The influence of aquatic macrophytes on the distribution and feeding habits of two *Asplanchna* species (*A. priodonta* and *A. herrickii*) in shallow wetlands in South Korea

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ABSTRACT

We tested the hypothesis that the spatial distribution and diet composition of Asplanchna *species might be affected by the presence of aquatic macrophytes in 33 wetlands in South Korea. We estimated the densities of* Asplanchna *and other rotifer and crustacean, together with environmental parameters, in both vegetated and open water zones, from May to June 2011. In the present study, two species of* Asplanchna, A. priodonta *and* A. herrickii, *were observed and significantly more abundant in open water zones lacking macrophytes. In particular, the density of* A. priodonta *was higher than that of* A. herrickii, *and the density of* A. priodonta *was strongly positively correlated with the area of open water. In addition, gut content analysis was used to determine their dietary preferences, with the finding that there was apparent differentiation in food source utilisation between the two* Asplanchna *species;* A. priodonta *consumed some protozoa, phytoplankton, and exclusively pelagic rotifer, while* A. herrickii *consumed primarily* Euglena. *In particular,* Keratella *and* Polyarthra *were most commonly consumed by* A. priodonta *in open water. Macrophytes represent a suitable habitat for epiphytic rotifer but not for pelagic rotifer; this characteristic drives pelagic rotifer such as* Asplanchna *towards open water and may be responsible for the significant negative correlation that we observed between macrophyte and* Asplanchna *densities.*

Key words: Aquatic macrophytes, Asplanchna, *diet composition, freshwater wetlands, rotifer distribution.*

INTRODUCTION

Freshwater macrophytes are one of the fundamental factors that influence the structure of freshwater ecosystems. Physically, macrophytes exert dramatic effects by participating in the construction of heterogeneous mosaics on different scales (O'Hare *et al*., 2006; Kuczynska-Kippen, 2007). The *habitat heterogeneity hypothesis* (Simpson, 1949; Lack, 1969) assumes that structurally complex habitats provide an increased availability of niches for resident animals and encourage diversity in exploiting environmental resources; this finally results in increased species diversity.

Some studies have suggested that macrophyte habitat structure mediates trophic interactions among various biological factors (Jeppesen *et al*., 1997; Pelicice and Agostinho, 2006), an aspect that has attracted interest regarding the role of macrophytes in determining predator-prey interactions. Macrophytes are commonly utilised as habitats or refuges for prey in freshwater ecosystems. In particular, the role of macrophytes in mediating zooplankton dynamics is well understood in freshwater ecosystems (Cazzanelli *et al*., 2008; Horppila *et al*., 2009). Furthermore, various predator-prey interactions, including those

between fish and zooplankton (mainly cladocerans and copepods), have been investigated within macrophyte habitats. Moreover, rotifer dynamics can be explained by predator-prey interactions within rotifer groups in macrophyte habitats. Because rotifer play a fundamental role (*i.e*., as primary consumer) in determining food web structures and functions, examining this relationship will improve our understanding of the patterns of rotifer biological diversity in relation to macrophyte density. Even though previous research has examined *Asplanchna*, a predatory rotifer species, in terms of its dynamics and the role that habitat plays in freshwater ecosystems, the predator-prey interactions within the rotifer community related to habitat heterogeneity has not been addressed.

Asplanchna is a typical omnivore that consumes phytoplankton, detritus, and rotifer (Thatcher *et al*., 1993). Species of this genus are one of the most voracious rotifer predators, often cannibalistic, and are capable of feeding on nearly all species of the family Brachinidae as well as other rotifer (Gilbert, 1980). Interestingly, *Asplanchna* species exhibit different dietary preferences, and consequently have different prey preferences. *Asplanchna priodonta* is known as typically omnivorous, but it often

prefers colonial algae and even cyanobacteria over zooplankton prey (Pociecha and Wilk-Woznisk, 2008). On the contrary, *Asplanchna herrickii* is a detritivore (Chang *et al*., 2010). Temporal variations in *Asplanchna*, including interannual or seasonal dynamics (Ortega-Mayagoitia *et al*., 2000; Havel *et al*., 2004), have demonstrated that various habitat environments and types/size of prey and predators influence its feeding strategies (Guiset, 1977; Nandini *et al*., 2003). Furthermore, hydrological stability (*i.e*., a relatively long water residence time) is also an important factor determining the density and feeding of *Asplanchna* (Ferrari *et al*., 1989). Among freshwater ecosystems, wetlands are relatively stable and provide structural heterogeneity conferred by macrophytes. Therefore, they are a suitable system for understanding the influence of macrophytes on the distribution and feeding habits of *Asplanchna*. Previous research on *Asplanchna* spp. has primarily focused on their population dynamics or on the basic characteristics of their feeding habits. On the other hand, the relationships between the feeding patterns of *Asplanchna* species and the characteristics of their surrounding habitats, with reference to predator-prey interactions, have not been fully scrutinised.

