Development of Indicator for Coastal and Estuarine Eutrophication Using Morphological Characteristics and Tissue N Content of Eelgrass, Zostera marina

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Since cultural eutrophication has the detrimental effects on estuarine and coastal ecosystems, recognition of early stage of nutrient over-enrichment is critical for effective managements of the ecosystems. Since released nutrients into coastal ecosystems are diluted and dissipated through tidal action and rapid uptakes by marine plants, monitoring of in situ nutrient concentrations may not be useful for detecting early eutrophication on coastal and estuarine ecosystems. To develop an effective indicator of cultural eutrophication using marine plants, tissue N content and area normalized leaf mass of eelgrass, Zostera marina were examined in Kosung Bay and Koje Bay on the south coast of Korea from June 2001 to April 2003. Eelgrass tissue N content exhibited obvious seasonal variations. Leaf N content was highest during winter and early spring and lowest during summer. Eelgrass tissue N content was higher at Kosung Bay site, which has higher sediment organic content, than at Koje Bay site. Area normalized leaf mass showed reverse trend of leaf N content, and consequently, eelgrass leaf N content and leaf mass exhibited strong negative correlation at both study sites. The results of the present study suggested that the ratio of eelgrass leaf N content to area normalized leaf mass can be applied to assess environmental nitrogen conditions on the coastal and estuarine ecosystems.

Key Words: Area normalized leaf mass, Coastal eutrophication, Eelgrass, Indicator, N content, Zostera marina

INTRODUCTION

Estuarine and coastal ecosystems receive enormous amounts of nutrients as a consequence of anthropogenic loading (Valiela et al. 1992; Short and Burdick 1996; Tomasko et al. 1996; McMahon and Walker 1998). Runoff from agricultural fields and domestic sewage is the major source of these nutrients. Nutrient over-enrichment on coastal and estuarine ecosystems can cause cultural eutrophication, which is a serious environmental problem in many coastal areas and estuaries. Coastal eutrophication impacts the structure and function of coastal ecosystems (Cambridge and McComb 1984; Cambridge et al. 1986; Valiela et al. 1990; Lapointe et al. 1994; Short and Burdick 1996; Short et al. 1996).

The algal blooms promoted by released anthropogenic nutrients into coastal areas change water quality and lead to oxygen depletion and resultant fish kills (Nixon et al. 1986; Taylor et al. 1999; Deegan et al. 2002). Since nutrient over-enrichment has the detrimental effects on estuarine and coastal ecosystems, recognition of early stage of cultural eutrophication is critical for effective managements of the ecosystems (Fong et al. 1994, 1998; Lee et al. 2004). Monitoring nutrient concentrations in water column has been used to predict nutrient over-enrichment in coastal and estuarine ecosystems (Lapointe et al. 1994). However, loaded anthropogenic nutrients into coastal ecosystems will be dissipated and diluted through tidal action, currents, and microbial and marine plant uptakes (Morgan and Simpson 1981; Short and McRoy 1984; Stapel et al. 1996; Terrados and Williams 1997; Lee and Dunton 1999b). Additionally, nutrient loadings into coastal ecosystems occur in pulses that are highly variable temporally (Peters et al. 1986). Thus, monitoring of environmental nutrient concentrations is probably not a useful method to detect cultural eutrophication in coastal and estuarine ecosystems.

There have been attempts to develop a robust indicator of coastal eutrophication using marine plants

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(Fong et al. 1994, 1998; Lee et al. 2004). Tissue nutrient constituents of marine plants reflect nutrient conditions of the places in which the plants grow (Atkinson and Smith 1983; Duarte 1990; Short et al. 1990; Fong et al. 1994; Lee and Dunton 1999a; Lee et al. 2004). Tissue nutrient content of macroalgae and seagrass integrates nutrient regime over a period of time. Thus, tissue nutrient content of marine plants may record continuous nutrient conditions experienced by the marine plants. Therefore, tissue nutrient content of macroalgae and seagrass is probably better indicator of environmental nutrient over-enrichment or eutrophication in coastal and estuarine ecosystems than water column nutrient concentrations (Fong et al. 1994, 1998; Lee et al. 2004).

