1 **Development of an integrated stress index to determine multiple**
2 **anthropogenic stresses on macrophyte biomass and richness in**
3 **ponds**

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7 **Abstract**

8 Ponds significantly contribute to local and regional biodiversity by supporting
9 wide-ranging communities of aquatic organisms. However, ponds are often disturbed
10 by intensive anthropogenic activities because of their accessibility. Hence, methods to
11 assess the effects of anthropogenic activities on ponds are of great important. In this
12 study, we developed an integrated stress index (ISI) to characterize the integrated
13 stress from different types of anthropogenic activities on ponds and then tested the
14 robustness of this index through sensitivity analysis. We also examined the
15 relationships between ISI and the species richness and biomass of different
16 macrophytes through regression analyses. Results showed that ISI significantly
17 negatively correlates with total species richness but significantly positively correlates
18 with total biomass. Emergent, floating-leaved, and submerged species exhibited
19 different responses to ISI. Moreover, ISI produced more significant effects on species
20 richness and biomass of different macrophytes than eutrophication. Therefore,
21 eutrophication is not the only stress to be treated in the area of study. These findings
22 provide pond restoration-useful insights into the relationships among inhibiting
23 anthropogenic activities, water level management, harvesting, and species

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composition.

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1. Introduction

Ponds significantly contribute to local and regional biodiversity by supporting wide-ranging communities of aquatic organisms (Gioria et al., 2010). Ponds also largely benefit human society because of their use in irrigation, drinking for cattle, water transportation, and tourist or recreational use (Søndergaard et al., 2005; Declerck et al., 2006; del Pozo et al., 2010). Therefore, ponds are intensively disturbed by different types of anthropogenic activities and face many ecological and environmental problems, such as nutrient enrichment (De Backer et al., 2012), water level drawdown (Usio et al., 2013), physical destruction (Biggs et al., 2005), and area losses (Curado et al., 2011). In particular, decreased diversity of aquatic macrophytes has been observed in ponds over the past few decades (Williams et al., 2004; Akasaka et al., 2010).

Anthropogenic activities around ponds are often represented as the proportions of different land-use types around ponds (Rolon et al., 2012; Raebel et al., 2012), as well as the properties of anthropogenic activities, such as population density, number of villages and docks, and grazing intensity (De Meester et al., 2005; del Pozo et al., 2010, 2011). These activities can greatly influence the growth, distribution, and community composition of aquatic macrophytes by altering many environmental factors (e.g., light availability, pH, and nutrient level) (Radomski and Perleberg, 2012; Karus and Feldmann, 2013). Specifically, agricultural production and residential development can cause water eutrophication, which eventually results in the loss of aquatic macrophyte species, especially submerged ones (Barker et al., 2008; Kolada, 2010; Sass et al., 2010). Excessive water extraction and desiccation related to agricultural practices also have been proved closely related to species loss (Chappuis et al. 2011). The construction of docks around ponds changes sediment quality and

slope in littoral zones, ultimately inhibiting the distribution of aquatic macrophytes (Radomski and Goeman, 2001). These docks can also increase boating activity, which directly destroys macrophytes through wave erosion (Alexander et al., 2008). Forests surrounding ponds may suppress the growth of aquatic macrophytes by litter accumulation and shading effects (Declerck et al., 2006). Residential development surrounding ponds also reduce macrophyte coverage because shoreline land owners often directly remove macrophytes to create beach conditions (Jennings et al., 2003; Hicks and Frost, 2011).

Aquatic macrophytes are key components in freshwater ecosystems (Søndergaard et al., 2010). They can stabilize sediments (Madsen et al., 2001), absorb and store nutrients (Dai et al., 2012), enhance water clarity (Søndergaard et al., 2010), and support other aquatic organisms (e.g., zooplankton, fish, and invertebrates) in freshwater ecosystems (Jeppesen et al., 2000). Thus, declines in the species composition and abundance of aquatic macrophytes because of intensive anthropogenic activities could exert lasting, substantial, negative effects on the biodiversity, structure, and function of pond ecosystems (Heino, 2002; Ribeiro et al., 2011). Accordingly, some ecological engineering approaches aiming to restore aquatic macrophytes need to be implemented in ponds.

Ecological engineering is defined as the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both, and the most fundamental concept of ecological engineering is self-design (Mitsch, 1996; Mitsch and Jørgensen, 2003; Odum and Odum, 2003). Ecological engineering is being practiced over a wide spectrum of approaches to restore wetlands with different ecological problems (Mitsch, 2012). For instance, a modest intervention (e.g., continual inflow of river water) has been proven for facilitating the introduction and optimization of species composition without planting by self-design in a created wetland ecosystem (Mitsch et al., 1998). Some ecological approaches including modified agricultural practices (e.g., reduced chemical fertilizer use) and landscape

restoration (e.g., creation and restoration of riparian buffers and wetlands) were proposed to restore wetlands in river basins affected by excessive nitrate–nitrogen inputs from increased fertilizer use (Mitsch and Day, 2006; Mitsch, 2014). The restoration of approximate original hydrological conditions by breaking dikes or removing impediments to flooding have been proven successful for the restoration of a marshland dramatically disturbed by upstream dams and water control structures (Mitsch and Gosselink, 2007; Mitsch, 2014).

