



# Isotopic values in oysters indicate elemental sources constrained by multiple gradients



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## ABSTRACT

Stable nitrogen and carbon isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) and elemental content (% nitrogen, % carbon) in oysters (*Crassostrea virginica*) grown by a network of 132 citizen–scientists (11,600 km<sup>2</sup>, 87.9 km<sup>2</sup> site<sup>-1</sup>) were examined to test effects of land use, salinity, flushing time, and oyster size on bioindication of human and/or animal nitrogen sources. Oyster  $\delta^{15}\text{N}$  sampled from shallow waters sites throughout Chesapeake Bay and its tributaries exhibited nested spatial patterns: (1) decreasing toward the mouth of Chesapeake Bay (1000s km<sup>2</sup>) and (2) decreasing, increasing, and not changing toward tributary mouths (100s km<sup>2</sup>). Distinct isotopic ‘signatures’ in tributaries were associated with the composition of land use, water quality in tributaries and freshwater streams, human and/or animal nitrogen sources, and marine vs. terrestrial nitrogen and carbon sources. Yet at 1000s km<sup>2</sup>, oyster  $\delta^{15}\text{N}$  varied with flushing time, salinity, and bioindicator size, thus constraining the upper extent for inferring nitrogen sources from bioindicator  $\delta^{15}\text{N}$  to the scale of gradients in these confounding physical and biological factors. Nevertheless, at 100s km<sup>2</sup> isotopic ‘signatures’ can be used to infer nutrient sources and transport mechanisms and might have implications for fishery management/enforcement. Ultimately,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in bioindicators distributed to citizen–scientists may add substantial value to existing and ongoing programs, networks, monitoring and databases, and might have some use for imputing data gaps where intensive water quality monitoring is lacking.

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## 1. Introduction

Stable isotopes in bioindicators in oysters and other species have been used to indicate anthropogenic nutrient sources, relative elemental contributions from marine vs. terrestrial ecosystems, and food web structure. Yet the factors constraining the scale to which these types of information can be reliably inferred from bioindicators has rarely been examined. Spatial patterns of nitrogen and carbon sources have previously been identified by oysters at 10s km<sup>2</sup> (Piola et al., 2006; Daskin et al., 2008; Fertig et al., in press) and 100s km<sup>2</sup> (Fertig et al., 2009), but not yet at 1000s km<sup>2</sup> scale subject to gradients of temperature, salinity, and water circulation rates. Achieving a high density of bioindicator sampling over

large geographic scales can be difficult yet augmented by access to a citizen–scientist network. In Chesapeake Bay, such a network of ‘Oyster Gardeners’ exists with a goal of culturing, rearing, and redistributing oysters for restoration.

Stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) are enriched (+5 to +8‰, Kendall et al., 2007) in sewage or animal waste due to isotopic fractionation by ammonia volatilization and/or denitrification at the source or by microbial processing employed by wastewater treatment facilities (Fry, 2006; McClelland and Valiela, 1998; Sweeny and Kaplan, 1980; Tucker et al., 1999). In oyster (*Crassostrea virginica*) tissues,  $\delta^{15}\text{N}$  has indicated sewage, septic, and manure nitrogen sources (e.g. Daskin et al., 2008; Fertig et al., 2009, 2013, in press; Piola et al., 2006) but is subject to seasonal variations associated with factors affecting growth, e.g. temperature, salinity (Lorrain et al., 2002; Fertig et al., 2010).

Stable carbon isotope values ( $\delta^{13}\text{C}$ ) are used to identify terrestrial (–22‰) and marine (–14‰) carbon sources (Valiela, 1995; Fry, 2006), due to differences in fractionation associated with C3 photosynthesis in marine phytoplankton and C4 photosynthesis in terrestrial grasses (–7 to –13‰; McMillan et al., 1980; Haddad and Martens, 1987; Goñi et al., 1997). Fractionation of  $\delta^{13}\text{C}$  also occurs

