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# Distribution and attachment characteristics of *Sida crystallina* (O.F. Müller, 1776) in lentic freshwater ecosystems of South Korea

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## Abstract

**Background:** Macrophytes are commonly utilised as habitat by epiphytic species; thus, complex macrophyte structures can support high diversities and abundances of epiphytic species. We tested the hypothesis that the presence of aquatic macrophytes is an important factor determining *Sida crystallina* (O.F. Müller, 1776) distribution.

**Results:** An ecological survey was conducted in 147 lentic freshwater bodies. *S. crystallina* was frequently observed, and its density was strongly associated with macrophyte abundance. *S. crystallina* was found on emergent plant species such as *Phragmites australis* and *Paspalum distichum*, attached to the stem surfaces by adhesive substances secreted by the nuchal organ. Thus, *S. crystallina* was more strongly attached to macrophytes than to other epiphytic cladoceran species. We found higher densities of *S. crystallina* in filtered water with increased macrophyte shaking effort (i.e. 10, 20, 40, or 80 times). *S. crystallina* attachment was not related to fish predation. Stable isotope analysis showed that *S. crystallina* utilises epiphytic organic matter (EOM) on macrophytes as a food source.

**Conclusions:** Consequently, *S. crystallina* seems to have a strong association with species-specific macrophyte biomass than with other cladoceran species, which may contribute to this species' predominance in various freshwater ecosystems where macrophytes are abundant.

**Keywords:** *Sida crystallina*, Aquatic macrophytes, Attachment characteristics, Stable isotope analysis, Lentic freshwater ecosystems

## Background

Aquatic macrophytes commonly occur in shallow aquatic ecosystems and can have dramatic effects on their physical structure (O'Hare et al. 2006; Smokorowski and Pratt 2007). Aquatic macrophytes give rise to heterogeneous spaces with varying degrees of structural complexity (Denny 1994; Findlay and Bourdages 2000). Some studies have suggested that vegetated beds with high structural heterogeneity provide small animals with refuges from predators and suitable spawning and foraging substrates, mediating trophic interactions among diverse organisms (Vieira et al. 2007; Thomaz et al. 2008). The effectiveness

of macrophytes as a refuges and/or habitat varies with their life form, density, and species (see review in Burks et al. 2002). In particular, macrophyte morphological characteristics have a significant bearing upon the availability of small animals as a food source, mediated via detritus trapping (Rooke 1984) and the growth of periphytic algae (Cattaneo et al. 1998). Thus, the presence of aquatic macrophytes facilitates increased abundance and diversity in aquatic animal communities.

Among the many animals utilising macrophyte habitats, freshwater cladocerans are well known to exploit macrophytes as habitats and/or refuges (Kuczyńska-Kippen and Nagengast 2006; Choi et al. 2014a). The majority of studies that have focused on the interactions between macrophytes and cladocerans have considered the influence of macrophytes on pelagic cladoceran species (Jeppesen et al.

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1998; Meerhoff et al. 2007). These studies argued that aquatic macrophytes are capable of providing suitable habitats for mainly pelagic cladocerans. However, pelagic species are continuously exposed to predators such as fish due to their frequent movement; thus, it would be relatively difficult for such pelagic species to develop higher abundances in freshwater ecosystems. In comparison with other aquatic systems, shallow wetlands are characterised by abundant aquatic macrophytes, and this abundance of habitat tends to attract more cladoceran species. These are known as epiphytic cladocerans (i.e. plant-attached species; Castilho-Noll et al. 2010; Gyllström et al. 2005; Kuczyńska-Kippen and Nagengast 2006) and are strongly affected by the abundance, morphology, and arrangement of plant species (Choi et al. 2014b). Unfortunately, epiphytic cladoceran distribution patterns and modes of macrophyte utilisation are still unclear, and their abundances are usually underestimated.

*Sida crystallina* (Cladocera: Sididae, O.F. Müller 1776) is a typical epiphytic cladoceran species occurring in temperate and tropical waters. They attach to aquatic macrophytes by means of maxillary glands and thus filter feed in fixed positions (Fairchild 1981). Compared with other cladoceran species, *S. crystallina* occurs at relatively high water temperatures (approximately 21 to 22 °C; Kotov and Boikova 1998) and is prevalent in temperate zones during summer (Balayla and Moss 2003). They are found in most shallow freshwater ecosystems in South Korea during summer (Choi et al. 2014c). Unfortunately, although the overall distribution of the species has been reported (Downing and Peters 1980; Lauridsen et al. 1996), its distribution patterns, attachment characteristics, and feeding habits have been insufficiently studied.

In this study, we investigated the distribution patterns of *S. crystallina* in lentic freshwater ecosystems in South Korea. We hypothesised that *S. crystallina* may prefer shallow wetland microhabitats in which macrophytes dominate and may utilise epiphytic algae growing on macrophyte stands as their food source. To test these hypotheses, we investigated (i) the influence of diverse physicochemical parameters and macrophytes on *S. crystallina* distribution and (ii) the nature of attachment of *S. crystallina* in relation to fish predation and food availability. We surveyed 147 lentic ecosystems in South Korea and recorded physicochemical parameters of water, macrophyte occurrence, and *S. crystallina* densities.