Similarly, different ecological engineering approaches are also required for pond restoration because they are widely distributed over a long anthropogenic stress gradient and often show diverse problems owing to anthropogenic stress (Gioria et al., 2010). Thus, an urgent demand to quantify anthropogenic stress for pond categorization and to explore changes in species composition along the stress gradient exists. However, a holistic perspective on the integrated stress of different anthropogenic activities remains lacking, and less is known about the effects of integrated anthropogenic stress on aquatic macrophytes in ponds.

Lake Baiyangdian was made up of 140 ponds before 1990s. However, the continuously decreasing water input, increased population, and rapid economic development have severely impacted this region (Zhong et al., 2005; Zhuang et al., 2011); the number of ponds is only less than 30 nowadays, accompanied by substantial species losses (Han and Cui, 2015). With the increase in awareness on ecological conservation and restoration, the local wetland management office has formulated several plans to regulate anthropogenic activities within this region, and eutrophication control is viewed as the main strategy for pond restoration. However, the integrated anthropogenic stress suffered by each pond has not yet been quantified in these plans, and effects of integrated anthropogenic stress on aquatic macrophytes in ponds have not yet been analyzed. To develop effective restoration strategies for different ponds, the integrated stress of different types of anthropogenic activities and the changes in aquatic macrophytes along a stress gradient are important to

quantitatively assess.

The present study aims to (1) develop an integrated stress index (ISI) and test its robustness, (2) analyze the relationship between aquatic macrophytes and ISI, and (3) summarize some implications for pond restoration.

2. Materials and Methods

2.1 Study area

Lake Baiyangdian (38°38' N, 115°54' E) is the largest macrophyte-dominated shallow lake in Baoding City, North China. Before the 1990s, the lake had an average water level of >8.5 m and comprised 140 ponds that were interconnected through water channels. Nowadays, the number of ponds is only less than 30 because of to continuously decreasing water input, as well as expansions of residential areas, docks, and cropland within this region over the past few decades. Most of these ponds are also isolated. Meanwhile, substantial species loss and large spatial variability of community structure among different ponds were also observed. In this study, we presented the results of a field survey on aquatic macrophytes in 20 ponds that were selected based on a gradient of surrounding anthropogenic stress (Fig.1).

[Fig.1 here.](#)

2.2 Data collection

2.2.1 Integrated stress

We developed an ISI to characterize the integrated stress of the following four types of anthropogenic activity around each pond: (1) residential development, (2) dock construction, (3) cropland reclamation, and (4) raised field construction. A raised field (1.0 m to 1.5 m higher than the water surface) was constructed by local farmers with pond sediment and planted with *Phragmites australis* for economic value. The ISI, which contains data on the area and property of the four types of anthropogenic activity, was calculated as follows:

$$ISI = \sqrt{P_{rd} + A_{rd}} + \sqrt{P_{dc} + A_{dc}} + \sqrt{P_{rc} + A_{rc}} + \sqrt{P_{cr} + A_{cr}} \quad (1)$$

where A is the area of an anthropogenic activity, P is the property of an anthropogenic

activity, *rd* means residential development, *dc* means dock construction, *rc* means raised field construction, and *cr* means cropland reclamation.

Area data were represented by the area proportions of the four types of anthropogenic activity around each pond within a 500 m distance from the pond edges. These data were obtained from digital land-use maps by using a geographical information system (ArcGIS 9.1, ESRI). The property data of the four types of anthropogenic activity were represented by population density, boat number, *P. australis* yield (dry mass of aboveground parts per square meter), and fertilizer use. Data on population density, boat number, and chemical fertilizer use were collected from the *Bureau of Statistics of Anxin County*. The yield of *P. australis*, which was harvested from a raised field in October 2011, was represented as dry mass of aboveground parts per unit area (m²). The aboveground parts of *P. australis* were harvested within a 1 m × 1 m quadrant around the 20 studied ponds, and then the samples were gently hand washed with deionized water and oven dried at 80 °C for about a week in laboratory until reaching constant weight. The total dry mass weight per square meter was calculated by summing.

We selected 500 m as the width of buffer zones for several reasons. Previous studies on the scale dependency of anthropogenic stress on ponds have revealed that anthropogenic activities in marginal zones exert more significant effects on aquatic macrophytes than activities in catchment areas (Declerck et al., 2006; Akasaka et al., 2010). In addition, property data in ISI (e.g., population density and chemical-fertilizer use) cannot be divided into different scales. The average distance between different ponds in the area of study was 500 m; a larger distance would have extended into the scope of neighboring ponds.

The ISI is additive because we did not have sufficient information on the interactions among multiple stressors, and interactions of these activities on the ecosystem (Allan et al., 2013). A square root transformation was applied to produce more even distribution and to reduce the influence of outliers. Variables that did not

fit the assumption of normality were transformed with $\ln(x+1)$ prior to analysis, and then were rescaled from 0 (zero or minimum observed value) to 1 (maximum observed value) using a max-min rescaling equation as follows:

$$X'_i = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}} \quad (2)$$

2.2.2 Macrophyte data

Macrophytes were sampled in late August 2011 when biomass was at its peak. Macrophyte occurrence throughout each pond was surveyed using a cruising boat and a rake. Macrophytes within each pond were sampled at eight sites to roundly represent species composition. At each site, four transections were placed perpendicular to the shoreline and extending a distance of 50 m from the shore. A 0.5 m $\times$ 0.5 m quadrant was placed along each transection at 5 m intervals. Within each quadrant, the species present were recorded to calculate species richness. The aboveground biomass of macrophytes within each quadrant was collected by handcrafting, returned to the laboratory, and dried at 80 °C for 48 h to calculate dry weight. Total biomass was calculated as the sum of dry weight of emergent macrophyte, submerged macrophyte, and floating-leaf macrophyte. The dry weight of emergent macrophytes at pond level was calculated as the sum of average dry weight of all emergent species present on a pond. The dry weight of submerged macrophytes and floating-leaved macrophytes were calculated using the same method. The biomass and species richness of total macrophytes and three macrophyte growth forms were selected as the response variables in subsequent analyses. All biomass data were $\ln$ -transformed prior to analysis.