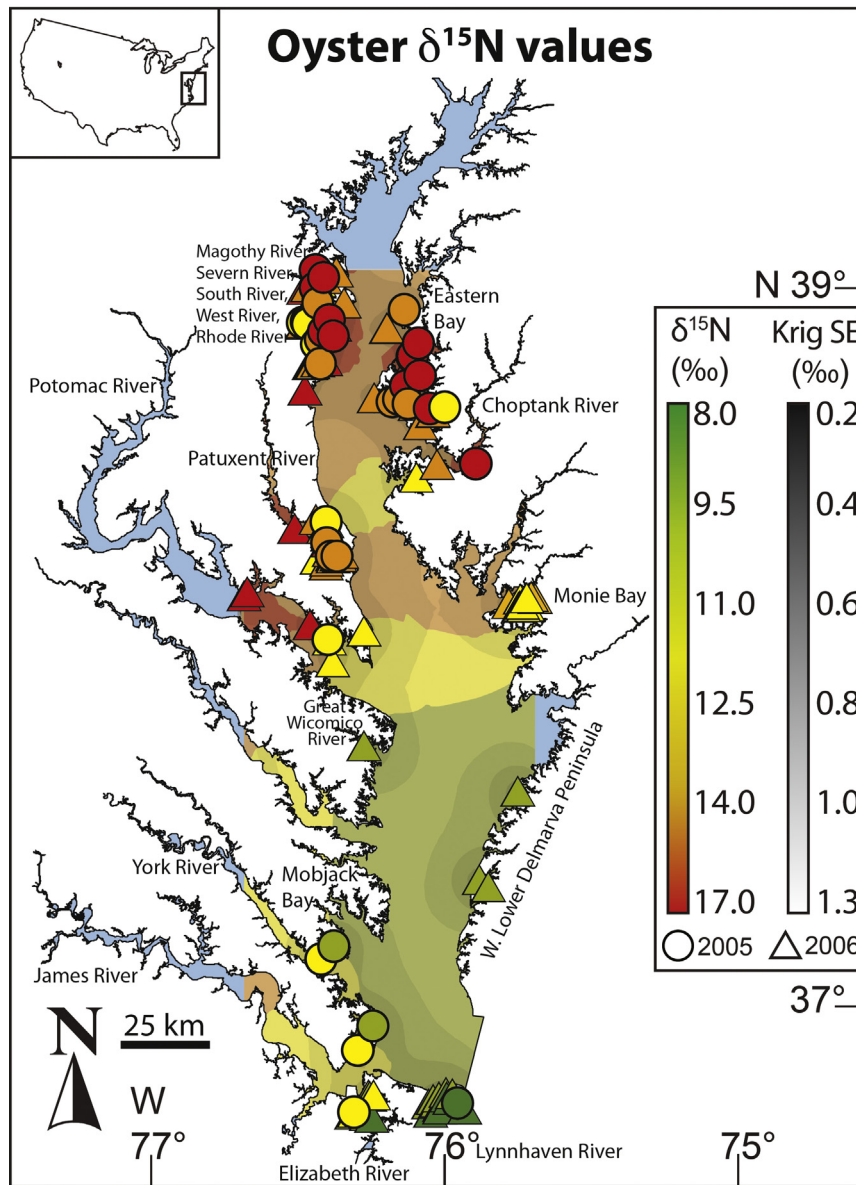
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**Fig. 1.** Map of mean oyster  $\delta^{15}\text{N}$  values in Chesapeake Bay during 2005 (circles) and 2006 (triangles) overlaid atop kriged interpolation (color gradient) and its standard error (black to white gradient). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

as a result of  $\text{CO}_2$  limitation during photosynthesis, phytoplankton bicarbonate assimilation, and post-depositional diagenesis (Fogel et al., 1992; Cornwell et al., 1996).

Physical factors such as temperature, salinity, and water circulation may affect oyster  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  due to variations in growth rates or elemental availability. Oysters grow more quickly in warmer, saltier waters (Dame, 1972a,b) and tissue isotopic values reflect dietary values modified via trophic transfer, growth, and repair (Fertig et al., 2010). Shorter exposure times to anthropogenic nitrogen sources due to rapid circulation may limit nutrient assimilation by bioindicators, which integrate  $\delta^{15}\text{N}$  signatures over time (Fertig et al., 2009, 2010, 2013). Therefore, the spatial and temporal scales at which gradients of these factors occur may be an important consideration for interpreting isotopic evidence in organismal tissue.

Identifying constraints and appropriate scales of isotopic approaches to detect elemental sources and growth locations for commercially relevant species has implications for monitoring, pollution assessment, and shellfishery management/enforcement.

Further, access and collaboration with a ‘citizen–scientist’ network such as the ‘Oyster Gardeners’ has the potential to add substantial value to scientific research. Therefore, goals of this study were to (1) test effects of land use, salinity, flushing time, and oyster size on  $\delta^{15}\text{N}$  values and (2) identify spatial patterns of sewage, septic, and animal manure inputs to Chesapeake Bay.

## 2. Methods and materials

### 2.1. Oyster collection and stable isotope analysis

Oysters (*C. virginica*) were grown for 9 months (September–May) in mesh bags or cages suspended above the bottom while tied to docks and tended by citizen–scientists. Surviving oysters were sampled in summer (June 2005, 2006) from 132 sites (37 sites in 2005, 95 sites in 2006) in Chesapeake Bay opportunistically located from Annapolis, MD (38°58′38″N 76°29′31″W) to Virginia

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