The relationship between diatom species and measured environmental variables was explored at different sites of Honghe wetland region located in northeastern China. Planktonic and epiphytic diatom assemblages in the wetland were identified from May to October of 2007 and 2008. Their relationships with environmental variables were examined using canonical correspondence analysis with forward selection and Monte Carlo permutation. The test revealed that each of the eight environmental variables, pH, total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved Oxygen (DO) and water temperature (WT) were statistical significant fractions of the variation in diatom taxa. This study reveals that DO was the most important environmental variables affecting diatom communities in the wetland. It also indicated that water quality in Honghe wetland were oligosaprobic and mesosaprobic.

Key words: Honghe wetland, environmental variables, canonical correspondence analysis, diatom communities, water quality.

INTRODUCTION

Wetland functions are defined as a process or series of processes that take place within a wetland. These include the storage of water, transformation of nutrients, growth of living matter and diversity of wetland organisms, and they have value for the wetland itself, for surrounding ecosystems, and for people. Functions can be grouped broadly as habitat, hydrologic or water quality, although, these distinctions are somewhat arbitrary and simplistic (Novitzki et al., 1996).

Algae are typically aquatic organisms that are able to record the environmental events that occur in a water body. This is due to their high sensitivity to environmental changes (Mariacristina and Antonio, 2006). The sensitivity of algae, especially diatoms, is commonly used in different ecological and paleoecological investigations (Stoermer and Smol, 1999; Ács et al., 2003). Diatoms are suggested to be indicators of environmental conditions (Round, 1991; Rimet et al., 2004). Their generation time is one of the quickest among bioindicators of water quality (Rott, 1991). They divide frequently and can thus indicate rapidly a change in water quality and can be used successfully in biomonitoring (Stevenson and Pan, 1999). The composition of diatoms communities can reflects an entire complex of ecological parameters at a particular site (van Dam, 1982). Diatom indices are very useful by regulatory bodies (Prygiel et al., 1999). Water managers use diatoms as one of the tools for assessing water quality, because they are present in all river sections (Mariacristina and Antonio, 2006). As bioindicators, they are used in environmental studies over a long time period (Stevenson and Pan, 1999). Presently, diatom indices are used routinely in different European countries to assess the biological quality of running waters (Prygiel et al., 1999).

Environmental conditions can influence the algae diversity of lotic systems by constraining particular species (Mäggi et al., 2001). Several studies have shown that certain parameters such as water velocity, water chemistry, water temperature and nutrient supply can affect
diatom species composition in a given diatom community (Biggs et al., 1998; Kann, 1978; Kuhn et al., 1981). Honghe wetland is located in the Sanjiang plain, at the northeast corner of Heilongjiang province. In 1984, it was approved by Heilongjiang provincial people's government to be the provincial nature reserve. In November of 1996, the state council approved it to be the national nature reserve. The convention on wetlands approved it as an international wetland in January of 2002. At present, it is one of the 36 internationally recognized wetlands in China. The aims of this experiment were to explore the major patterns of diatom communities collected from Honghe wetland and their relationships with eight measured environmental variables and to identify the most important environmental variables regulating patterns in the species composition of diatoms.

MATERIALS AND METHODS
Study area
Honghe wetland is located in Heilongjiang Province, China. It covers 21835 km² and lies between 133°34'28"-133°46'29"E and 47°42'18"-47°52'00"N (Figure 1). Nong and Wovlian Rivers flow through the region converging at the central area. The river is circuitous; the water current is slow and very marshy. The initial animal colonizers are typical of the cold temperate zone. In the region, there are 283 kinds of vertebrates. The plants grow in abundance, particularly hydrophytes. 1012 species of plants were identified there. They are 262 species of lichen and moss, 35 species of ferns and 715 species of seed of plants (Ma Shoujia, 2008). The sanctuary reserves the biggest marsh area in China-Sanjiang plain wetland. The warmest month is July with air temperature (average 22.4° C) and the coldest month is December or January (average -23.4° C). The wetland is usually covered by ice from November to April. The fauna includes animals of national importance such as, Ciconia boyciana, Ciconia nigra, etc. and the flora includes Acanthopanax senticosus, Juglans mandshurica, Astragalus membranaceus, etc.

