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Utilization of nitrate stable isotopes of *Chydorus sphaericus* (OF Müller) to elucidate the hydrological characteristics of riverine wetlands in the Nakdong River, South Korea

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Abstract

Background: This study aimed to identify NO_3^--N sources using the stable isotope $\delta^{15}N$ in *Chydorus sphaericus* (OF Müller), to investigate hydrological characteristics and nutrient states in artificial wetlands near the Nakdong River. *Chydorus sphaericus* is dominant in wetlands where aquatic plants are abundant, occurring in high density, and is sensitive to wetland water pollution, making it suitable for identification of NO_3^--N sources.

Results: NO_3^--N sources for each wetland were strongly dependent on hydrological characteristics. Wetlands with sewage or rainfall/groundwater as their main sources had high levels of NO_3^--N, whereas wetlands with surface water as their main input had comparatively lower levels. Since wetlands with sewage and rainfall/groundwater as their main water sources were mostly detention ponds, their inputs from tributaries or the main river stream were limited and nutrients such as NO_3^--N easily become concentrated. Changes in NO_3^--N levels at each wetland were closely associated with $\delta^{15}N$ of *C. sphaericus*. Interestingly, regression analysis also showed positive correlation between $\delta^{15}N$ of *C. sphaericus* and NO_3^--N level.

Conclusions: We conclude that the nitrate stable isotope ($\delta^{15}N$) of *C. sphaericus* can be used to elucidate the hydrological characteristics of riverine wetlands. This information is important for maintenance and conservation of artificial wetlands at the Nakdong River.

Keywords: Hydrological characteristics, Indicator species, Stable isotope analysis, Water pollution, Four Rivers Project, Nitrate contamination

Background

Nitrate contamination is a pervasive problem in various areas associated with agricultural fields, including the South Korean riverine wetlands (Kellman and Hillaire-Marcel 2003; Kim et al. 2015). Such contamination is especially concerning in tile-drained areas since large amounts of NO_3^--N in water can be discharged rapidly into wetlands (Kovacic et al. 2000; Green and Galatowitsch 2001, 2002). Agricultural use of both inorganic N

fertilizers and various animal waste fertilizers complicates the evaluation of various N fertilizer application types. Riverine wetlands and surrounding areas of inflow streams in South Korea consist mostly of agricultural lands and orchards from which there is an inflow of a diverse array of potential contaminants. In areas with multiple potential sources of contamination, measurement of their relative contributions may help to clarify the contamination processes.

Empirical studies suggest that stable nitrate isotopes can be used to discriminate between the sources of NO_3^--N in field settings because NO_3^--N compounds from different sources have characteristic isotopic ratios

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(Kellman and Hillaire-Marcel 2003; Lee et al. 2008). During the mineralization of dissolved organic N, nitrate stable isotopes are the same as those of the original organic N in the + 4 to + 9‰ range (Heaton 1986). The distinct differences in the nitrate stable isotopes means that NO_3^--N in waters originating from a combination of sources should be represented within the range of these samples if NO_3^--N behaves in a conservative manner during transport (Heaton 1986). This understanding has been the basis for the identification of NO_3^--N sources using natural abundances of $\delta^{15}N$ during studies on nitrate contamination (Wells and Krothe 1989). Normally, the stable isotopic value of NO_3^--N can be obtained from oxidizable carbon sources such as dissolved organic carbon (DOC). However, it is necessary to filter a large volume of water to prepare a sample for nitrate stable isotope measurement from DOC, and because wetlands have strong habitat heterogeneity as a standing environment, water must be collected at multiple points to achieve a representative measurement. Therefore, it is difficult not only to measure the nitrate stable isotopes that represent wetlands, but also to trace the changes in nitrogen sources.

