

Article

A Study of the Distribution of *Daphnia obtusa* and *Simocephalus vetulus* in Response to Varying Environmental Conditions Using Field and Microcosm Approaches

Jong-Yun Choi *  and Seong-Ki Kim

National Institute of Ecology Seo-Cheon Gun, Chungcheongnam Province 325-813, Korea; skkim@nie.re.kr

* Correspondence: jyc311@naver.com; Tel.: +82-41-950-5473

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Abstract: We investigated three shallow wetlands in South Korea to understand the spatial distribution of cladoceran species (*Simocephalus vetulus* and *Daphnia obtusa*) associated with different environmental variables, through field sampling, stable isotope analysis, and an experimental approach. The surface and bottom layer of the water was occupied by surface-dwelling and submerged macrophytes, respectively, and the two cladoceran species were distributed correspondingly to the macrophyte distribution pattern. The results of a stable isotope analysis showed cladocerans' large dependency on the particulate organic matter (POM). The microcosm experimental approach revealed that the life forms of macrophytes determined the vertical distribution of cladoceran species. A greater number of *S. vetulus* were found on the surface-dwelling macrophytes on the surface, whereas *D. obtusa* was more abundant in the bottom layer (only in submerged macrophytes) in all treatments. This distribution pattern was largely extended by predation. We identified that the varying distribution pattern would be due to the characteristic habitat utilization of each cladoceran species. Their different habitat use facilitated the coexistence of the two species. Significantly, the macrophytes were supporting the coexistence of the Cladocera species, and may play an important role in enhancing the biodiversity of the wetlands and sustaining its complex food web. The spatial distribution of two cladoceran species, especially those with restricted niches, allow us to understand biodiversity responses of wetland littorals under changing limnological regimes.

Keywords: cladoceran distribution; aquatic macrophytes; fish predation; food availability; microcosm experimental; stable isotope analysis

1. Introduction

Cladocerans play a crucial role in the functioning of freshwater wetland ecosystems [1]. Various diverse microbial loops form the dynamic food web in a wetland ecosystem. The patterns of cladoceran distribution should be primarily identified to investigate wetland functions. It is known that the distribution of cladocerans is affected by various biotic and abiotic factors such as microhabitat structures, predation, and food availability [2–4]. A large volume of literature suggests that predation and food stress often induce changes in life history, morphology, and behavior pattern of cladocerans [5–7]. Cladocerans living offshore must find the optimum strategy to minimize the risk of predation and to maximize the intake of resources [8]. In recent decades, there has been an increased interest in discovering some combination that determines the distribution pattern of cladocerans.

It is well-known that macrophytes provide a refuge from predation for both small fish and cladocerans such as *Daphnia* [9–12]. The effectiveness of macrophytes as a refuge varies in accordance with the life form, density, and species composition of these plants (see review in Burks et al. [13]).

Furthermore, the morphology of the macrophytes has a significant bearing upon the availability of zooplankton as a food source, because of detritus tapping [14], and the growth of periphytic algae [15]. Consequently, the development of various macrophytes in wetlands leads to diverse cladoceran communities, and supports abundant cladoceran populations [16,17]. The heterogeneous structure of the vegetated habitat has also been demonstrated to affect the abundance of cladoceran species [18]. Several studies have found that complex, mixed macrophyte beds harbored more cladocerans than water space occupied by simply structured vegetation (e.g., reed bed; [19–21]), due to the partitioning of niches within a given space [22]. Moreover, the complex structure of macrophytes can lower the predatory pressure by reducing predator feeding efficiency [12,23], and protects cladocerans against hydrological disturbance such as rainfall and high water velocity [24]. Additionally, the fecundity of cladocerans can increase because of a relatively higher availability of food in the complexly vegetated habitat, which results in the active selection of complex macrophyte species as cladoceran habitat [25].

The majority of studies that have focused on the interactions between macrophytes and cladocerans have considered the role of submerged macrophytes [17,26]. These studies argued that submerged macrophytes were capable of providing a suitable habitat for cladocerans (e.g., daphniids) due to an increase of refuge space in the water. In the past decade, in contrast to the previous examples, the importance of free-floating macrophytes has been emphasized in subtropical lagoons [27]. Plant-attached cladoceran species with a high biomass were also often found on floating-leaved macrophytes [28]. Unfortunately, the pattern of macrophyte utilization by plant-attached cladocerans is still unclear, and their abundance is usually underestimated. The information currently available regarding surface-dwelling macrophytes (i.e., free-floating and/or floating-leaved plants) is also focused on their utilization by mainly pelagic cladocerans [29,30]. In shallow water ecosystems, where macrophytes frequently dominate, the diversity of cladocerans is largely affected by the presence of plant-attached cladoceran species [18,31,32]. Previous research on plant-attached cladoceran distribution, which has focused on explaining causal relationships among macrophytes, plant-attached cladocerans, and other environmental factors (such as predation and food availability), has provided evidence for the importance of macrophyte type.

The primary objective of our study is to investigate the pattern of distribution of two different cladoceran species—a plant-attached species *Simocephalus vetulus* and a pelagic species *Daphnia obtusa*—associated with different morphological types of macrophyte as potential drivers of their distribution. We simultaneously considered food availability as well as the presence of predation. Three different approaches were taken in the study: (1) Field monitoring to identify the distribution patterns of the two cladoceran species in wetland ecosystems; (2) stable isotope analysis to verify the dependence of cladoceran species on food resources; and (3) microcosm experiment to elucidate the relationships among macrophytes, predation risk, and food availability. Furthermore, the significance of the coexistence of cladoceran species with different habitat preferences is discussed.

2. Materials and Methods

2.1. Study Sites and the Selection of Cladoceran Species

The wetlands monitored in this study are located in Southeastern South Korea. This area has more than 160 wetlands and diverse zooplankton species that inhabit the wetlands [33]. The main purpose of this study is to compare the distribution pattern of plant-attached and pelagic cladoceran species in association with macrophytes. We studied a database cataloging macrophyte development status and zooplankton species distribution in the wetlands to identify appropriate wetlands for the study. On the basis of a previous study [33], the two cladoceran species *S. vetulus* (plant-attached) and *D. obtusa* (pelagic) were most frequently reported. We found some wetlands that supported both species contemporarily and had excessively developed macrophytes. On the basis of this data, we finally selected three wetlands (Mokpo, Gahang, and Palak, Wetlands; Figure 1). The wetlands are very shallow and the surface area is almost completely covered by various macrophytes. The sizes of

wetlands are 1.84, 0.56, and 1.06 ha, respectively, and they are used as an agricultural water supply. The littoral zones were shallow (depth ranged between 0.5 and 0.8 m), and the central areas were deeper (depth ranged between 1.2 and 1.6 m). It has been reported that *Phragmites australis*, *Paspalum distichum*, *Zizania latifolia*, *Spirodela polyrhiza*, *Salvinia natans*, *Trapa japonica*, *Ceratophyllum demersum*, and *Hydrilla verticillata* are present in the study sites and developed in the littoral area. Figure 2 illustrates the schematic diagram of the current study.