The distribution characteristics of higher plants were chosen in the sampling sites, according to the actual station which was chosen in the sampling sites. We collected the sampling sites having sufficient representation, representing different ecotope types in Honghe wetland. Along with a handheld global positioning system (GPS), we established eight sampled sites (Figure 1), 1 to 4 sampling sites (s1, s2, s3, s4) covered up to 118 km in the wetland. Higher plants, such as Deyeuxia angustifolia Chang., Cyperaeae lasiocarpa Ehrh., Salix rosmarinifolia L., Comarum palustre L., Gentiana scabra Bunge were distributed in the area. From 5 to 8
sampling sites (s5, s6, s7, s8) covered 135 km in the reserve. Apart from sampling site 5 which was majorly reeds, other sampling sites include species such as *Cyperaceae pseudo-curaica* Schmidt, *Menyanthes trifoliata* L., *Salix rosmarinifolia* L., *Comarum palustre* L., *Lysimachia thyrsiflora* L., *Triadenum japonicum* Makino and *Pedicularis grandiflora* Fisch.

**Sample collection and chemical analysis**

From May to October of 2007 and 2008, diatom samples were collected (once a month from May to October) in Honghe wetland. Phytoplanktonic diatom samples were collected twice from the same sites. The collection was done from the surface of the river (depth 0.5 to 1.0 m) using plankton net (26 μm mesh size). Epiphytic diatoms were collected from at least five stones from the littoral parts of the sampling sites. The upper surface of stones was scratched with brush and the suspension in order to collect sample. The samples were stored in small plastic bottles (Kelly et al., 1998). If stones were absent in the study area, diatom samples were obtained by cutting 5 to 20 cm long pieces of submerged plants into plastic bottles, they were stored using Lugol's solution immediately after collection (Buczko and Acs, 1996, 1997).

During the course of this experiment, water temperature and pH were measured with standard instruments (pH B-4). Samples for water quality analyses were collected simultaneously with the diatoms samples. The water samples were stored in 500 ml bottles, kept in cool storage (4° C) and were transferred to the laboratory for analyses. The TOC, TN and TP were measured with Enovi instruments (ET99730). The BOD (BOD-system OxDirect Italy), COD (COD-Reaktor ET108) and DO (iodimetry) were determined by national standards.

**Diatom preparation, identification and counts**

Diatom valves were cleaned with concentrated hydrogen peroxide (H₂O₂ 40%) to eliminate organic matter and later with hydrochloric acid to dissolve calcium carbonates. After washing with distilled water, samples were mounted on a slide with Naphrax. Using light microscope (1000 magnification) at least 500 valves (per slide) were counted and identified to species. Identification of species was done according to Krammer and Lange-Bertalot (1999, 2000) and Krammer (2002). The counts was converted to relative abundance.

**Data analysis**

The diatom data was analyzed prior to statistical analysis and reduced to include only those species occurring in at least two samples with a relative proportion of 1% or more in at least one sample (Bennion, 1994). We used canonical correspondence analysis (CCA) (Ter Braak and Smilauer, 2002), to explain the distribution of the diatom communities along each environmental variable. PCAs, DCAs and CCAs were carried out using CANOCO 4.0 (Ter Braak and Smilauer, 1998). The data was analyzed with statistical analysis of factor and correlated by Microsoft office excel 2007.

