer potentially powerful additional proxies to unravel the complexity of DOC cycling in estuarine systems (Raymond and Bauer, 2001a). It should be noted, however, that the methodological difficulties in measuring isotope signatures in saline waters has resulted in a preference of many researchers to focus on various fractions of dissolved organic matter by ultrafiltration (typically with a cutoff size of 1–3 kDa; e.g., McCallister et al., 2004; Guo et al., 2009), or using solid-phase extraction (SPE) procedures (e.g., Dittmar et al., 2006). Such preparation techniques are intrinsically selective (i.e., based on size or affinity) with sometimes rather low and variable recoveries (e.g., Benner et al., 1997), and may therefore not reflect processes related to the entire DOC pool.

As the analytical difficulties in measuring $\delta^{13}C$–DOC are starting to be overcome (St-Jean, 2003; Bouillon et al., 2006, Lang et al., 2007; Osburn and St-Jean, 2007; Panetta et al., 2008), the number of case studies documenting $\delta^{13}C$–DOC variations in estuarine systems is slowly increasing (e.g., Coffin and Cifuentes, 1999; Raymond and Hopkinson, 2003; Kaldy et al., 2005; Bouillon et al., 2007a; Ralison et al., 2008). Initial studies have been critical in confirming the potential net inputs or losses of DOC in estuarine systems (Peterson et al., 1994) – some contrasting examples of DOC and $\delta^{13}C$–DOC
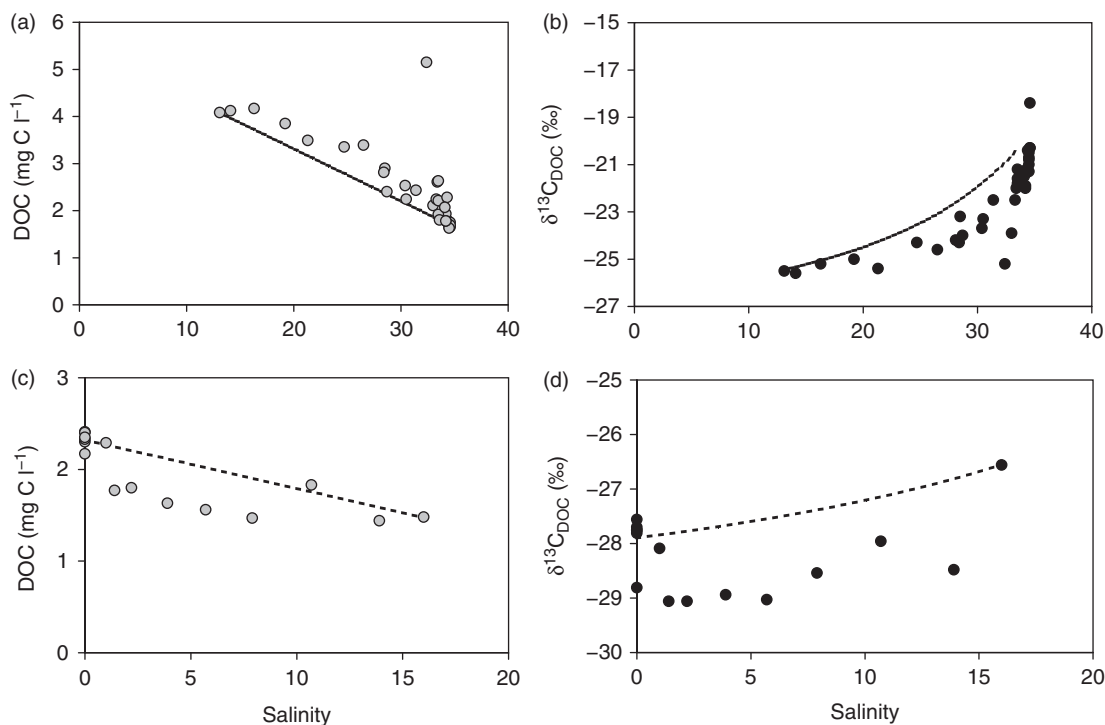
Figure 3   Examples of nonconservative behavior of dissolved organic carbon in estuaries. (a) and (b) Profiles of DOC and $\delta^{13}C_{DOC}$ from Mtoni (Tanzania), and (c) and (d) for the Tien estuary (Mekong Delta, Vietnam). Dotted lines represent conservative mixing scenarios. Data from S. Bouillon and A.V. Borges, presented earlier in Bouillon, S., Connolly, R., 2009. Carbon exchange among tropical coastal ecosystems. In: Nagelkerken, I. (Ed.), Ecological Connectivity among Tropical Coastal Ecosystems. Springer Science + Business Media NV, ch. 3, doi:10.1007/978-90-481-0. With kind permission of Springer Science + Business Media.

profiles (Figure 3) illustrate this. More recent data further indicate that the origin of POC versus DOC in estuarine systems can, in some cases, be distinctly different (e.g., Bouillon et al., 2007a; Ralison et al., 2008).

The addition of $\delta^{13}C$ (and/or $\Delta^{14}C$) data on DOC can also be exploited to complement mass-balance models on DOC cycling in estuarine systems; a more detailed description of such an approach can be found in Cifuentes and Eldridge (1998) and Raymond and Bauer (2001b).

Other isotopic tracers of dissolved organic matter have only rarely been employed. The feasibility and potential application of sulfur-stable isotopes ($\delta^{34}S$) has recently been demonstrated by Alling et al. (2008), but further developments and case studies are needed to fully evaluate the potential of this approach. Stable isotope analyses on dissolved organic nitrogen ($\delta^{15}N$–DON) are very scarce due to the methodological complexity in measuring $\delta^{15}N$–DON, and, to the best of our knowledge, no published data sets exist for any estuarine environment.

7.07.2.5   Inorganic Carbon Cycling in Estuaries

DIC in estuaries originates from multiple sources. The main determinant of $\delta^{13}C$–DIC variations in estuaries is the mixing of freshwater inputs and marine waters, whereby the former typically has more negative $\delta^{13}C$ signatures and the latter has a $\delta^{13}C$ signature close to 0‰ (Mook and Tan, 1991). The ^{13}C-depleted signatures in freshwater (i.e., riverine) DIC result mainly from the effects of mineralization, which contributes DIC with a signature close to that of the source organic matter (typically ranging

between –12‰ and –28‰ in case of terrestrial plants or SOM), and is also determined by the degree and relative dominance of silicate and carbonate weathering in the catchment. Silicate weathering in soils results in a DIC pool with a $\delta^{13}C$ signature expected to range between –18‰ and –5‰ (for C_3- and C_4-dominated sites, respectively), that is, similar to that of the organic matter mineralized within the soil, but taking into account additional fractionation during diffusion of CO_2 into the soil water phase (see Brunet et al., 2005). Atmospheric inputs or DIC resulting from carbonate dissolution would result in more enriched $\delta^{13}C$ signatures (>–5‰), as DIC produced by carbonate dissolution is composed of both soil CO_2 ($\delta^{13}C$, depending on C_3/C_4 abundance) and carbonates ($\delta^{13}C$ ~0‰, assuming ancient marine carbonates). Mixing between freshwater and coastal ocean water thus typically results in curvilinear $\delta^{13}C$–DIC gradients, whereby the shape of the curve is set not only by the difference in $\delta^{13}C$–DIC values between freshwater and marine end members, but also by the concentration difference; DIC concentrations in coastal waters are fairly constant (~2 mM), but DIC concentrations in rivers can vary widely depending on the catchment geology and weathering rates (e.g., Ludwig et al., 1996; Brunet et al., 2005). While the distribution of $\delta^{13}C$–DIC along estuarine gradients may be conservative or close to conservative in some systems (e.g., the western Scheldt estuary, see Mook and Tan, 1991; Gillikin et al., 2006b; Ahad et al., 2008), this is certainly not a general rule and nonconservative mixing has been observed in a wide range of systems. The nonconservative behavior of DIC in estuarine waters can result from different processes, each with a different effect on $\delta^{13}C$–DIC.

7.07.2.5.1 Photosynthesis

All aquatic primary producers show isotope fractionation during uptake and assimilation of inorganic carbon (CO_2 or HCO_3^-) during photosynthesis. While the degree of fractionation depends on a range of variables, such as the photosynthetic pathway used, whether the primary substrate is CO_2 rather than HCO_3^-, temperature, volume, and shape of the phytoplankton cells, water flow velocity, or growth rate, the net effect of photosynthesis is an enrichment in the residual DIC pool. Given the typically relatively high concentrations of DIC in estuarine waters, relatively high productivity combined with high residence times are required for the effect of photosynthesis to be clearly discernible. However, in a number of case studies, elevated δ^{13}C–DIC signatures appear to be linked to primary production, for example, Coffin and Cifuentes (1999) found that δ^{13}C–DIC values higher than expected for conservative mixing to occur in the Perdido estuary (USA) during periods of higher phytoplankton production, and the seasonal δ^{13}C–DIC data presented by Gillikin et al. (2006b) for the Scheldt estuary show higher δ^{13}C–DIC signatures during phytoplankton bloom periods.

7.07.2.5.2 CO_2 gas exchange

Estuarine systems are typically highly oversaturated in CO_2 (e.g., Abril and Borges, 2004); hence, CO_2 exchange between the water and atmosphere is typically directed toward the atmosphere. The different DIC species can be assumed to be in isotopic equilibrium, whereby CO_2 is ^{13}C-depleted relative to HCO_3^- and CO_3^{2-}, and, consequently, also relative to the total DIC pool. Degassing of CO_2 toward the atmosphere thus leads to enrichment in ^{13}C in the residual DIC pool. The effect of degassing of a certain amount of CO_2 will depend on the overall DIC concentration, the pH (because this determines the speciation of the different inorganic C forms, and thus, the isotope composition of each), and temperature and salinity, both of which influence the fractionation factors between the various DIC species (Mook et al., 1974; Zhang et al., 1995). Quantitative estimates of the influence of CO_2 exchange on δ^{13}C–DIC profiles in estuarine systems have been described by Coffin et al. (1994) and Miyajima et al. (2009). Coffin et al. (1994) provided a steady-state, one-dimensional model with advection–diffusion equations for closed system conditions (i.e., no CO_2 exchange), open system conditions (i.e., complete equilibration with atmosphere), and intermediate conditions, that is, those typically expected in estuarine systems. Miyajima et al. (2009) observed that the differences in DIC concentrations between observed DIC profiles and those predicted by conservative mixing were much smaller than the difference expected when estimating the addition of ^{13}C-depleted DIC (assumed to derive primarily from surrounding mangroves) based on the δ^{13}C–DIC differences, and give (simplified) estimates of how δ^{13}C–DIC data can provide estimates for the overall DIC inputs, assuming that Rayleigh-type distillation equations apply to the evolution of δ^{13}C–DIC during degassing.

7.07.2.5.3 Remineralization (in situ or via lateral inputs)

In estuarine systems where DIC does not show conservative behavior, it typically shows net inputs of DIC along the salinity gradient, and δ^{13}C–DIC gradients most frequently show more
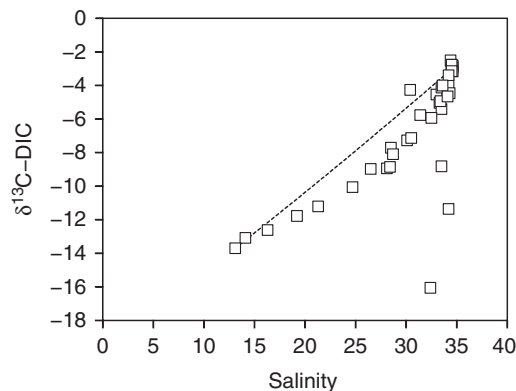
Figure 4 Estuarine profile of δ^{13}C–DIC along the mangrove-lined Mtoni creek, Dar es Salaam (Tanzania). The dotted line represents the theoretical conservative mixing line. The data deviating most from the conservative mixing line are from small drainage creeks in the intertidal flats. From S. Bouillon, unpublished data, September 2005.

^{13}C-depleted signatures than would be expected for conservative mixing (e.g., Atekwana et al. 2003, Bouillon et al., 2007b, Miyajima et al., 2009). Net inputs of ^{13}C-depleted DIC indicate the importance of respiration, as this contributes DIC with a δ^{13}C signature similar to that of the organic matter being respired. A typical example of a nonconservative δ^{13}C–DIC profile from mangrove-lined tidal creeks is illustrated in Figure 4. The net additions of DIC can be substantial and the most pronounced examples appear to come from estuarine systems with intertidal wetlands, such as salt marshes or mangroves. For such systems, several studies have demonstrated that DIC inputs in estuarine waters derive mostly from remineralization taking place in intertidal areas and subsequent lateral DIC fluxes toward the estuary proper (e.g., Cai and Wang, 1998; Cai et al., 1999; Raymond and Hopkinson, 2003; Bouillon et al., 2007c).