2.2.3 Trophic state

Several water samples were collected from the whole water column in each pond, and composite samples collected using a corer were preserved at 4 °C in the laboratory until analysis. We selected total phosphorus (TP) and total nitrogen (TN) as the indicators of trophic state for each pond and analyzed them through the cadmium reduction method and molybdate-ascorbic acid method, respectively, after persulfate

digestion in disposable polycarbonate bottles in an autoclave at 120 °C for 45 min (APHA, 1998). Secchi depth (SD) was adopted as an indicator for light availability. Other physicochemical variables such as, conductivity, dissolved oxygen (DO), mean depth (Z), suspended solids (SS), chlorophyll-a (chl a) were also measured.

2.3 Statistical analysis

2.3.1 Sensitivity analyses of ISI calculation methods

Our analysis required various anthropogenic activity data, each of which could influence the results. The robustness of ISI was verified by assessing its sensitivity from the following three aspects.

2.3.1.1 Sensitivity to data standardization approaches

The native units of area and property for different types of anthropogenic activity considerably varied. Thus, data standardization was performed to minimize the influence of extremely high values that could be spurious but influential. Data standardization was achieved using two approaches. One is Ln (x+1) transformation and max–min linear rescaling; the other is max–min linear rescaling without Ln (x+1) transformation. Each approach rescaled the values of each component of ISI to a 0-1 range. If the correlation coefficients between the results of the two approaches were high, then the ISI was deemed independent of the data standardization approaches.

2.3.1.2 Sensitivity to weights

Equal weights were assigned to the different types of anthropogenic activity, but the contribution of each type to the overall patterns of the ISI must still be assessed. Therefore, we tested the sensitivity of ISI to weights using two approaches. First, random weights were assigned to each type of anthropogenic activity to recalculate ISI, and the sum of all the weights was 1 (e.g. 0.25, 0.56, 0.09, 0.1, respectively). Meanwhile, the weights of area and property of each anthropogenic activity type were maintained at 1 and contributed equally to ISI. Then, the correlation coefficients between the original ISI and recalculated ISI were calculated by Pearson correlation analysis. Second, random weights were assigned to the area and property of each

anthropogenic activity type, and the sum of all the weights was 1 (e.g. 0.2 and 0.8 for the area and property of cropland reclamation, respectively; 0.3 and 0.7 for the area and property of dock construction, respectively). Meanwhile, the weights of each type of anthropogenic activity were maintained at 1 and contributed equally to ISI. Then, the correlation coefficients between the original ISI and the ISI with random weights were calculated by Pearson correlation analysis. To test whether or not the results of correlations were small probability events, we randomly assigned the weights 5000 times. Subsequently, the frequency counts of correlation coefficients (Pearson r) between the original ISI and the recalculated ISI were calculated. If the values of Pearson r were generally larger than 0.7, the ISI was deemed independent of the weight of each type of anthropogenic activity.

2.3.1.3 Sensitivity to data removal

To test whether or not ISI was only influenced by certain types of anthropogenic activity, we removed one of the four stressors from the ISI at a time and retained the three other stressors. The correlation coefficients between ISI with the four stressors and ISI with the three stressors were calculated. If the original ISI strongly correlated with the ISI with removals, then ISI was robust even after the removal of any individual stressor.

2.3.2 Effects of integrated stress and nutrient on aquatic macrophytes

To determine the effects of integrated stress and nutrient on aquatic macrophytes in ponds, regression analysis was performed. ISI, TN, and TP were considered the explanatory variables, respectively, and macrophyte data (species richness and biomass) were considered as response variables. Given that macrophyte response to anthropogenic stress and nutrient level often depend upon different growth forms, we analyzed the responses of total macrophytes and three macrophyte growth forms separately. The relationships between ISI and water physicochemical variables of pond water were also analyzed through Pearson correlation analysis.

3 Results

3.1 Characteristics of the 20 studied ponds

Stress from each anthropogenic activity type, biomass proportions, and physiochemical variables largely varied among the 20 ponds (Fig. 2 and Table 1). Species richness ranged from 6 to 23 across these ponds, and the average species richness per pond was 16.75 ± 1.13 (mean $\pm$ s.d.). The most common species found in over 10 ponds were *P. australis*, *Potamogeton pectinatus*, *Ceratophyllum demersum*, *Chara* spp., and *Typha angustifolia*.

[Fig. 2 here.](#)

[Table 1 here.](#)

3.2 Sensitivity analysis

The correlations were high between ISI values calculated by two data standardization approaches (Pearson $r = 0.8376$), indicating that the alteration of the data standardization approaches only resulted in small changes in ISI. Thus, we can conclude that ISI was independent of the data standardization approaches and we eventually adopted $\ln(x+1)$ transformation followed by max-min linear rescaling (Fig. 3).