## Methods

### Study sites

South Korea is located in East Asia and has a temperate climate. Four distinct seasons lead to the dynamic succession of biological communities in the freshwater ecosystems of South Korea. Annual mean rainfall is ca. 1150 mm, more than 60 % of which occurs from June to

early September (Choi et al. 2011; Jeong et al. 2007). Our study sites were located in south-eastern South Korea, along the middle and lower reaches of the Nakdong River. Historically, there were numerous riverine wetlands in this river basin (Son and Jeon 2002); however, large areas of wetland have vanished due to the expansion of human society (Burkett and Kusler 2000). The dominant land cover surrounding reservoirs is agricultural, and non-point source pollution continuously influences the study sites (Korean Ministry of Environment 2006).

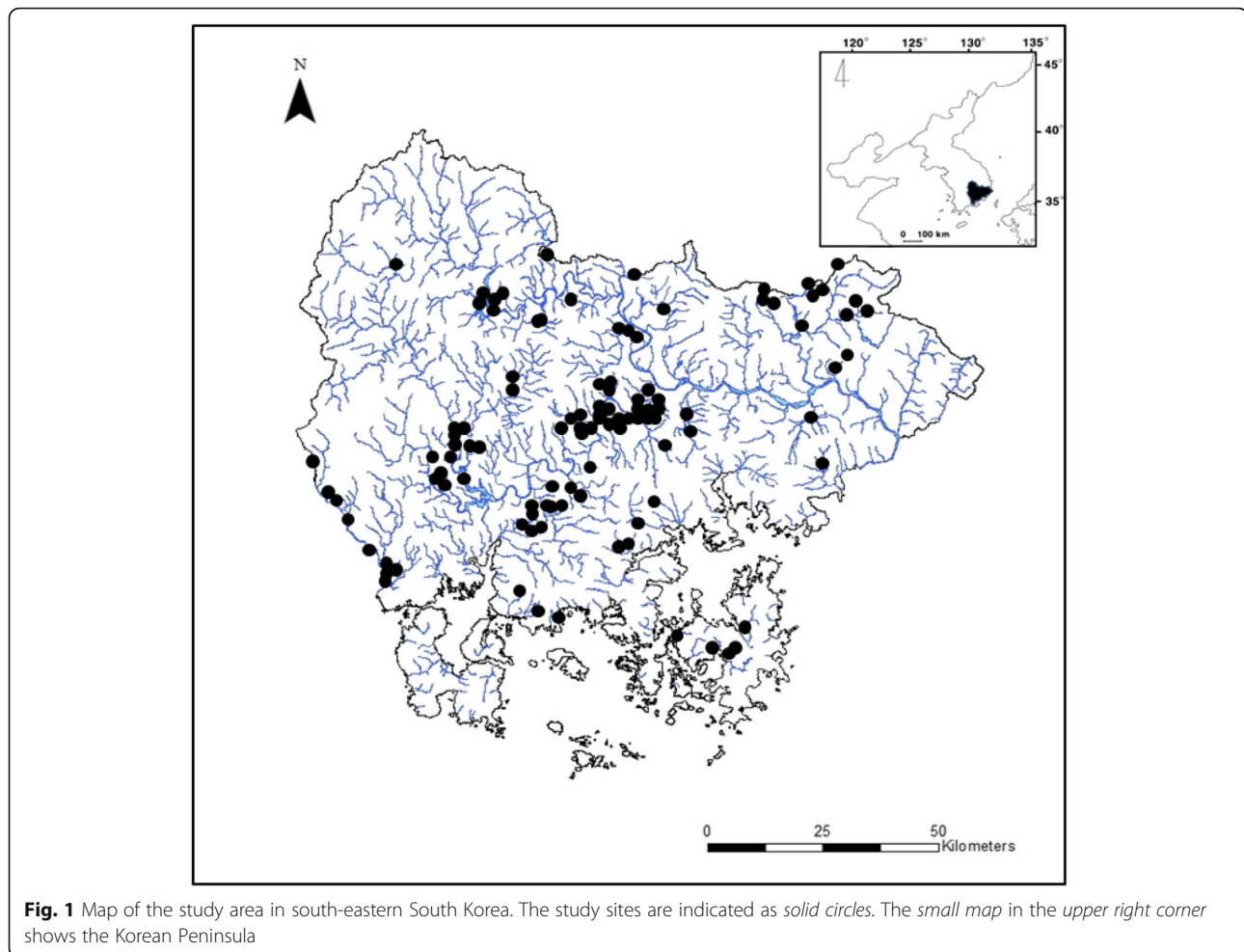
We investigated 147 lentic freshwater ecosystems in the river basin (wetlands, ponds, and reservoirs; see Fig. 1). The wetlands and shallow lakes are dominated by various macrophyte species; however, the development and growth of macrophytes is inhibited in reservoirs and some lakes due to their impermeable floors. In addition, some wetlands support only a few plant species because of high water levels and low nutrient concentrations. Therefore, the study sites encompassed a wide range of microhabitat characteristics (i.e. different types of lentic systems and different patterns in their constituent plant communities).

