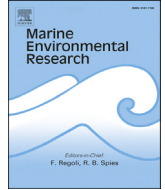




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## Characterizing seston in the Penobscot River Estuary

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## ABSTRACT

The Penobscot River Estuary is an important system for diadromous fish in the Northeast United States of America (USA), in part because it is home to the largest remnant population of Atlantic salmon, *Salmo salar*, in the country. Little is known about the chemical and biological characteristics of seston in the Penobscot River Estuary. This study used estuarine transects to characterize the seston during the spring when river discharge is high and diadromous fish migration peaks in the Penobscot River Estuary. To characterize the seston, samples were taken in spring 2015 for phytoplankton identification, total suspended matter (TSM), percent organic TSM, chlorophyll *a*, particle size (2  $\mu\text{m}$ –180  $\mu\text{m}$ ), particulate carbon and nitrogen concentrations, and stable carbon and nitrogen isotopes. The estuarine profiles indicate that TSM behaved non-conservatively with a net gain in the estuary. As phytoplankton constituted only 1/1000 of the particles, the non-conservative behavior of TSM observed in the estuary was most likely not attributable to phytoplankton. Particulate carbon and nitrogen ratios and stable isotope signals indicate a strong terrestrial, allochthonous signal. The seston in the Penobscot River Estuary was dominated by non-detrital particles. During a short, two-week time period, *Heterosigma akashiwo*, a phytoplankton species toxic to finfish, also was detected in the estuary. A limited number of fish samples, taken after the 2015 Penobscot River Estuary bloom of *H. akashiwo*, indicated frequent pathological gill damage. The composition of seston, along with ichthyotoxic algae, suggest the need for further research into possible effects upon resident and migratory fish in the Penobscot River Estuary.

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## 1. Introduction

The composition and characteristics of seston in an estuary can provide useful information about the spatial and temporal complexity of the particles within the estuary (Canuel et al., 1995; Carpenter et al., 2005; Dias et al., 2014; Hoffman et al., 2008; Huxel et al., 2002). The particles that make up the seston can be classified as autochthonous or allochthonous. In most estuaries, autochthonous carbon is in the form of phytoplankton, microphytobenthos, and higher plant fragments (Deegan and Garritt, 1997; Dias et al.,

2014; Hoffman et al., 2008). Allochthonous carbon sources can come from both marine, terrestrial, freshwater, and anthropogenic sources (Berto et al., 2013; Dalu et al., 2016). To better understand the sources and/or transformation of seston in an estuary, stable isotopes of  $\delta^{13}\text{C}$  (carbon) and  $\delta^{15}\text{N}$  (nitrogen), along with carbon/nitrogen (C/N) ratios, have been used as a “fingerprint” to characterize pools of seston (Dias et al., 2014; Hoffman et al., 2008; Middelburg and Nieuwenhuize, 1998; Pasquaud et al., 2007). The use of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes and C/N ratios as tracers of different pools of seston in an estuary is well documented (Fry, 2002; Middelburg and Nieuwenhuize, 1998; Smith and Epstein, 1970, 1971). Briefly, stable isotopes and ratios can be used as tracers because of large differences in these variables for marine-derived particles relative to terrestrial particles. For example, the average  $\delta^{13}\text{C}$  for terrestrial plants is  $-28\text{‰}$ ; whereas, for marine

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phytoplankton this variable is  $-21\text{‰}$  (Fry, 2002; Middelburg and Nieuwenhuize, 1998; Smith and Epstein, 1970, 1971). Terrestrial  $\delta^{15}\text{N}$  values range from  $-4\text{‰}$  to  $4\text{‰}$ ; whereas, marine aquatic sources range from  $6\text{‰}$  to  $10\text{‰}$  (Cloern et al., 2002; Dias et al., 2014; Peterson and Fry, 1987). Further discrimination of plant tissue versus alga composition in the seston of an estuary can be accomplished using the C/N ratio, with terrestrial plant material being carbon-enriched (C/N: 20–500) compared to algae (C/N 6–8) (Hedges et al., 1986). The differences in elemental C/N ratios and stable isotopes between terrestrial, marine, and anthropogenic sources have made them useful to determine if seston in an estuary is of autochthonous or allochthonous origin.

The Penobscot River Estuary (Fig. 1, Maine, USA) is well known for its anadromous and diadromous fish populations (Hayes and Kocik, 2014; Lipsky et al., 2016; Stevens et al., 2016; Waldman et al., 2016). For diadromous fish populations in the Penobscot River Estuary, the primary migration window is in spring (Saunders et al., 2006). Because this estuary is an important habitat for many fish and was subjected to pollution in the 1960s, numerous studies have characterized mercury in the Penobscot River Estuary (Merritt and Amirbahman, 2007, 2008; Panel, 2013; Santschi et al., 2017). Other biogeochemical research in the Penobscot River Estuary has focused on tidal movement (Geyer and Ralston, 2013; Haefner, 1967) and dissolved nitrogen, phosphate, and dissolved organic matter transport through the estuary (Barnard and Roesler, 2005, 2008). Little research, however, has been focused on characterizing the seston in the estuary. This study characterized seston along the Penobscot River Estuary during the spring (April through June) when peak fish migrations occurs.