**RESULTS**

**Physical and chemical variables**

During the period of this study, water temperature changed slightly at the eight sites in the same season. During sample collection from summer (July to August) to autumn (September to October), water temperature ranged from 1 to 21°C. In spring (May to June), average water temperature ranged from 10 to 14°C. The pH value was relatively stable in the eight sites and during the three seasons. The pH value only changed from 6 to 6.6, and did not change with seasons. DO changed conspicuously from 3.2 to 14.6 mg/L in different seasons. The lowest value was observed in July, at 3.2 mg/L; the highest value appeared in colder water temperature season, at 14.6 mg/L. A negative correlation occurred between the contents of BOD and DO. During the period of higher water temperature, BOD was also high (5 mg/L), while DO was low. During the period of lower water temperature, BOD was also low (1mg/L), while DO was high. High COD and TN values were detected in summer. The maximum values were seen in August, about 15.3 and 3.93 mg/L, respectively. Whereas, the minimum of COD was detected in June, about 11.2 mg/L, and that of the TN was in October, about 1.21 mg/L. TP changed significantly in different seasons, from 0.01 to 0.15 mg/L. The highest value was observed in June, while lowest value was observed in August. TOC values ranged from 3.97 to 5.13 mg/L. The lowest value was observed in May, while highest value was observed in July.

The entire parameters except pH changed significantly with season. The changes of the parameters were not significant in all the samples between the two years. There are no significant changes of physical and chemical parameters between these two years, but with obvious variation at each month (Table 1).

**Diatom assemblages**

Although, a total of 180 taxa (species and varieties) belonging to 22 genera were identified, only 128 were present in over 1% of the samples. Among them, *Pinnularia* (23 taxa), *Gomphonema* (20 taxa), *Eunotia* (18 taxa) and *Navicula* (15 taxa) were the most common genera. The distributions of the most abundant taxa is illustrated in Figure 2. The results show that the abundance of *Eunotia lunaris* in all samples were higher than other diatoms.

In Figure 2, the vertical axis is sampling sites, No. 1 to 8 representing the s1 to s8 on May, 2007, and No. 9 to 16 representing the s1 to s8 on May, 2008, and No. 17 to 24 representing the s1 to s8 on June, 2007, and so on. The horizontal axis is quantity of species. The composition of the diatom assemblage changed in different seasons, but occurrence were basically similar within the eight sites in the wetland. The most common taxa are *Eunotia tenella* Hustedt, *Gomphonema gracile* Ehr., *Gomphonema olivaceoides* Hust., *Gomphonema angustatum* var. *producta* Grun., *Navicula salinarum* Grun., *Nitzschia*
Neidium iridis
Melosira islandica pectinalis
Eunotia glacialis
Eunotia arcus radiosa
Hantz.

wetland. While in the colder season, Ehr., were observed in high proportions within the
Eunotia flexuosa
Amphora ovalis
shown in Table 2.

study area. The statistic of dominant diatoms species is
however, the richness at several stations (s1, s5 and s7)
significant differences were observed in species richness,
were investigated during the period of this research. No
Furthermore, the changes in the richness of diatoms
oligohalobinic species and slightly acidic species.

They are classified as low salinity and rich oxygen
diagram species of the Honghe wetland were
differences among species of diatom. The dominant
diatoms that were observed in each month were simila r
month. In May of 2007, 98 taxa were observed and then

edited during the period of this research. No significant differences were observed in species richness, however, the richness at several stations (s1, s5 and s7) collected in 2007 were slightly richer than those of 2008 (Figure 3).

The richness of each taxa were changed along the month. In May of 2007, 98 taxa were observed and then gradually rose to 113 taxa in July. In 2008, the species of diatoms that were observed in each month were similar to that of 2007, but the number was slightly higher and ranged from 93 to 110 taxa.

Although, the quantities of diatom species that were observed in these seasons were similar, there were differences among species of diatom. The dominant diatom species of the Honghe wetland were E. lunaris Grun. and Nitzschia clausii Hantzsch all over the year. They are classified as low salinity and rich oxygen species, so they can be used as indicator species in this study area. The statistic of dominant diatoms species is shown in Table 2.