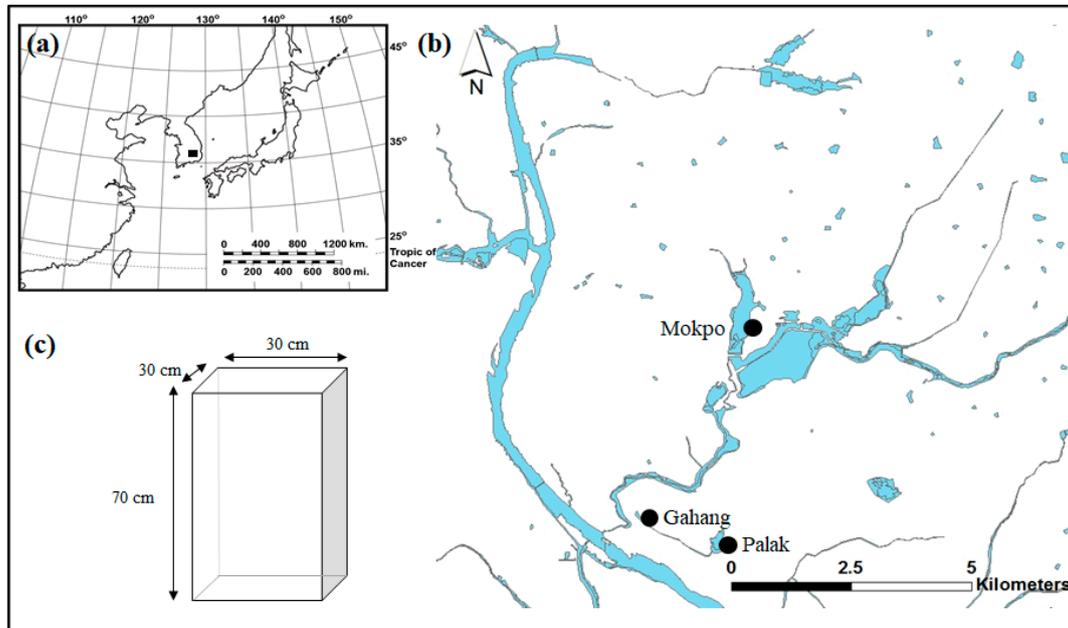


Figure 1. Map of the study sites. The sites located in southeastern South Korea are indicated by solid squares (■). (a) The Korean Peninsula, (b) the study sites (Mokpo, Gahang, and Palak Wetlands), and (c) water sampler design (water column of 20-L and size 20 × 30 × 70 cm). The sampling locations are identified as solid circles (●). The wetlands have shallow depths and are covered by various aquatic plant species.

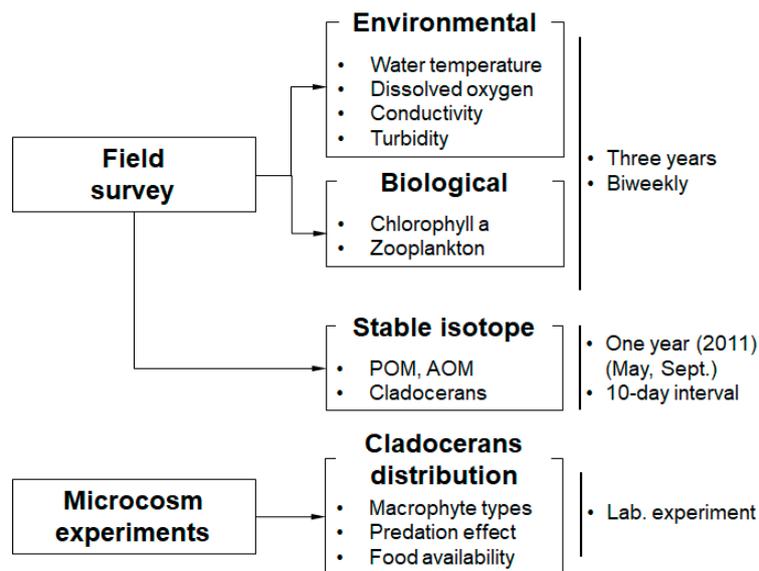


Figure 2. Schematic flowchart of the current study. Particulate organic matter (POM), and attached organic matter (AOM).

2.2. Field Data Collection

We established three locations for each wetland to collect water samples, and three sampling points per wetland site were monitored throughout the study period (March 2009–February 2012; biweekly interval). We also collected samples from the surface and the bottom of the water. Therefore, one sampling set produced a total of 18 water samples. Physicochemical parameters (water temperature, % saturation of dissolved oxygen, conductivity, pH, and turbidity) and biological factors (chlorophyll a) were measured from the water samples. A DO meter (Model 58, YSI Inc., Yellow Springs, OH, USA) was used to measure the water temperature and dissolved oxygen, and conductivity and pH were measured using a conductivity meter (Model 152, Fisher Scientific, Hampton, NH, USA) and pH meter (Orion Model 250A, Orion Research Inc., Boston, MA, USA), respectively. The water samples were taken to the laboratory to measure the concentration of chlorophyll a and turbidity. Turbidity was measured using a turbidimeter (Model 100B, HF Scientific Inc., Ft. Myers, FL, USA). The water samples were filtered through a mixed cellulose ester (MCE) membrane filter (Advantech; Model No., A045A047A; pore size, 0.45 μm), and the filtrate was used to determine the concentration of chlorophyll a based on Wetzel and Likens [34].

To obtain *S. vetulus* and *D. obtusa*, water from the surface and bottom was collected using a 20 L column sampler (length, 20 cm; width, 30 cm; height 70 cm). A total of 10 L of water was filtered through a plankton net (60 μm mesh) at each of the study points, and immediately fixed with formaldehyde (final concentration, ca 5%). The *S. vetulus* and *D. obtusa* were identified and counted using a microscope (ZEISS, Model Axioskop 40; $\times 200$ magnification), based on the classification key of Mizuno and Takahashi [35].

2.3. Stable Isotope Analysis

Stable isotope analysis was conducted to investigate the potential food source of cladoceran species. Particulate organic matter (POM, i.e., free or uncomplexed organic matter $>50 \mu\text{m}$; predominantly phytoplankton), attached organic matter (AOM, organic matter $>50 \mu\text{m}$ attached to stem and leave of aquatic macrophytes; predominantly periphytic diatom), and the two cladoceran species were sampled in the spring (May) and autumn (September) of 2011 during the study period. We conducted three samplings at each of the sampling points per month (i.e., in addition to the regular monitoring program; ca 10-day interval). We collected 5 L surface water samples ($n = 4$) per sampling. To process the POM samples, first, any micro- or macroinvertebrates were removed using a plankton net (32 μm mesh size), then the water samples were filtered through GF/F glass-fiber (pre-combusted at 500 $^{\circ}\text{C}$ for 2 h). The surface or submerged parts of the macrophytes (free-floating, floating-leaved, and submerged macrophyte species) and bottom substrate present at the study points were gently brushed in a tank filled with distilled water, in order to retain the AOM. Similar to the POM processing step, micro- or macroinvertebrates were also removed using the plankton net (32 μm mesh size).