The ISI with 5000-times randomized weights using two approaches were both highly correlated with the ISI with equal weights (Pearson r : mean = 0.7463, range 0.362 – 0.956), suggesting that weights had only a slight influence on ISI (Fig. 4). The ISI after removal of any stressor was also strongly correlated with the original ISI (mean Pearson r : = 0.9747) (Fig. 5), indicating that no individual stressor had substantial influence on the ISI.

[Fig. 3 here.](#)

[Fig. 4 here.](#)

[Fig. 5 here.](#)

3.3 Effects of integrated stress on water physicochemical variables and aquatic macrophytes

As shown in Table 2, TN was significantly correlated with ISI, whereas the correlation between ISI and TP was weak. Most of the other water quality indicators were weakly correlated with ISI (the Pearson coefficient r ranged within 0.11– 0.23; Table 2), with two exceptions, i.e., strong relationships between SD, SS, and ISI ($r = -0.71$ and 0.64 , respectively; Table 2).

[Table 2 here.](#)

ISI was significantly correlated positively to total biomass and was significantly correlated negatively to total species richness. It explained about 13% and 69% of the variances in total biomass and total species richness, respectively (Figs. 6a and 6b), as well as 50% and 41% of the variances in biomass and species richness of three macrophyte growth forms on average (Figs. 6c and 6d). The species richness of emergent and floating-leaved macrophytes decreased with increased ISI, and the species richness of submerged macrophytes showed a unimodal relation to ISI (Fig. 6d). The biomass of emergent macrophytes was significantly correlated positively to ISI, whereas the biomass of floating-leaved macrophytes was significantly correlated negatively to ISI (Fig. 6c). The relationship between biomass of submerged macrophytes and ISI was unimodal, and a slight increase was observed after an initial decrease (Fig. 6c).

Based on changes in total species richness, we classified the 20 ponds into three categories: slightly disturbed ponds ($ISI < 2.0$), moderately disturbed ponds ($2.0 < ISI < 3.9$), and highly disturbed ponds ($ISI > 3.9$). The species richness in the three categories of ponds ranged from 17 to 22 (mean $\pm$ s.d.: 20 ± 2.16 species; median values: 20.5 species), 11 to 23 (mean $\pm$ s.d.: 17.15 ± 3.67 species; median values: 17 species), 6 to 9 (mean $\pm$ s.d.: 7.33 ± 1.53 species; median values: 7 species), respectively. The highly disturbed ponds were often inhabited by *Phragmites australis*, *Potamogeton maackianum*, and *Chara* spp., and the moderately disturbed ponds were dominated by *Myriophyllum spicatum*, *Ceratophyllum submersum*, and *Potamogeton* spp., whereas the most common species in the slightly disturbed ponds were

Vallisneria. spiralis, *Utricularia vulgaris*, and *Chara* spp.

[Fig. 6 here.](#)

3.4 Effects of nutrient on aquatic macrophytes

The relationships among species richness, biomass, and TP concentration are shown in [Fig. 7](#). A significant linear relationship was found between total biomass and TP ([Fig. 7a](#)), whereas total species richness was insignificantly correlated with TP ([Fig. 7b](#)). Significantly binomial and unimodal relationships were found between the biomass and richness of submerged macrophytes and TP, respectively, whereas the biomass and species richness of floating-leaved macrophytes and emergent macrophytes were insignificantly correlated with TP ([Figs. 7c and 7d](#)).

[Fig. 8](#) shows that the correlations between total biomass and TN were insignificant ([Fig. 8a](#)), whereas total species richness was significantly correlated negatively with TN ([Fig. 8b](#)). Both biomass of emergent macrophytes and floating-leaved macrophytes were significantly correlated linearly with TN, whereas the fitted curve between biomass of submerged macrophytes and TN was binomial and had relatively weaker significance ([Fig. 8c](#)). Both species richness of emergent macrophytes and floating-leaved macrophytes were significantly correlated linearly with TN, whereas the relationship between species richness of submerged macrophyte and TN was insignificant ([Fig. 8d](#)).

[Fig. 7 here.](#)

[Fig. 8 here.](#)

4 Discussion

4.1 Effects of integrated stress on water physicochemical variables and aquatic macrophytes

This study primarily aims to develop an index (ISI) that can represent the integrated stress of different types of anthropogenic activities, as well as to investigate the effects of integrated stress on aquatic macrophytes. Sensitivity analysis proved that data standardization approaches, changes in random weights, and data removal

cause only slight changes on ISI. These results confirmed that our index is robust and can represent the integrated stress of all the anthropogenic activities studied.

Many studies found strong correlations between anthropogenic activities (e.g., agricultural activity, shoreline development, and urban land use) and nutrient concentrations in shallow lakes or ponds ([Akasaka et al., 2010](#); [Sass et al., 2010](#); [Moss et al., 2013](#)). However, in the present study, the correlations between nutrient concentrations and ISI were not as strong as those of previous reports, particularly for the correlation between TP and ISI. This phenomenon may be explained by the seasonal variations of nutrient concentrations, which were associated with farming periods. [Zhao et al. \(2011\)](#) found that the monthly averaged TN concentrations in Lake Baiyangdian varied from 2.56 mg/L to 5.6 mg/L, and the monthly averaged TP concentrations varied from 0.08 mg/L to 0.61 mg/L. The increased nutrient input mainly occurred in March during which farming and fertilization activities were intensified ([Li et al., 2012](#)). Our field sampling was conducted during late August; thus, the relatively decreased nutrient input may weaken the correlations between ISI and nutrient concentrations.