7.07.2.5.4 Carbonate dissolution

Assuming that carbonates in estuarine suspended matter derive from old marine carbonate deposits in the watershed, the δ^{13}C–DIC composition of DIC produced by carbonate dissolution is expected to be intermediate between that of the carbonates (i.e., ~0‰) and dissolved CO_2 (according to $CaCO_3 + CO_2 + H2O \rightarrow Ca^{2+} + 2HCO_3^-$). The latter is variable and depends on the overall initial δ^{13}C–DIC and the DIC speciation. Carbonate dissolution is likely only a minor process in the overall DIC dynamics in estuarine systems; so far, only the data by Bouillon et al. (2003b) from the Godavari estuary (India) show a likely influence of carbonate dissolution on water-column δ^{13}C–DIC signatures.

Additional information on DIC dynamics can also be provided by ^{14}C data, but, to our knowledge, this combination has only rarely been applied. Raymond and Hopkinson (2003) showed that the salt marshes along the Parker river estuary (USA) provide a source of ^{14}C-enriched DIC to the estuary (Figure 5), forming a convincing additional argument for the suggestion that tidal salt marshes are important in controlling water-column DIC and oxygen dynamics (Cai and Wang, 1998; Cai et al., 1999).

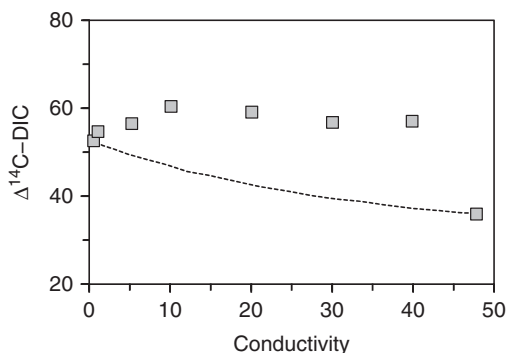**Figure 5** Estuarine profile of Δ^{14}C–DIC in the Parker estuary in July 2000. The dotted line shows the conservative mixing curve. Conductivity in mmho cm^{-1}. Reproduced with permission from Raymond, P.A., Hopkinson, C.S., 2003. Ecosystem modulation of dissolved carbon age in a temperate marsh-dominated estuary. Ecosystems 6, 694–705.

7.07.2.6   Inorganic Nitrogen Cycling in Estuaries

Nitrogen cycling in estuaries has been intensively studied, and is an important research area in view of the problems associated with eutrophication due to increased anthropogenic nitrogen inputs via rivers (Herbert, 1999; Hulth et al., 2005). Despite efforts to control the riverine anthropogenic N flux, a further increase is expected for the next decades (Bouwman et al., 2005). Stable isotope studies of the main DIN species (ammonium and nitrate) have important added value in studying the fate and cycling of DIN in estuarine systems, as the signatures of various sources of N are often distinct. Natural sources of DIN such as precipitation and soil-derived N are typically depleted in ^{15}N relative to anthropogenic sources (wastewater and manure), and the oxygen isotope composition of nitrate (δ^{18}O–NO$_3^-$) typically differs between nitrate in precipitation, fertilizers, and soil/anthropogenic nitrate. Thus, data from a range of different temperate river systems show an increase in δ^{15}N–NO$_3^-$ signatures with an increasing contribution of agricultural and urban land use in the catchment area (data compiled in Johannsen et al., 2008). Furthermore, nitrogen transformation processes such as nitrification, denitrification, or DIN assimilation by phytoplankton are associated with often strong fractionation effects, leading to distinct changes in δ^{15}N signatures, which can provide important clues on N transformation processes.

Cifuentes et al. (1989) were among the first to document δ^{15}N–NH$_4^+$ in estuarine systems, and observed large seasonal variations due to algal uptake, nitrification, and remineralization, with ranges of up to 30‰ at specific locations. Even larger variations have since been reported for the freshwater tidal reaches of the Scheldt estuary where seasonal variations of up to 60‰ have been recorded, with the intense nitrification and N uptake during phytoplankton blooms that are responsible for the high ^{15}N enrichments observed when NH$_4^+$ becomes depleted (De Brabandere et al., 2007). In other estuarine systems where N transformations are much less important, variations in δ^{15}N signatures of ammonia were fairly limited and deviated little from those predicted by conservative mixing (Ahad et al., 2006). For NH$_4^+$, the important N-transformation processes influencing their concentration result in an isotope enrichment in the residual NH$_4^+$ pool, and the patterns

observed in δ^{15}N–NH$_4^+$ in itself, therefore, do not lead to particularly new insights on ammonium cycling (Middelburg and Nieuwenhuize, 2001). For NO$_3^-$, in contrast, different processes have opposite effects on the δ^{15}N signatures of the NO$_3^-$ pool: denitrification and nitrate uptake by microorganisms lead to an enrichment of the residual NO$_3^-$ pool, while nitrification results in the addition of typically ^{15}N-depleted NO$_3^-$ (assuming that the NH$_4^+$ pools are not unusually enriched in ^{15}N). Dähnke et al. (2008) found surprisingly conservative behavior of nitrate concentrations in the Elbe estuary (Germany) over different seasons, and this was confirmed by the δ^{15}N signatures of NO$_3^-$. Other estuarine systems, however, do show clear evidence of nitrate processing in δ^{15}N–NO$_3^-$ signatures, even when the concentration profiles suggest conservative behavior (e.g., the Loire estuary, France, see Middelburg and Nieuwenhuize, 2001). There is particular scope for using the dual-isotope composition of nitrate (δ^{15}N and δ^{18}O), as the influencing factors and fractionation processes acting are different, but the application of this technique is, for the moment, still limited by the complexity of N cycling in estuarine systems, in particular when benthic N cycling is important, and by the lack of understanding of the processes affecting δ^{18}O–NO$_3^-$ produced during nitrification (e.g., see Wankel et al., 2009).

Information on the stable isotope composition of DIN not only is useful to study the cycling of DIN itself, but also has been successfully used in combination with δ^{15}N data of phytoplankton to estimate the relative contribution of different N sources to phytoplankton growth (York et al., 2007).

7.07.2.7   Identifying Microbial Carbon Sources Using Stable Isotopes

The inclusion of bacterial communities in stable isotope studies has evolved significantly during the past two decades. The first studies probing for carbon sources used by estuarine bacteria relied on bioassays, whereby filtered estuarine water samples were inoculated with a natural bacterial community and incubated prior to filtration of the resulting bacterial biomass for isotope analysis (Coffin et al., 1989; Hullar et al., 1996). These provided an important first step by confirming that the δ^{13}C signatures of bacteria were close to or similar to those of the organic substrates provided; however, the experimental conditions are not ideal to infer natural carbon sources as the exclusion of particulate organic matter may alter the carbon sources used during the incubation, and the more labile carbon sources might become limited during the time frame of the incubation period needed to obtain sufficient microbial biomass (up to 48 h). Coffin et al. (1990) were the first to extract nucleic acids from natural waters for stable isotope analyses, providing an indirect way to establish δ^{13}C signatures for natural bacterial communities, and results from nucleic acid extractions and bacterial incubations appear to match quite well (Kelley et al., 1998). Although part of the extracted nucleic acids could be of a different origin (e.g., from eukaryotic organisms or phototrophic bacteria), it has been confirmed that this contribution is typically minimal (McCallister et al., 2004), and this approach thus offers an elegant way to include bacterial communities in isotope studies. A particular advantage of this technique is that it is also suitable for ^{14}C analysis, which Cherrier et al. (1999) used to demonstrate that estuarine pelagic bacteria primarily relied on

modern organic carbon, despite the fact that the bulk estuarine DOC pool was relatively depleted in ^{14}C. The data of McCallister et al. (2004) also nicely demonstrate the added value of this additional tracer in estuarine systems, given the complexity and multitude of available sources.

By far the most frequently used approach, however, is the extraction of bacterial phospholipid-derived fatty acid (PLFA) markers, for subsequent analysis of δ^{13}C by gas chromatography–isotope ratio mass spectrometry (GC-IRMS), a technique, which has evolved rapidly since its first introduction (Pelz et al., 1997; Abraham et al., 1998; Cifuentes and Salata, 2001). This technique has been used in a range of estuarine and coastal systems including salt marshes, mudflats, mangroves, and seagrass beds (see review by Bouillon and Boschker, 2006, and in later work by Ralison et al., 2008) and in suspended matter along estuarine gradients (Boschker et al., 2005). A synthesis of data from coastal and estuarine sediments has indicated that bacterial communities generally show little evidence of selectivity and rely on contributions of various carbon sources in similar proportions to their occurrence in the bulk sediment organic carbon pool (Bouillon and Boschker, 2006). Only in sediments with low organic carbon contents do bacteria show a noticeable preference for ^{13}C-enriched carbon sources (mostly likely microphytobenthos). This does not imply that bacteria are generally nonselective, but indicates that, despite differences in lability, the expression of selectivity is typically only significant when the available bulk organic C pool is not dominated by more refractory terrestrial or macrophyte-derived C.

Data on pelagic bacterial communities are much scarcer. Cherrier et al. (1999) and McCallister et al. (2004), as mentioned above, demonstrated preferential use of more recent organic carbon by pelagic bacteria in the Santa Rosa Sound and in the Hudson and York river estuaries, although significant spatio-temporal variation was observed in the relative importance of terrestrial, marsh-derived, and *in situ* produced phytoplankton material in the latter two systems (McCallister et al., 2004). Boschker et al. (2005) provided a detailed data set on δ^{13}C signatures of DIC, POC, and various bacterial and algal PLFAs, and found a surprising similarity in the estuarine gradients of δ^{13}C$_{POC}$ and δ^{13}C of bacterial PLFA, despite the fact that algal PLFAs were much more depleted in the freshwater and low-salinity zones (Figure 6). This pattern indicates that bacterial communities largely consume organic matter as present in the bulk available organic carbon pool, in this case dominated by terrestrial carbon sources in the upper estuary and gradually shifting to a dominance of phytoplankton in the lower estuary.

7.07.3   Stable Isotopes as Tracers of Food-Web Structure

7.07.3.1   Introduction

An understanding of food webs is of fundamental importance in estuarine ecology. The impetus for food-web studies is often the desire to understand sources of nutrition supporting fisheries' production. Recently, however, it has also become clear that knowing the structure and processes of food webs is important in predicting the resilience of coastal ecosystems to human perturbations (Tewfik et al., 2005; Gascuel et al., 2008), and the evolution of ecosystem functioning in restored coastal wetlands (Howe and Simenstad, 2007).
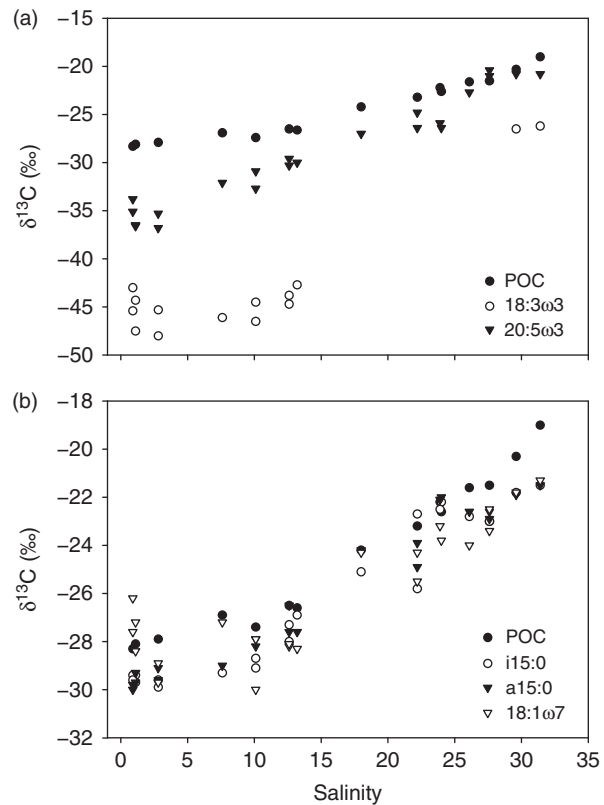
Figure 6   Profiles of δ^{13}C signatures in bulk suspended matter (δ^{13}C$_{POC}$) and selected microbial PLFA along the salinity gradient of the Scheldt estuary (modified from Boschker et al., 2005). (a) Data on POC and algal PLFA; (b) for POC and bacterial PLFA (note the difference in y-axis scale). Modified from Boschker, H.T.S, Kromkamp, J.C., Middelburg, J.J., 2005. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnology and Oceanography 50, 70–80. Copyright 2005 by the American Society of Limnology and Oceanography, Inc.

Food webs in estuaries are made complex by the potential inputs from upstream (riverine), downstream (oceanic), within estuary (e.g., seagrass and microalgae), and lateral sources (e.g., mangroves and salt marsh). This complexity is part of the reason that stable isotopes have been used so frequently to analyze trophic processes. Stable-isotope analysis has clear advantages over more traditional dietary methods such as stomach-content analysis, because

1. it incorporates spatial and temporal aspects that stomach-content analysis cannot realistically cover;
2. it can be used on microscopic organisms or soft-bodied animals that are difficult to include in stomach-content analyses; and
3. the stable-isotope signal tracks more or less faithfully, albeit with some fractionation shift, through different trophic levels.