POM and AOM samples were treated with 1 mol L⁻¹ HCL to remove any inorganic carbon. The samples were then rinsed with deionized distilled water to remove the acid. All samples were freeze dried and then ground with a mortar and pestle. All the powdered samples were maintained frozen (-70°C) until analysis. Carbon and nitrogen isotope ratios were determined using continuous-flow isotope mass spectrometry (CF-IRMS, model-ISOPRIME 100; Micromass Isoprime, GV Instruments Ltd., Manchester, UK). Prior to their analysis, the samples were then placed overnight in a sealed CF-IRMS through which 99.999% He was flowing at a few mL/min. Instrument linearity (dependence of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ on signal amplitude at the collectors) was tested daily and confirmed to be $<0.03\text{‰}/\text{nA}$ over the 1–10 nA range. $100 \pm 10 \mu\text{g}$ silver-encapsulated cellulose samples (no carbon added to samples inside capsules), producing approximately 4–6 nA signal at the collectors, were loaded in a 99-position zero-blank CF-IRMS and converted to a mixture of carbon monoxide, carbon dioxide, water, and hydrogen gases over glassy carbon chips in a quartz tube at 1080 $^{\circ}\text{C}$, within a stream of 99.999% carrier Helium flowing at 110 mL/min. Data are expressed as the relative per mil (‰)

difference between the sample and the conventional standards of Pee Dee Belemnite carbonate (PDB) for carbon and atmospheric N₂ for nitrogen, according to the following equation:

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

where X is ¹³C or ¹⁵N and R is the ¹³C:¹²C or ¹⁵N:¹⁴N ratio. A secondary standard of known relationship to the international standard was used as a reference material. The standard deviations of δ¹³C and δ¹⁵N for 20 replicate analyses of the Peptone (δ¹³C = −15.8 ‰ and δ¹⁵N = 7.0 ‰, Merck) standard were ±0.1 and ±0.2 (‰), respectively.

2.4. Microcosm Experiment

We performed separate microcosm experiment in order to further examine the effect of life form of macrophyte species (floating, floating-leaved, and submerged macrophyte), effect of fish predation risk, and availability of food sources on the two cladoceran species (*S. vetulus* and *D. obtusa*). We prepared rectangular parallelepiped glass tanks as microcosms (approximately 126 L in total volume; Figure 3). Since the experiment dealt with plant-attached cladocerans, the material of the tanks was critical. To prevent continuous attachment of *S. vetulus*, we constructed the edge of the tanks with a very smooth material.

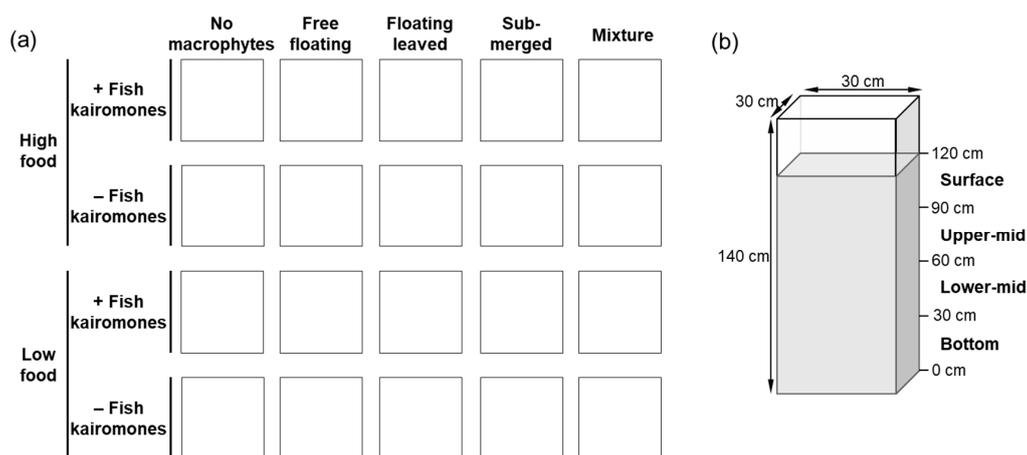


Figure 3. Design of the microcosm experiment. Three factors—macrophyte type, presence of fish predation, and food availability—were simultaneously considered to investigate the distribution of the two cladoceran species at four depths. (a) Allocation of experimental groups, (b) shape of microcosm tank, length, 30 cm; width, 30 cm; height 140 cm. The microcosm tanks were filled with Elendt M4 medium up to 120 cm, and the medium-filled space was separated into four layers according to depth.

Different types of macrophytes, including *Salvinia Natans*, *Trapa japonica*, and *Ceratophyllum demersum*, were transplanted into the experimental tanks. These macrophyte species are characterized by their morphological plasticity to represent each life form of macrophytes (free-floating, floating-leaved, and submerged macrophytes). In addition, these species of macrophytes are ubiquitous in the lentic freshwater ecosystems of South Korea. A total of five conditions—(1) no macrophytes, (2) free-floating, (3) floating-leaved, (4) submerged, and (5) mixture (three macrophytes species simultaneously)—were established using these macrophyte species. Free-floating macrophytes and leaves of floating-leaved species were present on the surface of the water (stems of the floating-leaved were allowed to extend to the bottom). The submerged macrophytes were placed near the bottom of the microcosm. The placement of the macrophytes in each of the experimental treatments was based on the measured biomass (wet weight) of the macrophytes and the number of individuals. We used same amount of macrophyte biomass for each of the experimental microcosm (free-floating, 30 g; floating-leaved, 25 g; submerged, 35 g; and mixture, 10 g of each macrophytes species). For each condition, either the presence or absence of the predation effect and availability of food source was applied.

For the fish treatment, we collected bluegill sunfish (*Lepomis macrochirus*, approximately 3–4 cm in length) from the Mokpo Wetland, and acclimated the fish in ElenDt M4 medium [36] for 24 h. To maintain the concentration of the chemical cue, we used 50 fish per 108 L of medium for each replication. The fish-exposed M4 medium was then filtered using a 30- μ m mesh net (different from the plankton net) to remove particulate matters. The experimental tanks assigned as “fish presence” were filled with this fish-exposed M4 medium, while we used clear M4 medium for the “fish absence” tanks. In the case of food availability, we provided *Chlorella vulgaris* as the food source for the cladocerans inhabiting the experimental tanks (M4 medium environment). The food phytoplankton species was maintained in an Eyela 2000T growth chamber at 10 °C, 5000 lux illumination, and 12L:12D light-dark cycle. The organic carbon content, calculated from the cell volumes of *C. vulgaris*, was considered in determination of the dose of food source [37]. We established two different conditions regarding food availability, i.e., high (2.5 mg carbon L⁻¹) and low (0.5 mg carbon L⁻¹), by providing different amounts of *C. vulgaris* to the cladocerans. The carbon content of the *C. vulgaris* cells was calculated based on Strathman [37].

The experimental tanks were filled with ca 99 L of ElenDt M4 medium, which was approximately 2/3 of the total tank volume (108 L). The microcosm experiment was performed in zooplankton incubators. The temperature and light conditions were maintained at 15 °C and 16L:8D to represent spring or autumn seasons in Korea. To represent the light conditions in the natural ecosystem, 20 photons were provided to the microcosms. To prevent lateral penetration of light, we sealed the microcosm tanks completely with black sheets. We divided the tanks into four different depths to examine the distribution pattern of *S. vetulus* and *D. obtusa*. The first 30 cm from the surface was defined as the “Surface Layer,” and the immediately subtending 30 cm layer was the “Upper-mid Layer,” the next 30 cm subtending layer was defined as the “Lower-mid layer,” and the final 30 cm was defined as the “Bottom Layer.” The following was the macrophytes placement: the free-floating and the floating-leaved species were located in the Surface Layer while the submerged macrophytes were in the Bottom Layer. A total of 100 individuals of each cladoceran species were used in the experimental tanks, and the experiment was separately conducted in accordance with the cladoceran species. For the cladoceran species collected from the Upo Wetlands, we used the next generation of the collected cladoceran individuals in the microcosm experiment. A total of ten replicates were used for each experimental treatment. We had sufficient acclimatization time (3h) after injection of the cladoceran individuals into each experimental treatment. We were certain that each microcosm was on the same trajectory or behaving in the same way prior to the introduction of the treatments. The individuals of the two cladoceran species were counted immediately after the black sheet was carefully removed from the microcosm tanks.

