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Stable N Isotopic Signatures in Bay Scallop Tissue, Feces, and Pseudofeces in Cape Cod Estuaries Subject to Different N Loads

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Scallops (*Argopecten irradians*) feed on particulates in estuaries, and their growth and survival may depend on the quality and quantity of food particles available (1, 2). To a significant degree, particle supply in shallow estuaries such as those on Cape Cod depend on rates of land-derived N load (3). Linkages between estuarine organisms and terrestrial loadings have been studied in various ways, including stable isotopic techniques. Isotopic fractionation leads to detectable shifts created by microbial transformations, trophic steps, as well as to differences due to source of the N (4, 5).

In this paper we apply isotopic analyses and experiments with introduced scallops to define the rate at which scallops acquire the signature of the estuary in which they are located; we examine whether scallop tissues differ from the signatures of pseudofeces and feces ejected by scallops, and whether differences in N-loading rates and sources to different estuaries result in corresponding differences in the signature of scallops within the estuaries. Finally, we use results of the introduced scallop experiments to see if differences in δ^{15}N signature acquisition are related to differences in growth or survival of the scallops.

We compared the acquisition of δ^{15}N signatures by scallops incubated in two estuaries of Waquoit Bay, Cape Cod, receiving different N inputs. Childs River (CR) has a loading rate of 601 kg N ha^{-1} y^{-1}. Sage Lot Pond (SLP) has a loading rate of 14 kg N ha^{-1} y^{-1}. The difference in N load between these estuaries is due to different levels of urbanization in their watersheds, and the differences in wastewater contributions to these two estuaries result in different isotopic signatures in the N entering the estuaries from land (5, 6). Juvenile scallops (40–50 mm) were obtained from Taylor Seafood, Fairhaven, Connecticut. In each estuary we placed four plastic-coated wire cages, each containing 20 scallops. Cages were secured 10 cm above the sediment surface in 1 m of water at mean low tide.

To monitor the acquisition of the δ^{15}N signature in tissue and ejecta over time, we removed one cage of scallops from each estuary on days 3, 6, 12, and 24. Animals were immediately placed in filtered seawater for 24 hours to clear their guts. Feces and pseudofeces were filtered through pre-ashed, 7-μm Whatman GF/F filters. Scallop tissue was dissected from the shell and dried at 60 °C overnight. Ejecta were acidified to remove carbonates, and samples not collected on filters were homogenized.

We determined the δ^{15}N signatures of potential food sources, particulate organic matter in water (POM, or seston) and sediments. In each estuary, we sampled the water column and sediments near the cages on days 0, 3, 6, 12, and 24. Water column samples were processed in the same manner as ejecta. The top 1 cm of sediment was sampled using a 5-cc syringe as a corer. We combined four sediment cores for each sample. Sediment samples were acidified and homogenized. All samples were analyzed using a Europa Scientific Integra mass spectrometer at the University of California-Davis.

To determine scallop growth over time, length of shells of animals from each cage were measured with vernier calipers accurate to 0.1 mm. The number of dead scallops per cage were counted on each collection day.

The δ^{15}N values of tissue from scallops grown in each estuary were initially 9.23% and during the course of the field incubation approached δ^{15}N values of POM in water and sediments, corrected by an expected trophic fractionation of 3% (4) (Fig. 1A, B). For example, if scallops in CR were feeding only on sediments, we extrapolate that the scallops, at the measured rate of change in tissue signature, would converge on the mean sediment signature (corrected by a 3% trophic fractionation) in 93 days. Similarly, if the scallops were feeding on only seston, the convergence would take place in 60 days. For the scallops in SLP, the convergence time would be shorter: 47 days and 36 days, respectively.

To examine whether scallops eject fractionated food particles, we compared the δ^{15}N signature of ejecta (feces + pseudofeces) to the δ^{15}N signature of food supply from each estuary. Lighter δ^{15}N signatures for food in SLP corresponded to lighter δ^{15}N signatures in ejecta from the scallops grown in SLP, while heavier δ^{15}N food signatures in CR corresponded to heavier ejecta signatures from the CR scallops (Fig. 1C). In both estuaries, the δ^{15}N signature of ejecta was equal to or heavier than that of potential food sources (Fig. 1C). In addition, δ^{15}N signatures of ejecta were lighter than δ^{15}N signatures of tissue in CR [8.75%–9.85% (Fig. 1A)] and SLP [0.07%–9.23% (Fig. 1B)]. The 2%–3% enrichment from food to ejecta agrees with trophic level fractionation reported in the literature. The relative similarity between the δ^{15}N signatures of seston and sediments makes