Chydorus sphaericus (OF Müller) has a wide distribution range (Illyová and Némethová 2005), is highly sensitive to environmental changes such as water pollution, and continuously uses phytoplankton as a food source; therefore, the nitrate stable isotopes of this species are suitable for the identification of NO_3^--N sources in wetlands. *Chydorus sphaericus* is often attributed to littoral areas where macrophytes are abundant (Ali et al. 2007; Choi et al. 2015), therefore, this species is probably better adapted to detrital food sources compared to large-bodied pelagic cladocerans (e.g., representatives of the *Bosmina* genus; Vijverberg and Boersma 1997). *Chydorus sphaericus* is very tolerant, not only to chemical changes such as dissolved oxygen, pH, and water temperature, but also to trophic level changes (Rybak and Błędzki 2010). Tavernini (2008) stated that a small-bodied zooplankton, such as *C. sphaericus*, is influenced by the level of eutrophication. Therefore, *C. sphaericus* is often considered to be an indicator species for the eutrophication of lakes in temperate climates (Kattel and Sirocko 2011).

Small water bodies can have different origins, which may reflect the age of the wetland or the characteristics of the bottom sediments, which in turn may directly influence a variety of physical and chemical features of the water and indirectly influence the in vivo formation (e.g., assimilation material) for the inhabiting organisms. Moreover, the characteristics of the catchment area of wetlands (e.g., anthropogenically modified, inflow of pollutants from tributaries) may also be of great importance in influencing the water quality and consequently the

structure of the inhabiting plant and animal communities (Wantzen and Junk 2000).

This study aimed to compare the nitrate stable isotopes of *C. sphaericus* among different types of riverine wetlands, representing different inflow channels of nitrogen source. *Chydorus sphaericus* has been hypothesized to be a very good indicator species, not only for trophic conditions and changes in pollutants in the aquatic environment, but also to reflect other environmental features such as the level of habitat heterogeneity, different types of catchment areas of a water body, and the various morphometric features of a wetland. We predicted that different concentrations of NO_3^--N in each wetland would be clearly related to $\delta^{15}N$ in *C. sphaericus*.

Results and discussion
Influence of chemical factors on hydrological characteristics
The forms and hydrological characteristics of artificial wetlands along the Nakdong River were diverse without being one-sided (Fig. 1; Table 1). Channel type formations, in the form of small streams, occurred most frequently. In the upper and lower parts of these wetlands, inlets and outlets supported efficient water conveyance due to their connection with the main river and tributaries, with riparian connectivity evaluated as being "good". Detention ponds, with water supplied by sewage, rainfall, and groundwater, rather than by surface water, often showed poor riparian connectivity. Riparian type wetlands located in the channel of the main river were supplied mainly by the river itself, with excellent riparian connectivity, but evaluated as "good" in this study. The soil type and topography of most study sites were found to be regosol and floodplain, respectively, with similarities due to their construction and subsequent flooding influence. Exceptions included wetlands 2 and 12, which represented a water channel (found within an existing stream) and an island, respectively.