2.5. Data Analysis

We calculated the statistical difference of environmental variables between the surface and bottom layers by means of Student’s t-test. The test was conducted in accordance with wetlands and seasons. In addition, we determined the statistical difference between isotopic signals using the Student’s t-test. The following cases were compared: (1) Environmental variables and the two cladoceran species, (2) AOM and POM, and (3) the season (spring and autumn).

We used one-way nested ANOVA ($\alpha = 0.05$) to analyze the statistical differences of the vertical distribution of cladoceran individuals’ in association with different treatments. As we established ten replicates for each experimental treatment, direct application of on-nested ANOVA may cause pseudoreplication problem (i.e., data homogeneity between sampling points should be ensured; [38]). Therefore, we set the depth layer in each of the microcosm tanks as the primary factor and the ten replication were regarded as nested subgroups. The null hypothesis for every treatment was “there was no difference in cladoceran individuals according to depth,” meaning that all of the cladoceran individuals would be distributed evenly in every experimental tank (i.e., the ratio of individuals in Surface, Upper Middle, Lower Middle, and Bottom layers = 1:1:1:1). Statistical significance indicated that cladoceran individuals were not distributed evenly with depth. The tendency of aggregation could be detected by comparing the number of individuals found in each of the layers.

3. Results

3.1. Limnological Characteristics and the Distribution of the Two Cladoceran Species

Table 1 lists the environmental variables measured at the study sites. Water temperature exhibited the strongest seasonality; it was the highest in the summer (surface, $25.6 \pm 2.4\%$; bottom, $24.8 \pm 2.3\%$) and lowest in the winter (t-test, $p < 0.05$). The % saturation of dissolved oxygen varied depending on the temperature; summer (surface, $38 \pm 27.8\%$; bottom, $32 \pm 26.1\%$) and autumn (surface, $34 \pm 26.2\%$; bottom, $28 \pm 19.1\%$) showed low saturation, whereas it was relatively high in spring (surface, $101 \pm 38\%$; bottom, $96 \pm 25.7\%$) and winter (surface, $84 \pm 31.8\%$; bottom, $86 \pm 27.3\%$). Turbidity was the highest in autumn (surface, $56 \pm 36.8\%$; bottom, $43 \pm 32.7\%$), but the average turbidity of each of the other seasons was similar. Chlorophyll a was below $20 \mu\text{g/L}$ from spring to autumn, and was high in winter (surface, $39 \pm 25.6\%$; bottom, $32 \pm 23.5\%$). Conductivity and pH did not show a distinct seasonal variation.

Table 1. Seasonal variation of environmental variables measured in surface and bottom layer at each wetland. WT, water temperature ($^{\circ}\text{C}$); DO, %saturation of dissolved oxygen; Cond., conductivity ($\mu\text{S cm}^{-1}$); Tur., turbidity (NTU); Chl.a, chlorophyll a ($\mu\text{g L}^{-1}$). Each season consisted of three months: Spring, March to May; summer, June to August; autumn, September to November; winter, December to next February. Mp, Mokpo; Gh, Gahang; Pl, Palak Wetland.

Sites	Variable	Spring (n = 15)		Summer (n = 18)		Autumn (n = 18)		Winter (n = 18)	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Mp	WT	16.3 ± 5.1	16.1 ± 4.2	25.6 ± 2.4	24.8 ± 2.3	16.1 ± 5.8	15.9 ± 4.9	4.9 ± 1.7	4.6 ± 4.6
	DO	101 ± 38	96 ± 25.7	38 ± 27.8	32 ± 26.1	34 ± 26.2	28 ± 19.1	84 ± 31.8	86 ± 27.3
	Cond.	408 ± 134	435 ± 125	420 ± 152	428 ± 169	361 ± 81	349 ± 101	483 ± 131	452 ± 147
	pH	7.8 ± 0.4	6.9 ± 0.2	7.1 ± 0.5	6.7 ± 0.6	7.2 ± 0.5	6.9 ± 0.4	7.7 ± 0.3	7.2 ± 0.6
	Tur.	36 ± 14.6	25 ± 18.9	27 ± 14.8	21 ± 10.5	56 ± 36.8	43 ± 32.7	39 ± 25.6	32 ± 23.5
	Chl. a	13.8 ± 4.6	5.3 ± 1.7	18 ± 5.4	9.4 ± 0.4	18 ± 3.2	8.4 ± 1.3	32 ± 2.6	18 ± 1.7
Gh	WT	16.7 ± 5.7	16.3 ± 4.3	25.9 ± 1.6	25.4 ± 2.1	16.9 ± 5.8	16.4 ± 5.2	3.4 ± 2.6	3.6 ± 1.4
	DO	96 ± 40.3	82 ± 34.5	26 ± 11.6	21 ± 10.4	35 ± 17.1	27 ± 20.3	73 ± 37.4	69 ± 31.5
	Cond.	341 ± 66.7	375 ± 72.4	320 ± 243	336 ± 264	275 ± 156	286 ± 196	384 ± 246	402 ± 351
	pH	7.6 ± 0.4	7.1 ± 0.6	6.8 ± 0.4	6.1 ± 0.8	6.9 ± 0.6	6.8 ± 0.4	7.8 ± 0.4	7.3 ± 0.5
	Tur.	26 ± 14.6	20 ± 16.4	28 ± 20.6	24 ± 18.2	31 ± 21.2	24 ± 16.3	28 ± 13.4	23 ± 14.6
	Chl. a	25 ± 2.3	17 ± 2.3	36 ± 4.1	18 ± 2.7	31 ± 2.7	25 ± 5.1	43 ± 4.1	25 ± 2.5
Pl	WT	17.3 ± 5.6	17.5 ± 4.8	26.8 ± 1.8	26.4 ± 1.6	18.2 ± 5.9	18.0 ± 5.7	4.7 ± 2.7	4.6 ± 2.5
	DO	97 ± 62.3	91 ± 56.4	20 ± 31.2	16 ± 25.1	23 ± 14.3	20 ± 16.1	90 ± 43.2	84 ± 46.7
	Cond.	289 ± 117	296 ± 135	238 ± 118	256 ± 138	269 ± 184	287 ± 167	348 ± 216	358 ± 187
	pH	7.4 ± 0.6	7.2 ± 0.8	6.9 ± 0.4	7.1 ± 0.8	7.3 ± 0.3	7.5 ± 0.7	7.7 ± 0.4	7.4 ± 0.8
	Tur.	29 ± 17.1	23 ± 15.4	27 ± 14.8	23 ± 17.3	40 ± 14.7	34 ± 17.8	31 ± 24.7	27 ± 12.8
	Chl. a	14 ± 3.4	9.1 ± 2.6	32 ± 3.5	18 ± 2.7	27 ± 4.8	13 ± 2.4	33 ± 3.7	31 ± 2.7

Water temperature, dissolved oxygen, conductivity, and pH showed minor variation between the surface and the bottom layers. In contrast, a higher magnitude of turbidity and chlorophyll a was recorded on the surface of the water. Among the environmental variables, physicochemical parameters (water temperature, dissolved oxygen, conductivity, pH, and turbidity) were not significantly different between the surface and bottom layer, but the biological factors (chlorophyll a) were significantly different between the surface and bottom layers (t-test, $p < 0.01$; see Table 2).

In most cases, a higher density of zooplankton was found in summer; the wetlands were dominated by *Alona rectangula*, *Chydorus sphaericus*, and *Diaphanosoma brachyurum*. The growing seasons (i.e., spring and autumn) were dominated by *S. vetulus* and *D. obtusa*, and the density of the remaining zooplankton species was very low (approximately 13% of total zooplankton).