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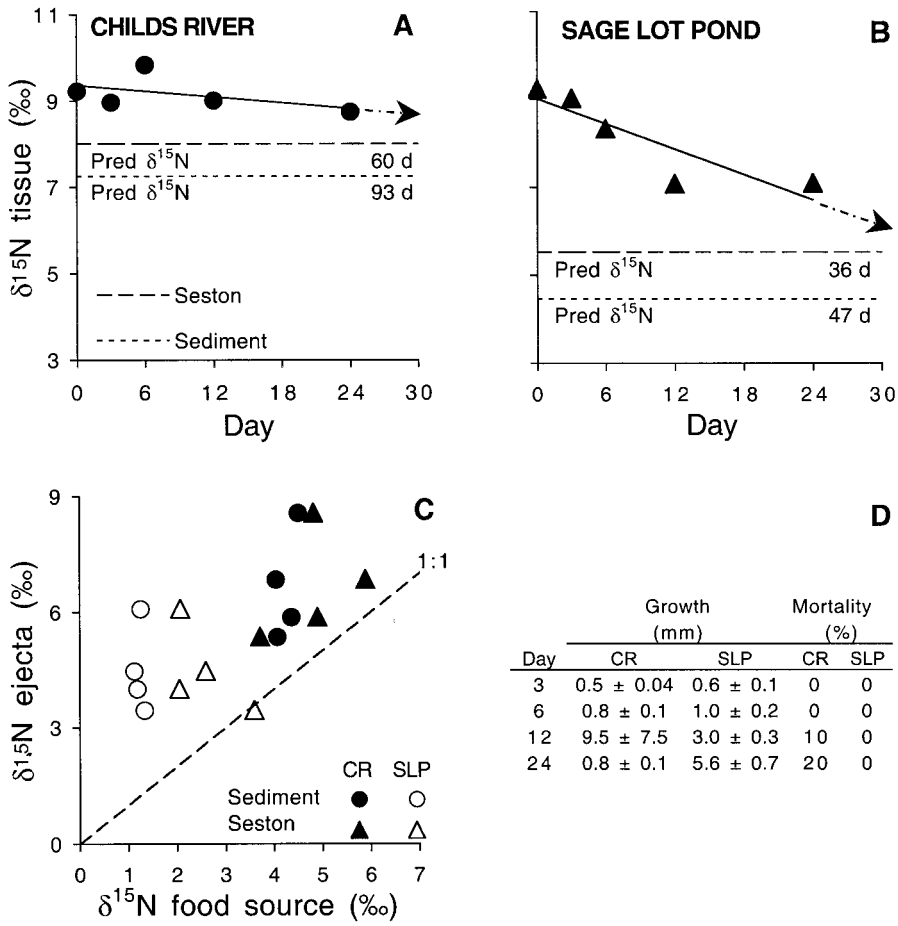

Figure 1. *(A, B) $\delta^{15}N$ signature of tissue from scallops grown in Childs River (A) and Sage Lot Pond (B)* vs. *time. CR regression:* $y = -0.02x + 9.37$, $F = 1.08$ *ns. SLP regression:* $y = -0.10x + 9.01$, $F = 10.85$**. Predicted $\delta^{15}N$ signature lines for tissue are derived from mean seston and sediment signatures, +3% to correct for trophic shift. The lines represent predicted ultimate tissue signatures for scallops assuming exclusive consumption of either food source. (C) $\delta^{15}N$ signature of scallop ejecta (feces + pseudofeces) is generally heavier than that of food sources (seston and sediment). (D) Mean (± std. error) scallop growth (measured as cumulative change in shell length) and mortality over time, in each study estuary. Mean growth was calculated using a subsampling of the individuals in the cage (*$n = 10$*).*

it difficult, however, to determine which food source contributed most to the diet of scallops during this study.

The faster rate at which SLP scallops approached predicted $\delta^{15}N$ signatures of their food sources (Fig. 1A, B) may be related to the faster growth of scallops in SLP (Fig. 1D). SLP scallops grew more quickly and achieved greater length than CR scallops (Fig. 1D). Mean growth rates (from incremental growth data) are 0.24 ± 0.03 mm/day for SLP, and 0.14 ± 0.01 mm/day for CR. In addition, no scallops in SLP died during the study, whereas those in CR reached 20% mortality by day 24 (Fig. 1D). The data suggest that conditions in CR were less favorable for scallops than conditions in SLP. This could be related to lower water quality in CR (7), which could have lowered feeding rate and possibly altered the rate of internal turnover of nitrogen within the scallop tissue.

Scallop $\delta^{15}N$ signatures moved toward the signatures of their presumed food sources at a rate suggesting they would converge with trophic-shift-corrected $\delta^{15}N$ food signatures in 1–3 months of feeding. Material ejected by scallops had heavier $\delta^{15}N$ signatures than potential food signatures but lighter than tissue signatures. The increased wastewater N load in CR coincided with a slower convergence of tissue signatures to trophic-shift-corrected food signatures, lowered growth, and increased mortality.