The weaker correlation between TP and ISI than that between TN and ISI may be attributed to the nutrient sources. [Moss et al. \(2005\)](#) found that TP concentrations remained high in shallow lakes recovering from heavy fertilization, indicating that TP concentration was not only determined by sewage effluent and agricultural runoff but was also affected by the sediment release as temperature increases during summer. The TN concentration in shallow lakes, where nutrient sources are diffused, was more closely related to fertilizer activities and sewage effluent than TP ([Kronvang et al., 2005](#); [Moss et al., 2013](#)).

The stronger correlations among SD, SS, and ISI than those among other water physicochemical variables and ISI can be explained as follows. [Pennings et al. \(2008\)](#) reported that poor light availability may not only be ascribed to eutrophication but also to increased suspended solids, which mainly resulted from sediment resuspension

induced by wave action and boating activity (Anthony and Downing, 2003; James et al., 2004), as well as bank soil erosion caused by tourist and recreational activities (Loiselle et al., 2002; Kolada, 2010). In the present study, the areas of the dock for tourism/fishing and the number of boats were included in the developed ISI. Therefore, the increases of suspended solids in water with intensive anthropogenic activities may contribute much to the strong correlations among SD, SS, and ISI.

Both significantly linear and quadratic fitted relationships were found between integrated stress and aquatic macrophytes. Changes in species and biomass can be driven by trophic state, light availability, and physical destruction; all these effects may be altered by integrated anthropogenic stress. Thus, the integrated anthropogenic stress may be considered important in determining macrophyte biodiversity. Our major findings showed different responses among three macrophyte growth forms with increasing ISI; these changes in response may also directly lead to alterations in total biomass and total species richness. The relationships between ISI and three macrophyte growth forms can be explained as follows.

A unimodal relationship was observed between the richness of submerged macrophytes and ISI. The richness of submerged macrophytes showed a positive increase when ISI was in the range of $0.22 < \text{ISI} < 2.63$ but decreased when ISI exceeded 2.63. This result suggests that moderately disturbed ponds support more submerged species than slightly and highly disturbed ones, and this phenomenon is directly caused by changes in dominant submerged species along the stress gradient. The dominant submerged species in slightly disturbed ponds were *Utricularia vulgaris*, *Vallisneria spiralis*, and *Chara* spp. Among of them, *U. vulgaris* and *V. spiralis* often present fragile root systems; hence, they are easily affected by increasing anthropogenic stress (del Pozo et al., 2010); moreover, *Chara* spp. are generally regarded as clear water indicators and often constrained by light attenuation caused by increasing anthropogenic stress (Kimber et al., 1995; Alexander et al., 2008). These characteristics result in the disappearance of the above species in

moderately disturbed ponds.

Compared with the species described above, the dominant submerged species in moderately disturbed ponds mainly include some tolerant species, such as *Potamogeton perfoliatus*, *P. malaianus*, *P. lucens*, *P. pectinatus*, *Myriophyllum spicatum*, and *Ceratophyllum demersum*. The specific adaptation strategies of these species allow them to withstand anthropogenic stress. For instance, *P. pectinatus* can elongate its shoots and form a canopy in the upper water layer, whereas *C. demersum* is often characterized as a rootless species living just below the water surface (Alexander et al., 2008; Pennings et al., 2008a; del Pozo et al., 2010). Thus, these species are not restricted by poor light availability caused by anthropogenic stress, and the presence of these species contributed largely to the increased species richness of submerged macrophytes.

The dominant submerged species in highly disturbed ponds were only *Chara* spp. and *Potamogeton maackianum*. Re-colonization of *Chara* as ISI increases ($ISI > 3.9$) contradicts several studies showing *Chara* spp. as one of the first submerged species to disappear in a degradation aquatic ecosystem (van den Berg et al., 2003; Coops et al., 2007). This phenomenon may be attributed to two reasons. First, our results showed that water transparency was negatively correlated with ISI; thus, colonization and growth of *Chara* seemed to be impossible in highly disturbed ponds ($ISI > 3.9$) with poor light condition. However, the colonization of submerged macrophytes depend largely upon the water depth but not on light intensity (Håkanson and Boulion, 2002; Wang et al., 2005). In the case of very shallow lakes, such as the Dutch border lakes and Polish lowland lakes, water depth, instead of other factors, would determine the occurrence of submerged macrophytes (Coops et al., 2007; Pennings et al., 2008; Kolada, 2010). The average water depth in highly disturbed ponds was only 0.75 ± 0.11 m, and the average transparency was 0.48 ± 0.13 m; hence, the ratio of transparency and water depth was approximately 0.64. Van den Berg et al. (2003) reported that the critical value of transparency for *Chara* colonization was around 0.4

m in shallow lakes (1 m to 2 m depth). Therefore, we can infer that light may reach the middle and lower layers of water column in highly disturbed ponds and then promote colonization of *Chara*. spp. Second, different *Chara* species have different environmental requirements. More than 70 *Chara* species were found in China, indicating that the ecological amplitude of *Chara*. spp is wide, and some *Chara* species can withstand poor light condition through elongation of internode and stem (Wang et al., 2014).