### Monitoring strategy

The target species *S. crystallina* is known to prefer relatively high water temperatures (approximately 21 to 22 °C; Kotov and Boikova 1998) and is prevalent in temperate zones during summer (Balayla and Moss 2003). Based on this information, we monitored study sites in summer (June to July 2012). At each site, three sampling points were established in the littoral zone.

Physicochemical parameters were measured and *S. crystallina* collected at each sampling point. Water temperature, dissolved oxygen, conductivity, pH, chlorophyll a, and turbidity were measured at each site. Water samples were collected at a depth of 0.5 m. We used a DO meter (YSI DO meter; Model 58, YSI Research Inc., OH, USA) to measure water temperature and dissolved oxygen. Conductivity and pH were measured using a conductivity meter (YSI Model 152; Yellow Springs Instruments, Yellow Springs, OH, USA) and pH meter (Orion Model 250A; Orion Research Inc., Boston, MA, USA). Turbidity and chlorophyll a concentration were measured in the laboratory. Turbidity was measured using a turbidimeter (Model 100B; Scientific Inc., Ft. Myers, FL, USA). The water samples were filtered through mixed cellulose ester (MCE) membrane filters (Advantech; Model No., A045A047A; pore size, 0.45 µm), and chlorophyll a concentration was ascertained based on the methodology of Wetzel and Likens (2000).

At each sampling point, we took an additional 10 L of water for zooplankton collection from the surface layer (to a depth of 0.5 m), using a 10-L column sampler. This water was filtered through a plankton net (68-µm mesh size), and the filtrate was preserved in sugar formalin



(final concentration 4 % in the form of aldehyde). *S. crystallina* and other zooplankton species were identified and counted using a microscope (ZEISS, Model Axioskop 40;  $\times 200$  magnification) using the classification key of Mizuno and Takahashi (1991).

To investigate attachment characteristics of *S. crystallina*, we additionally collected *S. crystallina* from stems and leaves of macrophytes at six sites where high abundances of *S. crystallina* were observed. We established five quadrats ( $0.5 \text{ m} \times 0.5 \text{ m}$ ) along the littoral zone at each of these sites and counted all *S. crystallina* within each quadrat. We did not include emergent organs of macrophytes above the water surface (i.e. stalks and flowers) because *S. crystallina* inhabit underwater environments. The submerged parts were handled carefully to prevent *S. crystallina* from accidentally detaching. *S. crystallina* were kept alive using filtered wetland water. Small animals including zooplankton were removed from 2 L of water using a plankton net ( $32 \mu\text{m}$  mesh size), and the filtered water was stored in 5-L tanks. This water was used as temporary storage for epiphytic species including *S. crystallina*. Collected macrophytes were shaken in the tank to detach *S. crystallina*

(for the detaching process, see Sakuma et al. 2002). *S. crystallina* on the plants were detached by shaking 10, 20, 40, and 80 times. After collection of *S. crystallina* at the study sites, macrophyte samples were carried to the laboratory and dried at  $60 \text{ }^\circ\text{C}$  for 2 days. Epiphytic species, including *S. crystallina*, were filtered from the water using a  $68 \mu\text{m}$  mesh net and immediately fixed with sugar formalin (final concentration 4 % in the form of aldehyde). We counted numbers of *S. crystallina* using a microscope (ZEISS, Model Axioskop 40;  $\times 200$  magnification). Densities of *S. crystallina* attached to plants were expressed as number of individuals per gramme dry weight of macrophyte ( $\text{ind. g}^{-1} \text{ dw}$ ).

#### Microcosm experiment

To understand how the attachment characteristics of *S. crystallina* influence fish predation, we conducted additional microcosm experiments. Approximately 200 *S. crystallina* adult individuals with similar life-history traits (body size and condition of clutch) were selected. These *S. crystallina* individuals were acclimatised for approximately 48 h in a stock culture environment

(Elendt M4 medium; Elendt 1990). To simulate the fish predation, we used fish chemical cues (De Meester and Cousyn 1997) in this microcosm experiment. A total of five fish species were considered, *Micropterus salmoides* (Lacepède 1802), *Lepomis macrochirus* (Rafinesque 1819), *Pseudorasbora parva* (Temminck and Schlegel 1846), *Rhinogobius brunneus* (Temminck and Schlegel 1845), and *Misgurnus anguillicaudatus* (Cantor 1842). We collected samples of these fish at the study sites where we obtained *S. crystallina* using a 7 mm × 7 mm cast net and a 5 mm × 5 mm scoop net. To obtain fish chemical cues from the collected fish, we allocated each fish species to one of five tanks, and five individuals of each fish species were acclimatised in each tank for 24 h (tanks filled with 10 L Elendt M4 medium).

The experiment was designed as follows: a total of six groups—control (no predation) and five experimental groups by using each of the fish species. First, we prepared 60 500-mL beakers (10 beakers per experimental group) and filled each with 300 mL of clear M4 medium. We put five *S. crystallina* individuals in each beaker and allowed them to acclimatise to the new environment for 30 min. During the acclimatisation period, we prepared the fish chemical cues used in the experiment: the fish-exposed M4 medium was filtered using a 30 µm mesh net (used only in this experiment and not used for field plankton collection) to remove particulate matter. Before introducing the resulting fish chemical cues to the experimental beakers, we very carefully marked the position of each *S. crystallina* individual on the outer surface of every beaker. Then, we injected 200 mL of fish chemical cue into each beaker. For the control group, unexposed, clear M4 medium was added. The *S. crystallina* individuals were allowed to respond to the changed environment for 30 min, and then, we investigated the number of moved individuals.