Seagrass morphology is also strongly linked to environmental nutrient availabilities (Short 1983, 1987; Udy and Dennison 1997; Lee and Dunton 2000; Lee et al. 2004). A strong correlation between sediment nutrients and eelgrass leaf morphology has been reported (Short 1983). Seagrass characterized by short and narrow leaves grew in low sediment nutrient conditions, while plants exhibiting long and wide leaves were found in high sediment nutrient areas. Additionally, seagrass morphological characteristics significantly responded to environmental nutrient manipulations (Short 1987; Udy and Dennison 1997; Lee and Dunton 2000). Therefore, seagrass morphological characteristics have been suggested for use as an indicator of environmental nutrient levels (Short et al. 1990; Fourquarean et al. 1997; Lee et al. 2004). Area normalized leaf mass (mg dry wt cm$^{-2}$ leaf area) of eelgrass exhibited a strong negative relationship with leaf tissue nitrogen content, and this relationship has been used to construct a nutrient pollution indicator (NPI), a tool for early detection of eutrophication (Lee et al. 2004). The ratio of eelgrass leaf N content to leaf mass provided a robust and sensitive early indicator of coastal eutrophication (Lee et al. 2004).

Eelgrass, Zostera marina is the most abundant seagrass species, widely distributed throughout all coasts of the Korean peninsula (Shin and Choi 1998; Lee and Lee 2003). In addition, seagrass can take up inorganic nutrient through leaf tissues from the water column, and the nutrient uptake is reflected in seagrass tissue nutrient content (Iizumi and Hattori 1982; Thursby and Harlin 1982, 1984; Short and McRoy 1984; Stapel et al. 1996; Terrados and Williams 1997; Lee and Dunton 1999b). Therefore, eelgrass is probably one of ideal candidates for a bioindicator of nutrient over-enrichment on the coasts of the Korean peninsula. In the present study, potential possibility of eelgrass morphology and tissue N content for detection of environmental N conditions was examined on the south coast of the Korean peninsula. Water column and sediment pore water dissolved inorganic nitrogen (DIN) concentrations, eelgrass tissue N content, and area normalized leaf mass were monitored from two bay systems on the south coast of Korea.

![Fig. 1. Study sites in Kosung Bay and Koje Bay on the south coast of Korea.](image)

**MATERIALS AND METHODS**

**Study sites**

The study sites were located in Kosung Bay and Koje Bay on the south coast of Korea (Fig. 1). The study was conducted on monotypic meadows of eelgrass, Zostera marina with an average water depth of about 2m. Eelgrass tissue N content, area normalized leaf mass, water temperature and water column and sediment pore water DIN concentrations were monitored monthly from June 2001 to April 2003. The Kosung Bay site has muddy sediments, while the Koje Bay site was characterized by high sand content in sediments.

**Water temperature and DIN concentrations measurements**

Water temperature was measured using Hobo data-logger (Onset Computer Corp.) encased in a waterproof underwater housing every 15 min and averaged daily during the experimental periods. Four replicate surface water samples were collected to determine water column DIN concentrations. Concentrations of NH$_4^+$ were
determined using standard colorimetric techniques following the alternative method of Parsons et al. (1984). Concentrations of NO$_3^-$+NO$_2^-$ were determined after running through a column containing copper coated cadmium, which reduces NO$_3^-$ to NO$_2^-$. To measure sediment pore water DIN concentrations, 6-10 replicate sediment samples were collected randomly to a sediment depth of about 13 cm with a corer. Samples were placed on ice and frozen pending lab analyses. Sediment pore water was obtained by centrifugation (5000 × g for 15 min), and pore water DIN concentrations were determined after dilution with low nutrient seawater. To determine sediment organic content, oven-dried sediments were burned at 550°C for 2 h, and sediment organic content was calculated from the loss of sediment weight. Shells were removed from the sediments before combustion.

**Area normalized leaf mass and tissue N content measurements**

Area normalized leaf mass was determined on 10 cm long leaf sections of the second or third youngest leaves. Epiphytes on the leaf tissues were removed completely, and leaf widths were measured to obtain the leaf section areas. The cleaned leaf sections were dried at 60°C to a constant weight, and the leaf mass was calculated as dry weight per leaf area (mg dry wt cm$^{-2}$ leaf area; Lee and Dunton 2004).