Changes in dominant submerged species in ponds with increasing stress also result in a quadratic relationship between ISI and biomass of submerged macrophytes . The results demonstrated that biomass of submerged macrophytes significantly decreases within a certain threshold limit of $0.22 < \text{ISI} < 2.63$ and presents a positive trend when ISI exceeds 2.63. An initial decrease in biomass of submerged macrophytes with increasing ISI is attributed to the replacement of *Chara* spp. by *Potamogeton* spp., as the latter often accumulates less biomass than the former because of its canopy-like growth form (Bakker et al., 2010). Furthermore, *Potamogeton* spp., *M. spicatum*, and *C. demersum* which dominated in moderately disturbed ponds often concentrate biomass in the upper water layer, where they are easily disturbed by anthropogenic activities, resulting in decreased biomass. As the ISI range increases from 2.0–3.9 to 3.9–5.3, re-colonization of *Chara* spp. in highly disturbed ponds forms a dense cover accumulating a relatively larger biomass than that achieved by canopy-like growth form and rootless species; this development contributes to a slight increase in biomass of submerged macrophytes after its initial decrease.

The biomass of emergent macrophytes was significantly positively related to ISI, whereas species richness of emergent macrophytes was significantly negatively related to ISI; the fitted curves of both variables showed linear features. Increases in biomass of emergent macrophytes along the ISI gradient may be fundamentally attributed to the increasing dominance of *P. australis*, particularly in highly disturbed

ponds. However, some studies reported that *P. australis* biomass decreases with increasing anthropogenic stress because this species often colonizes in littoral zones; hence, *P. australis* is easily damaged by physical destruction, such as direct removal, trampling by cattle, and grazing pressure (del Pozo et al., 2011; Hicks and Frost, 2011). The most common physical destruction in the area of study is mechanical harvesting for *P. australis*, which is mainly conducted on raised fields rather than in ponds. Thus, limited physical destruction cannot decrease the biomass of *P. australis*. Shallow water depths in the 20 ponds (mean $\pm$ s.d. = 1.21 ± 0.35 m) and plastic modification of *P. australis* allow this species to proliferate all over the ponds and out-compete other species for abiotic factors (e.g., light and nutrients), which contribute to the decreased richness of the three macrophyte growth forms (Karunaratne et al., 2003; Deegan et al., 2007; Mäemets et al., 2010). Considering that most of the highly disturbed ponds were eutrophic in nature (TP concentration > 0.035 mg/L) and with respect to the great capacity of *P. australis* for absorbing nutrients in its tissues (Karunaratne et al., 2004), this species often accumulates in large biomass and contribute largely to organic matter accumulation in pond sediments because of their slow decomposition rate (Ágoston-Szabó et al., 2006; Lan et al., 2012; Li et al., 2013). Our survey results indicated that the average dry mass of *P. australis* was $1072 \text{ g}\cdot\text{m}^{-2}$, and 48% detritus mass that still remained after 1 year of decomposition (Li et al., 2013), from which we can estimate that up to $515 \text{ g}\cdot\text{m}^{-2}$ detritus remained in the sediment. At present, harvesting for *P. australis* is mainly conducted on raised fields rather than in ponds. Therefore, we recommend that the coverage of harvesting should also involve *P. australis* distributed in highly disturbed ponds.

The biomass and species richness of floating-leaved macrophytes were relatively lower than those of emergent and submerged macrophytes. The floating-leaved species characterized as flexible and narrow leaves, such as *Brasenia schreberi*, *Nymphaea stellata*, and *Trapa maximowiczii*, often offer weak resistance against wave erosion; hence, they are easily disturbed by breaking and mechanical damage owing

to anthropogenic activities, thereby decreasing biomass and species richness (Radmoski and Goeman, 2001; Madsen et al., 2001; Alexander et al., 2008). Only floating-leaved species with thick, wide, and stiff leaves, such as *Nymphoides peltatum* and *Trapa bispinosa*, offer strong resistance to disturbance and thrive in highly disturbed ponds.

Although the species richness of submerged macrophytes benefits from moderate anthropogenic stress, decreases in total species richness and increases in total biomass along the stress gradient indicate the obvious simplification of species composition and the excessive growth of emergent macrophytes. Rasmussen and Anderson (2005) indicated that agricultural and urban land use practices around a lake cause shifts in the dominance of submerged macrophytes toward the dominance of emergent macrophytes and even the complete collapse of the macrophyte community. Changes in aquatic macrophytes in the present study showed similar trends along the gradient of integrated stress.

4.2 Effects of nutrient on aquatic macrophytes

Compared with integrated stress, TP only produced significant effects on total biomass, as well as species richness and biomass of submerged macrophytes; this parameter was not significantly related to species richness and biomass of the emergent and floating-leaved macrophytes. A significant but weak unimodal relationship between TP concentration and species richness of submerged macrophytes was observed in this study, and more submerged species were found in meso-eutrophic ponds (0.0462 – 0.1393 mg/L) than in other pond types. Our findings are sound with the study of Pennings et al. (2008b), in which a unimodal relationship was observed between species richness of submerged macrophytes and TP concentration in 1147 lakes from 12 European states. In contrast with this finding, Jeppesen et al. (2000) and Kosten et al. (2009) found a monotonic negative relationship between submerged richness and TP concentration. These differences may be explained by the absence or reduced number of oligotrophic lakes that may

yield monotonic rather than unimodal relationships between TP concentration and submerged richness.