In this study, we examined 33 wetland ecosystems to understand the relationship between aquatic macrophytes, prey rotifer, and predation by *Asplanchna* spp. on other rotifer. We hypothesised that the presence of aquatic macrophytes would have a negative impact on *Asplanchna* predation and might affect the diversity and density of prey rotifer. In addition, we also hypothesised that i) aquatic macrophytes determine the distribution patterns of *Asplanchna* in wetland ecosystems; ii) prey rotifer (pelagic and epiphytic) in wetlands present different patterns between vegetated and open water zones because of the unequal densities of predators (*i.e*., *Asplanchna*); and iii) density changes in prey rotifer cause a shift in the food selection patterns of *Asplanchna* spp.

METHODS

Study site

South Korea is located in the East Asian region, and has a temperate climate with four distinct seasons. The annual average rainfall is approximately 1150 mm; freshwater ecosystems in this country experience a summer maximum in rainfall (more than 60% of annual rainfall occurs from June to early September; Jeong *et al*., 2007). The wetlands monitored in this study are located in south-eastern Korea, within the central and lower reaches of the Nakdong River. Historically, numerous riverine wetlands dominated the river basin, but urbanisation has led to a reduction in the area and number of wetlands; this has also been observed in other countries (Burkett and Kusler, 2000).

Currently, 146 wetlands are present in the river basin and this study selected 33 of them. We used two selection criteria to analyse *Asplanchna* predation in relation to macrophytes: i) the average depths of the study sites should be broadly similar; and ii) zones of vegetated water and open water should be simultaneously present. Consequently, we selected 33 wetlands in the river basin for analysis (Fig. 1; Supplementary Tab. 1). The areas of the studied wetlands varied from 1321 to 5234 m^2, most of which were being utilised for agricultural water supply at the time this study was conducted. Littoral zones were shallow (depths ranging from 0.5 to 0.8 m), but central areas were deeper (depths ranging from 1.2 to 1.6 m), resulting in a clear littoral zonation of aquatic macrophyte development (abundant only in littoral zones). Altogether, five aquatic macrophytes were commonly observed at the study sites, namely, *Phragmites australis* (Cav.), *Paspalum distichum* L.*, Zizania latifolia* Griseb.*, Trapa japonica* Flerov., and *Scirpus tabernaemontani* Gmel.*,* all of which were abundant in the littoral area only.

Monitoring strategy

We surveyed the wetlands from late May to early June 2011 (over a period of two weeks), before the occurrence of the summer monsoon. This was to avoid any disturbance of the distribution of aquatic macrophytes. We established six sampling points in each of the 33 wetlands to collect water samples: three points were in vegetated zones and three were in open water zones. The sampling points were randomly selected based on virtual grids constructed over the maps of the wetlands.

Water temperature, dissolved oxygen, conductivity, turbidity, and chlorophyll *a* concentration were measured at each of the wetlands. Water samples were collected at a depth of 0.5 m in both the vegetated and open water zones. We used a DO meter (YSI DO meter; Model 58) to measure water temperature and dissolved oxygen; conductivity was measured using a conductivity meter (Fisher Conductivity Meter; model 152). Turbidity was measured using a turbidimeter (Model 100B). Water samples were filtered through a Mixed Cellulose Ester (MCE) membrane filter (Advantech; Model No., A045A047A; pore size, 0.45 μm), and chlorophyll *a* concentration was measured based on Wetzel and Likens (2000). The area of open water was measured using a Geographic Information Systems (GIS) program (ArcGIS 9.3) and a digital map (National Geographic Information Institute, 2005; 1:25,000). Additionally, we sampled an additional 10 L of water for zooplankton (rotifer, cladoceran, and copepod) collection from the surface layer (0-0.5 m depth), using a 10 L column sampler. This water was filtered through a plankton net (32-μm mesh size) for estimating the density of plankton, after which the plankton were preserved in formaldehyde (final concentration: ca. 5%). The zooplankton were identified and counted at genus level using a microscope (ZEISS, Model Axioskop 40; 200× magnification), based

on the classification key supplied by Mizuno and Takahashi (1999).