#### Data analysis

We used two-way ANOVA ( $\alpha = 0.05$ ) to analyse how the density of *S. crystallina* varied with shaking procedure (i.e. 10, 20, 40, and 80 times) and collection site. Differences in *S. crystallina* movement among fish predation treatments were analysed statistically using one-way ANOVA. Furthermore, the relationships between *S. crystallina* density and environmental variables were tested using stepwise multiple regression. All statistical analyses, including stepwise multiple regression and ANOVA, were conducted using the statistical package SPSS for Windows ver. 14.

#### Stable isotope analysis

Stable isotope analysis was conducted to identify the food sources of *S. crystallina*. Particulate organic matter (POM), epiphytic organic matter (EOM), and *S. crystallina* individuals were sampled at six different sites where *S. crystallina*

was abundant. To process the POM samples, any small animals were first removed using a plankton net (32 µm mesh size), and then, the water samples were filtered through GF/F glass-fibre (pre-combusted at 500 °C for 2 h). The surfaces of submerged parts of macrophytes from each study point were gently brushed in a tank filled with distilled water, in order to obtain the EOM. Similar to the processing of POM, micro- and macroinvertebrates were removed using the plankton net (32 µm mesh size). *S. crystallina* individuals were isolated using a micropipette.

POM and EOM samples were treated with 1 N HCl to remove inorganic carbon and rinsed with distilled water to remove the acid. *S. crystallina* samples were not acidified to remove inorganic carbon, because acidification affects nitrogen values (Pinnegar and Polunin, 1999). All samples were freeze-dried and homogenised with a mortar and pestle, and the powdered samples were kept frozen (−70 °C) until analysis. Carbon and nitrogen isotope ratios were determined using continuous-flow isotope mass spectrometry. Dried samples (ca. 1 mg for *S. crystallina* samples and 1.5 mg for POM and EOM) were combusted in an elemental analyser (Euro EA 3000 Elemental Analyzer, Eurovector SPA., Milano, Italy), and the resultant gas (CO<sub>2</sub> and N<sub>2</sub>) was introduced to 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 atmospheric N<sub>2</sub> for nitrogen according to the following equation:

$$\delta X(\text{‰}) = \left[ \left( R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000 \quad (1)$$

where  $X$  is <sup>13</sup>C or <sup>15</sup>N and  $R$  is the <sup>13</sup>C:<sup>12</sup>C or <sup>15</sup>N:<sup>14</sup>N ratio. A secondary standard (Peptone) of known relation to the international standard was used as a reference material. Standard deviations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for analyses with 20 replicates of Peptone standard were ±0.1 and ±0.2 (‰), respectively.

To determine which of the two food sources (POM and EOM) was assimilated more readily by *S. crystallina*, we used two-source isotope mixing models. The carbon isotope values of POM and EOM significantly differed among sites (see 'Results'). The model was defined as:

$$\begin{aligned} \delta^{15}\text{C}_M &= f_X(\delta^{15}\text{C}_X + \Delta^{15}\text{N}) \\ &+ f_Y(\delta^{15}\text{C}_Y + \Delta^{15}\text{C}); 1 \\ &= f_X + f_Y \end{aligned} \quad (2)$$

where  $X$ ,  $Y$  and  $M$  represent the two food sources and a mixture of the two, respectively;  $f$  represents the proportion of N from each food source in the consumer's diet; and  $\Delta^{15}\text{C}$  is the assumed trophic fractionation (i.e. the change in  $\delta^{15}\text{C}$  over one trophic step from prey to predator; Phillips and Gregg 2001). Trophic fractionation was

assumed to be constant at either 3.4 or 2.4 % (Minagawa and Wada 1984).

## Results

### Physicochemical parameters, macrophytes, and zooplankton

There were few differences in physicochemical characteristics of water among the study sites (Table 1). Although some study sites had exceptionally high or low values, the coefficients of variation (CV; standard deviation/mean  $\times$  100 %) were lower than 100 %. Conductivity had the highest CV, but this was only approximately 66.9 %.

Macrophyte species composition and dry weight differed among study sites. *Paspalum distichum* L. dominated most of the study sites; a total of 10 species of macrophyte were found (*Phragmites australis* Trin. (Cav.), *P. distichum*, *Zizania latifolia* Griseb., *Scirpus tabernaemontani* Gmel., *Spirodela polyrhiza* L., *Salvinia natans* L., *Trapa japonica* Flerov., *Ceratophyllum demersum* L., *Hydrilla verticillata* (L.F.) Royle, and *Nymphoides indica* (L.) Kuntze).

A total of 122 species of zooplankton were identified (86 rotifers, 27 cladocerans, and 9 copepods). The highest abundance of zooplankton was 4135 ind. L<sup>-1</sup>, followed by 3736 ind. L<sup>-1</sup>. *Lecane hamata* (Stokes 1897), *Polyarthra vulgaris* (Carlin 1943), *Chydorus sphaericus* (O.F. Müller 1785), and *Diaphanosoma brachyurum* (Lievin 1848) were recorded frequently.