Dried leaf tissues from leaf mass samples and rhizome tissues from the first to sixth youngest nodes were used to determine plant tissue N content. Dried plant tissues were ground using mortars and pestles, and approximately 2-3 mg of ground tissues was placed into a tin boat for determination of eelgrass tissue N content using a CHN elemental analyzer (Flash EA1112).

**Statistics**

All values were reported as means ± 1 standard error (SE). Statistical analyses were performed on a microcomputer using a general linear model procedure (SAS). Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics prior to analysis. Significant differences in eelgrass tissue N content and area normalized leaf mass between sampling stations and among sampling times were tested using a two-way ANOVA. When significant differences were observed between variables, the means were analyzed by a Tukey multiple comparison test to determine where the significant differences occurred.

![Fig. 2. Seasonal changes in water temperature at Zostera marina beds in Kosung Bay and Koje Bay from June 2001 to April 2003.](image)

Area normalized leaf mass was correlated with eelgrass tissue N content.

**RESULTS**

**Water temperature**

Water temperature showed obvious seasonal trends at both study sites, but water temperature at Kosung Bay exhibited stronger seasonal trend than at Koje Bay (Fig. 2). Water temperature at Kosung Bay site ranged from 1.6°C in January 2003 to 31.7°C in July 2001, while water temperature at Koje site ranged from 8.3°C in late January 2003 to 26.9°C in August 2002. Water temperature during summer was higher at Kosung Bay site than at Koje Bay site, but water temperature during winter was lower at Kosung Bay site than at Koje Bay site.

**Water column and sediment pore water DIN concentrations**

NH$_4^+$ concentrations in water column were significantly (P < 0.001) higher at Kosung Bay site than at Koje Bay site (Fig. 3A). Water column NH$_4^+$ concentrations significantly (P < 0.001) changed with sampling time, but did not show distinct seasonal trends at both experimental sites. Water column NO$_3^-$+NO$_2^-$ concentrations were also significantly (P < 0.001) higher at Kosung Bay site than at Koje Bay site (Fig. 3B). Water column NO$_3^-$+NO$_2^-$ concentrations at Kosung Bay site were remarkably high after the rain. NO$_3^-$+NO$_2^-$ concentrations in water column were similar at two study sites during non-raining periods. NO$_3^-$+NO$_2^-$ concentrations in water column also varied significantly
Fig. 3. Water column NH$_4^+$ (A) and NO$_3^-$+NO$_2^-$ (B) concentrations in Kosung Bay and Koje Bay sites from June 2001 to April 2003.

Fig. 4. Sediment pore water NH$_4^+$ (A) and NO$_3^-$+NO$_2^-$ (B) concentrations in Kosung Bay and Koje Bay sites from June 2001 to April 2003.

(P < 0.001) with sampling time, but did not show any obvious seasonal trends.

Sediment pore water NH$_4^+$ concentrations at Koje Bay site were significantly (P < 0.001) higher than those at Kosung Bay site (Fig. 4A). Average NH$_4^+$ concentration at Koje Bay site was 316 µM in sediment pore water, while average concentration at Kosung Bay site was 181 µM. Sediment pore water NH$_4^+$ concentrations were highly fluctuated, but showed no obvious seasonal trends at both study sites. NO$_3^-$+NO$_2^-$ concentrations in sediment pore water were also significantly (P < 0.001) higher at Koje Bay site than at Kosung Bay site (Fig. 4B). Mean pore water NO$_3^-$+NO$_2^-$ concentration was 4.3 µM at Koje Bay site, and was 1.2 µM at Kosung Bay site. NO$_3^-$+NO$_2^-$ concentrations in sediment pore water were also significantly (P < 0.001) varied with sampling time, but showed no seasonal trend.

Sediment organic content, however, was significantly higher at Kosung Bay site than at Koje Bay site (Fig. 5). Mean sediment organic content was 9.71% at Kosung Bay site, and 4.78% at Koje Bay site. Sediment organic content did not show an obvious seasonal trend at both study sites.