One of the goals of this study was to compare the food preferences of *Asplanchna* species. Therefore, we classified potential prey rotifers into two habitat groups, pelagic and epiphytic, according to Sakuma *et al.* (2002), Gyllström *et al*. (2005) and Choi *et al.* (2012). Supplementary Tab. 2 summarizes the information about the habitat groups based on the aforementioned references. We applied the following criteria: i) if a genus is identified as either *pelagic* (the term *planktonic* was replaced with *pelagic* in the current version) or *epiphytic* in all three references, then the genus was identified as is; ii) if a genus is differently identified (*e.g.,* one reference indicate the genus as pelagic, but the others do as epiphytic; or more than one reference indicates the genus as *ambiguous*), then we identified the genus as *ambiguous*.

For *Asplanchna* diet analysis, commercial bleach (ca. 0.2 mL; containing sodium hypochlorite) was added to suspensions of isolated *Asplanchna* individuals to extract their trophi and gut contents and to dissolve the organic matter attached to their body surfaces (Schoeneck *et al.*, 1990). The treatment with bleach enabled us to extract the trophi of *Asplanchna* as well as remains from prey items (such as other rotifer, phytoplankton and protozoa). Among organs or tissues of living things, hard chitinous structures can remain for a longer time when bleach is treated, but the other soft tissues may be dissolved quickly. Therefore, we applied bleach to the samples following suggestion from Chang *et al.* (2010), under very careful consideration not allowing complete dissolution of samples (empirically, sample identification should be completed within 30 to 120 s after bleach treatment; more than 180 s may cause loss of samples; Kwang-Hyeon Chang, *personal communication*). We conducted gut analysis of all *Asplanchna* individuals collected from each wetland, and all of the prey found in the guts were identified and quantified. Identification of the trophi of both
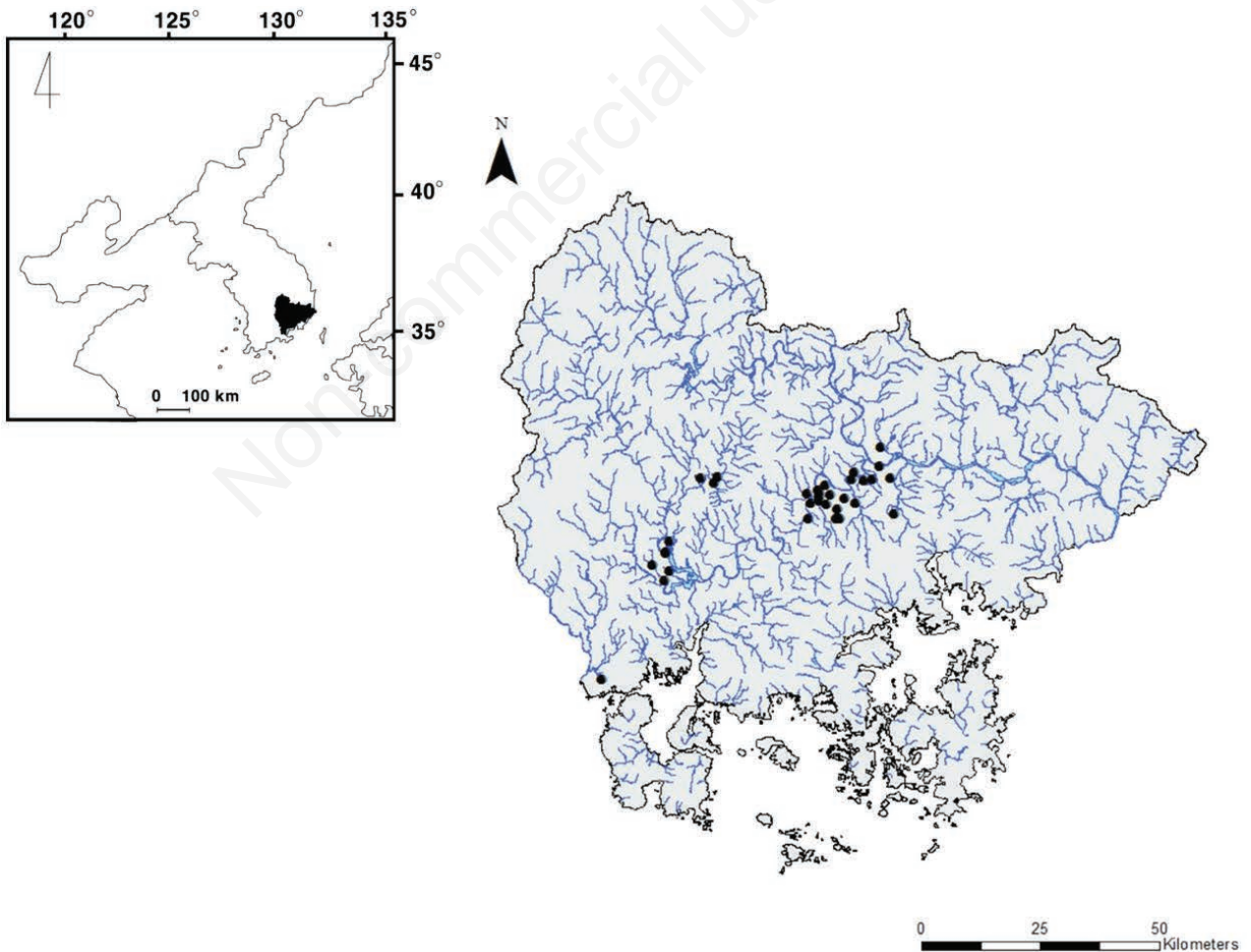
Fig. 1. Map of the study area in south-eastern South Korea. The study sites are indicated as solid circles (●). The small map in the upper left corner indicates the Korean Peninsula.