A negative relationship between biomass of submerged macrophytes and TP concentration was observed, and the biomass in ponds with lower TP concentrations (0.02 mg/L to 0.08 mg/L) was larger than that in ponds with higher TP concentrations (>0.8 mg/L). This finding indicates that although sufficient nutrients can promote primary production of the submerged species, higher TP concentrations in water may also induce excessive growth of phytoplankton algae, which can decrease light availability through shading effects and inhibit photosynthesis of the submerged macrophytes (del Pozo et al., 2010; Kolada, 2010). TP concentration did not show a significant relationship with emergent macrophytes because these species can extend their leaves or culms above the water surface; hence, they are not restricted by decreased light availability with increasing TP concentration (del Pozo et al., 2011). Although no significant relationship existed between TP concentration and floating-leaved macrophytes, we found that the dominant floating-leaved macrophytes changed from clear water indicators (e.g., *Euryale ferox* and *Nymphaea stellata*) to eutrophic water indicators (e.g., *Lemna minor* and *Salvinia natans*), likely because of increased nutrient levels (Egertson et al., 2004; Alexander et al., 2008).

TN produced more significant effects on species richness and biomass of aquatic macrophytes than TP. Nitrogen limitation owing to denitrification in anaerobic sediments in shallow lakes may act as an important cause for this phenomenon (Moss et al., 2013). Furthermore, rooted aquatic macrophytes may acquire P from the sediments; thus, the effects of TN on biomass and species richness of aquatic macrophytes were more significant. TN can affect aquatic macrophytes through various ways. For instance, high TN concentration can decrease light availability and inhibit the production of submerged macrophytes by shading effects from growth of blue-green algae and periphyton (Romo et al., 2007; del Pozo et al., 2010). Moreover, high ammonium concentrations can adversely affect the growth and diversity of

emergent macrophytes at low light availabilities (Clarke and Baldwin, 2002). The species richness of submerged macrophytes was not significantly correlated with TN, probably because TN in summer may be a poor surrogate for N loading and influence on macrophyte communities, and other factors including grazing of fish (Hilt et al., 2006), water movement (Madsen, 2001), and especially light availabilities may affect the presence of submerged species (Kolada, 2010). Thus, nitrogen is also of great importance for diversity of aquatic macrophytes in pond, and there may be an argument for N control by relieving anthropogenic activities.

The lower significance level and less value of adjusted R^2 show that nutrient concentration is ascribed to less variance of species richness and biomass than that of the ISI, although eutrophication is considered as the main stress to be treated in the area of study. Thus, species losses of aquatic macrophytes should be attributed not only to eutrophication, as has been considered in the past decade. Focusing solely on eutrophication may also cause another problem: restoration strategies aimed at relieving anthropogenic stress will mainly concentrate on the prohibition of certain anthropogenic activity type that is closely related to nutrient input (i.e., cropland reclamation) and ignore other anthropogenic activity types. Sensitivity analysis proves that all anthropogenic activities are equally important and aquatic macrophytes may be affected by the integrated stress. Therefore, restoration strategies aimed only at relieving one or a few stressors could fail to restore macrophyte richness because of negative effects from other stressors, and all anthropogenic activities around a pond need to be evaluated.

4.3 Implications for pond restoration

The results showed that the index we developed is robust to represent the integrated stress of various anthropogenic activities affecting ponds. We hope that the use of this index in the following aspects will be useful for pond restoration.

First, pond categorization based on ISI values and changes in dominant species along the ISI gradient suggested different restoration strategies for the three categories

of ponds. According to the *Planning for Lake Baiyangdian Natural Reserve of Hebei Province (2010-2030)* and the *Special Planning for Wetland Conservation and Restoration of Anxin County (2010-2030)*, only a simple regional division for regulating anthropogenic activity was proposed as follows: a core zone where natural ecosystems should not be disturbed unless absolutely necessary, a buffer zone where development of organic agriculture and tourism are permitted, and an experimental zone where scientific research can be conducted. However, anthropogenic stress around each pond has not been quantified, and the classified restoration strategies for different categories of ponds have not been formulated. On the basis of the negative relationship between ISI and species richness, we suggested that the anthropogenic activities around highly ($ISI > 3.9$) and moderately disturbed ponds ($2.0 < ISI < 3.9$) should be prohibited immediately. Changes in dominant species along the ISI gradient indicated that proper hydrology design may be adopted as an additional ecological engineering approach to optimize species composition in highly and moderately disturbed ponds, which have been proven effective in wetlands creation and restoration through self-design (Mitsch et al., 1998; Mitsch and Jørgensen, 2003). In highly disturbed ponds, the excessive growth of *P. australis* was the main problem to be solved, and the lower water level ($\text{mean} \pm \text{S.D.} = 0.75 \pm 0.11 \text{ m}$) may be considered an important cause to this phenomenon. Therefore, increasing the water level through water replenishment may be adopted to inhibit germination and proliferation of *P. australis* (Karunaratne et al., 2004; Brix et al., 2014). In moderately disturbed ponds, the dominant species such as *Potamogeton* spp., *C. demersum*, and *M. spicatum* were proven to play less important roles in preventing phytoplankton growth and reducing sediment resuspension (Hilt et al., 2006). Hence, the re-establishment of low-growing species, such as *Chara* spp. and *V. spiralis*, which contribute largely to stabilize sediment and improve water quality, should be considered (Gulati et al., 2008). Decreasing the water level during spring may be adopted as a main approach to re-establish low-growing species, by which light

availability can be improved, and seed germination of the low-growing species can be promoted (Crisman et al., 2005; Zhang et al., 2014). The amplitude for decreasing the water level should be determined by the light requirements of the low-growing species, and the transparency level in slightly disturbed ponds may be viewed as a reference. In slightly disturbed ponds, we recommend anthropogenic stress should be mitigated gradually, and the conservation ethic should be shared by local managers and farmers by recognizing the pond ecosystem values as important aquatic habitat and water resource (Mitsch and Jørgensen, 2003).