*S. crystallina* was observed at 81 out of 147 sites. From the observation, *S. crystallina* was mostly found in shallow wetlands where macrophytes were present. Stepwise multiple regression showed that density and distribution of *S. crystallina* were clearly related to macrophyte biomass (d.f. for regression, residuals, and total = 2, 143, 146, respectively;  $F = 94.32$ ,  $P = 0.001$ , see Table 2 for independent variables). In addition, *S. crystallina* density was related to water depth and chlorophyll a concentration. However, other environmental parameters did not have significant influence on *S. crystallina* distribution or abundance.

**Table 1** Mean macrophyte dry weights and physicochemical parameters measured at the study sites

Variable	Units	Max	Min	Mean $\pm$ SD	CV (%)
Macrophyte biomass	gdw	114.1	0	42.8 $\pm$ 23.1	68.4
Water depth	m	231	14	78.4 $\pm$ 40.6	51.7
Water temperature	°C	27.1	22.5	25.6 $\pm$ 2.5	11.2
Dissolved oxygen	%	217.2	21.6	87.9 $\pm$ 34.2	38.2
Conductivity	$\mu\text{s cm}^{-1}$	746.7	83.6	187.8 $\pm$ 125.6	66.9
pH	–	8.8	6.3	7.8 $\pm$ 0.6	9.2
Chlorophyll a	$\mu\text{g L}^{-1}$	42.8	2.1	25.8 $\pm$ 9.0	35.2
Turbidity	NTU	24.0	1.33	9.4 $\pm$ 6.0	64.5
Total nitrogen	mg L <sup>-1</sup>	13.2	1.4	7.3 $\pm$ 2.6	25.4
Total phosphorous	mg L <sup>-1</sup>	123.2	23.6	76.7 $\pm$ 32.5	36.7

**Table 2** Summary of stepwise multiple regression between *Sida crystallina* abundance (response variable) and physicochemical parameters (explanatory variables)

Response variable	Explanatory variables	$B_j$	$t$	$P$
<i>Sida crystallina</i>	Constant	27.201	-2.488	0.014
	Macrophyte biomass (g)	2.469	12.287	0.000
	Water depth (mm)	0.748	3.735	0.005
	TP (mg L <sup>-1</sup> )	-1.794	-2.185	0.031

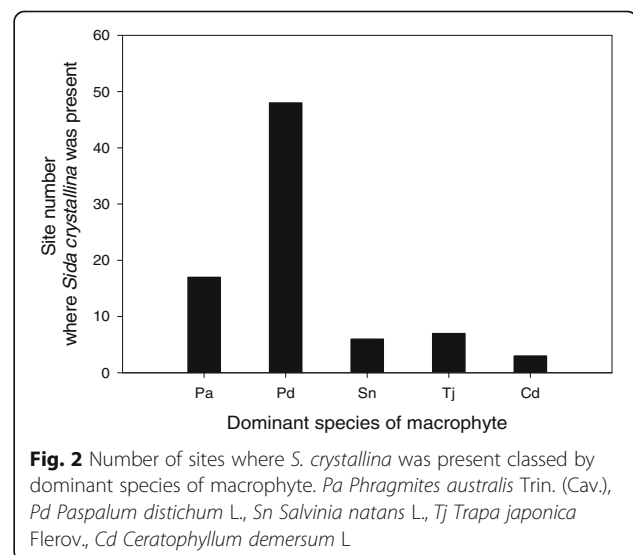
Data were transformed prior to analysis using either the arcsine-square root (proportion of agricultural land) or log (all other variables) transformation

Interestingly, *S. crystallina* was frequently observed in sites where emergent plants such as *P. australis* and *P. distichum* dominated (Fig. 2). In particular, sites dominated by *P. distichum* supported higher densities of *S. crystallina*. By contrast, *S. crystallina* densities were low in sites where free-floating (*S. natans*), floating-leaved (*T. Japonica*), and submerged (*C. demersum*) macrophyte species dominated.

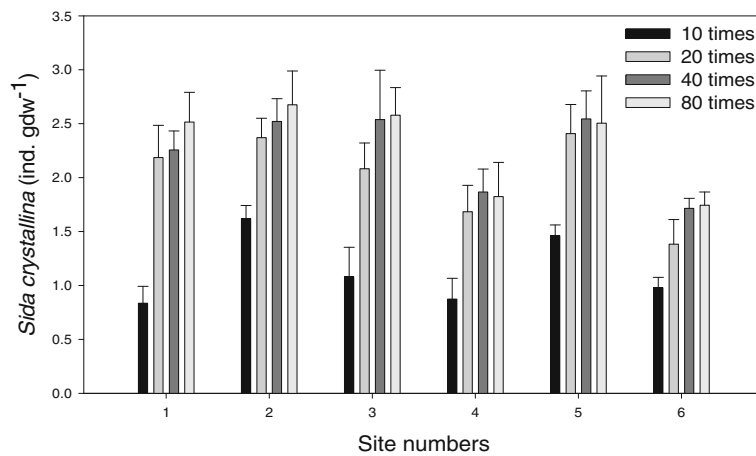
### Attachment characteristics of *S. crystallina*

Numbers of *S. crystallina* detached from macrophytes differed with shaking effort at each site (Fig. 3). Two-way ANOVA revealed that *S. crystallina* counts were significantly affected by both shaking effort (10, 20, 40, or 80 times; d.f. = 3,  $F = 265.98$ ,  $P < 0.05$ ) and sites (between total 6 sites; d.f. = 5,  $F = 101.43$ ,  $P < 0.05$ ). *S. crystallina* counts were proportional to the shaking effort, but the increase in counts tailed off as the efforts increased. This phenomenon was observed at all six study sites.