Asplanchna and prey rotifer was done according to Jersabek *et al*. (2003). Prey phytoplankton and protozoa in *Asplanchna* guts were identified to the genus level based on Yamaji (1991).

Data analysis

We used one-way nested ANOVA (α=0.05) to analyse statistical differences of environmental parameters, densities of two *Asplanchna* species, and other rotifers (both pelagic and epiphytic), between vegetated and open water zones. As we established three sampling points for each zone in each wetland, direct application of one-way nested ANOVA may cause pseudoreplication problem (*i.e.*, data homogeneity between sampling points should be ensured; Hurlbert, 1984). Therefore we set the two zones as the primary factor and the three replication (*i.e.*, sampling points) were regarded as nested subgroups for each zone. For the environmental parameters, total 99 samples per zone was obtained (33 wetlands×3 sampling points=99 samples; 99 samples×2 zones=total 198 samples). Furthermore, the relationship between the density of *A, priodonta* species and environmental variables were tested with stepwise multiple regression. All statistical analyses, including stepwise multiple regression and ANOVA, were conducted using the statistical package SPSS for Windows ver. 14). In order to assess prey selectivity, we compared the relative frequencies of rotifers in the water samples with those in *Asplanchna* guts.

Regression analysis was used to examine the following relationships: i) area of vegetated and open water zones in the studied wetlands and the distribution of *A. priodonta* in these zones; ii) density of *Asplanchna* and prey rotifers (*i.e.*, pelagic and epiphytic rotifers, respectively; genus identified as *ambiguous* were not included in the analysis); and iii) counted prey rotifers in *Asplanchna* guts and density in water samples. We tested for linear, exponential, inverse, power, and logistic functions, to determine an equation generating the best curve fit. Among the regression results, the curve-fitting equation that returned the highest determination coefficient was selected to explain the observed relationships. For the regression analysis investigating relationship between density of *Asplanchna* and prey rotifers, we used sum of sample data (density of zooplankton) that were obtained from three sampling points in open water zone, to avoid pseudoreplication problem. Moreover, not only the regression analysis aforementioned but also stepwise multiple regression utilized only data from the wetlands with presence of *Asplanchna*.

RESULTS

The monitored parameters showed little difference between the two studied zones (*i.e.*, vegetated and open water zones) among the 33 wetlands (Tab. 1; Supplementary Tab. 1). In result of one-way nested ANOVA analysis (Tab. 2), the environmental parameters were not significantly different between the two zones. Subgroups (*i.e.*, three sampling points) for all of the environmental parameters also did not show statistical difference.

Two *Asplanchna* species, *A. priodonta* and *A. herrickii*, were identified in the wetlands, found in total 18 wetlands among 33 wetlands. *Asplanchna priodonta* was observed in all wetlands where *Asplanchna* appeared, whereas *A. herrickii* co-occurred with *A. priodonta* in only three wetlands among 18 wetlands. When those 18 wetlands were used in the one-way nested ANOVA to figure out the distribution pattern of *A. priodonta*, a clear difference of distribution was observed. Even though, *Asplanchna priodonta* were found in both vegetated- and open water zones, they were more abundant in open water zones than vegetated zones based on homogeneous distribution between sampling points (Tabs. 1 and 2). In contrast, *A. herrickii* was observed in only open water zones. Rotifers were observed in all of the investigated wetlands, and density of rotifer was clearly different between vegetated zones and open water zones (Tabs. 1 and 2). The density of pelagic rotifers was not different between the two zones, but epiphytic rotifer densities were significantly different (Tab. 2). No statistical difference was found between sampling points. Vegetated zones supported not only pelagic rotifers but also epiphytic rotifers. In open water, the rotifer species composition was dominated by pelagic types such as *Keratella* and *Polyarthra*, but other genera such as *Anuraeopsis*, *Ascomorpha*, *Filinia*, and *Kelicottia*, also known as pelagic type rotifers, were less abundant. Cladocerans and copepods were detected in most of the investigated wetlands, but their densities were lower than those of rotifers (Tab. 1).