Elgrass tissue N content and area normalized leaf mass

Elgrass leaf tissue N content showed significant (P < 0.001) seasonal variation at both study sites (Fig. 6A). Leaf N content was highest (3.8-4.1%) at Kosung Bay site,
Fig. 6. Nitrogen content of eelgrass leaf (A) and rhizome (B) tissues in Kosung Bay and Koje Bay sites from June 2001 to April 2003.

and 2.7-2.9% at Koje Bay site) during winter and early spring and lowest (2.0-2.2% at Kosung Bay and 1.5-1.6% at Koje Bay) during summer. N content of eelgrass leaf tissues was significantly (P < 0.001) higher at Kosung Bay site than at Koje Bay site. Mean leaf N content was 2.9% at Kosung Bay site, and 2.3% at Koje Bay site.

Rhizome N content also showed significant (P < 0.001) seasonal variation at both sites (Fig. 6B). N content of rhizome tissues was highest during late fall and lowest during spring. Rhizome N content was also significantly higher at Kosung Bay site than at Koje Bay site. Mean rhizome N content was 1.8% at Kosung Bay site and 1.2% at Koje Bay site.

Area normalized leaf mass significantly (P < 0.001) varied with sampling time (Fig. 7). Leaf mass was highest during late summer and lowest during spring at both study sites. Leaf mass at Kosung Bay site ranged from 1.96 mg dry wt cm⁻² leaf area during March 2003 to 5.18 mg dry wt cm⁻² leaf area during June 2002, while it ranged from 2.29 mg dry wt cm⁻² leaf area during April 2002 to 4.65 mg dry wt cm⁻² leaf area during July 2001 at Koje Bay site. Mean values of leaf mass were 2.98 and 3.32 mg dry wt cm⁻² leaf area at Kosung Bay and Koje Bay sites, respectively.

Fig. 7. Area normalized leaf mass from the study sites in Kosung Bay and Koje Bay from June 2001 to April 2003.

Fig. 8. Area normalized leaf mass and leaf N content in Kosung Bay (A) and Koje Bay (B).

**Relationships between eelgrass tissue N content and leaf mass**

Area normalized leaf mass showed reverse trend of leaf N content at both sites (Fig. 8). In general, leaf mass was highest during the periods of lowest leaf N content, and lowest leaf mass during the periods of highest leaf N
content. Eelgrass leaf N content exhibited negative relationships with area normalized leaf mass at both study sites (Fig. 9). Slopes and y-intercepts of regression lines for these two parameters were similar at two study sites.

The ratio of eelgrass leaf N content to leaf mass showed significant (P < 0.001) seasonal variation at both study sites (Fig. 10). The ratios were highest during winter and spring and lowest during summer. The ratios were significantly higher at Kosung Bay site than at Koje Bay site.

DISCUSSION

Because cultural eutrophication leads to harmful effects on coastal and estuarine ecosystems, early detection of coastal eutrophication is critical for effective management of coastal and estuarine areas. There have been attempts to develop an effective indicator of nutrient over-enrichment for early detection of cultural eutrophication (Shubert 1984; Pong et al. 1994, 1998; Lee et al. 2004). Morphological and physiological characteristics of marine plants such as macroalgae and seagrasses have been used as an indicator of coastal or estuarine eutrophication, because these plant characteristics reflect the nutrient regime experienced by the plants (Shubert 1984; Pong et al. 1994, 1998; Lee et al. 2004). In the present study, eelgrass tissue N content and area normalized leaf mass were examined to employ these features as a bio-indicator of early stage of cultural eutrophication in coastal and estuarine ecosystems.

Since tissue N content of opportunistic macroalga, Enteromorpha intestinalis, more rapidly responded to environmental nitrogen enrichment than plant growth or biomass changes, tissue N content of the algae has been suggested to be a better indicator of environmental nitrogen conditions than any other algal characteristics (Fong et al. 1998). There have been tried to infer environmental nutrient conditions from seagrass tissue nutritional content (Atkinson and Smith 1983; Duarte 1990; Fourquarean et al. 1992; Lee et al. 2004). Eelgrass leaf N content responded within 2 weeks to water column N enrichment during mesocosm experiments (Lee et al. in preparation). In the present study, eelgrass tissue N content was significantly higher at Kosung Bay site than
at Koje Bay site. This result implies that eelgrass plants in Kosung Bay site were exposed to higher nitrogen availability than plants at Koje Bay site. However, sediment pore water DIN concentrations were significantly higher at Koje Bay site than at Kosung Bay site.