Interestingly, *S. crystallina* attachment was not affected by simulated fish predation (one-way ANOVA, d.f. = 5,  $F = 0.236$ ,  $P = 0.945$ ; Fig. 4). We counted the number of moved individuals of *S. crystallina* following exposure to fish chemical cues, but almost no individuals moved in any experimental group, including the control.



**Fig. 2** Number of sites where *S. crystallina* was present classed by dominant species of macrophyte. Pa *Phragmites australis* Trin. (Cav.), Pd *Paspalum distichum* L., Sn *Salvinia natans* L., Tj *Trapa japonica* Flerov., Cd *Ceratophyllum demersum* L.



**Fig. 3** Numbers of *Sida crystallina* detached from macrophyte stands based on the shaking effort (10, 20, 40, and 80 times) at each study sites

Figure 5 shows the organ used by *S. crystallina* for attachment. They attached to substrata using adhesive anchors that are connected to the cuticle by anchor threads.

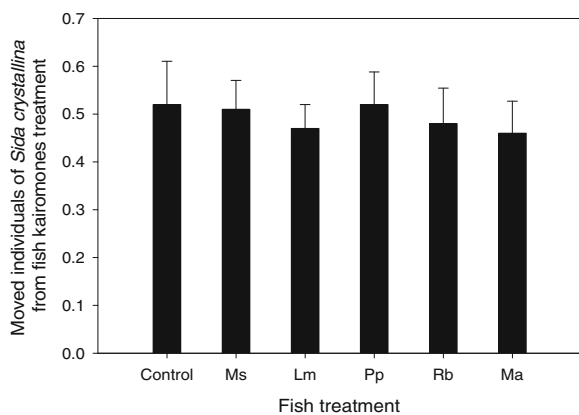
**S. crystallina food sources**

The results of stable isotope analysis indicated potential food sources of *S. crystallina*. *S. crystallina*  $\delta^{13}\text{C}$  values indicated a bias in the composition of their food (between SPOM and EPOM) and reflected differences in the  $\delta^{13}\text{C}$  values of SPOM and EPOM (Fig. 6). At all sites, *S. crystallina* was more dependent on EPOM on macrophyte surfaces than SPOM. Although  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *S. crystallina* and EPOM differed among sites, a common relationship between the grazer and its diet could be assumed for all sites (a fractionation coefficient of 1 ‰ was used per trophic step for carbon isotopes

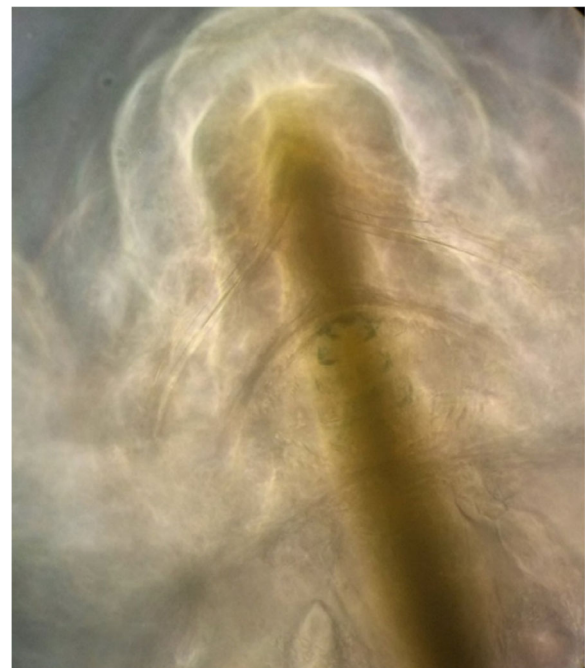
and 2~3 ‰ per trophic step for nitrogen isotopes). Moreover, when the contributions of the two potential food sources to *S. crystallina* diet were calculated from isotope analyses, the contribution of EPOM (average, 83 %) was higher than that of SPOM (average, 17 %), according to the two-source mixing model.