The result of the stepwise multiple regression allowed us to examine the influence of environmental parameters on the density and distribution of *A. priodonta* (Tab. 3; *A. herrickii* was not included in the analysis due to very low density). Form the observation, *Asplanchna* species were found only from open water zones, therefore stepwise multiple regression was applied to the *Asplanchna* data of the open water zones. The total density of *A. priodonta* displayed strong relationship with the area of open water zones and the density of pelagic rotifers (r^2=0.907, d.f. for regression, residuals, and total=2, 29, and 32, respectively; *F*=91.44, P=0.001; Tab. 3 for independent variables). The correlation value between *A. priodonta* and the area of open water was particularly strong and show a positive relationship between *A. priodonta* density and the area of open water (Fig. 2). Relatively similar density of *A. priodonta* between vegetated zones of the wetlands would cause statistical insignificance. Analysis of the gut contents of 317 individuals of *Asplanchna* (*A. priodonta*,

Tab. 1. Mean, standard deviation, standard error, and range in zooplankton density and environmental parameters, across the selected study sites. The unit of zooplankton density is ind. L^{-1}.

Type	Variable	Vegetated zones			Open water zones		
		Mean±SE	SD	Range	Mean±SE	SD	Range
Environmental parameters	WT (°C)	23.7±0.6	3.2	14.0	23.7±0.6	3.3	13.9
	DO (mg L^{-1})	7.2±0.5	2.7	13.4	8.1±0.4	0.4	2.5
	Cond. (μS cm^{-1})	241.5±24.1	138.9	598.3	244.7±24.5	140.7	591.0
	Tur. (NTU)	10.2±2.0	12.0	50.4	11.7±2.6	15.2	74.0
	Chl. *a* (μg L^{-1})	15.5±1.6	9.1	35.0	19.4±1.8	10.1	36.7
Asplanchna	*A. priodonta*	2.6±0.8	4.6	17.5	65.7±19.7	112.9	354.0
	A. herrickii	-	-	-	15.4±11.0	63.1	343.8
Pelagic rotifer	*Anuraeopsis*	25.0±2.1	11.9	61.3	17.5±7.7	44.2	192.5
	Ascomorpha	21.3±0.6	0.6	8.8	0.3±0.3	1.5	8.8
	Brachionus	41.7±6.7	38.5	183.7	18.6±5.8	33.5	131.3
	Filinia	20.6±10.1	57.9	332.5	2.7±2.1	12.3	70.0
	Kelicottia	-	-	-	0.8±0.8	4.6	26.3
	Keratella	47.6±24.1	138.3	796.3	123.0±31.6	181.7	953.8
	Polyarthra	44.9±7.3	42.0	192.5	67.9±14.1	81.2	393.8
Epiphytic rotifer	*Cephalodella*	-	-	-	1.9±1.4	7.8	43.8
	Colurella	26.9±2.2	12.5	43.8	1.1±0.5	2.9	8.8
	Euchlanis	30.9±3.6	20.4	105.0	1.9±0.6	3.6	8.8
	Lepadella	31.9±4.6	26.4	131.3	2.1±0.9	4.9	17.5
	Leacane	37.2±4.7	27.1	96.3	5.3±1.9	10.7	52.5
	Testudinella	27.2±1.7	9.6	35.0	1.6±0.7	4.1	17.5
	Trichotria	49.2±10.8	62.3	323.8	19.4±5.0	28.5	122.5
Ambiguous rotifer	*Mytilina*	33.5±5.9	34.1	175.0	2.7±1.2	6.7	26.3
Crustacean	Cladocerans	25.4±4.2	24.3	105.0	12.6±4.6	26.4	140.0
	Copepods	25.6±3.8	21.8	105.0	12.2±2.2	12.5	35.0

WT, water temperature; DO, dissolved oxygen; Cond., conductivity; Tur., turbidity; Chl. a, *chlorophyll* a.

Tab. 2. One-way nested ANOVA results for the effects of main groups (shown as Habitat in the table: vegetated- and open water zone) and subgroups (*i.e.*, shown as Sampling point) on environmental parameters, two *Asplanchna* species, and other rotifers (both pelagic and epiphytic).