During the study periods, *S. vetulus* and *D. obtusa* appeared contemporaneously at all of the study sites (Figure 4). They were mainly observed in the late spring (Apr. to Jun.) and autumn (Sept. to Nov.), with similar densities. However, *S. vetulus* and *D. obtusa* showed different spatial distribution. *S. vetulus* was present in densities on the surface, whereas it was relatively scarce in the bottom layer. In contrast, *D. obtusa* was abundant in the bottom layer but present in relatively low

densities at the surface. This distribution pattern of the two cladoceran species was consistent in every study site. The majority of the environmental variables did not have a significant influence on the changes in distribution of the two cladoceran species. Several variables, including temperature and chlorophyll a, showed a significant effect; however, the relationship was not common to all the study sites, and therefore probably anecdotal.

Table 2. *t*-test results to compare difference of environmental variables between surface and bottom layer. WT, water temperature (°C); DO, percent of (%) dissolved oxygen; Cond., conductivity (µS/cm); Tur., turbidity (NTU); Chl.a, chlorophyll a (µg/L). d.f, degrees of freedom. Mp, Mokpo; Gh, Gahang; Pl, Palak Wetland.

Sites	Variable	Spring (n = 15)			Summer (n = 18)			Autumn (n = 18)			Winter (n = 18)		
		t	d.f	p									
Mp	WT	0.29	28	0.77	0.48	36	0.63	-0.41	36	0.68	0.53	36	0.60
	DO	-0.86	28	0.39	-0.73	36	0.49	-0.59	36	0.57	-0.64	36	0.54
	Cond.	0.56	28	0.58	0.42	36	0.67	0.67	36	0.51	0.58	36	0.56
	pH	-0.97	28	0.35	-0.62	36	0.55	-0.48	36	0.63	-0.31	36	0.74
	Tur.	1.25	28	0.19	1.18	36	0.23	0.86	36	0.40	1.17	36	0.25
	Chl. a	4.26	28	0.00	3.95	36	0.00	4.12	36	0.00	4.35	36	0.00
Gh	WT	0.43	28	0.67	0.58	36	0.59	0.52	36	0.59	0.45	36	0.63
	DO	-0.67	28	0.53	-0.76	36	0.50	-0.73	36	0.48	-0.67	36	0.53
	Cond.	0.38	28	0.71	0.47	36	0.63	0.45	36	0.63	0.52	36	0.63
	pH	-0.85	28	0.43	-0.93	36	0.38	-0.76	36	0.49	-0.68	36	0.54
	Tur.	1.07	28	0.28	1.23	36	0.21	1.18	36	0.21	1.24	36	0.21
	Chl. a	4.61	28	0.00	5.31	36	0.00	5.68	36	0.00	4.83	36	0.00
Pl	WT	0.36	28	0.70	0.44	36	0.68	0.46	36	0.63	0.51	36	0.61
	DO	-0.75	28	0.48	-0.65	36	0.55	-0.72	36	0.51	-0.63	36	0.56
	Cond.	0.66	28	0.51	0.78	36	0.48	0.74	36	0.52	0.74	36	0.50
	pH	-0.89	28	0.41	-0.85	36	0.44	-0.85	36	0.44	-0.78	36	0.48
	Tur.	1.26	28	0.20	1.17	36	0.24	1.34	36	0.12	1.37	36	0.13
	Chl. a	4.66	28	0.00	5.12	36	0.00	5.48	36	0.00	4.85	36	0.00

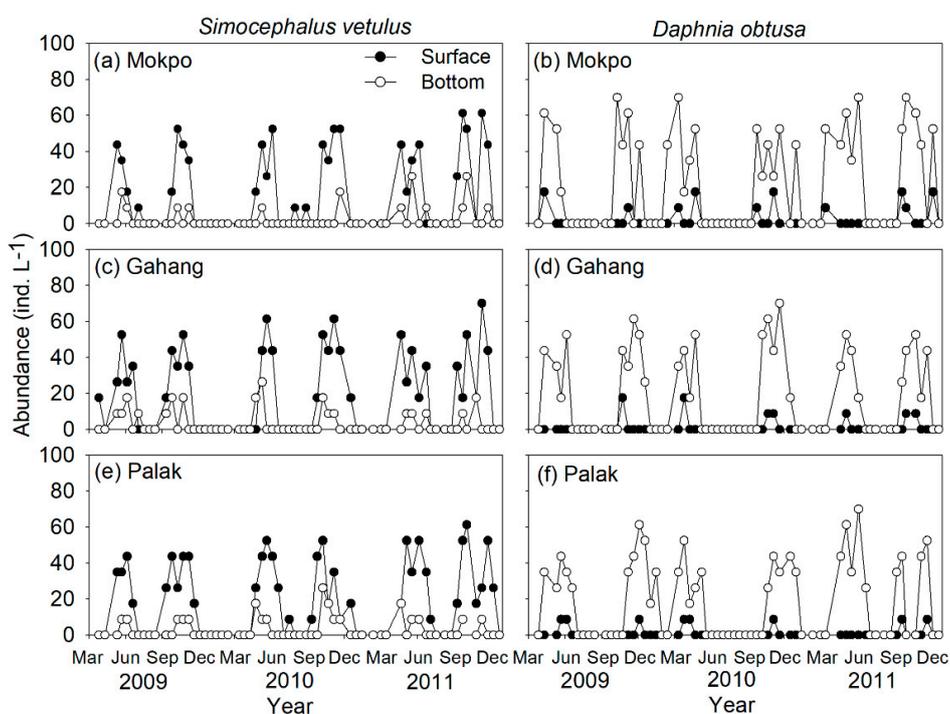


Figure 4. Spatial and temporal distribution pattern of *Simocephalus vetulus* and *Daphnia obtusa* at each study site during the study period (March 2009 to February 2012; three years). Panels (a), (c), (e) show changes in *S. vetulus* distribution in the surface and bottom of Mokpo, Gahang, and Palak wetlands. Panels (b), (d), (f) show the same for *D. obtusa*.

3.2. Influence of POM and Attached Microalgae on the Two Cladoceran Species

The $\delta^{13}\text{C}$ values of AOM were higher than those of POM in all sites and in both seasons (Figure 5). The $\delta^{13}\text{C}$ values of AOM and POM were not significantly different between study sites (AOM, $t = -0.5$, $p = 0.69$; POM, $t = -0.3$, $p = 0.73$), however they were significantly higher in autumn than in spring (AOM, $t = -3.8$, $p = 0.03$; POM, $t = -4.3$, $p = 0.01$). The $\delta^{13}\text{C}$ values of POM from the water in the surface layer were lower than those of the benthic POM. The AOM from macrophytes (mainly from free-floating or floating-leaved species) was lighter than the benthic AOM. AOM from submerged species could not be detected. In contrast to the $\delta^{13}\text{C}$ pattern, $\delta^{15}\text{N}$ did not show any distinct pattern in relation to depth or seasons in all the study sites.