## 2. Methods

### 2.1. Study area

The Penobscot River Estuary is approximately 25 km long (Fig. 1), with a watershed area of 20,109 km<sup>2</sup>, 78% of which is forested (Cronan, 2012). Seawater enters from the Gulf of Maine and proceeds northward into Penobscot Bay and up into the estuary. River discharge and diurnal tidal variations (mean tide = 2 m) cause large spatial and temporal variations in the estuarine salinity structure. Historically, higher flows ( $>800\text{ m}^3/\text{s}$ ) occur in April and May, with lower flows ( $<200\text{ m}^3/\text{s}$ ) in the summer (Cronan, 2012). During high-flow conditions and neap tides (spring/fall), the along-estuary salinity profile has a salt wedge structure with strong vertical gradients and a strong front at the landward limit of salt (Geyer and Ralston, 2013).

### 2.2. Field sampling

Estuarine water samples were taken on 23 April (neap and incoming tide), 12 May (neap and outgoing tide), and 30 June of 2015 (spring and outgoing tide). When sampling along a tidal gradient in which salt water can enter from only one location (Penobscot Bay), the station closest to the seawater source is referred to as the seawater end member, and the freshwater source (Penobscot River) is referred to as the river end member (Fry, 2002; Liss, 1976; Loder and Reichard, 1981). In April, 16 sites were sampled, and in May and June, four more stations were added for more detailed sampling at the river end member (two stations) a station near the input of Marsh Stream, and a station in the vicinity of Verona Island (Fig. 1). For each study site, the seston was characterized in the estuary by taking samples for the following measurements: (1) the identification of phytoplankton species present, (2) total suspended material concentration (TSM), (3) % organic material in TSM, (4) chlorophyll *a* concentrations, (5) size fraction

of detritus from 2 to 180  $\mu\text{m}$ , (6) phytoplankton, bacteria, and aggregated bacteria counts, (7) particulate carbon and nitrogen, and (8) the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the seston.

For all field samples, a 1-l Go-Flo<sup>2</sup> sampling bottle was used to obtain surface water samples (0.5 m). At each station, a YSI Model 85 was used to obtain dissolved oxygen (accuracy  $\pm 0.3\text{ mg l}^{-1}$ ), salinity (accuracy  $\pm 0.1$ ), and temperature (accuracy  $\pm 0.1^\circ\text{C}$ ). Salinity was measured using the practical salinity scale which is a conductivity ratio with no units. On site, the water in the Go-Flo sampling bottle was placed in a 1-l amber polypropylene bottle. Before subsampling, the 1-l amber bottle was inverted several times to ensure that the sample was well-mixed. On-site subsamples for live phytoplankton, preserved phytoplankton, and size-fractionation were taken immediately. The remaining water in the 1-l amber polypropylene bottle was stored in a cooler with ice for further processing at the laboratory (chlorophyll *a*, TSM, stable-isotope samples, and particulate carbon and nitrogen). Samples were processed within two hours of collection.

As riverine particles pass through an estuary, numerous reactions can occur as they encounter seawater. Physical properties (freshwater inputs) and biogeochemical processes (i.e., inputs, recycling, and removal) can influence particle properties before riverine particles reach the ocean (Howe and Simenstad, 2011; Riera, 2007). Assuming first-order mixing, when a constituent is plotted against salinity, information about the removal (exponential), inputs (quadratic), and mixing (linear) can provide insight into how an estuary acts as a reaction vessel for fresh/seawater mixing (Fry, 2002). Coinciding with first-order mixing models, when only physical mixing processes are controlling a constituent's movement through an estuary, then a straight line with the linear correlation close to 1 would connect the two end members, and the constituent would be classified as conservative (Fry, 2002; Liss, 1976; Loder and Reichard, 1981; Menzel, 1970; Readman et al., 1986). The further the linear correlation moves away from 1, the more likely it is that other processes might be affecting the constituent as it moves through the estuary, i.e., indicating non-conservative behavior (Loder and Reichard, 1981; Officer and Lynch, 1981). With no biological or chemical processes acting on it, a constituent will behave conservatively (i.e., salinity); whereas, a constituent that is biologically or chemically reactive (i.e., chlorophyll *a*) may behave non-conservatively. When the constituent falls below the straight line, removal is thought to be occurring; for values above the line, a source (i.e., groundwater inputs, production) is indicated (Loder and Reichard, 1981; Officer and Lynch, 1981). Because there is variability (i.e., surface runoff, groundwater input) in an estuary, if the natural variability of a constituent deviates by more than 10% from the theoretical dilution line, it can be classified as non-conservative (Liss, 1976). Based upon these recommendations, a correlation coefficient of a constituent greater than 0.70 was classified as conservative and less than 0.70 was classified as non-conservative in the present study. Most of the data indicated non-conservative behavior; therefore, all constituents were plotted versus distance to determine if there was an identifiable source or sink. The riverine end member was Station 1 at 0 km, and the seawater end member was Station 20 at 25 km.

### 2.3. Phytoplankton analysis

Water samples (100 ml) were collected and fixed immediately with Lugol's solution (Thronsen, 1978) at a final concentration of 2%. Samples were evaluated under a Zeiss Axio Observer Inverted

<sup>2</sup> Mention of a trade name or product does not imply endorsement by the National Marine Fisheries Service.

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