In the warmer season, mesotrophic indicators such as
Amphora ovalis Kütz., Cyclotella meneghiniana Kütz.,
Eunotia flexoua Kütz., Navicula pupula Kütz., Navicula radiosa Kütz.,
Pinnularia braunii Cl. and Pinnularia viridis Ehr., were observed in high proportions within the wetland. While in the colder season, Cyclotella lunata W.,
Eunotia arcus var. bidens Grun., Eunotia curvata Kütz.,
Eunotia glacialis Meist., Eunotia incise W., Eunotia pectinialis var. minor Rabh.,
Gomphonema gracile Ehr., Melosira islandica O. Müll.,
Navicula pupula var. capitata, Neidium iridis var. ampliatum Cl., Nitzschia gracilis
Hantz., Pinnularia braunii var. amphi cephalia Hust.,
Pinnularia viridis var. minor Cl. and Stauro nephel...
(Whitton and Rott, 1996; Prygiel et al., 1999). The use of algae as bioindicators has been discussed for almost 100 years. The first indicator system was published by Kolkwitz and Marsson (1908). Diatoms are important contributors of the primary production in shallow aquatic ecosystems (Wetzel, 1990). Diatom assemblages are also well known to be sensitive to organic pollution and eutrophication, making them particularly good
bioindicators for monitoring pollution in different parts of the world (Ector et al., 2004).

Although, a total of 180 taxa were identified in Honghe wetland, the distributions of the abundant taxa were observed with the different seasons. In summer, *Tabellaria fenestrate* Kützing, *Tabellaria flocculosa* Kützing etc. were dominant taxa. The dominant taxa in autumn were *Gomphonema olivaceoides* Hust., *Pinnularia divergens* W.Smith, *Pinnularia stomatophora* Cleve, etc. The general species composition of the diatom communities recorded in the Honghe wetland was investigated in accordance with Tilman's (1984) report which states that the dominant taxa were observed in summer and autumn. Although, seasonal change results in diatom species variation, the richness within two years has no significant difference due to little distinct environmental variation, all of
which manifests the fact that the water quality in Honghe wetland stays stable on the whole. Meanwhile, we found that the diatom communities were similar in all sites except in sampling site 5. The abundances of diatoms at sampling site 5 are higher than other sites. It maybe associated with
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Figure 2.

Continue

The CCA detects variation patterns within the species data that can be explained best by the environmental variables (Ter Braak, 1986). The length of the environmental variable axes showed that water temperature, DO and TN were driving factors associated with the seasonal variation of diatom composition in the wetland. In the left of the CCA biplot, with the lower DO and the higher water temperature, diatom species, such as Navicula seminulum Grun., Tabellaria flocculosa Kützing, Tabellaria fenestrate, Asterionella formosa Kützing, Tabellaria flocculosa, and Diploneis spp., were prominent. These species are known to have purification effects on nitrogen and phosphorus in water bodies (Hua et al., 2006; Wang et al., 2003; Liu, 2004; Zhao et al., 2009). The distribution of plenty of higher plants such as reed. Reeds are known to have purification effects on nitrogen and phosphorus in water bodies (Hua et al., 2006; Wang et al., 2003; Liu, 2004; Zhao et al., 2009).
observed. This result was similar to that of Semina (1972), where the aforementioned diatom species occurred in the lower DO and the higher water temperature. Also, observed in the region were mesotrophication species such as, *Cocconeis placenta* Hust., *Cymbella aspera*,...
Figure 3. Richness change of the diatom in different sampling stations.

Table 2. Statistic of dominant diatoms in Honghe Wetland.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Dominant species</th>
<th>Month</th>
<th>Year</th>
<th>Dominant species</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>2007</td>
<td>Gomphonema gracile Ehr. 36%</td>
<td>August</td>
<td>2007</td>
<td>Tabellaria fenestrate Kütz. 43%</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Eunotia tenella Hustedt 40%</td>
<td></td>
<td>2008</td>
<td>Tabellaria flocculosa Kütz. 38%</td>
</tr>
<tr>
<td>June</td>
<td>2007</td>
<td>Pinnularia viridis Ehr. 43%</td>
<td>September</td>
<td>2007</td>
<td>Pinnularia viridis Ehr. 44%</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Navicula salinarum Grun. 36%</td>
<td></td>
<td>2008</td>
<td>Nitzschia gracilis Hantzsch 44%</td>
</tr>
<tr>
<td>July</td>
<td>2007</td>
<td>Tabellaria fenestrate Kütz. 50%</td>
<td>October</td>
<td>2007</td>
<td>Pinnularia divergens W.Smith 36%</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Tabellaria flocculosa Kütz. 38%</td>
<td></td>
<td>2008</td>
<td>Gomphonema olivaceoides Hust. 37%</td>
</tr>
</tbody>
</table>