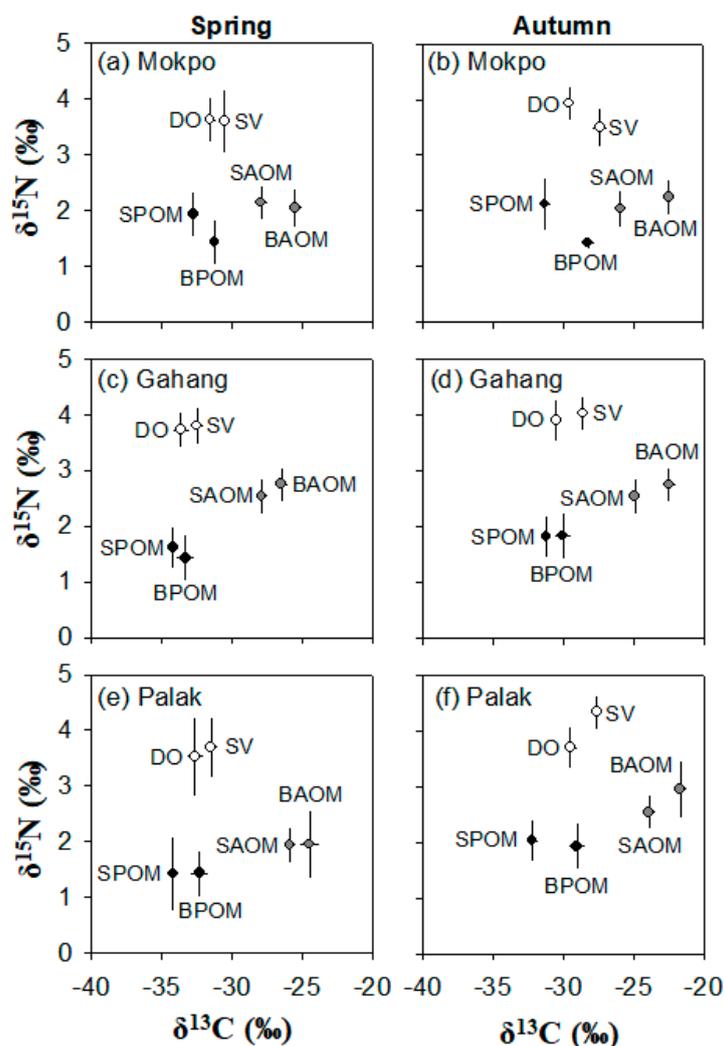


Figure 5. Carbon and nitrogen isotope plots at three wetlands from spring and autumn, 2011. Each symbol represents the mean signature of samples; error bars indicate ± 1 standard deviation ($n = 4$ or 5). Closed symbols are for the samples collected from the water surface, open symbols are for samples collected from the bottom. Surface particulate organic matter (SPOM); bottom particulate organic matter (BPOM); surface attached organic matter (SAOM); bottom attached organic matter (BAOM); *Simocephalus vetulus* (SV); *Daphnia obtusa* (DO).

In all the study sites, the $\delta^{13}\text{C}$ values of *S. vetulus* and *D. obtusa* in autumn were significantly higher than those in spring ($t = -3.6$, $p = 0.04$), which correspond to the seasonal changes in $\delta^{13}\text{C}$ of POM and AOM. However, site-dependent differences in *S. vetulus* and *D. obtusa* $\delta^{13}\text{C}$ were not significant. The $\delta^{13}\text{C}$ values for *D. obtusa* were significantly lower than those for *S. vetulus* ($t = -2.8$, $p = 0.04$). This aspect was common to all the study sites in both seasons. The $\delta^{15}\text{N}$ values of *S. vetulus* and *D. obtusa* did not show any statistically significant differences according to sites and seasons.

On the basis of these results, it was reasoned that the examined cladoceran species were largely dependent on POM rather than AOM. *D. obtusa* tended to depend exclusively on POM. Similarly, *S. vetulus* also depended on POM, but AOM also made a marginal contribution to isotope signatures of *S. vetulus*. The $\delta^{13}\text{C}$ of *D. obtusa* was consistently lower than that of *S. vetulus*, and *D. obtusa* was located closer to the POM in terms of $\delta^{13}\text{C}$ signature. This distribution pattern was observed in all study sites and in both seasons.

3.3. Spatial Distribution of the Two Cladoceran Species in the Microcosm Experiment

In the microcosm experiment, *S. vetulus* and *D. obtusa* showed different spatial distribution patterns in each treatment. The presence of macrophytes affected the distribution of the cladoceran species (Figure 6, Table 3). In the case of *S. vetulus*, statistically significant vertical distribution was found when macrophytes were present. Additionally, presence of the predation effect facilitated aggregation of *S. vetulus* individuals in the surface layer. In contrast, all the treatments induced significant vertical distribution of *D. obtusa*.

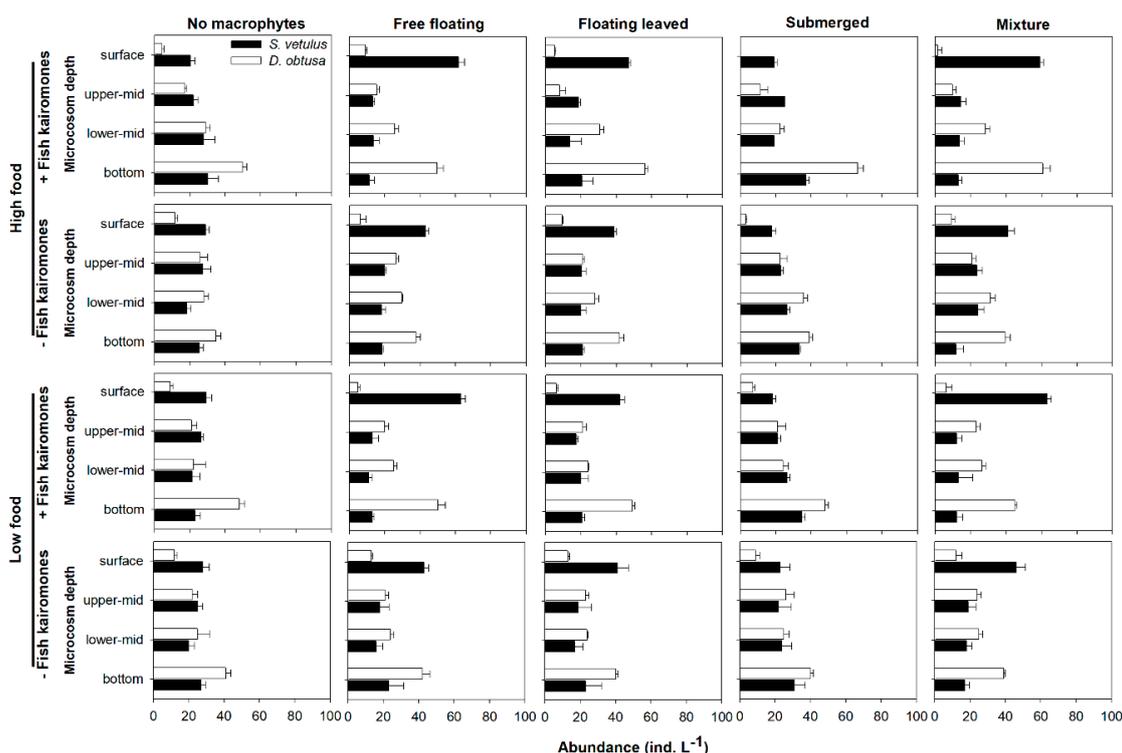


Figure 6. The vertical distribution of *Simocephalus vetulus* and *Daphnia obtusa* in the microcosm experiment, in relation to different treatments (macrophyte type, fish presence, and food availability in each of microcosm experiment).

Table 3. One-way nested ANOVA results for the effects of main groups (d.f, 3; shown as layer in the table; surface, upper, lower, and bottom) and subgroups (d.f, 9; shown as replicates in each of microcosm experiment) on two Cladocera species in association with three factors (macrophyte types, fish kairomones, and food availability in microcosm experiment). Pre., presence; ab. absence; Rep. replicates. Bold letter indicate values that are statistically significant.