7.07.3.2   Food Webs in Temperate Estuaries

Much of the early stable-isotope work in coastal systems was done in the temperate salt-marsh estuaries of the USA. The

theoretical framework was that outwelling of intertidal primary production in the marshes sustained a higher rate of secondary production in deeper estuarine and coastal waters (Odum, 1979), and this was the focus of many studies. Certainly, the massive production of salt-marsh macrophytes could be detected in the tissue of animal consumers. However, it was rarely the dominant source of carbon for animals living on the marsh, and was often difficult to detect at all for animals in adjacent waters (Haines, 1976). Microalgae on the marshes (Sullivan and Moncreiff, 1990) and possibly in the water column (Haines and Montague, 1979), seem to be more important than the marsh plants themselves. This same pattern of measurable, but not dominant, marsh grass utilization in and immediately around marshes, reducing to negligible marsh utilization in adjacent waters, has been confirmed for invertebrates and fish in estuaries from South Africa (Schlacher and Wooldridge, 1996; Paterson and Whitfield, 1997) and southern Australia (Svensson et al., 2007).

By combining the use of C, N, and S isotopes, it was ultimately shown that: (1) upstream riverine sources played no significant trophic role and (2) the relative balance of marsh and algal sources varied for different species of animals depending on their feeding mode and location within the estuary (Peterson and Howarth, 1987). This study highlighted, for the first time, the usefulness of incorporating sulfur isotopes into estuarine studies. Sulfur continues to show promise for resolving food-web questions, which cannot be answered using C and N (Connolly et al., 2004), and this will be made easier as ecologists come to terms with the different spatial and temporal scales over which sulfur isotopes vary in estuaries. The early isotope studies highlighted an unexpected complexity in estuarine food webs. The spatial and temporal variation of these food webs meant that the isotope tests of the outwelling theory (which hypothesizes a large trophic role for carbon produced in tidal wetlands for adjacent coastal ecosystems) were never quite conclusive, and this issue is being tackled today with new isotope methodologies (see Section 7.07.3.4).

Isotope studies have also helped resolve food webs in and around seagrass meadows. The carbon produced by seagrasses and algae growing on seagrass (epiphytic algae) is greater than the amount that can be used by consumers within the meadows. Although some of the excess production is sequestered in sediments within the meadow, carbon is also available for transport with currents to other habitats (Duarte and Cebrian, 1996). Isotope studies have shown that not only does carbon from the meadows support in situ animals (e.g., Vizzini et al., 2002) but it also supports invertebrates and fish in adjacent habitats (see Figure 7; Connolly et al., 2005a, 2005b; Melville and Connolly, 2005).

Out of this work rose the conundrum of whether the food web was sustained predominantly by the seagrass itself (macrophytes) or by epiphytic microalgae on the seagrass. Most studies have reported greater contributions from algae rather than from macrophytes (Kitting et al., 1984; Moncreiff and Sullivan, 2001), although, in some cases, the similarity in signatures of seagrass and associated microalgae prevents resolution (e.g., Loneragan et al., 1997).

In estuaries without dominant intertidal macrophytes, isotope studies helped force a reevaluation of the role of microphytobenthos, which is now known to play a major role in supporting secondary production (Middelburg et al., 2000). In still other systems, such as the New Zealand fjords, isotope analysis has shown that terrestrial forest litter can be the major source of nutrition for upper estuarine animals (Mcleod and Wing, 2007).

7.07.3.3   Food Webs in Tropical Estuaries

One of the dominant themes in food-web studies on tropical estuarine/coastal ecosystems is analogous to the discussion on temperate salt marshes discussed earlier, and concerns the potential role of mangrove productivity in sustaining nearshore production. Following the pioneering work of Rodelli et al. (1984) in Malaysia, a large number of studies have found
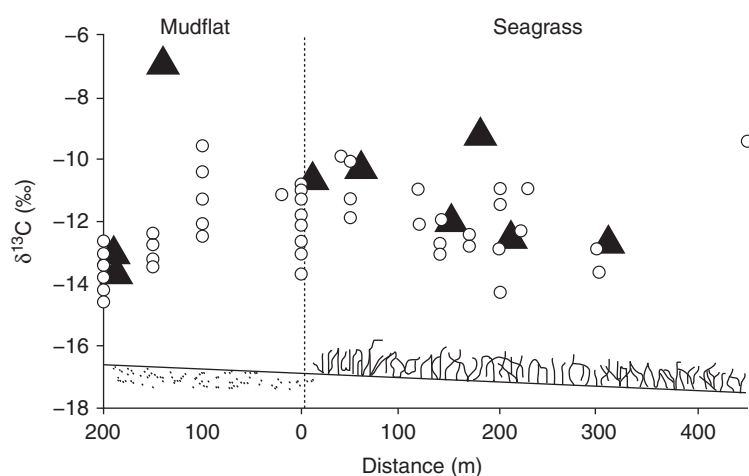
Figure 7   The importance of carbon from seagrass meadows to food webs both in the meadows and over adjacent mudflats. Data are $\delta^{13}C$ ratios for invertebrates (circles) and sediment detritus (triangles) collected at different distances into seagrass meadows (right side) and over adjacent mudflats (left side). Invertebrates are pooled data for the three most common invertebrate species (a carnivorous polychaete worm, a detritivorous polychaete worm, and an omnivorous shrimp), all of which showed the same pattern. Detritus values show that particulate matter available to detritivores, even on the mudflat, is largely from seagrass. From Connolly, R.M., Gorman, J.D., Guest, M.A., 2005a. Movement of carbon among estuarine habitats and its assimilation by invertebrates. Oecologia 144, 684–691.

gradients of increasing $\delta^{13}C$ with increasing distance from mangrove forests. Interpretation of these data, however, suffers from the inability to rule out a similar gradient in primary producers (e.g., phytoplankton), which results in little or no convincing evidence for a trophic role of mangroves in supporting adjacent food webs (discussed in Bouillon et al., 2008). A more recent study by Granek et al. (2009) indicates a measurable contribution of mangrove-derived C to the diet of certain sessile species of reef invertebrates, albeit at a very close distance to the mangrove edge.

Within the intertidal zone, however, stable isotope studies have also been instrumental in pointing out the surprising diversity in food sources and specialization in mangrove ecosystems. Rather than clustering close to the signatures of the dominant vegetation (mangroves), stable-isotope signatures of invertebrate consumers show an unexpectedly large range, indicating that invertebrates extensively rely on less conspicuous food sources such as imported organic matter, microphytobenthos, and epiphytic algae (reviewed in Bouillon et al., 2008). Moreover, invertebrates with symbiotic relationships were clearly identified based on their unusual stable-isotope signatures.

Recent isotope studies of landlocked estuaries with limited or intermittent connection to the sea have revealed a different food-web structure. Invertebrates and fish in these estuaries rely predominantly on nutrients from fringing vegetation such as mangroves (Hadwen et al., 2007; Abrantes and Sheaves, 2008). Isotope analysis has also demonstrated an unexpected coupling of the benthic and pelagic components of such an estuary, where jellyfish comprise a very large standing biomass that dominates the plankton seasonally. Stomach-content analysis showed that the diet of jellyfish was dominated numerically by small plankton such as copepods, upon which they feed continuously. Isotope analysis of jellyfish tissue revealed, however, that most of the carbon was actually assimilated from a small number of much larger individuals of species such as ghost shrimp that emerge from the benthos into the water column only at night (Pitt et al., 2008).

7.07.3.4   Tracking Sewage Nitrogen into Food Webs

Estuaries are being fundamentally transformed under pressure of the rapidly increasing human populations in coastal areas, especially through eutrophication from the deposition of nitrogen from sewage. Due to the volatilization of ammonia (the dominant initial form of inorganic N in sewage inputs) and subsequent nitrification and denitrification reactions, the residual inorganic N pool from sewage is typically enriched in ^{15}N compared to inorganic N from natural sources (McClelland and Valiela, 1998). Nitrogen-isotope analysis is proving to be one of the most robust proxies of sewage pollution in coastal waters (Peterson, 1999; Costanzo et al., 2001, 2003). Levels of $\delta^{15}N$ in various ecosystem components in coastal systems with anthropogenically impacted watersheds are typically elevated compared to more pristine systems, and there are now dozens of studies exploring the potential of this approach to document the spatial or seasonal extent of sewage impacts, or the recovery of ecosystems in response to decreased anthropogenic N inputs (e.g., McClelland and Valiela, 1998; Fry, 1999; Gartner et al., 2002; Abreu et al., 2006). Nitrogen-isotope signatures can provide fine-scale resolution of sewage

impact on food webs (Schlacher et al., 2005), and have been used in conjunction with histopathology to demonstrate the adverse impacts of sewage on fish health (Schlacher et al., 2007). They can also show how different components of food webs respond once nitrogen inputs from sewage are reduced; a year after nitrogen reduction in subtropical Australian estuaries, the sewage signal had depurated from algae and shore-crab tissues, whereas the signal was still clearly evident in mangrove trees (Pitt et al., 2009). Nitrogen-isotope analysis shows, too, that sewage nitrogen can be flushed out of the estuary in plume water and incorporated into coastal fisheries food webs (Connolly et al., 2009).

7.07.3.5   Models and Statistics

Early stable-isotope studies used only one element (generally carbon) and data were analyzed by visually estimating the proximity of the consumer of interest to potential sources on a line (e.g., Nichols et al., 1985). To overcome the lack of separation of the carbon-isotope signatures of some autotrophs, bi-plots were constructed using two elements. The demonstration of food-web patterns by referring to bi-plots of C and N isotope ratios was, for many years, the most common form of analysis in estuarine studies. This method is usually limited to qualitative conclusions, however, and it was this drawback that led to rapid advances in statistical processing and modeling.

One advance was to turn to advantage the spatial and temporal variation in source-and consumer-isotope signatures that bedeviled some studies. Kitting et al. (1984) were the first to do this, by plotting carbon signatures of the two potential sources, seagrass and epiphytes, against those of the shrimp consumers, separately for each of their sites. Their demonstration that shrimp signatures tended to match those of epiphytes rather than seagrass was taken as evidence that epiphytes made a substantial contribution to shrimp nutrition. This qualitative demonstration was taken a step further by Melville and Connolly (2003), who developed a two-dimensional (e.g., C and N) correlation statistic for formal, probabilistic hypothesis testing of spatial (or temporal) variation of sources and consumers.

Another advance on graphic analysis has been the quantification of potential source contributions using mathematical mixing models. Initially, these simply provided a more accurate measurement of percent contributions of sources to a consumer (Szepanski et al., 1999). These were then modified to use the variation around the mean source and consumer signatures to provide confidence limits around the estimate of each source contribution (Phillips and Gregg, 2001). Models were developed further to account for concentration-dependent assimilation, for example, for situations where sources have different C:N ratios (Phillips and Koch, 2002), something that has found useful application in estuaries (Wilson et al., 2009).

Even these more advanced mixing models were restricted to situations with a unique mathematical solution. This meant that the number of potential sources that could be used in a model was limited to one more than the number of elements used (e.g., in a two-element study, up to three sources could be used). This can be a limitation for studies in estuaries, where the number of potential sources is particularly large

(Bouillon et al., 2008). The IsoSource model (Phillips and Gregg, 2003), which examines the entire distribution of feasible solutions in such cases where no unique solution exists, was the first proposed model to provide objective quantitative estimates in such situations, and was thus quickly taken up by estuarine researchers (e.g., Melville and Connolly, 2003). Other simulation routines have been developed since (e.g., Lubetkin and Simenstad, 2004), but Isosource remains a widely used mixing model (Benstead et al., 2006). While the original version of IsoSource does not take differences in element concentrations into account, modified versions have been used to overcome this shortcoming (e.g., Granek et al., 2009). One of the other limitations of IsoSource, however, is that it models mean values of source and consumer signatures, rather than the distribution of actual values, and that it does not account for variability or uncertainty in the fractionation factors used. Bayesian modeling approaches are becoming more popular to address these issues, and several models have recently been proposed, which include variability and sensitivity analyses as improvements on IsoSource, such as MixSIR (Moore and Semmens, 2008; Semmens et al., 2009) and stable isotope analysis in R (SIAR) (Parnell et al., 2008).

Finally, natural-abundance stable-isotope data offer many more possibilities than just visual interpretation of source contributions or more quantitative estimates of possible source contributions. There is much scope in further developing quantitative metrics to interpret stable isotope data in terms of the ecological niches, community-wide food-web structure and trophic diversity (e.g., see Layman et al. (2007) and Newsome et al. (2007) and subsequent discussion by Hoeninghaus and Zeug (2008) and Layman and Post (2008)), and circular statistics to quantitatively compare differences in food-web structure across time and/or space (Schmidt et al., 2007).

7.07.3.6 Dealing with Fractionation Issues in Food Web Studies

Isotopic fractionation, one of the processes described in Section 7.07.2, has proved to be an ongoing issue in estuarine food-web studies. Biochemical and physiological processes in the production and transfer of C, N, and S within food webs result in changes in isotope ratios between trophic levels. Adjustments for fractionation are required before applying most types of statistical analysis or modeling (although not for the spatial analyses mentioned above, which is an advantage of those techniques). Meta-analyses of fractionation results from numerous studies have shown that there is some regularity to the pattern of fractionation, with a greater fractionation shift for N than for C or S (Peterson and Fry, 1987; Vander Zander and Rasmussen, 2001; McCutchan et al., 2003; Caut et al., 2009). The actual degree of fractionation for an animal in a specific situation, however, is affected by several factors including age (Overman and Parrish, 2001), food availability (Barnes and Jennings, 2007), and food quality (Adams and Sterner, 2000).