Pinnularia gracillima Gregory, Gomphonema acuminatum Ehr., Cymbella dilicatula, etc. (Rimet et al., 2007). In the right, Surirella angustata Kütz., Pinnularia borealis Ehrenberg, Pinnularia stomatophora Cleve and Nitzschia clausii Hantzsch etc. were observed in this region with higher DO and lower water temperature. The acidobiotic diatoms, Eunotia tenella Hustedt, Eunotia exigna Grun. Eunotia flexuosa Kütz., Eunotia valida Hust., Surirella angustata Kütz. also occurred. Because the eight sampling sites are close to each other and ecological environment of the two sampling sites are similar, the environmental parameter of sampling sites did not obviously change. But the monthly diatom species has significant changes.

Diatoms, as pH indicators, were applied to trace the acidification history of lakes in several paleolimnological studies (Battarbee, 1984; Cameron et al., 1999). It is also used in determining the pH in running waters (van Dam and Mertens, 1995; Coring, 1996). According to the pH classification by Van Dam et al. (1994), acidobiotic species to alkalibiontic species were found in this study. The typical acidobiotic and acidophilous species included Cymbella affinis Kütz., Eunotia tenella Hustedt, Eunotia exigna Grun., Eunotia flexuosa Kütz., Eunotia valida Hust., Gomphonema acuminatum Ehr., Navicula subulissima Cl., Pinnularia gibba Ehr., Pinnularia viridis Ehr., Surirella angustata Kütz., etc., but some alkaliophilus diatoms such as Cymbella tumida, Gomphonema angustatum var. producta Grun., Nitzschia palea W. Smith, Navicula neoventricosa Hust., Rhopalodia gibba, Tabellaria flocculosa Kützing etc. were also found.