Food	Fish	Species	Variance	Macrophytes									
				No		Free-Floating		Floating-Leaved		Submerged		Mixture	
				F	P	F	P	F	P	F	P	F	P
High	Pre.	<i>S. vetulus</i>	Layer	2.51	0.20	231.5	0.00	51.41	0.00	3.56	0.86	198.1	0.00
			Rep.	0.11	0.95	0.22	0.90	0.13	0.94	0.20	0.91	0.34	0.86
		<i>D. obtusa</i>	Layer	113.2	0.00	104.3	0.00	104.2	0.00	0.11	0.00	295.1	0.00
			Rep.	0.25	0.88	0.15	0.93	0.12	0.95	0.27	0.89	0.09	0.99
	Ab.	<i>S. vetulus</i>	Layer	1.83	0.45	18.5	0.00	4.92	0.00	3.14	0.07	26.8	0.00
			Rep.	0.14	0.92	0.28	0.89	0.18	0.92	0.16	0.92	0.17	0.92
		<i>D. obtusa</i>	Layer	3.21	0.06	23.4	0.00	36.1	0.00	67.4	0.00	30.2	0.00
			Rep.	0.12	0.95	0.13	0.94	0.21	0.90	0.01	0.99	0.13	0.94
Low	Pre.	<i>S. vetulus</i>	Layer	1.48	0.54	241.3	0.00	5.52	0.00	3.18	0.06	174.3	0.00
			Rep.	0.09	0.96	0.35	0.86	0.13	0.94	0.19	0.93	0.2	0.91
		<i>D. obtusa</i>	Layer	80.1	0.00	123.4	0.00	98.3	0.00	90.1	0.00	71.3	0.00
			Rep.	0.49	0.78	0.31	0.84	0.27	0.90	0.35	0.86	0.16	0.93
	Ab.	<i>S. vetulus</i>	Layer	1.94	0.39	38.4	0.00	21.6	0.00	2.64	0.18	5.57	0.00
			Rep.	0.14	0.94	0.16	0.94	0.12	0.95	0.26	0.88	0.34	0.85
		<i>D. obtusa</i>	Layer	26.7	0.00	28.4	0.00	14.7	0.00	29.7	0.00	6.42	0.00
			Rep.	0.21	0.90	0.12	0.95	0.23	0.89	0.31	0.87	0.20	0.91

S. vetulus responded more strongly to the types of macrophytes present. This species was distributed almost uniformly with depth in the “No macrophytes” treatment. However, the presence of “floating macrophyte” or “floating-leaved macrophyte” induced more individuals of *S. vetulus* to aggregate on the surface layer (0 to 30 cm). This pattern was also found in the “mixture” treatment. In contrast, the “submerged macrophyte” treatment attracted a small number of *S. vetulus* individuals to the bottom layer, but the effect was much smaller than that for the surface-dwelling macrophyte. In contrast, *D. obtusa* tended to aggregate in the bottom layer even though there was a slight difference in the distribution of individuals in relation to the type of macrophyte.

When the availability of food and presence of predators were considered simultaneously, both the cladocerans responded strongly to the types of macrophytes available in the experimental microcosms. Even though the general pattern of distribution was largely determined by the types of macrophytes, cladoceran species were affected by the presence of predators. The fish predation condition seemed to stimulate the aggregation of *S. vetulus* on the surface when the free-floating macrophyte was available in the microcosm. This pattern was also observed in the “mixture” treatment. Even though the “floating-leaved” treatment resulted in more individuals in the surface layer compared to the “no macrophytes” treatment, it was to a lesser degree compared to the “free-floating” or “mixture” treatments. In the case of the “submerged” treatment, a minor increase of *S. vetulus* individuals in the bottom layer was detected under the fish predation condition. The individuals of a species tended to disperse when fish predation was not a factor. Interestingly, the availability of food affected the distribution pattern of *S. vetulus*. They were more abundant in the middle layer in the “low food” compared to the “high food” treatment.

The other cladoceran species, *D. obtusa*, exhibited a different pattern of distribution compared to *S. vetulus*. The presence of fish predation resulted in a positive tendency for bottom aggregation; however, this pattern weakened under the “fish absence” condition. The “submerged” treatment attracted *D. obtusa* individuals to concentrate in the bottom layer, which was also observed in the “mixture” treatment. Although food availability did not have a clear influence on *D. obtusa* distribution, they were more dispersed, like *S. vetulus*, under the “low food” treatment compared to the “high food” treatment.

4. Discussion

4.1. The Impact of Macrophytes on Cladoceran Species Distribution

Even though *S. vetulus* and *D. obtusa* occur contemporaneously at the study sites, they occupy different depths. In the wetlands, *S. vetulus* tended to reside in the surface layer rather than the bottom and *D. obtusa* utilized the bottom layer as its primary habitat. Species of macrophytes such as *S. polyrhiza*, *S. natans*, and *T. japonica* dominated the aquatic vegetation on the surface of the water, whereas submerged macrophyte species (*Ceratophyllum demersum* and *Hydrilla verticillata*) developed the macrophyte bed on the bottom layer. It is known that submerged macrophytes are an important habitat for small animals (e.g., fish or some macro-invertebrates, see [39,40]); they are a particularly suitable habitat for cladoceran species in freshwater ecosystems [41,42]. The complex structure and large volume of space occupied by submerged macrophytes can conspicuously decrease the foraging activity of predators such as fish [12].

However, it was difficult to observe the plant-attached cladoceran species in the submerged macrophyte bed in the current study. The majority of pelagic cladoceran species utilize submerged macrophytes as their primary habitat and free-floating macrophytes used as an alternative refuge when submerged macrophytes were not sufficiently presented [43]. A few studies have reported that plant-attached cladocerans often inhabit stands of floating-leaved macrophytes with high biomass [28]. Even though submerged macrophytes provide a microhabitat with complex structure, they are more easily agitated by wind and water currents than surface-dwelling plants (i.e., free-floating or floating-leaved macrophytes; [44]). This allows plant-attached cladocerans to use surface-dwelling macrophytes. Furthermore, free-floating macrophytes provide a complex network of root systems that are able to hold large amounts of food, which attracts some of the cladoceran species [45]. The greater abundance of potential food sources (phytoplankton represented by chlorophyll a) on the water surface than on the bottom layer would elucidate the circumstance in the current study.

On the basis of the aforementioned relationship, we can infer that the coexistence of different types of macrophytes has led to the high diversity of cladoceran species. Plant-attached cladoceran species exploit the food sources from surface, from the macrophytes' stems and leaves. Therefore, macrophytes that form stable structures and have large surface areas are advantageous to plant-attached cladoceran species. In contrast, pelagic species need more space to take refuge from predators. Different requirements by various cladoceran species can be satisfied when surface-dwelling and submerged macrophytes are contemporaneously available. Consequently, the development of various macrophytes in the wetlands has brought about the coexistence of *S. vetulus* and *D. obtusa*.

4.2. Effect of Predation and Food Availability on Cladoceran Spatial Distribution

We conducted microcosm experiment to clarify the interaction between three treatments (i.e., predation effect, food availability, and macrophyte types) and the cladoceran species. The results confirm the field observations in the current study as well as evidence obtained in previous studies. The spatial distribution pattern of the two cladoceran species was largely determined by the different types of plant life forms. This pattern became more apparent when predation stress was introduced (see Figure 6). However, the availability of food sources did not bring about distinct changes, compared to the other factors.