The best solution is to determine situation-specific fractionation rates for key species experimentally, so that the correct adjustment can be made prior to analysis. Typically, in estuarine studies, this has not, or cannot, be done, and adjustments must be based on estimates of trophic levels and the expected extent of fractionation (McCutchan et al., 2003; Caut et al., 2009). The most recent meta-analyses of experimental fractionation data

(Caut et al., 2009) revealed fairly consistent differences in dietary shifts for $\delta^{13}C$ between mammals, birds, fishes, and invertebrates, and also lower fractionation of ^{15}N for fish and invertebrates when compared to mammals and birds. Surprisingly, they also reported relationships (although not significant in all cases) between the degree of isotope fractionation and the actual stable-isotope signature of the diet. Unfortunately, mixing models can be highly sensitive to these adjustments and the larger and more variable adjustments required for ^{15}N can render this element less useful for source identification in mixing models (Connolly et al., 2005b).

7.07.4 Stable Isotope Enrichment Experiments

The relatively large number of potential sources of primary production in estuaries means that there is often an overlap of source-isotope ratios. A novel means of distinguishing among food sources has been developed for situations where analysis of natural isotopic ratios cannot separate their contributions. One or more putative sources are spiked, or labeled, with an artificially enriched isotope ratio (e.g., ^{13}C and ^{15}N). Any contribution of the artificially enriched source to the diet of consumers is detected as a shift in isotopic ratios of consumers. It is because most tracer experiments in estuaries use enrichment of the uncommon, heavy isotope that these studies are referred to as enrichment experiments; however, the experiments can also be done using artificial depletion of the uncommon isotope (or enrichment of the common isotope, see Mutchler et al. (2004) for an example in seagrass meadows).

The methods were initially applied in freshwater systems using either carbon- or nitrogen-isotopic enrichment (e.g., Hall, 1995). The first application in estuaries was the demonstration by Winning et al. (1999) that the normally similar nitrogen-isotope ratios of seagrass and its epiphytic algae could be artificially separated using enrichment methods. Although the experimental procedures are much more difficult than natural abundance surveys, they lead to stricter testing of hypotheses and a higher level of certainty about trophic links. The number of enrichment experiments in estuaries has increased rapidly over the last decade, matched by the rate of exciting revelations about food webs. Deliberate tracer experiments in estuaries have ranged from simple process-rate measurements (typically under closed conditions, e.g., incubation bottles or cores) to more complex *in situ* labeling experiments.

One of the most basic applications of stable-isotope-tracer experiments is the determination of uptake or assimilation rates of certain elements. A typical example is the determination of primary production rates using ^{13}C-labeled bicarbonate additions as an alternative to the more traditional ^{14}C method (e.g., Hama et al., 1983; Dauchez et al., 1995; Pimenov et al., 2008) – despite the simplicity of the technique, this approach is still surprisingly little used in comparison to other methods such as dark/light O_2 evolution, ^{18}O labeling, or ^{14}C incorporation. The particular advantages of using ^{13}C rather than ^{14}C (besides the obvious benefits of not working with radioactive tracers) is the scope to extend simple measurements of particulate production by phytoplankton to DOC production (through measurements of ^{13}C labeling in DOC), estimates of group- or taxon-specific primary production by looking at ^{13}C incorporation in specific algal biomarkers (PLFA or other

membrane lipids; see e.g., Wuchter et al. (2003) and Dijkman et al. (2009)), and longer-term tracing of the fate of pelagic primary production into heterotrophic microbial communities and/or higher trophic levels. The latter approach has been applied extensively in lake systems (e.g., Carpenter et al., 2005; Kritzberg et al., 2006), but, on the mesocosm scale, is also applicable in estuarine waters (e.g., Van den Meersche et al., 2004).

In intertidal areas, ^{13}C-labeled substrates have been applied successfully to trace the fate of microphytobenthic production on mudflats (Herman et al., 2000; Middelburg et al., 2000), salt marshes (Carman and Fry, 2002), mangroves (Oakes et al., 2010), and sandflats (Lee et al., 2011). Probably, ^{13}C-labeling experiments will play a critical role in quantifying chemo-autotrophic production and its fate in estuarine sediments and in the water column (e.g., see de Bie et al., 2002; Knief et al., 2003).

The use of ^{15}N-labeled substrates has been more widespread in estuarine environments, and is used widely to quantify denitrification rates by the isotope-pairing technique (Nielsen, 1992). Benthic or pelagic N uptake rates by microbial communities can also be quantified using ^{15}N-labeled substrates. Furthermore, the advent of compound-specific stable-isotope analyses has recently enabled a distinction to be made between ^{15}N assimilated by heterotrophic and autotrophic communities, by determining ^{15}N uptake in the bacterial marker D-alanine (Veuger et al., 2005, 2007; Cook et al., 2007; Veuger and Middelburg, 2007). ^{15}N labeling with inorganic N substrates has been used successfully in field experiments in estuarine and tidal freshwater marshes (Hughes et al., 2000; Tobias et al., 2001, 2003; Gribsholt et al., 2005, 2009). Work from salt-marsh creeks shows that these experiments work even for higher trophic levels (Galvan et al., 2008). Carman and Fry (2002) used dual labeling (C and N) to demonstrate differences in feeding strategies among meiofauna species that were not evident based on natural-abundance isotope data.

Analysis of isotope-enrichment data has been made more quantitative through the use of numerical modeling. Early enrichment experiments could only quantify contributions if the experiment continued for long enough to achieve isotopic equilibrium, something unlikely in most experiments, particularly those involving larger animals. Hamilton et al. (2004) solved this problem in a freshwater system using a compartment modeling approach, in a computer program called WinSAAM that avoided the need to wait for tissue-isotope ratios to reach equilibrium. Another compartment-modeling approach, using the equations in FEMME, has been used to determine nutrient pathways of bacterial carbon in a benthic-estuarine food web (van Oevelen et al., 2006). These approaches can estimate precisely the contributions of enriched versus nonenriched sources.

7.07.5   Stable Isotopes as Tracers of Animal Movement

7.07.5.1   Introduction

Another major use of stable-isotope analysis in estuaries is to determine the movement of animals, especially patterns of regular migration or ontogenetic movements. The concept relies on two conditions being met:

1. food sources in different places having different isotope signatures and
2. animals remaining long enough in any one place to assimilate enough food to take on the isotope signature of that place.

The use of stable-isotope analysis to study animal movement has only recently been reported in estuaries. There has been a longer history of use for terrestrial mammals and birds (Rubenstein and Hobson, 2004; Crawford et al., 2008), and these terrestrial studies highlight the potential in estuarine systems. Some recent intelligent work tracking juvenile Atlantic salmon in freshwater lakes has also been done in Canada, where broad movements between tributaries and lakes measured using stable-isotope analysis have been successfully combined with more detailed, finer-scaled measures using tagging and telemetry (Cunjak et al., 2005).

In estuaries, work falls into three categories of study: movements in and out of estuaries, movements among habitats within estuaries, and site fidelity of animals.

7.07.5.2   Movements in and out of Estuaries

This work resolves the question of when animals enter or leave brackish estuarine waters for either fresh or truly marine waters adjacent to the estuary. The boundary between fresh- and saltwater provides a variety of chemical signals, more broadly than stable isotopes, and these have been quite widely used to track fish migration. The most commonly used signal is the relative concentration of strontium (Sr)–barium, which can be detected in the otoliths of fish. The otolith elemental composition can, therefore, provide a reliable indicator of one or more shifts between fresh- and saltwater during the life of fish (Gillanders et al., 2003).

In stable-isotope analysis, resolution of animal movements relies on differences in isotope signatures between either freshwater and estuarine, or estuarine and marine food sources. In many cases, freshwater systems are dominated by terrestrial plant inputs and the whole food web takes on a depleted carbon-isotope signature. This can be different from the estuarine signatures where the estuary is inhabited by, for example, plants such as seagrasses with their more enriched carbon-isotope signatures. Carbon-isotope signatures in marine systems, especially planktonic systems, hover around a value of –20‰ (Riera and Richard, 1996). These differences have been used to monitor the movements of fish in and out of estuaries.

A good example of how stable isotope analysis provides evidence of migration is the movement of the Asian catfish from estuarine to freshwater reaches of the Mekong River in Laos (Hogan et al., 2007). Strontium concentrations in catfish otoliths suggested upriver migrations, and these were confirmed by showing that fish caught in freshwater reaches had taken on a more enriched marine carbon-isotope signature (–18‰) in their flesh during prior feeding in the estuary (Hogan et al., 2007).

Animal movements between estuarine and marine waters using stable-isotope analysis of fish tissue have been demonstrated only recently. Theoretically, the extent to which the fish-tissue ratio varies between the two extremes of estuarine and marine signals will explain when a fish was recruited (Herzka, 2005). An attempt to use isotope analysis of fish

otoliths of juvenile plaice in the Firth of Forth, Scotland, concluded that juvenile plaice ratios did not match sediment ratios, and, thus, benthic prey ratios, well enough to reliably hind-cast where adults had come from (Augley et al., 2007). The use of carbon ratios in fish tissue has been more successful at calculating recruitment by juvenile herring and sprats from the North Sea into the Schelde estuary (Guelinckx et al., 2006). This work was further advanced conceptually using similar data on a sand goby from the same estuary. Using modeled rates of tissue turnover between the estuarine and marine end members, a very informative isotopic clock could be used to calculate when fish moved into the estuary (Guelinckx et al., 2008).

7.07.5.3   Movements among Habitats within Estuaries

There are several good examples of stable-isotope analysis being used to demonstrate animal movements among habitats within estuaries. Following the pioneering study of Fry (1981), documenting shrimp migration patterns in coastal systems has been a frequent case study of this approach (e.g., Fry et al., 1999; Riera et al., 2000). Fry et al. (2003) analyzed stable-isotope data on the shrimp *Farfantepenaeus aztecus* in a salt-marsh system in Louisiana, USA. They collected juvenile shrimp from adjacent marsh, pool, and open water sites during an intensive, fine-scaled survey. Their novel analysis compared isotope values of shrimp to the values of food sources specific to these microhabitats. They were able to infer how long shrimp had been in each microhabitat from the extent of the mismatch between shrimp and food values (Figure 8). Greater dissimilarity was interpreted as recent arrival by shrimp. The stable isotope results helped build a scenario of ontogenetic migration through less-favored open-water habitats to ponds within the salt marsh that provide better-quality nursery sites (Fry et al., 2003). More recently, Leakey et al. (2008) used stable-isotope analysis in the Thames estuary, UK, to delineate subpopulations of juvenile sole. First, they used a multivariate statistical technique, discriminant function analysis, on C, N, and S isotope ratios to demonstrate distinctly different isotope characteristics of invertebrate prey in estuarine and coastal areas. The correlation of sole isotope values against these distinct invertebrate signals led to the discovery of previously unidentified subpopulations of these juvenile fish, an important fisheries species.

A similar strategy has been proposed for pelagic estuarine waters (Suzuki et al., 2008). In the Chikugo estuary in Japan, planktonic crustaceans that form the main prey of juvenile Japanese bass show a well-defined gradient in C isotope values, from –28‰ in the upper estuary to –18‰ in the marine-dominated lower estuary. Suzuki et al. (2008) have proposed that movements of juvenile bass can, in future, be measured against this consistent, predictable relationship in prey values.

Stable-isotope analysis has also proven very effective in tracking fine-scale movements of coral reef fish over short periods between adjacent seagrass and mangrove habitats (Cocheret de la Morinière et al., 2003). This work, in the Caribbean, is not estuarine, but the soft-sediment habitats are similar to those found in estuaries, and the success of this work further highlights the potential of stable-isotope analysis for movement studies in estuaries.

7.07.5.4   Demonstration of Site Fidelity

Stable-isotope studies have produced convincing demonstrations of site fidelity in fish. These studies have principally aimed at determining estuarine food webs, but have ultimately been used to conclude that fish move surprisingly little, remaining in the same estuary, and in particular reaches of estuaries. The first such example was from the extensively modified Nerang estuary on the Gold Coast, Australia. Snub-nosed garfish are omnivorous, obtaining the bulk of their energy from seagrass or algae, and their protein from invertebrate prey (Waltham and Connolly, 2006). Carbon-isotope values of garfish differ enormously in fish collected from seagrass meadows (–12‰), and adjacent algal-dominated canals (–19‰). This striking difference is consistent over different seasons and years (Waltham and Connolly, 2006), showing that individuals in these two adjacent habitats form subpopulations that do not mix over timescales of weeks to months (Connolly, 2003).

Site fidelity was also demonstrated during a study of sewage nitrogen incorporation into food webs in estuaries along the east coast of Australia (Schlacher et al., 2005). The enriched N-isotope signature typical of treated sewage provides a convenient tracer. Fish incorporated the enriched N-isotope values in the mid-estuarine reach of the Maroochy estuary, adjacent to the main sewage outfall. For both the benthic-feeding whiting and the more pelagic silver biddy, this pattern was highly consistent, both with distance up the Maroochy estuary and between this estuary and the nearby unpolluted Noosa estuary (Schlacher et al., 2007). Once again, this pattern was consistent over different seasons and years, demonstrating distinct subpopulations with little mixing over the time it takes to incorporate N-isotope signatures (Figure 9).