**Discussion**

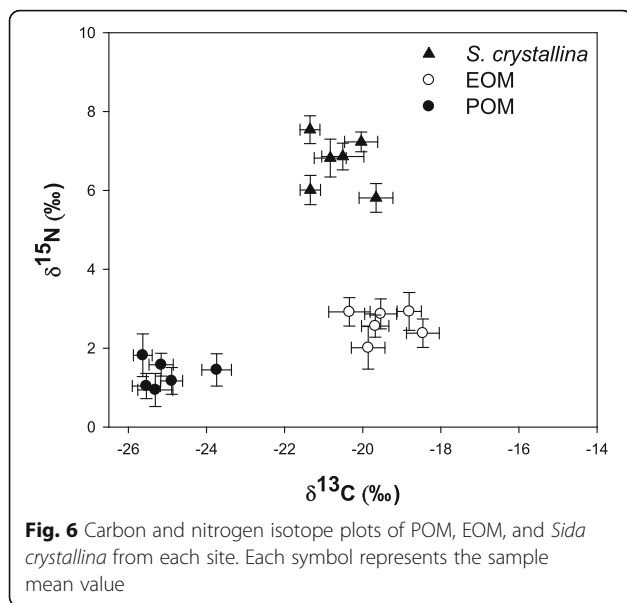
In this study, *S. crystallina* distribution showed a strong relationship with macrophyte biomass. Among cladoceran species, *S. crystallina* is well known to have an epiphytic character and mainly attaches to stem and leaf surfaces of



**Fig. 4** Number of *Sida crystallina* individuals to have moved in response to simulated predation by five fish species. *Ms* *Micropterus salmoides* (Lacepède, 1802), *Lm* *Lepomis macrochirus* (Rafinesque, 1819), *Pp* *Pseudorasbora parva* (Temminck and Schlegel, 1846), *Rb* *Rhinogobius brunneus* (Temminck and Schlegel, 1845), *Ma* *Misgurnus anguillicaudatus* (Cantor, 1842)



**Fig. 5** Adhesive anchors of *Sida crystallina*



macrophytes. Thus, *S. crystallina* was not frequently observed in ecosystems where macrophytes were absent or not abundant. We found distribution patterns of *S. crystallina* varied with dominant macrophyte species. Moss et al. (1998) suggested that some epiphytic zooplankton species could attain high biomasses in free-floating and floating-leaved macrophyte beds. However, macrophyte species occupy limited space in the water (mostly at the water surface) and are mainly utilised as habitat by small species (i.e. rotifers; Choi et al. 2014c). Although submerged macrophytes make a large contribution to aquatic habitat complexity, the leaves and stems of submerged macrophytes are more easily agitated by wind and water currents than those of other plant species (Vermaat et al. 2000). As a result, they are less suitable for the attachment of epiphytic species. Some reports suggest that submerged macrophytes are mainly used by pelagic zooplankton, such as daphnids, as daytime refuges (Lauridsen and Lodge 1996; Burks et al. 2002). In contrast, emergent macrophyte species are tightly fixed in place and thus are suitable for attachment of large epiphytic species such as *S. crystallina*. Although emergent macrophytes are known to have simpler structure than other aquatic plant species (Choi et al. 2014b), it seems emergent plants are important as habitat for *S. crystallina*. In particular, *S. crystallina* was more abundant in ecosystems where *P. distichum* dominated than those dominated by other emergent macrophyte species (e.g. *P. australis*). We suggest that *S. crystallina* prefers *P. distichum* because it has a more complex structure (more diversified arrangement of stems and leaves) than *P. australis*. In addition, *S. crystallina* distribution was related to water depth and total phosphorus. Deeper water may increase the volume of water available to be occupied by macrophytes and thus support greater densities of *S.*

*crystallina*. In addition, *S. crystallina* mostly occurred in aquatic environments where nutrient levels were relatively low. In such wetland, macrophytes are thought to make a large contribution to nutrient removal and improve water quality (Sooknah and Wilkie 2004). Thus, the positive relationship between *S. crystallina* and macrophyte biomass can explain the negative relationship with total phosphorus. However, further investigation is needed to better understand the relationships between *S. crystallina* and nutrient status.

From the results of an additional experiment on the interactions between *S. crystallina* and macrophytes, we found densities of *S. crystallina* on macrophyte varied with shaking effort (10, 20, 40, or 80 times) in filtered wetland water. We collected more *S. crystallina* individual as the number of shakes, suggesting that *S. crystallina* was strongly attached to the macrophytes. Similarly, Sakuma et al. (2002) compared densities of some epiphytic rotifer and cladoceran species on plants after shaking for different numbers of times and found large numbers of *Lecane* and *Collotheca* remained on plants even after shaking 50 times. However, numbers of the epiphytic cladoceran genus *Alona* did not vary with shaking effort. Some crustaceans, such as conchostracans or cladocerans, have nuchal organs named maxillary glands, which secrete excreta (Thorp and Covich 2001) that are used to attach to substrate surfaces. In particular, *S. crystallina* has well developed nuchal organs, allowing it to firmly attach to leaf and stem surfaces of macrophytes. Therefore, we considered that this strong attachment of *S. crystallina* to macrophytes caused the differences in observed densities with shaking effort. Moreover, *S. crystallina* populations had statistically different demographic structures among sites.