The chemical factor largely depended on the hydrological characteristics of each wetland (Fig. 2). The highest levels of NO_3^--N were found in wetlands fed by sewage sources, with similar results found across spring and autumn. The sewage inflows occur from agricultural land and residential areas rather than from tributaries or the main river. Previous studies have suggested that wetlands or streams influenced by agricultural land or animal farming are prone to eutrophication or water pollution (Euliss and Mushet 1999; Woli et al. 2002). Wetlands with rainfall/groundwater sources had similarly high NO_3^--N levels. These wetlands generally had poor inlet and outlet functions and low water conveyance efficiency, which makes them highly prone to NO_3^--N enrichment. Meanwhile, wetlands supplied by
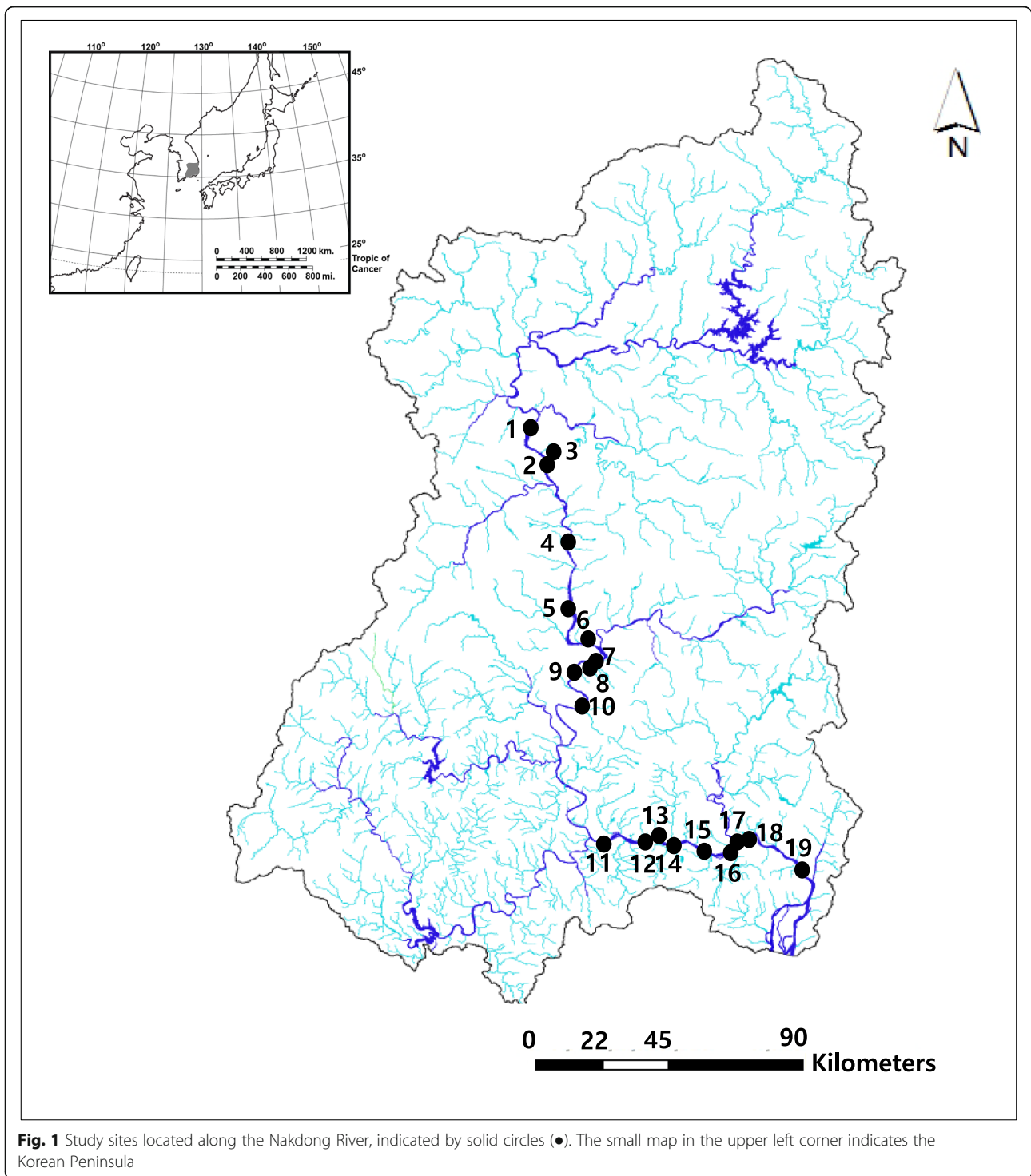
Fig. 1 Study sites located along the Nakdong River, indicated by solid circles (●). The small map in the upper left corner indicates the Korean Peninsula

surface water had lower NO_3^--N, due to greater flow efficiency precluding its local concentration.