The interaction between the types of macrophytes and predation stress can be explained as follows. Most cladoceran species avoid predation to increase their rate of survival [5,46,47]. Under potential predation stress, two species in the experiment explored the appropriate refuge. The presence of free-floating macrophytes successfully fulfilled the role of a refuge for *S. vetulus*, and submerged macrophytes did the same for *D. obtusa*. In contrast, food availability had a negligible influence in determining the individual distribution of cladocerans. It is known that cladoceran species do not explore food resources; instead they are filter feeders [48]. Even though the availability of food sources

is a crucial factor for population growth, its availability in the entire water body is not a deterministic factor. Rather, localized food availability where cladocerans are aggregated is crucial for their survival.

The changes in distribution pattern in relation to environmental factors have been considered here. The plant-attached species (i.e., *S. vetulus*) was distributed almost uniformly in the microcosm where no macrophytes were provided; however, *D. obtusa* always tended to aggregate in the bottom layer. Concurrently, Rinéberg [49] found that the daphnids were close to the bottom in an enclosure experiment. Daphnids stay in the deep layer during the day to avoid predators such as fish, and they migrate toward the surface layer to acquire food at night [50,51]. The bottom-dwelling character of *D. obtusa* would cause consistently higher number of individuals in the bottom layer in the current experiment. *S. vetulus* individuals, however, were moving continuously in the microcosm when macrophytes were not present. Since we adopted a slippery material for the microcosm tanks to prevent possible attachment to the wall, *S. vetulus* explored the relevant substrate for attachment, resulting in continuous movement.

4.3. The Role of Macrophytes Life Form for Two Cladoceran Species

The primary factors that determine the distribution of cladocerans are the presence of macrophytes and the effect of predation. The absence of macrophytes inhibits the survival of plant-attached as well as pelagic cladoceran because of their increased vulnerability to predation. It is impossible for pelagic cladoceran such as *D. obtusa* to find refuge, and the lack of a microhabitat hampers the survival of plant-attached cladoceran (e.g., *S. vetulus*) as well.

On the other hand, the presence of macrophytes can bring about some unique practical cases. Pelagic cladocerans can survive in a wetland ecosystem composed exclusively of submerged macrophytes since it is their preferred refuge. Despite the fact that plant-attached species prefer surface-dwelling macrophytes, they can also utilize submerged macrophytes as their habitat. In this case, predation pressure facilitates this pattern, i.e., gathering in the submerged macrophyte bed to avoid predation, as confirmed by our experiment. Consequently, it is expected that both pelagic and plant-attached cladoceran compete for the same food sources (i.e., POM shown in the stable isotope analysis). However, a wetland ecosystem with exclusively surface-dwelling macrophytes may lead to a different situation. Free-floating or floating-leaved macrophytes are the preferred habitat for plant-attached cladocerans (they depend more on the former type of macrophytes), and the rate of survival of the plant-attached cladocerans increases. However, pelagic cladoceran species are expected to face predation directly, which may cause the disappearance of the species. In this case, the diversity of cladocerans decreases and competition for food resources is not expected.

The optimum environment for the coexistence of the two cladoceran groups is the contemporaneous development of various types of macrophytes. On the basis of the current experiment, field survey, and stable isotope analysis results, plant-attached and pelagic cladocerans use surface-dwelling and submerged macrophytes, respectively. The spatial separation of the two different cladoceran species decreases the probability of competition for food resources, and they maintain a relatively higher rate of survival in their preferred microhabitats.

An interesting phenomenon was observed in our experimental results, that is, food resource availability did not influence the vertical distribution of cladocerans. We suspect that the availability of the preferred microhabitat and predation pressure may be more significant than the availability of food sources. If unthreatened by the lack of nutrition, the cladocerans may attempt to survive and avoid predation in their preferred habitats. The “low food supply” treatment in the experiment caused a slight increase in the number of individuals in the middle layers; however, the general distribution pattern was maintained. Therefore, it is apparent that the shortage of food did not induce vigorous movement of cladocerans. It is noted that starvation has an impact on the vertical distribution, but this was not tested in our study; therefore, the possible coaxing effect into food exploration cannot be determined. However, an increase in movement also increases the rate of detection by predators,

which in turn may threaten the survival of the population. This should be examined in a different experimental setup.

4.4. Macrophyte Utilization by the Two Cladoceran Species

In this study, we found that the two cladoceran species were distributed in different layers (surface vs. bottom) of wetlands where macrophytes had developed. There are two possible explanations for this distribution pattern. First, coexistence of the two cladoceran species was due to their mutually exclusive dependency on different macrophyte species. Their coexistence in the same wetland was possibly due to their divergent habitat preference, which led to more dispersed use of the layers of the water. Cladoceran species compete with each other for their food resources [52,53], and the seasonality of the zooplankton species is a result of an adaptation to avoid possible competition for the same food resource [54,55]. We found that two cladoceran species depended on similar food sources (i.e. POM than AOM) through stable isotope analysis. This consumption pattern implies that the presence of various macrophytes might alleviate potential food competition, allowing the two cladoceran species to coexist.

This pattern of coexistence might be influenced by predation pressure. We observed that *S. vetulus* was more abundant in the surface-dwelling macrophyte bed. This habitat preference of *S. vetulus* primarily coaxed the species into aggregating in the free-floating or floating-leaved macrophytes, and presence of predators in the wetlands promoted this aggregation pattern. A similar phenomenon was found in the *D. obtusa* distribution, but the situation was slightly different from that of *S. vetulus*. The submerged macrophyte beds strongly disrupted fish foraging [56]; therefore, the beds were a suitable refuge for pelagic cladoceran species (e.g., daphniids). The abundance of *D. obtusa* in the bottom with submerged macrophytes can be explained by the hypothesis. However, a potential food source (i.e., chlorophyll a) was largely retained on the surface rather than the bottom, and the presence of predators cornered the species into the submerged bed. On the basis of the similar density of *D. obtusa* to *S. vetulus* in the wetlands (see Figure 4), we assume that the pelagic species would select the most efficient strategy to sustain its population. In the “submerged” macrophytes treatment, the “fish presence” combination, distribution aspect of *D. obtusa* in the “low food” treatment was slightly different from that in the “high food” treatment. This implies that some individuals may attempt to move to other spaces at the risk of predation. Some previous studies maintained that the surface-dwelling macrophytes were not appropriate for pelagic species [29,30,57,58]; therefore, the small number of *D. obtusa* in the wetlands could be due to this movement pattern. We can conclude that continuous exposure to fish predation in wetlands enforce a different spatial distribution of the two cladoceran species, and the complexity of macrophyte composition supports the coexistence of various cladoceran species.

To summarize, our results clearly demonstrate that the spatial distribution of the two cladoceran species (*S. vetulus* and *D. obtusa*) is attributed to differences in the macrophyte species. Each of the cladoceran species utilized the macrophytes in distinguishable ways. Submerged macrophytes were utilized as a refuge by pelagic cladocerans (*D. obtusa*), and surface macrophytes (i.e., free-floating and/or floating-leaved macrophytes) were suitable for epiphytic cladocerans (*S. vetulus*). Even though, the two cladoceran species relied on similar food sources, the different habitats of the two cladoceran species enabled their coexistence. Macrophytes support the coexistence of Cladocera species, and play an important role in enhancing the wetland biodiversity and sustaining complex food webs. Moreover, we suggested that different habitat utilization of two cladoceran species implies that it is necessary to discover optimal macrophytes species to satisfy both aspects of esthetic and ecological function in wetlands restoration or management. The introduction of proper macrophyte species for restoring or creating wetlands, in order to not only increase biodiversity in the wetland, but also to sustain an ecologically healthy food web.

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