Although, water column and sediment pore water DIN concentrations have been used as an indicator of environmental nitrogen conditions, turnover rates of the nitrogen pool and the regeneration of NH$_4^+$ are more important factors that determine the degree of the nitrogen availabilities. Sediment NH$_4^+$ pools in seagrass beds have been reported to have rapid turnover rates ranging 0.3 to 6 days (Capone 1982; Moriarty et al. 1985; Boo et al. 1986). Bacterial sulfate reduction in sediments of seagrass beds is important in terms of the regeneration of sediment nutrients (Hines and Lyons 1982; Holmer and Nielsen 1997). In the present study, sediment organic content was significantly higher at Kosung Bay site than at Koje Bay site. This trend implies higher inorganic nitrogen regeneration rates in Kosung Bay site than in Koje Bay site. Sediment organic content and eelgrass tissue N content showed a positive relationship, while eelgrass tissue N content exhibited reverse relationship with sediment pore water DIN concentrations in this study. These results suggest that in situ nutrient concentrations in water column or sediment pore water are not effective indicators to predict nutrient conditions in coastal or estuarine ecosystems. Sediment organic content and nutrient regeneration rates probably better represent environmental nutrient conditions than in situ nutrient concentrations.

Lee et al. (2004) showed that leaf N content and area normalized leaf mass of eelgrass, Zostera marina reflected environmental nitrogen conditions, and exhibited a strong negative correlation. It was demonstrated that the ratio of eelgrass leaf N content to leaf mass was a more sensitive indicator of early changes in environmental nitrogen conditions than either characteristics alone, and the ratio was suggested as a nutrient pollution indicator (NPI), a tool for early detection of cultural eutrophication. In the present study, area normalized leaf mass exhibited reverse trend of leaf N content at both study sites; highest leaf mass during the periods of lowest leaf N content, and lowest leaf mass during the periods of highest leaf N content. In addition, eelgrass leaf N content and area normalized leaf mass showed significant negative correlations in this study. These results suggest that the nutrient pollution indicator (ratio of eelgrass leaf N content to leaf mass) probably can be applied to predict environmental nitrogen conditions on the coasts of the Korean peninsula.

In contrast to terrestrial plants, seagrasses can take up nutrients from water column and sediment through leaves and root, respectively (Iizumi and Hattori 1982; Thursby and Harlin 1982, 1984; Short and McRoy 1984; Stapel et al. 1996; Terrados and Williams 1997; Lee and Dunton 1999b). Therefore, seagrass tissue nutrient content reflects the nitrogen availabilities in both water column and sediments. Tissue N content and leaf mass measurements of rooted seagrass plants on the sediments probably provide valuable information to assess environmental nitrogen availabilities on coastal and estuarine ecosystems. Cultural eutrophication, however, will be mainly caused by over-enriched nutrient in the water column. The contributions of sediment and water column nitrogen on eelgrass morphology and tissue N content cannot be separated for naturally growing eelgrass plants. Tissue N content and leaf mass of the naturally growing eelgrass plants in the present study represent nitrogen conditions in both sediment and water column. Therefore, to use tissue nutrient content and morphological characteristics of eelgrass, Zostera marina as an indicator of estuarine and coastal eutrophication, eelgrass shoots should be deployed hydroponically on the place.

The main purpose of the present study was to examine and assess eelgrass tissue N content and area normalized leaf mass, which could represent environmental nitrogen conditions. Results of this study demonstrated strong and consistent relationships between eelgrass leaf mass and leaf N content in both study sites. Therefore, the ratio of eelgrass leaf N content and leaf mass appears to represent environmental nitrogen conditions, and could be used as an indicator for coastal and estuarine eutrophication.

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