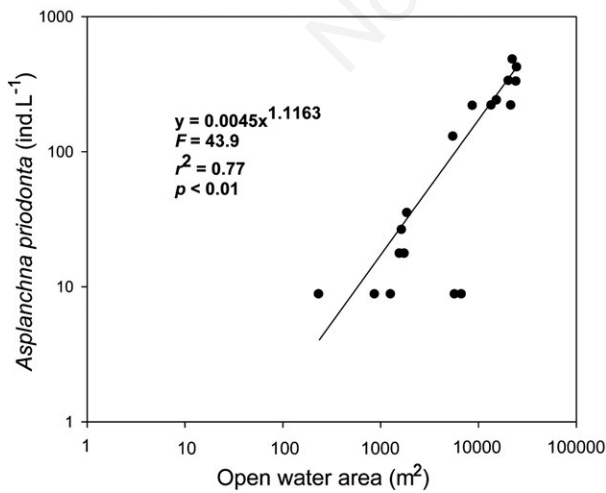| Type	Source of data	Component of variance	df	Residual df	F	P
Environmental parameters	WT (°C)	Habitat	1	192	0.11	0.95
		Sampling point	4	192	0.20	0.91
	DO (mg L^{-1})	Habitat	1	192	3.03	0.07
		Sampling point	4	192	0.04	0.99
	Cond. (μS cm^{-1})	Habitat	1	192	0.19	0.93
		Sampling point	4	192	0.07	0.98
	Tur. (NTU)	Habitat	1	192	0.49	0.78
		Sampling point	4	192	0.05	0.98
	Chl. *a* (μg L^{-1})	Habitat	1	192	2.94	0.09
		Sampling point	4	192	0.35	0.86
Asplanchna	*A. priodonta*	Habitat	1	102	32.16	0.00
		Sampling point	4	102	0.31	0.88
Rotifer	Total rotifer	Habitat	1	192	2.47	0.18
		Sampling point	4	192	0.05	0.98
	Pelagic rotifer	Habitat	1	192	1.61	0.41
		Sampling point	4	192	0.01	0.99
	Epiphytic rotifer	Habitat	1	192	240.34	0.00
		Sampling point	4	192	0.06	0.97

df, degree of freedom; WT, water temperature; DO, dissolved oxygen; Cond., conductivity; Tur., turbidity; Chl. a, *chlorophyll* a.

259; *A. herrickii*, 58) revealed that the two *Asplanchna* species had different diets in the study sites (Fig. 3). *Asplanchna priodonta* consumed more diverse prey to *A. herrickii*, and rotifers, phytoplankton, and protozoa were found from *A. priodonta*'s guts. In contrast, *A. herrickii* consumed only *Euglena*; no other prey items were found. In the case of rotifer predation by *A. priodonta*, this predator consumed primarily *Keratella* and *Polyarthra*, even though *Polyarthra, Keratella,* and *Trichotria* (sorted by relative frequency) were the most frequent species at the study sites (Fig. 4).
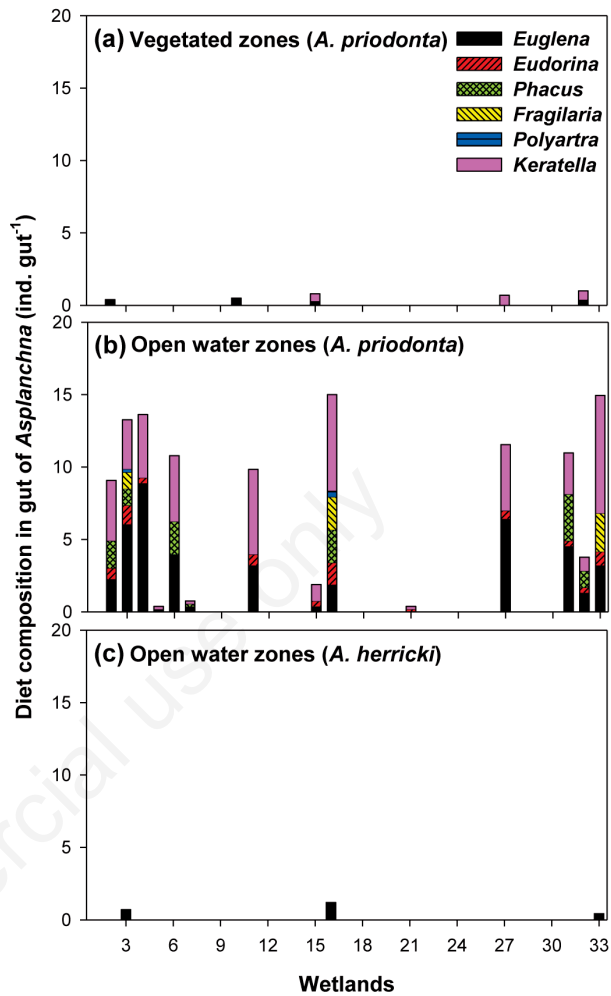
Regression analysis (Fig. 5) clearly showed the relationships between average densities of *A. priodonta* and the prey rotifer species in the water (pelagic, epiphytic, *Keratella*, and *Polyarthra,* respectively). A power function generated the highest determination coefficient (Tab. 4). Pelagic rotifers in vegetated zones were not correlated to *A. priodonta* density, but they were negatively correlated