Riparian connectivity categorizations, i.e., as good, moderate, or poor, showed similar characteristics. Wetlands with poor riparian connectivity mostly consisted of detention ponds with little input from tributaries or the main river. As a result, nutrients such as NO_3^--N may become enriched to high concentrations. In contrast, wetlands with excellent riparian connectivity, such as channels and riparian types, have robust interactions with the main river or tributaries, and thus minimal NO_3^--N enrichment. However, some channel type wetlands were assessed to have moderate riparian connectivity but had high NO_3^--N levels. These wetlands formerly had good inlet and outlet

Table 1 Local-scale hydrological characteristics in each studied wetland in the Nakdong River basin

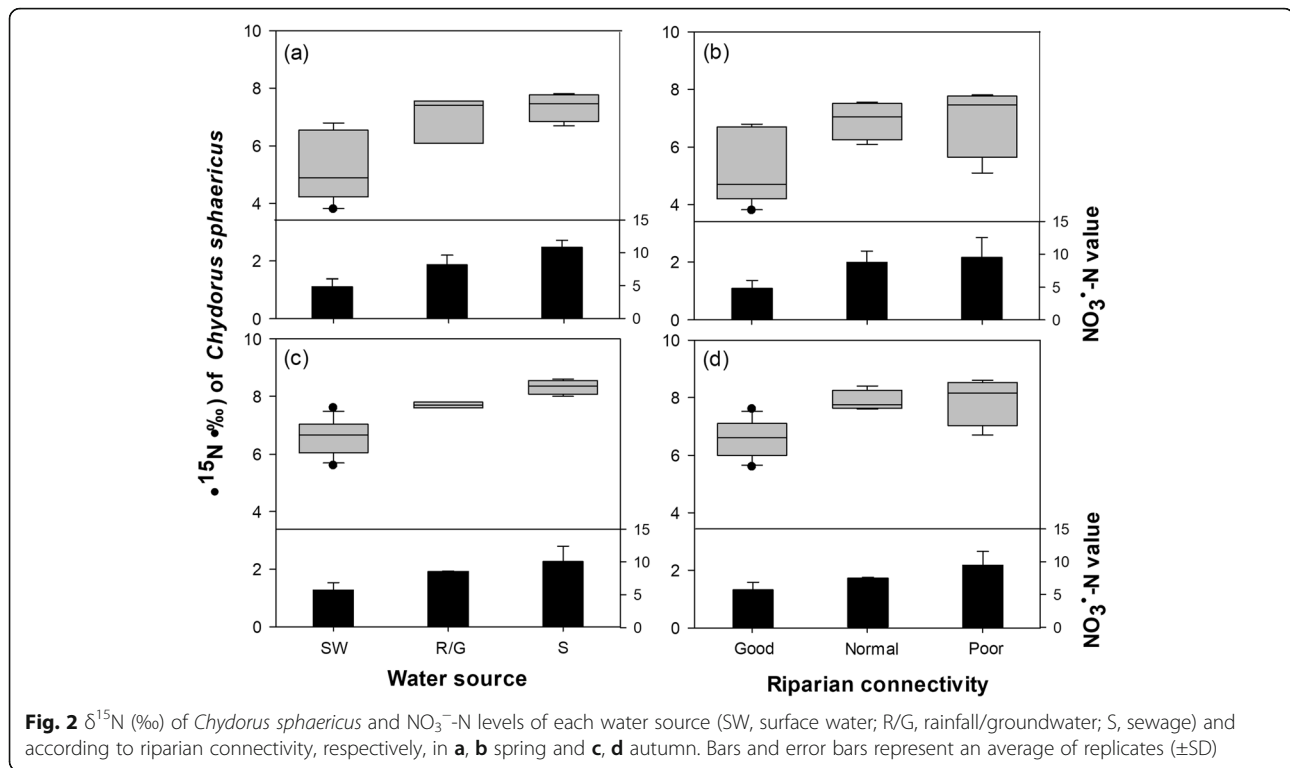| Num.	Type	Water source	Riparian connectivity	Soil type	Topography	Edge index	Area (m^2)	NO$_3^-$-N (spring)	NO$_3^-$-N (autumn)	Chydorus sphaericus (spring)	Chydorus sphaericus (autumn)
1	Channel	Surface water	Good	Regosol	Flood plain	0.035	44,083	4.8	5.2	4.7	6.6
2	Detention pond	Sewage	Poor	Regosol	Water channel	0.018	8347	10.6	8.3	6.7	8.4
3	Riparian	Surface water	Good	Regosol	Flood plain	0.025	87,000	3.2	4.5	4.2	5.9