7.07.5.5   Advantages of Stable Isotopes for Studying Animal Movements

Stable-isotope studies of animal movements have several advantages over tagging techniques. The animals essentially tag themselves, gaining distinct stable-isotope signatures merely by feeding. No intrusive tagging is necessary, thus saving the substantial effort and cost of traditional tagging (Begg et al., 1997). Probably even more importantly, it also means that even the smallest animals can be studied; the difficulty of tracking larval and early juvenile fish has severely hampered fisheries research in estuaries (Gillanders et al., 2003).

As stable-isotope studies of animal movements become more frequent, we predict that several formerly irresolvable questions will be answered. Differences in carbon, nitrogen, and sulfur isotope ratios either among estuaries (e.g., on a latitudinal scale; Rubenstein and Hobson, 2004) or within estuaries (e.g., between benthic and pelagic, or shallow and deep) should prove very useful. These types of study are closely related to food-web research, but the different objective requires different spatial and temporal intensity of sampling. For small animals, sampling usually results in mortality; hence, rare or endangered species will not be suitable candidates. However, for larger animals, only a blood sample, a scale or a tissue biopsy might be needed, and there is enormous potential for increased use of stable isotopes in movement studies.
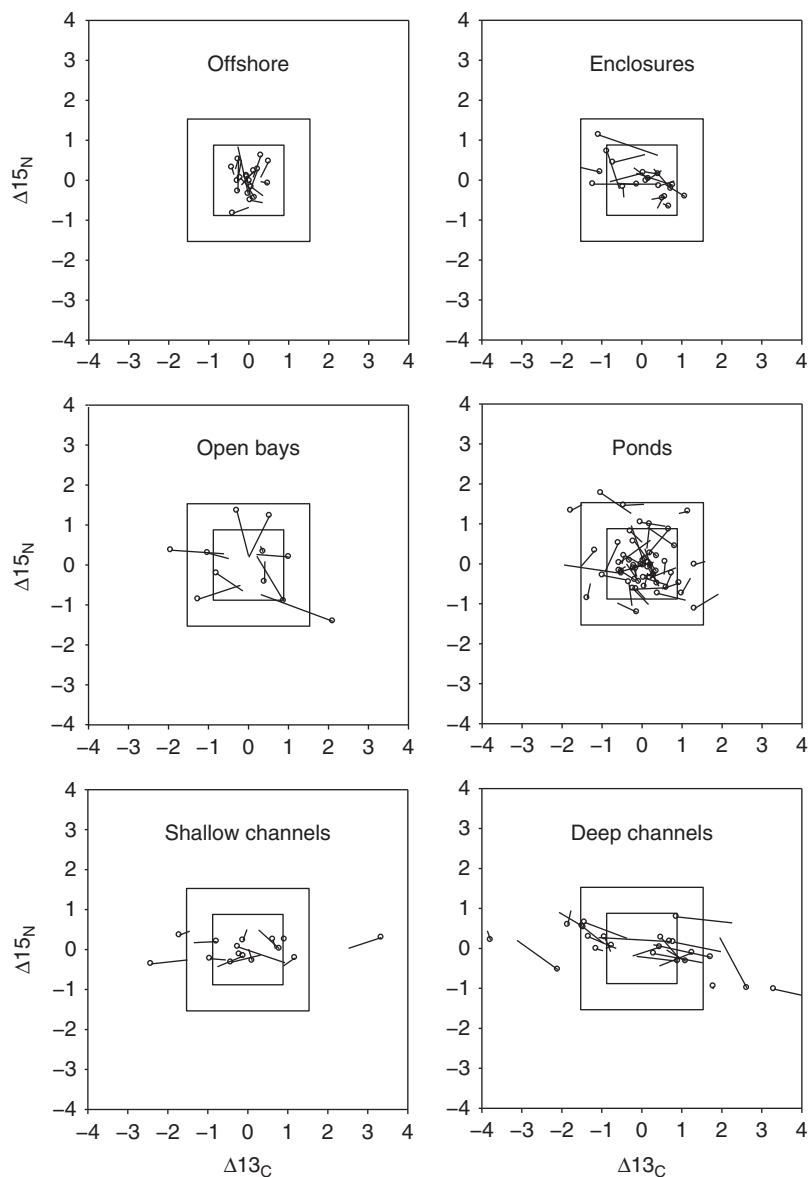
Figure 8 Isotope analysis of diet switching as a residency measure in the shrimp *Farfantepenaeus aztecus* in six microhabitats in a Louisiana salt marsh. Each shrimp is represented by an open circle and a line, with the circle representing isotope values of the long-term diet (isotope analysis of shrimp) and the opposite end of the line representing the most recent diet (isotope analysis of food sources in that microhabitat). Two boxes are given for reference that include all offshore residents (small box) or all enclosure and offshore residents (large box). Residents have both long-term past and recent past isotopes values within a box, specialists have long-term past isotopes within the box but recent past isotopes outside the box, and immigrants have long-term past isotopes outside the box but recent past isotopes within the box. Reproduced with permission from Fry, B., Baltz, D.M., Benfield, M.C., Fleeger, J.W., Gace, A., Haas, H.L., Quinones-Rivera, Z.J., 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries 26, 82–97.

7.07.6   High-Resolution Stable Isotope Records: Biological Archives of Past Environmental Conditions

Carbonate-secreting organisms such as mollusks (e.g., bivalves and gastropods) or corals produce carbonate in sequential layers through time. Therefore, they have the potential of storing information about estuarine conditions throughout the time in which they grew. Both the analysis of the variations in the width of these growth lines (sclerochronology) and their geochemistry (sclerochemistry; see Gröcke and Gillikin, 2008) can provide records of past estuarine environmental conditions. Foraminifera and Ostracoda are generally short lived, but can

be sampled from sediment cores and provide data on past conditions, with timescales ranging from 10^7 to 10^2 years, with decadal or even annual resolution (e.g., Ingram and Sloan, 1992, Cronin et al., 2002a, 2002b), but not usually with seasonal resolution. Bivalves, on the other hand, can offer snapshots of several years at very high temporal resolution (daily and even sub-daily; e.g., Wurster and Patterson, 2001; Lazareth et al., 2007) because they exhibit very high growth rates and thereby provide adequate material to sample at high resolution. Bivalves can have shell-extension rates ranging from 0.2 to 2 cm yr^{-1} (e.g., Gillikin et al., 2005c) and even up to 6 cm yr^{-1} (Cerrato and Keith, 1992; Goewert and Surge, 2008). The isotope
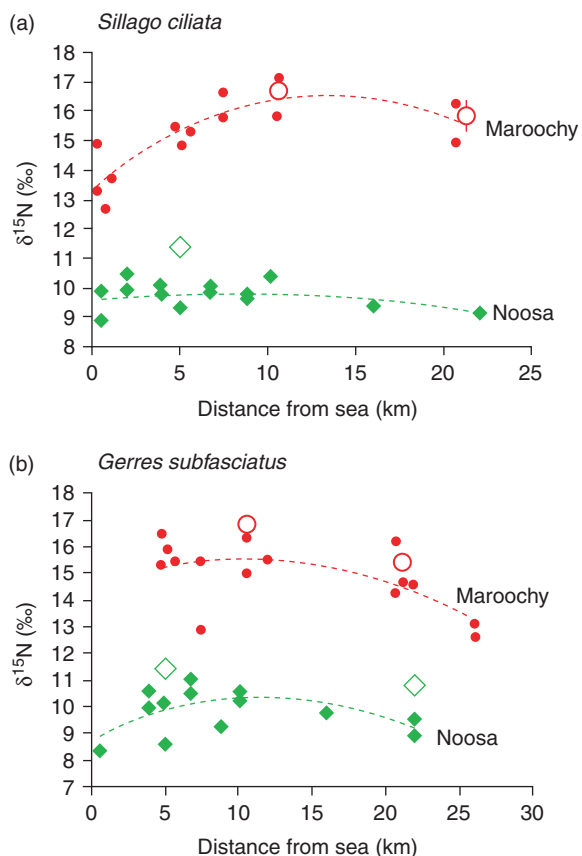
Figure 9 Variation in stable nitrogen isotope ratios along the main axis of three estuaries in Queensland, Australia, in two species of fish (a) Summer Whiting and (b) Silver Biddy. Small symbols are data from 2000 to 2001 (Schlacher et al., 2005), compared with data obtained in 2006 denoted by large symbols for each estuary (error bars for 2006 data are smaller than symbol plot sizes). From Schlacher, T.A., Mondon, J.A., Connolly, R.M., 2007. Estuarine fish health assessment: evidence of wastewater impacts based on nitrogen isotopes and histopathology. Marine Pollution Bulletin 54, 1762–1776.

geochemistry of biological carbonates has long been known to reflect the environmental conditions under which the animal grew (Epstein et al., 1953; Mook and Vogel, 1968; Mook, 1971). The isotopes most extensively studied in biological carbonates are oxygen and carbon. However, more recently, isotopes of Ca as well as trace metals (e.g., Mg, Sr, and B) have been investigated (Hemming and Hanson, 1992; Ingram and Sloan 1992; de Villiers et al., 2005). Moreover, mollusk shells contain up to 5% organic matter in and between the carbonate crystals (Marin and Luquet, 2004; Rueda and Smaal, 2004). Isotope data from shell organic matter hold similar information as in organic tissues, but unlike the tissue material, the information from the shell organic matter can be extended back through time (O'Donnell et al., 2003). Isotopes of C, N, S, and H in shell organic matter can be relatively easily analyzed.

This section will discuss the use of these aforementioned proxies to investigate past estuarine functioning, with a focus on estuarine bivalve shells. Biological carbonates can provide information on past estuarine water temperature, salinity, carbon cycling, pollution, nitrogen cycling, productivity, and riverine discharge, as well as the timing of biological invasions.

7.07.6.1   Controls on Shell $\delta^{18}O$

In 1947, Urey determined that the oxygen-isotope fractionation between carbonates and water is temperature dependent. Subsequently, Epstein et al. (1953) found that the oxygen-isotopic signature recorded in mollusk shells ($\delta^{18}O_{Shell}$) not only reflects the temperature of crystallization, but also reflects the oxygen-isotopic signature of the water ($\delta^{18}O_{water}$) within which they formed. Freshwater is typically more depleted in ^{18}O than is seawater due to Rayleigh fractionation during precipitation (see Dansgaard, 1964), and $\delta^{18}O_{water}$ behaves conservatively with salinity (i.e., the relationship between salinity and $\delta^{18}O_{water}$ is typically linear). Furthermore, shell mineralogy also affects the isotopic composition of the carbonate; bivalves are known to precipitate either of the two polymorphs of calcium carbonate, that is, calcite or aragonite (or both), and calcite is depleted in ^{18}O by about 0.6–1.0‰ relative to aragonite (Tarutani et al., 1969; Kim and O'Neil, 1997; Böhm et al., 2000; Kim et al., 2007). Taking all the above-mentioned factors into account, $\delta^{18}O_{shell}$ values can provide information about past water temperature, salinity, freshwater discharge, and precipitation.

Ever since Urey's (1947) discovery, scientists started to develop empirical paleo-temperature equations, which were determined by sampling organisms grown under different temperatures with known $\delta^{18}O_{water}$. The two most popular empirically derived paleo-temperature equations for mollusks are the equations developed by Epstein et al. (1953) (modified by Anderson and Arthur, 1983) for calcitic bivalves:

$$T(°C) = 16–4.14^*(\delta^{18}O_{Shell}−\delta^{18}O_{water}) + 0.13^*(\delta^{18}O_{Shell}−\delta^{18}O_{water})^2 \qquad [3]$$

and the equation developed by Grossman and Ku (1986) for aragonite mollusks:

$$T(°C) = 20.6–4.34^*(\delta^{18}O_{Shell}−(\delta^{18}O_{water}−0.2)) \qquad [4]$$

where $\delta^{18}O_{Shell}$ is the $\delta^{18}O$ value of CO_2 (vs. Vienna Peedee Belemnite (VPDB)) liberated from the reaction between carbonate and phosphoric acid at 25 °C, and $\delta^{18}O_{water}$ is the $\delta^{18}O$ value of CO_2 (vs. Vienna Standard Mean Ocean Water (VSMOW)) equilibrated with water at 25 °C. There are other more recent equations (see Kim et al., 2007 for aragonite and Thébault et al., 2007 for calcite), but those shown above are by far the most frequently used. However, it should be noted that use of these equations in this form can result in a systematic error when $\delta^{18}O_{water}$ is less than −10‰ (SMOW) such as in freshwater systems. In these cases, the '1000 ln (α)' form should be used (see Dettman et al., 1999). Dettman et al. (1999) used the data from Grossman and Ku (1986) to relate temperature directly to the fractionation factor for aragonite:

$$1000 \ln(\alpha) = 2.559(10^6 T^{−2}) + 0.715 \qquad [5]$$

where T is degrees Kelvin and α is the fractionation between water and aragonite described by

$$\alpha \text{ aragonite/water} = (1000 + \delta^{18}O_{aragonite})/(1000 + \delta^{18}O_{water}) \qquad [6]$$

where both aragonite and water are on the VSMOW scale. A similarly formatted equation for calcite is available from Kim and O'Neil (1997) (0.25 should be subtracted from

measured $\delta^{18}O$calcite depending on the acid fractionation factor used (see also Chauvaud et al., 2005):

$$1000 \ln(\alpha) = 18.03(10^3 T^{-1}) - 32.42 \qquad [7]$$

Although there have been a few reports of mollusks precipitating carbonate out of O-isotope equilibrium (based on the aforementioned equations) (Fastovsky et al., 1993; Fenger et al., 2007; Thébault et al., 2007; Ford et al., 2010; Furguson et al., 2011; García-March et al., 2011), it does seem that most bivalves precipitate near thermodynamic equilibrium (see Wefer and Berger, 1991; Dettman et al., 1999; Kennedy et al., 2001; Lécuyer et al., 2004; Chauvaud et al., 2005; Kim et al., 2007; Wanamaker et al., 2007; and many more), and the empirical paleo-temperature equations based on mollusks have been used with much success. Moreover, many of the studies suggesting disequilibrium did not characterize water $\delta^{18}O$ very well; hence, the possibility that these mollusks are actually in equilibrium still remains.