Second, the improvement of pond connectivity may be considered as another restoration strategy to offset the anthropogenic stress represented by ISI. The hydrologically open system is an important design consideration for wetland restoration because wetlands that are open enough can receive continual input of propagules through floods and inflow of pumped river water (Mitsch, 1998). However, most of the studied ponds were weakly connected or even isolated from each other, especially in the dry season. To date, both connectivity and quantity of ponds have significantly decreased compared with those in the 1990s, as accompanied by substantial species losses of aquatic macrophytes with intensive anthropogenic stress and decreasing water input. This phenomenon suggests that connectivity among the ponds can affect biodiversity through exchange of propagules and spread of species (Demars and Harper, 2005; Akasaka et al., 2010). Therefore, ecological engineering approaches aiming to improve pond connectivity should be implemented by local managers. For instance, water channels among ponds may be reconnected. Such channels were proven to play a positive role in diversity and water quality among wetland networks (Zhang et al., 2011). Returning anthropogenic land use alterations (e.g., cropland and residence around ponds) to ponds can also be implemented to improve connectivity through increase of pond density (Moilanen and Nieminen, 2002), which was obviously regarded as another main factor for the species richness of aquatic macrophytes (Gledhill et al., 2007).

In addition to the abovementioned points, some aspects need to be further investigated about this index (ISI). Specifically, long-term monitoring of anthropogenic stress within this region should be conducted to obtain an exact changing trend, which may be helpful to analyze the changes in aquatic macrophytes responding to anthropogenic stress over time. The contribution of pond connectivity to offset anthropogenic stress and maintain biological diversity is of great interest for pond restoration. However, the relationship between connectivity and aquatic macrophyte diversity and the effects of related measures to improve connectivity should be further evaluated.

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FIGURES

Fig.1. Study area within Anxin County, Baoding City, China (a), and location of the sampled ponds (b).

Fig.2. Boxplots of stress from each anthropogenic activity type (a), and proportions of biomass for three macrophyte growth forms (b). Box is the interquartile range (25% and 75% percentiles), the vertical endpoints are not longer than 1.5 times the interquartile range, and the horizontal line within the box is the median.

Fig.3. Pearson's correlation analysis between two ISI values with different data standardization approaches.

Fig.4. Pearson's correlation analysis between value of original ISI with equal weights and value of recalculated ISI with random weights. (a) Random weights were given to each anthropogenic activity type and all the random weights summed to 1.0. (b) Histogram of Pearson's correlation coefficients from 5000 times randomizations of the weights for each anthropogenic activity type. (c) Random weights were given to area and property of each anthropogenic activity type and the two random weights were summed to 1.0. (d) Histogram of Pearson's correlation coefficients from 5000 times randomizations of the weights for area and property of each anthropogenic activity type.

Fig.5. Pearson's correlation analysis between value of original ISI and value of recalculated ISI after remove individual stressors. (a) removing residential development; (b) removing dock construction; (c) removing cropland reclamation; (d) removing raised field construction.

Fig.6. Relationships between ISI and (a) total biomass; (b) total species richness; (c) biomass of the three macrophyte growth forms; (d) species richness of the three macrophyte growth forms .

Fig.7. Relationships between TP concentration and (a) total biomass; (b) total species richness; (c) biomass of the three macrophyte growth forms ; (d) species richness of the three macrophyte growth forms .

Fig.8. Relationships between TN concentration and (a) total biomass; (b) total species richness; (c) biomass of the three macrophyte growth forms ; (d) species richness of the three macrophyte growth forms.

TABLES

Table.1 Mean, standard deviation (s.d.) and range for variables characterizing the pond physicochemical status.

Variable	Mean±SD	Range
Surface area (hectares)	65±4.6	23-172
Mean depth (m)	1.21±0.35	0.72-1.53
pH	8.24±0.18	7.38-8.39
Dissolved oxygen(mg/L)	3.39±1.03	3.21-12.6
Conductivity (us/cm)	480±231	45-1187
Total nitrogen (mg/L)	5.78±7.78	0.96-21.7
Total phosphorus (mg/L)	0.082±0.086	0.0183-0.3726
Secchi depth (m)	0.76±0.15	0.37-1.46
Chlorophyll a (ug/L)	35 ± 58	4.21-169.3
Suspended solids (mg/L)	17.4 ± 10.5	5.7-135

Table 2 Pearson r between ISI and water physicochemical variables

pH	DO	Con	TN	TP	SD	Chl-a	SS
0.11	0.14	0.27	0.65	0.22	-0.71	0.23	0.64

The correlation r values above 0.6 are shown in bold type.