4	Detention pond	Rainfall/groundwater	Poor	Regosol	Flood plain	0.041	22,590	7.0	8.5	6.1	7.7
5	Channel	Surface water	Good	Regosol	Flood plain	0.036	10,400	5.4	6.4	6.1	7.1
6	Detention pond	Rainfall/groundwater	Poor	Regosol	Flood plain	0.029	4630	7.6	8.6	7.4	7.8
7	Riparian	Surface water	Good	Regosol	Flood plain	0.015	15,000	3.3	4.7	3.9	5.6
8	Riparian	Surface water	Good	Regosol	Flood plain	0.018	24,168	4.1	5.0	4.3	6.2
9	Channel	Sewage	Moderate	Regosol	Flood plain	0.027	38,628	11.8	12.5	7.6	8.3
10	Riparian	Surface water	Good	Regosol	Flood plain	0.019	75,000	3.8	4.3	3.8	6.0
11	Channel	Surface water	Good	Regosol	Flood plain	0.035	390,000	5.2	5.9	5.1	6.7
12	Detention pond	Sewage	Moderate	Regosol	Island	0.011	60,456	9.5	7.8	7.3	8.0
13	Channel	Surface water	Good	Regosol	Flood plain	0.029	426,551	5.8	6.2	6.8	7.2
14	Channel	Surface water	Good	Regosol	Flood plain	0.064	17,500	6.4	7.3	6.7	6.8
15	Channel	Surface water	Moderate	Regosol	Flood plain	0.021	122,000	5.2	5.9	5.1	6.7
16	Detention pond	Rainfall/groundwater	Poor	Regosol	Flood plain	0.068	33,700	9.8	8.5	7.6	7.6
17	Channel	Surface water	Good	Regosol	Flood plain	0.018	147,400	6.8	7.9	6.8	7.6
18	Channel	Sewage	Moderate	Regosol	Flood plain	0.021	68,579	11.5	11.6	7.8	8.6
19	Riparian	Surface water	Good	Regosol	Flood plain	0.031	34,024	4.1	5	4.3	6.2