While most bivalves typically precipitate their shell in oxygen-isotope equilibrium, this is not the case for all biogenic carbonates. Despite the fact that corals and bivalves both calcify rapidly from an internal fluid with varying pH, they show vastly different vital effects; with corals generally precipitating out of isotopic equilibrium. The term 'vital effect' has been applied to biogenic carbonates that are apparently not formed in isotopic equilibrium (Urey et al., 1951). Different explanations for the vital effect have been proposed: 'kinetic effects' and 'carbonate ion effects'. Kinetic effects can cause depletions in ^{18}O relative to equilibrium when $CaCO_3$ precipitation is fast enough to allow precipitation of HCO_3^- and/or CO_3^{2-} before equilibration with H_2O

(McConnaughey, 1989a, 1989b). As both C and O are on the same molecule, kinetic effects will act on both isotopes and cause a correlation between the two (Figure 10; McConnaughey, 1989a). However, it should be noted that $\delta^{18}O_{water}$ and $\delta^{13}C$–DIC can also (and will likely) covary in estuaries (Figure 11), which would also cause a correlation between these two isotopes in shells. 'Carbonate ion effects' involve equilibrium between $CaCO_3$ and the individual inorganic carbon species (CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}) of the DIC, the abundance of which is pH dependent. The carbonate model has been proposed for both foraminifera (Spero et al., 1997; Zeebe, 1999) and corals (Adkins et al., 2003), but not yet for bivalves. Interestingly, Spero et al. (1997) discussed the pH of the external medium and both Adkins et al. (2003) and Rollion-Bard et al. (2003) discuss the pH at the internal calcification site. Nevertheless, McConnaughey suggests that these effects can still be explained by purely kinetic effects (Cohen and McConnaughey, 2003; McConnaughey, 2003).

7.07.6.2 Biogenic Carbonates as Paleo-Thermometers

Having a good estimation of the $\delta^{18}O_{water}$ at a high temporal resolution is crucial when calculating temperature from $\delta^{18}O_{shell}$, especially in estuarine conditions. The fact that coastal regions are highly dynamic in nature, and the stable-isotope ratios in carbonates are dependent on the isotope ratio of the water, which co-varies with salinity, make these areas difficult for isotope paleo-thermometry. In addition to the problem of variable salinity, variable or multiple-source freshwater end members will cause changes in the salinity–$\delta^{18}O_{Water}$ relationship (e.g., groundwater and surface water). This makes using salinity to determine
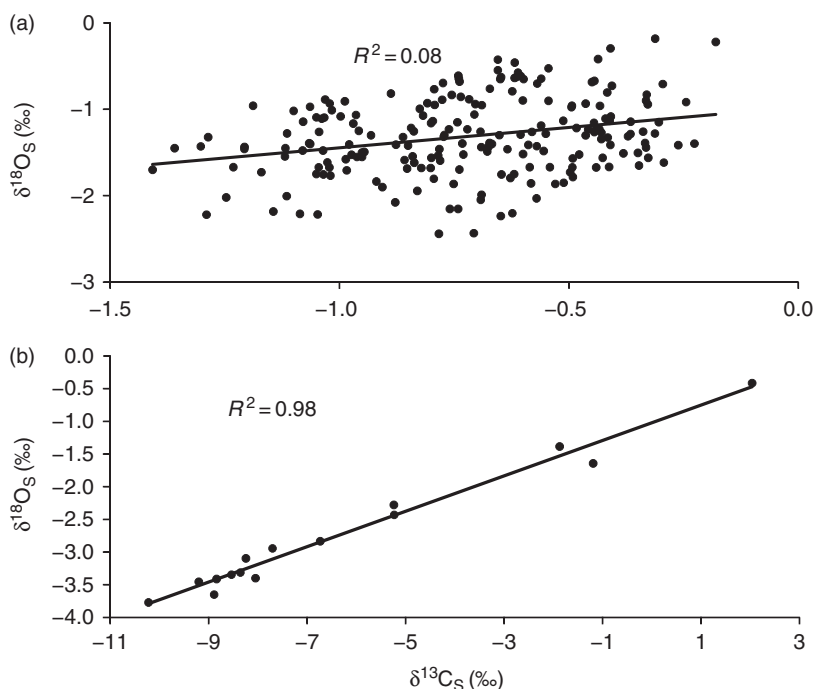
Figure 10 (a) Regression between $\delta^{18}O_S$ and $\delta^{13}C_S$ for a bivalve shell (*Saxidomus giganteus*) shell and (b) a coral from the Galapagos Islands. *S. giganteus* data from Gillikin, D.P., De Ridder, F., Ulens, H., Elskens, M., Keppens, E., Baeyens, W., Dehairs, F., 2005b. Assessing the reproducibility and reliability of estuarine bivalve shells (*Saxidomus giganteus*) for sea surface temperature reconstruction: implications for paleoclimate studies. Palaeogeography, Palaeoclimatology, Palaeoecology 228, 70–85; and coral data from McConnaughey, T.A., 1989a. ^{13}C and ^{18}O isotope disequilibrium in biological carbonates. 1. Patterns. Geochimica et Cosmochimica Acta 53, 151–162.
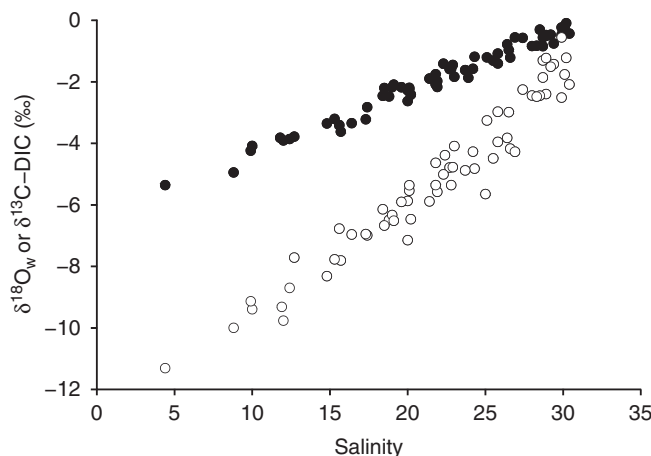
Figure 11 Relationship between salinity and $\delta^{18}O_{Water}$ (filled symbols) and $\delta^{13}C_{DIC}$ (open symbols) in Schelde (the Netherlands) waters sampled at least monthly over a full year. The simple linear regressions are $\delta^{18}O_{Water}$ = Salinity * 0.20 ($\pm$0.01) – 6.31 ($\pm$0.20) (R^2 = 0.97, $p < 0.0001$, $n = 63$) and $\delta^{13}C_{DIC}$ = Salinity * 0.39 ($\pm$0.03) – 13.71 ($\pm$0.57) (R^2 = 0.94, $p < 0.0001$, $n = 63$). $\delta^{18}O$ data from Gillikin, D.P., 2005. Geochemistry of Marine Bivalve Shells: The Potential for Paleoenvironmental Reconstruction. Ph.D. Thesis, Vrije Universiteit Brussel, Belgium, p. 258; and $\delta^{13}C$ data from Gillikin, D.P., Lorrain, A., Bouillon, S., Willenz, P., Dehairs, F., 2006b. Stable carbon isotopic composition of *Mytilus edulis* shells: relation to metabolism and $\delta^{13}C$ of DIC and phytoplankton. Organic Geochemistry 37, 1371–1382. $\delta^{18}O$ data available on the Global Seawater Oxygen-18 Database at http://data.giss.nasa.gov/o18data/

$\delta^{18}O_{Water}$ prone to errors. For example, Ingram et al. (1996a) developed a salinity–$\delta^{18}O_{Water}$ relationship for San Francisco Bay based on measurements taken along a salinity gradient over 1 year ($\delta^{18}O_{Water}$ = Salinity * 0.32 ($\pm$0.01) – 10.90 ($\pm$0.23) (R^2 = 0.98, $p < 0.0001$, $n = 64$)). The prediction intervals on this relationship are rather large for $\delta^{18}O_{Water}$ (~2‰ at a salinity of 27; see Gillikin et al., 2005b); thus, using this relationship to calculate temperatures from shells by determining $\delta^{18}O_{Water}$ from salinity data will result in large errors (on the order of ~8 °C). Comparable errors were also found on the slope and intercept for a salinity–$\delta^{18}O_{Water}$ relationship from the Schelde estuary (The Netherlands; see **Figure 11**). Klein et al. (1997; their Fig. 1) illustrate the error that can occur when assuming a constant $\delta^{18}O_{water}$ value, and how this error is different depending on the value of the freshwater $\delta^{18}O$ end member.

A new proxy that circumvents the $\delta^{18}O_{water}$ problem is 'Clumped isotope thermometry' ($\Delta47$), which provides independent temperature estimation from carbonates (see Eiler, 2007). The clumped isotope method is based on the principle that 'clumping' of doubly substituted isotopologs of rare heavy isotopes (e.g., ^{13}C, ^{18}O) is temperature dependent. The amount of clumped isotopes is locked into the carbonate, with the heavy pair being slightly more stable and abundant at lower crystallization temperatures. The method is independent of source water because the amount of doubly substituted isotopologs is compared to the distribution of these clumped pairs in a high temperature gas with a random (stoichiometric) distribution of clumped isotopes (Ghosh et al., 2006; Schauble et al., 2006). $\Delta47$ calibration work on bivalves is ongoing (e.g., Henkes et al., 2010; Affek, 2011).

7.07.6.3 Biogenic Carbonates as Paleo-Discharge or Salinity Indicators

Despite the fact that paleo-thermometry in estuaries is challenging, the $\delta^{18}O$ values of estuarine mollusk shells can provide other environmental information. Ingram et al. (1996b, 1996c) used both O and C isotopes in different species of bivalve shells

obtained from sediment cores in San Francisco Bay to reconstruct the salinity of the Bay over several millennia (see further for more discussion on $\delta^{13}C_{shell}$). In both studies, the authors analyzed whole shells or sections of shells, and integrated most of the growing season. Moreover, they assumed the same average temperature for all the samples analyzed, and attributed changes in shell isotopes to changes in salinity over the millennia. While their methods were crude compared to today's standards, it certainly was pioneering and they were able to detect large-scale changes in salinity and, thus, river discharge. Dettman et al. (2004) conducted a similar (but high-resolution) study in the Gulf of California with the aim of determining paleo-discharge of the Colorado River. Today, the Colorado River discharge is entirely consumed by humans and only reaches the Gulf of California during the wettest years. By developing a mixing model between marine and freshwater end members, Dettman et al. (2004) were able to rather convincingly reconstruct known discharges from bivalve shell $\delta^{18}O$, thereby giving strong support to their paleo-discharge calculations. Dettman et al. (2004) used micromilling (micrometer-scale milling of shell carbonate using a dental drill bit) and sclerochronology to assign dates to their carbonate samples, and to obtain high-resolution shell $\delta^{18}O$ profiles from the first few years of the bivalve's life (to avoid time averaging). They then assumed similar temperature seasonality between years and attributed changes in shell $\delta^{18}O$ to changes in riverine discharge. This high-resolution method avoids unknowns involved with analyzing whole shells.