Attachment of *S. crystallina* was not affected by fish predation. Pelagic species such as daphnids actively move to avoid predators, but epiphytic species are relatively less influenced by predation. Epiphytic cladoceran species are more sensitive to food availability than predation, and their distribution is related to food sources. For example, Sakuma et al. (2004) suggested that epiphytic chydorid cladocerans such as *Alona* migrated from the reed zone to the submerged macrophyte zone in summer and may select food-rich habitats and migrate seasonally. However, *S. crystallina* is vulnerable to fish predation and periods of high population density are often short (Nurminen et al. 2001; Balayla and Moss 2003). Nurminen et al. (2007) also reported that predation by fish has a strong effect on the migration of *S. crystallina*. They have relatively larger than other epiphytic cladocerans (*Alona*, *Chydorus*, and *Pleuroxius*) and thus can be easily captured by predators. However, the lack of response of *S. crystallina* to simulated predation may indicate adaption to long-term movement

patterns, such as diurnal migration (vertical and horizontal migration; Zaret and Suffern 1976; Burks et al. 2002). Therefore, we conclude that *S. crystallina* was not influenced by fish chemical cues in the short term.

The stable isotope analysis indicated that *S. crystallina* are affected more by availability of EPOM on macrophyte stands than that of SPOM in the water. Their habit is to attach to aquatic macrophytes by means of the maxillary gland and thus filter feed from a fixed position (Fairchild 1981). Therefore, we suggest that *S. crystallina* consumes more EPOM than SPOM. Consumption of EPOM by *S. crystallina* plays an important role in the freshwater food web. Epiphytic materials on macrophyte stands are transported across food webs to epiphytic species such as *S. crystallina* (Schindler and Scheuerell 2002, Takai et al. 2002), and the trophic interactions can be interpreted as another route in food webs of freshwater ecosystems with developed macrophytes. Multiple trophic interactions can contribute not only to increased biodiversity in wetlands but also to sustaining an ecologically healthy food web.

In this study, we found that *S. crystallina* was more abundant in relatively less eutrophicated ecosystems where macrophytes were dominant. However, agricultural land surrounds most lentic freshwater ecosystems in South Korea, and non-point pollution sources continuously influence these ecosystems. Even though these ecosystems were frequently dominated by macrophytes, they had high nitrogen and phosphorus concentrations. Moreover, the development and growth of macrophytes is inhibited in some reservoirs due to their impermeable floors. We suggest that it is for these reasons that *S. crystallina* was not frequently observed in lentic freshwater ecosystems of South Korea. *Diaphanosoma* and *Simocephalus* have similar habitat requirements to *S. crystallina*, and they actively utilise macrophytes as habitat and refuges, resulting in greater abundances when macrophytes with complex structures, such as free-floating and submerged macrophytes, coexist (Lauridsen et al. 1996; Stansfield et al. 1997). *S. crystallina* was less dependent on such complexity and coexistence, but rather macrophyte biomass, water quality, and food availability. In addition, *S. crystallina* preferred emergent macrophytes that have relatively simple structures and thus is more vulnerable to predation by fish. Even though *S. crystallina* utilises emergent macrophytes as habitat, such plants did not play the role of refuges from fish predation. Moreover, *S. crystallina* did not show any avoidance behaviour to simulated fish presence. These habitat utilisation characteristics of *S. crystallina* may underlie its restricted distribution in lentic freshwater ecosystems of South Korea. Moreover, the species' preference for high water temperatures may make a significant contribution to their restricted distribution. Further investigation is needed to better understand the

importance of macrophytes, fish predation, food availability, and water temperature in sustaining growth and development of *S. crystallina* populations.

## Conclusions

*S. crystallina* was most frequently observed in shallow wetlands, and density was strongly associated with macrophyte biomass. Emergent macrophytes (*P. australis* and *P. distichum*) were preferred by *S. crystallina* to other plant species. Although free-floating or submerged macrophytes make a large contribution to aquatic habitat complexity, because they are more easily agitated by wind and water currents, they were not suitable for attachment of *S. crystallina*. Empirical studies report that some epiphytic species utilise stem and leaves surfaces of free-floating macrophytes as habitat, but this is true of most small species (e.g. those belonging to *Alona*, *Chydorus*, and *Pleuroxus*). The space occupied by free-floating macrophytes in the water is relatively small; thus, it is difficult for the large *S. crystallina* to use free-floating macrophytes. They attach to stem and leaf surfaces of macrophytes by means of secreted glues from the nuchal organ. Therefore, *S. crystallina* can more firmly attach to macrophytes than other epiphytic cladoceran species. We found greater densities of *S. crystallina* as the shaking effort increased. However, attachment of *S. crystallina* to substrate surfaces was not affected by simulated predation. Some studies suggest that predation threats by fish have strong effects on *S. crystallina*, but *S. crystallina* did not immediately respond to such threats in this study. The results of stable isotope analysis showed that *S. crystallina* primarily utilises EPOM as its food source. *S. crystallina* consumed food source through filter feeding from a fixed position on macrophyte stands, attached by means of the maxillary gland. Consequently, *S. crystallina* abundance seems to be more strongly correlated with macrophyte biomass than other cladoceran species. This may contribute to this species' predominance in various freshwater ecosystems where macrophytes dominate.

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## Authors' contributions

JYC carried out the field studies (collection and experiment), participated in the sequence alignment, and drafted the manuscript. KSJ participated in the design of the study and performed the statistical analysis. SKK and SHS participated in the sequence alignment. GJJ conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.



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