functions and interaction with the main river at the time of construction, but these functions had diminished due to the sedimentation of soil and plant remains. In the future, these wetlands are likely to develop into detention ponds whose water source is supplied by rainfall/groundwater rather than by the main river. Therefore, active management is required to maintain the function of wetlands corresponding to their construction purpose.

Relationship between δ^{15}N (‰) of *Chydorus sphaericus* and NO$_3^-$-N

NO$_3^-$-N levels measured in each wetland were closely related to δ^{15}N in *C. sphaericus* (Figs. 2 and 3). Differing levels of NO$_3^-$-N according to the water source and riparian connectivity also showed a similar pattern with δ^{15}N in *C. sphaericus*. This signified that the NO$_3^-$-N level of each wetland could be indicated by *C. sphaericus*. In general, the δ^{15}N ratio allows the discrimination of trophic levels and can provide information about the processing of nitrogen and its sources (Peterson and Fry 1987). In freshwater ecosystems, biological communities accumulate pollutants relatively easily via the food web and show a nitrate stable isotope ratio that is highly correlated with pollutants, such as organohalogen compounds (Kidd et al. 1998; Power et al. 2002). Swanson et al. (2003) suggested that the higher the trophic levels of the food chain, the higher the bodily concentrations of nitrate-

related pollutants. Anthropogenic pollutants generally have heavy nitrate stable isotopes compared to organic matter that is produced naturally (Lake et al. 2001), and the δ^{15}N levels in animals influenced by these pollutants tend to be high (Wayland and Hobson 2001; Lee et al. 2013). Nutrients often enter surface waters from diffuse or non-point sources associated with surface runoff and from point sources typically associated with intensive farming activities (Knight et al. 2000). Microcrustaceans that use phytoplankton as their major food source, including cladocerans or copepods, reflect changes in the nitrate stable isotope ratio of outer-origin organic materials within the habitat (Cole et al. 2011), leading to a close link with anthropogenic pollutants (Alcorlo and Baltanás 2013). In the present study, high levels of NO$_3^-$-N in water and δ^{15}N of *C. sphaericus* were mainly observed in wetlands with poor riparian connectivity and sewage sources. Phytoplankton, which naturally accrue non-point source pollution, serve as the main food source of *C. sphaericus* (i.e., as primary consumers), which, therefore, enables a sensitive response to such pollution. Regression analyses in the present study also indicated a positive correlation between δ^{15}N of *C. sphaericus* and NO$_3^-$-N levels (Fig. 3).

However, the changes in δ^{15}N levels in *C. sphaericus* in response to the nitrate levels in water do not occur in a short period of time. The δ^{15}N of *C. sphaericus* was not found to be sensitive to periodic changes in nitrate

Fig. 2 $\delta^{15}N$ (‰) of *Chydorus sphaericus* and NO_3^--N levels of each water source (SW, surface water; R/G, rainfall/groundwater; S, sewage) and according to riparian connectivity, respectively, in **a**, **b** spring and **c**, **d** autumn. Bars and error bars represent an average of replicates (±SD)
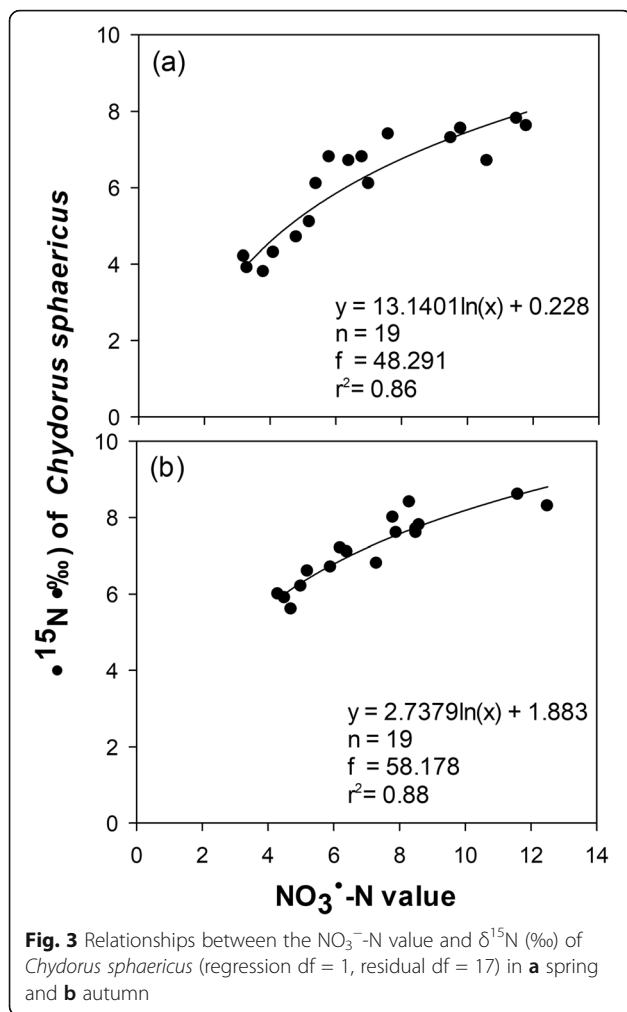
levels in each wetland. In general, phytoplankton absorb the ammonia ion in the DIN pool selectively rather than the nitrate ion, during which the fractionation is 10–20‰ for the ammonia ion, 5–10‰ for the nitrate ion, and 1‰ for the nitrite ion. The nitrogen isotope ratio of phytoplankton is, therefore, determined using the mass balance of all of these nitrogen sources. Zooplankton, including *C. sphaericus*, ingest phytoplankton as a food source and are subjected to fractionation of 2–3‰ by the effect of the trophic pathway. Thus, the relationship between zooplankton and the nitrate ion of the nitrogen isotope ratio can be established if phytoplankton absorb only nitrate ion in an ammonia-free water environment. However, such conditions rarely occur in wetland and river ecosystems, except in nutrition-depleted oceanic ecosystems in which ammonia is not consumed by phytoplankton in the surface water and nitrate is continuously supplied via upwelling from the deep-sea bottom. In the present study, the positive relationship between NO_3^--N in water and $\delta^{15}N$ of *C. sphaericus* could reflect the environmental changes in the wetlands over a long period of time in a stable condition. Therefore, this relationship can be only applied to elucidate the hydrological characteristics of riverine wetlands