Tab. 3. Summary of stepwise multiple regression aimed to predict density of *A. priodonta* (response variable) with respect to environmental parameters (explanatory variables) in open water zones. Data were transformed prior to analyses using either the arcsine-square root (proportion agricultural land) or log (all other variables) transformation.
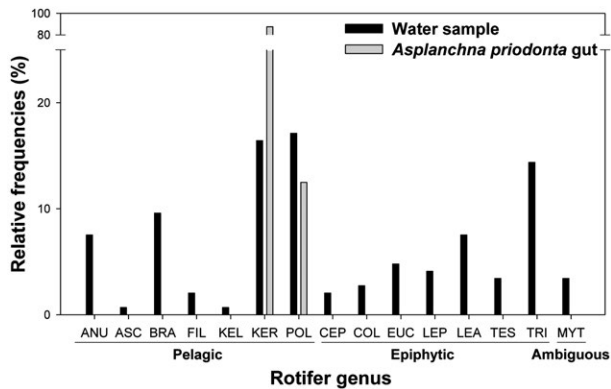
Explanatory variables	B_j	t	P
Constant	49.327	1.823	0.079
Area (m²)	0.015	11.115	0.000
Pelagic rotifer density (ind. L^{-1})	-0.125	-2.222	0.035

Fig. 3. Gut content of *Asplanchna priodonta* and *Asplanchna herrickii* in vegetated and open water zones.

Fig. 2. The relationship between the area of open water zones and the density of *Asplanchna priodonta* (n=18; regression df, 1; residual df, 16).

Fig. 4. Relative frequencies (%) of prey rotifer genera in wetland water samples and *Asplanchna priodonta* gut contents. ANU, *Anuraeopsis*; ASC, *Ascomorpha*; BRA, *Brachionus*; FIL, *Filinia*; KEL, *Kelicottia*; KER, *Keratella*; POL, *Polyarthra*; CEP, *Cephalodella*; COL, *Colurella*; EUC, *Euchlanis*; LEP, *Lepadella*; LEA, Leacane; TES, *Testudinella*; TRI, *Trichotria*; MYT, *Mytilina*.

with the density of the predator *A. priodonta* in open water zones. The density of prey pelagic rotifers in open water declined as *A. priodonta* density increased (Fig. 5). Meanwhile, *A. herrickii* did not display any statistically significant correlation with prey rotifer density in open water zones (not shown in the figure). There was no significant correlation between epiphytic rotifers and the density of either of the two *Asplanchna* species, in both vegetated and open water zones. Among prey rotifer genera, *Keratella* and *Polyarthra* displayed negative correlation with *A. priodonta* density (Fig. 5 c,d). Fig. 6 illustrates the relationships between prey density (*Keratella* spp. and *Polyarthra* spp.) in water samples and in the gut contents of *Asplanchna*. The density of *Keratella* in the water samples was strongly negatively correlated to the number of *Keratella* trophi in *Asplanchna* guts. However, *Polyarthra* did not show any statistically significant relationship.