The $\delta^{18}O_{water}$ value can also be calculated if the 'shutdown temperature' of a particular species is known, that is, the temperature below or above which many bivalves precipitate little if any shell material (e.g., Dettman et al., 1999; Goodwin et al., 2001; Surge et al., 2001; Schöne et al., 2003a). For example, Surge et al. (2001) have shown that the eastern oyster (*Crassostrea virginica*) does not produce shell above 28 ± 2 °C. Goodwin et al. (2001) found that the estuarine clam *Chione cortezi* does not produce shell below ~17 °C or above ~31 °C. Therefore, in *C. cortezi*, the minimum $\delta^{18}O_{shell}$ value should represent ~31 °C and the maximum $\delta^{18}O_{shell}$ value should

represent ~17 °C, both of which can then be used to solve for $\delta^{18}O_{water}$. Considering that the maximum and minimum $\delta^{18}O_{shell}$ values are known and the temperature associated with these data points can be estimated (from the shutdown temperature), then the $\delta^{18}O_{water}$ can be solved for using the paleo-temperature equations listed above. This $\delta^{18}O_{water}$ value can then be used as a salinity indicator or possibly to determine large-scale changes in precipitation or water sources (e.g., rain, glacial melt, or groundwater). Dettman et al. (2004) used a similar technique, also based on growth hiatuses, to calculate the freshwater $\delta^{18}O_{water}$ end member from freshwater mussel shells. Although this technique can provide robust data, it does have its caveats. Other parameters such as salinity, food availability, mean annual temperature, turbidity, and ontogenic stage can affect shell growth; however, temperature does seem to have the strongest impact of all the variables (see Goodwin et al., 2001). If these events are unknown, shutdown temperatures could be different than expected (Dettman et al. (2004) actually used shell hiatuses caused by turbidity to calculate $\delta^{18}O_{water}$).

Another potential problem with this method is that shutdown temperatures can be somewhat variable; hence, errors are certainly associated with this calculation, and only large changes in calculated $\delta^{18}O_{water}$ can be confidently determined. For example, the $\pm$ 2 °C range in shutdown temperature reported by Surge et al. (2001) results in a possible range of $\delta^{18}O_{water}$ of 1‰. This range might be due to methodological error caused by time averaging (or is species specific). Using higher-resolution techniques, Schöne et al. (2003a) report about an 1 °C range in shutdown temperatures during the second year of growth for the bivalve *Phacosoma japonicum*. This shutdown temperature will most likely change through ontogeny (see Goodwin et al., 2003; Schöne et al., 2003a); hence, only the earliest years of growth should be used. Moreover, as bivalves age, they typically reduce annual shell growth; therefore, time averaging may become a problem in slower-growing specimens. Time averaging occurs when shell growth slows and the shell-sampling interval remains the same, resulting in the same sample size (e.g., a 300-μm drill hole) representing (and averaging) more time (see Goodwin et al., 2003, 2004). Time averaging will thus bring the amplitude of the $\delta^{18}O_{Shell}$ cycle closer to the mean and the minimum and maximum $\delta^{18}O_{Shell}$ values will be underestimated.

Finally, the mean annual temperature or the temperature range seems to dictate the minimum growth temperature in many species of bivalves. For example, Weidman et al. (1994) calculated that shutdown temperature of *Arctica islandica* was about 6 °C for individuals that grew offshore of Rhode Island (USA) where the temperature ranges from 4 to 16 °C, whereas Schöne et al. (2005) showed that the same species grew in waters as cold as 4.5 °C near Iceland where cooler water temperature ranged from 5 to 9 °C. In another example, Gillikin et al. (2009a) showed that the shutdown temperature of the blue mussel *Mytilus edulis* was about 8 °C in the Schelde estuary in the Netherlands, but Wanamaker et al. (2007) successfully grew the same species at 4 °C in an aquarium. This example probably had to do with food availability as they were fed *ad libitum* in the laboratory. In conclusion, shutdown temperatures can be useful to calculate $\delta^{18}O_{water}$ only in circumstances where the species of interest and the environment of interest are well characterized and where the variability in $\delta^{18}O_{water}$ is large.

Another possibility of obtaining paleo-$\delta^{18}O_W$ values could be to measure fluid inclusions in bivalve shells, assuming that this fluid was in equilibrium with ambient water in which the bivalves grew. However, Lécuyer and O'Neil (1994) found that fluid inclusions in the shells of six bivalve species were not in oxygen-isotope equilibrium with ambient water, but had higher $\delta^{18}O$ values (6–18‰ higher than the environmental water). They postulated that inclusion waters in shells represent remnants of metabolic fluids produced by the mantle. In contrast, Lécuyer et al. (2004) found that the $\delta^{18}O$ values of calcification fluids were not vastly different than those in surrounding waters. Therefore, these values probably do not represent metabolic fluids. Nevertheless, inclusion waters in shells probably cannot help solve the paleo-$\delta^{18}O_W$ problem nor be used to obtain other environmental information.

Strontium-isotope ratios in biogenic carbonates can also be used as a proxy for the mixing of fresh and marine waters in estuaries (e.g., Ingram and Sloan, 1992). In theory, Sr isotopes should provide an advantage over $\delta^{18}O_{shell}$ because there is no temperature effect, and there is no fractionation when the Sr isotopes are incorporated into the $CaCO_3$ skeleton. However, analytical uncertainties on the $^{87}Sr/^{86}Sr$ measurements and the high cost of analysis actually make $\delta^{18}O_{shell}$ a more reliable paleo-discharge proxy (see Dettman et al., 2004). Moreover, as Sr is a trace element in carbonates, it is more prone to diagenesis than $\delta^{18}O_{shell}$. Sr concentrations in diagenically altered fossil bivalve shells have been shown to either decrease (Brand and Veizer, 1980; Kaufman et al., 1996) or increase (Walls et al., 1977; Gillikin et al., 2005a), which probably alters the original $^{87}Sr/^{86}Sr$ signal recorded in the shell, but not necessarily the O and C isotope values (see Gillikin et al., 2005a, 2007).

7.07.6.4 Using $\delta^{18}O_{shell}$ Cycles to Determine Bivalve Growth

Finally, $\delta^{18}O_{shell}$ can also be used to determine growth rate, which, in turn, can be used as a proxy for different environmental variables such as food availability (Witbaard, 1996; Witbaard et al., 2003), phytoplankton blooms (Lorrain et al., 2000), temperature (Schöne et al., 2002; Strom et al., 2004), and salinity (Schöne et al., 2003b). Although visual growth lines are present in most species, the nature of their periodicity cannot be assumed and needs to be determined (e.g., whether daily or tidal). Shells growing toward the marine end member of estuarine waters will typically have annual cycles in their $\delta^{18}O_{shell}$ values (shells from the freshwater end member may or may not have this cyclicity depending on when the rainy season occurs). This cyclicity can be used to infer the growth rate of the shell (e.g., De Ridder et al., 2004; Goodwin et al., 2009), which can be used to decipher the periodicity in growth lines (e.g., Veinott and Cornett, 1996; Goodwin et al., 2001). The last $\delta^{18}O_{shell}$ cycle also provides information on when the bivalve died. If the shells were collected from archaeological middens, this information can be used to determine how and at what time during the year early people were utilizing estuarine resources (Shackleton, 1973). Growth information can also show when an animal started to grow, which can be useful in determining when an exotic species was first introduced to a particular estuary. Goodwin et al. (2010) collected the first *Crassostrea gigas* shells found in San Francisco Bay, and, by using annual $\delta^{18}O_{shell}$ cyclicity, determined that the introduction happened about 6 years prior to their discovery and that at least two recruitment events took place. Considering the above examples, oxygen isotopes in estuarine mollusk shells can provide a wealth of information when used in the right context.

7.07.6.5   Carbonate δ^{13}C:DIC or Bivalve Metabolism?

Mollusk-shell δ^{13}C is more difficult to interpret than δ^{18}O$_{shell}$. McConnaughey and Gillikin (2008) recently reviewed the topic of carbon isotopes in mollusk shells, and we refer the interested reader to this article for an in-depth discussion on controls on shell δ^{13}C.

Early work suggested that skeletal carbon originates directly from DIC in seawater (Mook and Vogel, 1968; Killingley and Berger, 1979; Arthur et al., 1983). As δ^{13}C$_{DIC}$ is related to salinity (Figure 11), anthropogenic carbon inputs, productivity, and respiration, δ^{13}C$_{Shell}$ was proposed as a proxy for these environmental variables.

According to the experiments of Romanek et al. (1992) on inorganic carbonates, the equilibration ^{13}C fractionation for aragonite relative to HCO$_3^-$ ($\varepsilon_{carbonate - bicarbonate}$) is +2.7 ± 0.6‰ and is +1.0 ± 0.2‰ for calcite. Therefore, to calculate equilibrium values, the values above are simply added to the δ^{13}C value of DIC in seawater, which is mainly composed of HCO$_3^-$. However, Dillaman and Ford (1982), Swart (1983), Tanaka et al. (1986), McConnaughey et al. (1997), Furla et al. (2000), Lorrain et al. (2004), Gillikin et al. (2005b, 2006b, 2007), and others have proposed that the process of calcification utilizes carbon from two reservoirs, seawater DIC and metabolic DIC, with the latter composed of respiratory CO$_2$. The external source of carbon, seawater DIC, typically has a δ^{13}C value close to +1‰, whereas the internal carbon source, metabolically derived CO$_2$, has a highly depleted ^{13}C isotopic signature similar to the respiring tissues (about −20 to −25‰; Nier and Gulbransen, 1939; Craig, 1953; Fry, 2002; Bouillon et al., 2004; Gillikin et al., 2006b, 2007). The incorporation of such carbon results in lower δ^{13}C$_{Shell}$ values, obscuring the signal derived from water δ^{13}C$_{DIC}$. Tanaka et al. (1986) first suggested that up to 85% of the carbon in bivalve shells was metabolic in origin, but McConnaughey et al. (1997) have moderated this idea, suggesting that, in aquatic invertebrates, less than 10% of respired CO$_2$ is incorporated in the shell, resulting in only small decreases of shell δ^{13}C (<2‰) with respect to equilibrium values. However, using the model of McConnaughey et al. (1997), Gillikin et al. (2007, 2009b) have shown more than 30% of the shell carbon can have metabolic origins in some bivalve species.

Strong ontogenic decreases in δ^{13}C$_S$ are evident in many bivalves (Kennedy et al., 2001; Elliot et al., 2003; Gillikin et al., 2007, 2009b; McConnaughey and Gillikin, 2008). Lorrain et al. (2004) proposed a simple model where this ontogenic δ^{13}C$_{Shell}$ decrease is caused by an increase in the amount of respiratory CO$_2$ produced by the bivalve as it increases in size relative to the amount of carbon needed for shell growth. This model is based on the relationship between metabolic rate and body size: as bivalves grow, their absolute metabolism increases while shell growth slows. Therefore, more metabolic CO$_2$ will be available while the amount of carbon needed for shell growth is reduced, resulting in more metabolic carbon being incorporated into the shell. However, this is apparently species specific. While in some species, strong ontogenic decreases in δ^{13}C$_{Shell}$ have been noted (Krantz et al., 1987; Kennedy et al., 2001; Keller et al., 2002; Elliot et al., 2003; Lorrain et al., 2004; Freitas et al., 2005; Gillikin et al., 2007, 2009), in others, there is no discernible decrease (Buick and Ivany, 2004; Gillikin et al., 2005b, 2006b). It is still largely unknown what species of DIC mollusks use to build their shell. It is known that CO$_2$ passes more easily through biological membranes than bicarbonate (Gutknecht et al., 1977) and that CO$_2$ in equilibrium with DIC will be about ~8‰ lighter than HCO$_3^-$ at 25 °C (Zhang et al., 1995). Therefore, if CO$_2$ is entering the animal in a disproportionate amount, the shell will appear more negative than the δ^{13}C–DIC (see McConnaughey and Gillikin, 2008 for a more detailed discussion on this topic).

Although metabolic C can complicate the δ^{13}C$_{Shell}$ profile, δ^{13}C$_{Shell}$ can still be a useful indicator of environmental conditions. It is known that δ^{13}C$_{DIC}$ has large seasonal fluctuations due to respiration, photosynthesis, carbonate dissolution/precipitation, etc. (see previous sections). However, in well-flushed estuaries with short residence times, δ^{13}C$_{DIC}$ may follow a simple linear relationship with salinity, especially in salinities above 25 (Figure 11 and Mook and Vogel, 1968; Mook, 1971; Surge et al., 2001; Fry, 2002; Gillikin et al., 2006b). Furthermore, it is well known that freshwater input is often depleted in ^{13}C (Mook, 1971). Therefore, a drop in salinity will undoubtedly cause a decrease in δ^{13}C$_{DIC}$ and subsequently in δ^{13}C$_{Shell}$. Although Mercenaria shells can contain more than 30% metabolic C, shell δ^{13}C values from estuarine clams were significantly more negative than marine clams (Gillikin et al., 2007). The δ^{13}C$_{Shell}$ profile obtained from a transplantation experiment also clearly illustrates this (Figure 12, see also McConnaughey and Gillikin, 2008). The shell in Figure 12 was transplanted from
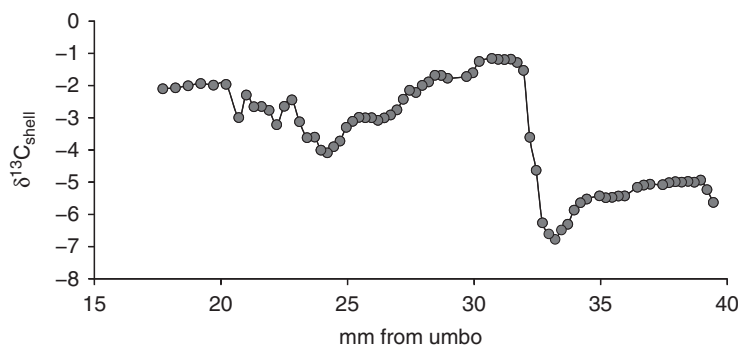
Figure 12   Carbon isotope signature recorded in a *Mytilus edulis* shell transplanted from a marine site (salinity ~35) to an estuarine site (salinity ~20). The change is probably more abrupt, but time averaging slightly smoothes the signal. Data from Gillikin, D.P., Dehairs, F., Lorrain, A., Steenmans, D., Baeyens, W., André, L., 2006a. Barium uptake into the shells of the common mussel (*Mytilus edulis*) and the potential for estuarine paleo-chemistry reconstruction. Geochimica et Cosmochimica Acta 70, 395–407.