Eutrophication can be defined as an intensified accumulation of diatom biomass generally due to an increase in nutrients (primarily phosphorus and nitrogen) (Vollenweider, 1989). The importance of primary nutrients; P and N in lotic ecosystems are not fully understood (Wetzel, 1983). Nutrients status played an important role in the distribution of diatom species. A large number of taxa are strongly related to TP concentrations. In the lower TP concentrations habitats, species such as Cyclotella lunata, Eunotia incisa, Eunotia pectinialis var. undulata, Eunotia lunaris Grun., Eunotia...
Figure 4. Biplot of environmental variables and diatom species in a CCA of the Honghe wetland. Selected species codes are as follows: na-vi = Navicula viridula Kütz., go-ac = Gomphonema acuminatum Ehr., na-sa = Navicula salinarum Grun., cy-tu = Cymbella turgidula Grun., ha-vi-vi = Hantzschia vivax var. vivax, ni-gr = Nitzschia gracilis Hantzsch, ha-am = Hantzschia amphioxys W. Smith, pi= = Pinnularia viridis Ehr., eu-lu = Eunotia lunaris Grun., ni-pe = Nitzschia perminuta Peragallo, eu-pr = Eunotia praerupta Ehr., na-ne = Navicula neoventricosa Hust., eu-fl = Eunotia flexuosa Kütz., eu-ar-bi = Eunotia arcus var. bidens Grun., go-he = Gomphonema hebridense Gregory, go-au = Gomphonema auritum Kützing, co-pl-eu = Cocconeis placentula var. euglypta, pi-di-me = Pinnularia divergens var. media, pi-fi-no = Pinnularia tirolensis, pi-gi = Pinnularia gibba Ehr., go-ol = Gomphonema olivaceoides Hust., st-ph = Stauroeis phoenicenteron Ehr., go-lo-su = Gomphonema longiceps var. subclavata f. gracilis Hust., pi-bo-bo = Pinnularia borealis var. borealis, su-an = Surirella angustata Kütz., ni-cl = Nitzschia clausii Hantzsch, pi-st = Pinnularia stomatophora Cleve, na-ra = Navicula radiosa Kützing, cy-na-no = Cymbella naviculiformis, go-gr = Gomphonema gracile Ehr., go-tr = Gomphonema truncatum Ehrenberg, eu-te = Eunotia tenella Hustedt, eu-va = Eunotia valida Hust., pi-di = Pinnularia divergens W. Smith, pi-br = Pinnularia brevicostata Cleve, eu-fa = Eunotia faba Ehrenberg, rh-gi-gi = Rhopalodia gibba O. Müller, na-aq = Nacicula aquaedurae, pi-sa = Nitzschia parvala, co-pl-ps = Cocconeis placentula var. pseudolineata, ca-am = Caloneis amphibiaena Cl., co-pl= = Cocconeis placentula Hustedt, cy-gr = Cymbella gracilis Cleve, cy-as = Cymbella aspera, ha-am-f. = Hantzschia amphioxys f. capitata F. Müll., na-se = Navicula seminulum Grun., go-an-pr = Gomphonema angustatum var. producta Grun., na-su = Navicula subundulata Cl., pi-br = Pinnularia borealis Cleve, etc. were observed. Most of which are oligotrophic indicators. However, in the higher TP concentrations habitats, mesotrophic diatom assemblages such as Amphora ovalis Kütz., Cyclotella meneghiniana Kütz., Gomphonema acuminatum Ehr., Gomphonema constrictum Ehr., Stauroeis aniceps Ehr., etc. were recorded. Our result is similar to Thomas (1989), who stated that Gomphonema parvulum Grunow occurred in the freshwater, but this result is inconsistent.
with the findings of Leska and Cynthia, (2002), who states that *Gomphonema parvulum* Grunow was observed in polluted water. *G. parvulum* Grunow was observed in both waters because it has a broad range of ecological habitats (Thomas, 1989).

Diatoms have a relatively short life cycle, therefore they respond rapidly to eutrophication and provide detailed information on nutrient changes (Winter and Duthie, 2000; Soininen and Niemela, 2002). The distributions of most oligotrophic diatoms were associated with species such as, *Caloneis amphissaena* Clr., *Cymbella affinis* Kütz., *Gomphonema truncatum* Ehr., *Gomphonema gracile* Ehr., *Gomphonema olivaceoides* Hust, *Hantzschia amphiocystis* Smith, *Navicula centroptera* Kütz., *Navicula cryptocephala* var.* centroptera* Kütz., *Navicula cryptocephala* var.* veneta* Rabh., *Navicula graciloides* Mayer, *Navicula laevissima* Kütz., *Navicula minima* Grun., *Navicula salinarum* Grun., *Navicula subulissima* Clr., *Nitzschia clausii* Hantzsch, *Nitzschia palea* Smith, *Nitzschia parva* var.* parva* Grun., *Navicula neoventricosa* Hust, *Stauroesia aniceps* Ehrenberg, *Tabellaria flocculosa* Kütz. and *Tabellaria fenestrata* Kütz. in the wetland from different seasons. The classification of water quality in Honghe wetland was affirmed as the good trophic state by the abundance of oligotrophic species in diatom communities.

In conclusion, this report is a pilot study on the composition of diatom assemblages and their distribution characteristics in Honghe wetland. The examinations of diatoms in the water bodies have shed more light on diatom biodiversity in this protected area. The results showed that environmental change affect diatom species composition, meanwhile the structure of diatom indicated environmental change. Trophic classification of water environment, the identification of diatom assemblages and environmental parameter, revealed that the water quality in Honghe wetland is both oligotrophic and mesotrophic.

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