The riverine wetland in the present study was constructed in 2011 as a component of river maintenance efforts; however, the wetland environment and hydrological regime are regularly affected by factors such as sedimentation, structure transformation, and human

disturbance. Inlet and outlet deterioration and continuous inflow of sewage accelerate pollutant enrichment in wetlands and influence the growth and distribution of biological factors. Nevertheless, information about wetlands, such as animal distributions and water quality measurements, is rarely collected and few plans have been developed for the systematic management and conservation of such ecosystems. Furthermore, maintenance and budget provisions are not included in business statements for stream implementation projects, making proactive management unfeasible. From this perspective, the nitrate stable isotopes of *C. sphaericus* are comparatively easier and more accessible indicators of wetland water pollution and eutrophication. As a result, the level of nutrient inflow in each wetland can be easily identified by continuously monitoring the nitrate stable isotopes of *C. sphaericus*, permitting methodical management of water pollution and eutrophication to secure wetland habitat functions. This monitoring system can also be applied to wetlands created near the Geum, Han, and Yeongsan Rivers, as well as in other areas of South Korea.

Conclusions
The riverine wetland sites in the present study were constructed in 2011 as a component of river improvement efforts, but these sites have consistently been modified by sedimentation, structural transformation, and human disturbance since their construction. We found that the local-scale hydrological conditions of each wetland led to

Fig. 3 Relationships between the NO_3^--N value and $\delta^{15}N$ (‰) of *Chydorus sphaericus* (regression df = 1, residual df = 17) in **a** spring and **b** autumn

different levels of NO_3^--N and $\delta^{15}N$ in *C. sphaericus*. Channel and riparian type wetlands have strong connections with the main river and tributaries and a high water conveyance efficiency, resulting in low NO_3^--N content. However, the detention ponds fed by sewage inflow or rainfall/groundwater had a higher NO_3^--N content. Although the nitrate stable isotope measurement of DOM or POM, which directly influences the NO_3^--N, are more effective for monitoring changes, the collection and analysis of these factors are relatively difficult. The different NO_3^--N contents of each wetland were closely related to $\delta^{15}N$ in *C. sphaericus*, and the regression analyses also revealed a positive correlation between $\delta^{15}N$ of *C. sphaericus* and NO_3^--N content. In addition, *C. sphaericus* has a high biomass and dominates wetlands where aquatic plants are abundant in high density, which makes their collection and analysis convenient. The positive relationship between the NO_3^--N content in each wetland and $\delta^{15}N$ in *C. sphaericus* indicates that *C. sphaericus* can be used to elucidate the hydrological characteristics of riverine wetlands (e.g., water quality changes).

Materials and methods
Site description and hydrological conditions
The study sites monitored in the riverine wetlands are located in the middle and lower reaches of the Nakdong River. The Nakdong River Basin was heavily modified by the Four Large River Projects, which involved the construction of eight large weirs across the river in 2011 to maintain a minimum channel depth and to facilitate water supply. Furthermore, most riverine areas have been modified for human use, e.g., through the construction of parks, parking lots, and other amenities. Historically, there were numerous riverine wetlands in the river basin, but human activities have led to modifications or losses in their morphologies and habitat structure. A total of 147 artificial wetlands were constructed in four river basins (Ministry of Land, Transport, and Maritime Affairs 2014). We selected 16 riverine wetlands located in the middle and lower areas of the Nakdong River.