a marine to an estuarine site, resulting in the abrupt drop in $\delta^{13}C_S$ values. Moreover, $\delta^{13}C$ in M. edulis shells growing along a salinity gradient shows a clear salinity response (Mook and Vogel, 1968; Mook, 1971; Gillikin et al., 2006b). Gillikin et al. (2006b) noted a nearly 1:1 relationship between shell $\delta^{13}C$ and $\delta^{13}C_{DIC}$ for shells growing within a protected estuary, but shells from an unprotected site did not plot on this line. They proposed that mussels at this site had higher metabolic rates due to the high wave action, and thus incorporated more metabolic carbon into their shells. This is a phenomenon that has been noted in fish ear bones (otoliths), with active swimming being correlated with decreased otolith $\delta^{13}C$ (Sherwood and Rose, 2003). Therefore, ecosystem energy can also play a role in shell $\delta^{13}C$. Nevertheless, if a modern calibration can be done on a species (such as M. edulis), and animals from similar types of environments are compared (e.g., protected vs. high energy), shell $\delta^{13}C$ values can be used in conjunction with $\delta^{18}O_{shell}$ values to obtain an indication of salinity. Smaller-scale fluctuations in shell $\delta^{13}C$ values can also provide environmental information, but they must be interpreted with caution. For example, Goodwin et al. (2010) noted a sharp peak in shell $\delta^{13}C$ values from several oysters growing in San Francisco Bay, which appear to correlate with strong phytoplankton blooms. Phytoplankton blooms are known to enrich the waters in ^{13}C (see Section 7.07.2.5), which is the most parsimonious explanation for this peak found in several shells. In summary, the interpretation of $\delta^{13}C_{shell}$ values largely depends on context. At times, it may be straightforward, and, on other occasions, it may depend on too many factors to tease apart.

7.07.6.6   Shell Organic Matrix Stable Isotopes

In addition to calcium carbonate, bivalve shells also contain up to 5% organic matter (Marin and Luquet, 2004; Rueda and Smaal, 2004). The stable isotope composition of this organic matter has also been used to obtain information about past environments. O'Donnell et al. (2003) measured $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in both the soft tissues and shell organic matter of modern M. mercenaria. They found that the difference ($\Delta_{tissue\ -\ shell}$) was minimal for $\delta^{13}C$ (0.1‰) and slightly larger for $\delta^{15}N$ (0.7‰), and $\delta^{34}S$ (1.8‰). Using these offsets ($\Delta_{tissue\ -\ shell}$), they were able to extract information about the diet of these bivalves from the Quaternary. Carmichael et al. (2008) found a larger $\Delta_{tissue\ -\ shell}$ in M. mercenaria shell $\delta^{15}N$ than O'Donnell et al. (2003) reported, with shells being about 2.4‰ more ^{15}N-depleted than soft tissues. They also found large differences between shells from wastewater-impacted estuaries and more pristine estuaries (with shells from impacted sites being more enriched in ^{15}N).

Data regarding the $\delta^{13}C$ values of fossil bivalve respiration can also be estimated from shell organic matrix and assist in understanding the influence of metabolic carbon to possibly help to decipher past $\delta^{13}C_{DIC}$. For example, if the $\delta^{13}C$ of metabolic CO_2 is known, and if the percent metabolic carbon incorporation is constant and known, then the $\delta^{13}C_{DIC}$ can be calculated. Furthermore, compound-specific carbon-isotopic analysis also can provide dietary, environmental, and diagenetic information (CoBabe and Pratt, 1995; CoBabe and Ptak, 1999; O'Donnell et al., 2007; Risk et al., 2009). However, more knowledge of species-specific metabolic carbon incorporation is needed. For example, Stott (2002) could not find a relationship between shell organic matter $\delta^{13}C$ and diet $\delta^{13}C$ in a snail

(Helix aspersa). δD data from shell organics can also provide environmental information. Carroll et al. (2006) found that shell organic δD and shell carbonate $\delta^{18}O$ plot on a line that reflects the isotopic composition of the waters in which the bivalve lived. This has great potential as a proxy for water isotopes, which would help with paleo-discharge or paleo-temperature studies discussed earlier. The analysis of stable isotopes in shell organic matter is a promising field and has received some attention in studies on foraminifera (Stott, 1992; Shemesh et al., 1993; Maslin et al., 1996, 1997), but remains relatively unstudied in mollusks.

There are many methods to extract organic matter from shells for isotopic studies, and most involve acidification to remove the carbonate (e.g., O'Donnell et al., 2003; Carmichael et al., 2008). This is required for carbon, but not for nitrogen, or hydrogen isotopes. Carroll et al. (2006) analyzed δD in freshwater mussel-shell carbonate without treatment (aside from equilibration with the atmosphere for exchangeable hydrogen). Similarly, $\delta^{15}N$ can be analyzed by directly combusting untreated shell material (Dietz, 2008; Rowell et al., 2010; Versteegh et al., 2011). Simple tests, where organic matter with known $\delta^{15}N$ values (International Atomic Energy Agency-N1 (IAEA-N1) and acetanilide) were added to pure $CaCO_3$, illustrated that the carbonate has no effect on the isotopic analysis of $\delta^{15}N$ down to samples with as little as 20 µg N (Versteegh et al., 2011). This is important because acid treatments are known to alter the $\delta^{15}N$ values of organic matter (e.g., Lohse et al., 2000; Kennedy et al., 2005). Shell organic-matrix geochemistry is an exciting new direction of research that should produce interesting results in the coming years.

7.07.6.7   Nontraditional Stable Isotopes: New Potentials for Environmental Proxies

Strontium isotopes in bivalve shells have long been known to be an excellent indicator of salinity and water source (Ingram and Sloan 1992; Vonhof et al., 1998, 2003; Holmden and Hudson, 2003), with no vital effects occurring (Reinhardt et al., 1999). Since the arrival of new mass-spectrometer technology (e.g., multicollector inductively coupled plasma-mass spectrometry; MC-ICP-MS), other nontraditional isotopes (traditional isotopes being H, C, O, N, and S) have been receiving increasing attention.

Boron isotopes have been recently shown to be an indicator of pH in foraminifera (Sanyal et al., 2001) and corals (Hönisch et al., 2004). This is due to the pH influence on the availability of isotopically light $B(OH)_4^-$ versus $B(OH)_3^-$ (Hemming and Hanson, 1992). However, Pagani et al. (2005) showed the presence of vital effects, which complicate the use of this proxy in foraminifera.

Calcium isotopes ($\delta^{44}Ca$) in inorganic aragonite and cultured planktonic foraminifera are positively correlated to temperature (Nägler et al., 2000; Gussone et al., 2003); however, a more recent study has highlighted problems with this proxy in foraminifera from core-top sediments (Sime et al., 2005). Moreover, unknown $\delta^{44}Ca$ composition of past waters and species-specific Ca-isotope fractionation make this a difficult proxy to interpret (Immenhauser et al., 2005; Steuber and Buhl, 2006). A recent study on M. edulis (Heinemann et al., 2008) investigated the $\delta^{44}Ca$ composition of many components involved in biomineralization and also concluded that there is a strong biological

fractionation with Ca-isotopes, but made many erroneous assumptions. For example, they took one sample of estuarine water and assumed it was representative of the water in which the mussel was growing (in estuaries, water geochemistry is highly variable), and they sampled the inner calcification space (the extrapallial fluid; EPF) and compared it to the outer growth layer (which is precipitated by the outer EPF; see McConnaughey and Gillikin (2008) for more discussion on this).

Magnesium isotopes have been used to gain insight on the δ^{26}Mg ratio of past seawater. de Villiers et al. (2005) have shown that the δ^{26}Mg ratio of seawater allows important inferences to be made about the relative contribution of different lithologies to the global continental weathering flux, particularly carbonate versus silicate weathering. They also suggest that echinoderm skeletons can serve as archives of seawater δ^{26}Mg, allowing the reconstruction of past weathering fluxes. However, this proxy also is species specific. For example, Chang et al. (2004) reported that δ^{26}Mg ratios in coral aragonite are similar to seawater indicating little biologic influence on Mg incorporation; however, in contrast, there was a large difference between foraminiferal calcite and seawater, indicating a biological fractionation. Planchon et al. (2007) found that δ^{26}Mg values of estuarine waters were very similar to marine waters and were also similar to internal fluids in the clam Ruditapes philippinarum (all around –0.8‰), but that shells were dissimilar. The marine shell was –1.9‰ and the estuarine shell was –4.2‰ indicating a biological effect, which is somehow dependent on salinity. Hippler et al. (2009) also found similar results for M. edulis shells from the western Dutch Wadden Sea with shells being 2.5 to 4.2‰ lighter than seawater, and shells from lower salinity showing larger fractionations.

Studies involving these isotopes (and others, e.g., lithium isotopes, Marriott et al., 2004) are rather new and offer promising results for both understanding bivalve biomineralization and paleo-estuarine reconstruction.

7.07.6.8 Multiproxy Approaches

Oxygen and C isotopes in biological carbonates are powerful tools for reconstructing past estuarine conditions. Combined, they are even more robust. If $\delta^{13}C_{Shell}$ becomes more negative due to a pulse of freshwater, $\delta^{18}O_{Shell}$ should also show a similar response. If more proxies such as isotopes of shell-organic matrix, or nontraditional isotopes are added and agree, the conclusions drawn become more robust.

In addition to isotope studies, element concentrations of biological carbonates (or element-to-calcium ratios) also were originally thought to have great promise as environmental proxies. However, many studies have shown this not to be true for most elements. Sr/Ca and Mg/Ca seem to have strong biological or kinetic effects associated with them (e.g., Purton et al., 1999; Vander Putten et al., 2000; Gillikin et al., 2005c; Lorrain et al., 2005; Surge and Lohmann, 2008; Wanamaker et al., 2008). Barium-to-calcium ratios, on the other hand, seem to hold great promise (Stecher et al., 1996; Vander Putten et al., 2000; Gillikin et al., 2006a, 2008; Thébault et al., 2009). Gillikin et al. (2006a) established that the Ba/Ca$_{shell}$ signal should be broken into two components: (1) the background or baseline Ba/Ca$_{shell}$ signal, which records ambient water Ba/Ca ratios (which are, in turn, related to salinity) and (2) the Ba/Ca peaks. These Ba/Ca$_{shell}$ peaks have been difficult to understand, with some species from some locations apparently exhibiting a relationship between Ba/Ca$_{shell}$ and phytoplankton blooms (Thébault et al., 2009), and others not (Gillikin et al., 2006a, 2008). Finally, lithium-to-calcium ratios also seem to be a potential temperature proxy (Marriott et al., 2004), but work on this proxy in bivalves has only begun (Thébault et al., 2009). If these nonisotopic proxies prove to be robust, they will also add to the confidence with which we can interpret the biological carbonate record.

Increased confidence may be gained by combing different organisms and proxies. Different species of bivalves, or better yet, bivalves and other sources of biogenic carbonates such as foraminifera (e.g., Pekar et al., 2004) can help disentangle which proxy signals are controlled by environmental or biological processes. Recent work has been combining both marine and terrestrial proxies such as corals and tree rings (e.g., D'Arrigo et al., 2006) or tree rings and bivalves (e.g., Black et al., 2009) to produce more robust climatic reconstructions. In estuaries, local environmental changes may be detected by using tree rings and bivalves as well. Trees are not often associated with estuaries in temperate regions, but mangroves line many of the world's tropical and subtropical estuaries. Certain mangrove species have recently been shown to have annual growth rings (Menezes et al., 2003; Verheyden et al., 2004a; Chowdhury et al., 2008; Estrada et al., 2008), which allow time-resolved samples to be taken. As mangroves use surface-soil water rather than groundwater as their water source (Lin and Sternberg, 1994), the $\delta^{18}O$ values of their stem cellulose have the potential to record the mixing between freshwater and seawater in the estuary, and thus can provide information about paleo-discharge or precipitation (Sternberg and Swart, 1987; Ish-Shalom-Gordon et al., 1992). In addition, $\delta^{13}C$ values of their stem cellulose can provide information on water-use efficiency related to precipitation and/or freshwater flow into the estuary (Verheyden et al., 2004b). Verheyden et al. (2004b) found that although there were complications caused by post-photosynthetic fractionation processes of storage materials such as starch, the $\delta^{18}O$ and $\delta^{13}C$ values of bulk wood responded strongly to freshwater inputs caused by El Niño rains. This field remains relatively unexplored, but both isotope- and wood-anatomical studies have the potential to record long-term (~100 years) changes in estuarine cycling (cf. Verheyden et al., 2005; Maingi, 2006; Schmitz et al., 2006), especially when combined in a multiproxy approach, such as with bivalve shells.

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