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Age Structure of the Pleasant Bay Population of *Crepidula fornicata:* A Possible Tool For Estimating Horseshoe Crab Age

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Crepidula fornicata, the common slipper shell, lives on rocks, horseshoe crabs (*Limulus polyphemus*), and other hard surfaces, often in stacks of one animal atop another. Unlike many other gastropods, they tend to remain sessile, and as they grow, their shells contour to the substrate (1). The association between horseshoe crabs and *C. fornicata* offers the possibility to use the slipper shell as a tool to determine the ages and average lifespan of horseshoe crabs (2). Knowing this information would be helpful for trying to understand horseshoe crab ecology for use in conservation efforts.

It is difficult to directly estimate horseshoe crab age because horseshoe crabs lack any hard parts that could be sectioned and analyzed for growth rings. Their chitinous exoskeleton is molted with decreasing frequency until a theoretical "terminal molt" (3). There are also a variety of sizes within visually estimated age classes because growth is very slow or stops in adults (3).

Other methods have been suggested for aging horseshoe crabs, including qualitative aging criteria and tagging studies. From the results of tagging studies it has been estimated that horseshoe crabs live 9 to 12 years before maturity and 5 to 7 years as adults, for a total lifespan of 14 to 19 years (4). These age spans are consistent with the prediction of Botton and Ropes (2) based on laboratory work using *C. fornicata* as a proxy for horseshoe crab age.

C. fornicata could indicate age of host horseshoe crabs if 1) horseshoe crabs have a terminal molt or do not molt often as adults, 2) *C. fornicata* remain on the same horseshoe crab, and 3) *C. fornicata* age can be determined with some degree of accuracy (5). It is also assumed that *C. fornicata* attach to a host horseshoe crab as soon as the new cuticle hardens.

Botton and Ropes (2) used a regression proposed by Walne (1) of *C. fornicata* length to age to quantitatively estimate the ages of horseshoe crabs. These *C. fornicata* were used to formulate this regression without comparison to a local population of horseshoe crabs, since the *C. fornicata* data was from England and horseshoe crabs were not measured at all.

In this study we measured shell length of *C. fornicata* and prosomal width of *Limulus polyphemus* in Pleasant Bay, Chatham, Massachusetts. We measured 496 crabs and their corresponding *C. fornicata,* with the number of *C. fornicata* per crab ranging widely from 1 to 30, with an average of 4 per crab. From these data we fitted cohorts of *C. fornicata* to a size-frequency distribution. We also related size of *C. fornicata* to prosomal width of *L.*

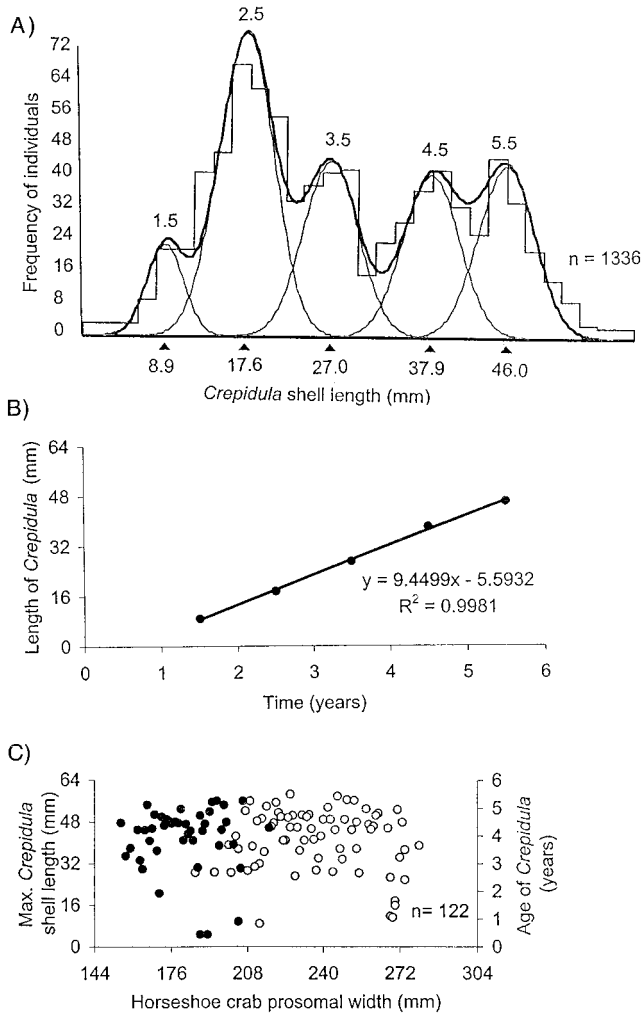

Figure 1. *(A) Cohorts of Pleasant Bay population of* Crepidula fornicata: *8.9 mm (~1.5 y), 17.6 mm (~2.5 y), 27.0 mm (~3.5 y), 37.9 mm (~4.5 y), and 46.0 mm (~5.5 y). (B)* C. fornicata *length* vs. *age; extrapolation data from Botton and Ropes (2). (C) Length of largest* C. fornicata *on horseshoe crabs of different prosomal width. Filled circles (●) represent males,open circles (○) represent females.*