The local-scale hydrological characteristics of each wetland were analyzed using a field survey and satellite map (Table 1). Wetlands were classified into three types according to their form and construction purpose: channel, detention pond, or riparian. The channel type was similar to a small stream constructed by retaining the form of a previous stream or furcating it from the main river. Detention ponds were a puddle form, without inlets or outlets. Riparian wetlands were distributed throughout the littoral area in the river channel. Water sources were also classified into three types: surface water, which flowed in from the main river or tributary; sewage, which consisted of contaminated water flowing through a drainage gate; and rainfall/groundwater, which fed wetlands without inflow from the main river or tributaries. Riparian connectivity was used to evaluate the resulting water conveyance efficiency from the main river or tributaries, whereby poor connectivity indicated minimal to nonexistent inlet/outlet functions, good connectivity indicated excellent inlet/outlet functions, and moderate connectivity indicated equivalent inlet and outlet functions. The soil type, topography, edge index, and area of each wetland were measured using a Geographic Information Systems (GIS) program (ArcGIS 10.1; ESRI, Redlands, CA, USA) and a digital map (National Geographic Information Institute 2005; scale 1:25,000).

Collection and stable isotope analysis of *Chydorus sphaericus*
For *C. sphaericus* collection and NO_3^--N measurement, we randomly selected three sampling points based on virtual grids constructed over the maps of each study site. Water samples (10 L) were collected at each point for enumeration and stable isotope analysis of *C. sphaericus* in May and October 2017. Water samples of 5 L were filtered through a 32-μm mesh plankton net and the filtrates were

preserved in sugar formalin with a final formaldehyde concentration of 4% (Haney and Hall 1973). *Chydorus sphaericus* individuals were sorted from the remaining 5 L samples using a micropipette for stable isotope analysis. Identification and enumeration of *C. sphaericus* was performed using a Zeiss Axioskop 40 (Zeiss, Göttingen, Germany) at × 200 (Mizuno and Takahashi 1999).

Water samples were stored on ice immediately after collection and were filtered at 0.45 μm in the laboratory within hours of sampling. The filtered samples were frozen until analysis of NO_3^--N. Nitrate concentrations were analyzed with an Autoanalyzer using cadmium reduction (EPA Method, N87-0065). The lower limit of detection in most cases was $0.025\ mg\,L^{-1}$ N; because samples were often below this limit, a value of 0.0125 $mg\,L^{-1}\ NO_3^-$-N was assigned to samples below the limit of detection when averaging the series.

Since acidification has detrimental effects on nitrogen values, stable isotopic samples of *C. sphaericus* were not acidified to remove the inorganic carbonates (Pinnegar and Polunin 1999). All samples were freeze-dried, homogenized with a mortar and pestle, and stored at − 70 °C prior to analysis. Nitrogen isotope ratios were determined using continuous-flow isotope mass spectrometry. Dried *C. sphaericus* samples (ca. 1 mg) were combusted in an elemental analyzer (EuroVector SpA, Milan, Italy) and the resultant N_2 gas was injected into an isotope ratio mass spectrometer (CF-IRMS, IsoPrime) in a continuous flow using a helium carrier. Data were expressed as the relative concentration (‰) difference between sample and conventional standards of Pee Dee Belemnite carbonate (PDB) for carbon and air N_2 for nitrogen according to the following equation:

$$\delta^{15}N\ (‰) = \left[\left({}^{15}N{:}^{14}N_{sample} / {}^{15}N{:}^{14}N_{standard} \right) - 1 \right] \times 1000$$

A secondary standard of known relation to the international standard was used as the reference. Standard deviations of $\delta^{15}N$ for analyses with 20 replicates of peptone standard were ± 0.1 and ± 0.2‰, respectively.

Data analysis

We used regression analysis to examine the relationship between nitrate stable isotopes in *C. sphaericus* and NO_3^--N levels in each study site. We tested the linear, exponential, inverse, power, and logistic functions to determine an equation that generates the best curve fit. The curve-fitting equation that returned the highest determination coefficient was selected to explain the observed relationships. All statistical analyses, including the regression analyses, were conducted using the statistical package SPSS for Windows v. 20.

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