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# Anthropogenic nitrogen input traced by means of $\delta^{15}N$ values in macroalgae: Results from in-situ incubation experiments

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#### **Abstract**

The macroalgae species *Fucus vesiculosus* (Phaeophyta), *Polysiphonia* sp., and *Ceramium rubrum* (Rhodophyta) originally grown at an unpolluted brackish site of the southern Baltic Sea were incubated for 10 and 14 days at 12 stations along a salinity gradient in a highly polluted estuary. We have expected an adaptation of the initially low  $\delta^{15}N$  values to the higher ones within the incubation period. In addition to the macroalgae the  $\delta^{15}N$  values of  $NO_3^-$  were measured to evaluate fractionation processes of the source nitrate. Inside the estuary,  $\delta^{15}N$ - $NO_3^-$  values were 6.2–9.7‰, indicating anthropogenic nitrogen sources. The red macroalgae adequately reflected the nitrate isotope values in the surrounding waters, whereas for *F. vesiculosus* the results were not that clear. The reasons were assumed to be higher initial  $\delta^{15}N$  values of *F. vesiculosus* and presumably a too slow nitrogen uptake and growth rate. The method of macroalgae incubations seems suitable as a simple monitoring to study the influence of anthropogenic nitrogen loading in an estuarine environment.

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#### 1. Introduction

Increased urbanization and excessive use of agricultural fertilizers over the last decades led to high nitrogen loads in many rivers and the resulting eutrophication in coastal areas became a serious, worldwide problem. High nitrogen loads into river-systems are often associated with elevated  $\delta^{15}N$  values (>8%) in dissolved inorganic nitrogen (DIN), particulate organic matter (POM) and macroalgae (Heaton, 1986). Nitrate

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and ammonium originating from human and animal waste usually show  $\delta^{15}$ N values >10% (Wassenaar, 1995; Kendall, 1998) because of fractionation processes during transformation from one N-species to another. Rivers with low anthropogenic N load usually show  $\delta^{15}$ N values in DIN, POM and macroalgae <8% (McClelland and Valiela, 1998; Mayer et al., 2002), which reflects nitrate and ammonium sources from atmospheric deposition or nitrate from nitrification in pristine soils.

A common method to estimate and track these anthropogenic N inputs in rivers is based on direct measurements of  $\delta^{15}$ N in nitrate and ammonium, which require long-lasting preparation procedures. An alternative method is the measurement of  $\delta^{15}$ N values in the tissues of macroalgae, grown in polluted rivers or

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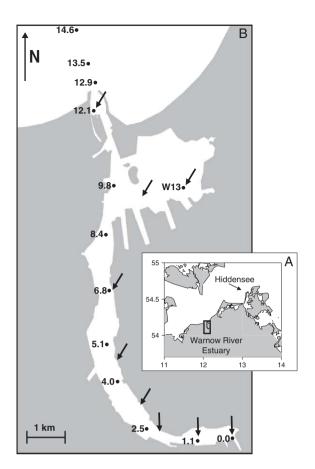


Fig. 1. Location of the Warnow River Estuary and the island of Hiddensee in the southern Baltic Sea (A). (B) shows a map of the sampled stations (sites are marked as distance from dam in km). The arrows indicate the stations where macroalgae were sampled.

estuaries (Costanzo et al., 2001; Gartner et al., 2002; Savage and Elmgren, 2004). Macroalgae only show small or no fractionation during uptake of nitrogen (Högberg, 1997), and thus directly reflect the  $\delta^{15}$ N value of the riverine DIN. In this study  $\delta^{15}$ N values in macroalgae from an unpolluted brackish site were incubated in a nitrogen rich estuary. With the additional determination of  $\delta^{15}$ N-NO<sub>3</sub> values, the hypothesis was tested if  $\delta^{15}$ N values in macroalgae tissue directly reflect the  $\delta^{15}N$  value of the river nitrate. Additional determination of C/N ratios and N content of the macroalgae was done to test whether the macroalgae increase the N content of the tissue if N is available in excess. Incubations were carried out in February/March and May, lasting 14 and 10 days, respectively. Furthermore, determinations of  $\delta^{15}N$  values of naturally grown macroalgae in the estuary were used to provide additional information on the natural variation in the estuary.

#### 2. Material and methods

#### 2.1. Study site

The Warnow river-system is located in northeastern Germany and discharges into the Baltic Sea (Fig. 1A). With a length of 149 km and a catchment area of 3270 km<sup>2</sup> it is the second largest river-system in Mecklenburg-Vorpommern. Land use is dominated by agriculture (63%) and forests (24%). 7% of the catchment is urban area and 6% covered with water. Total input of nitrogen into the river is 4071 tons year<sup>-1</sup> (Pagenkopf, 2001) which results in concentrations of  $> 250 \,\mu\text{M NO}_3^$ in the inner estuary. The estuary itself is 15 km long, flows through the city of Rostock, and is separated from the river with a dam (Fig. 1B). Mixing of the river water with seawater in the estuary strongly depends on wind strength and direction. Generally, strong northerly winds push seawater into the estuary, resulting in increased salinity inside. Winds from southerly directions push the river-water out of the estuary, consequently leading to reduced salinity. The water levels in the outer part of the estuary during the incubation experiments are shown in

The port of Rostock is located in the north eastern part of the estuary and a shipping channel passes through the whole estuary.

#### 2.2. Collection of macroalgae

Macroalgae were collected near shore in 0.5-1 m water depth at the west coast of the Island of Hiddensee (Fig. 1A) in the southern Baltic Sea 4 days prior to incubation. Macroalgae grew at salinity >7 and nitrate concentrations between 0 and 4  $\mu$ M. For the first incubation experiment the brown macroalga *Fucus vesiculosus* and the red macroalga *Polysiphonia* sp. were collected, and for the second incubation F.

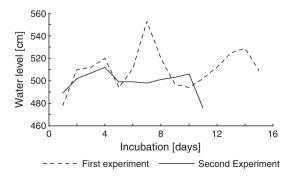


Fig. 2. Water level in the outer part of the estuary during the incubation experiments.

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