DISCUSSION

Influence of macrophytes on *Asplanchna* and prey rotifers

Macrophytes are known to provide a suitable habitat and refuge for small animals (mainly zooplankton) in freshwater ecosystems (Castilho-Noll *et al.*, 2010). This may explain the high density of rotifers observed in vegetated zones in the current study. The complex microhabitat structure provided by various macrophytes species hinders predator activity; therefore, they assist zooplankton in avoiding predators such as juvenile fish and macroinvertebrates (Dionne and Folt, 1991; Burks *et al.*, 2006; Thomaz *et al.*, 2008). In this scenario, it is to be expected that the density of prey rotifers in vegetated zones would be higher than that in open water. Predatory copepod as well as competitive cladoceran including *Daphnia* to rotifers (Gilbert, 1988; MacIsaac and Gilbert, 1989; Adrian
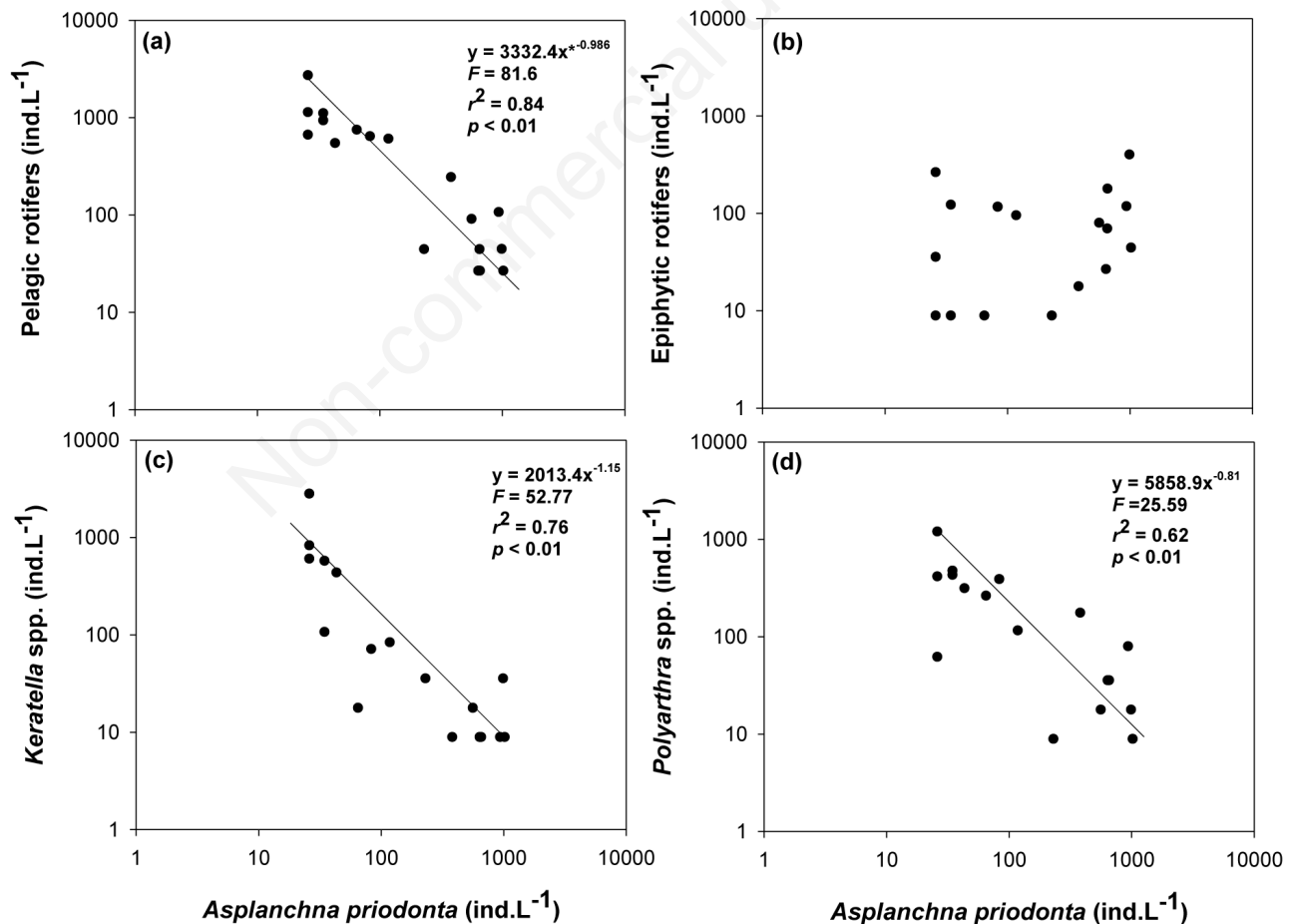

Fig. 5. The relationship between *Asplanchna priodonta* density and prey rotifers in open water zones (regression df=1, residual df=16). (a) Prey pelagic rotifers; (b) prey epiphytic rotifers; (c) *Keratella* spp. in water samples; and (d) *Polyarthra* spp. in water samples. n=18 for each panel.

and Frost, 1993; Brandl, 2005; Diéguez and Gilbert, 2011) rarely appeared in the vegetated zones of the study sites, which might affect larger density of prey rotifers in the vegetated zones.

In our study, macrophytes supported a relatively high abundance of epiphytic rotifers, but this was not the case for pelagic rotifers. Epiphytic rotifers can attach to substrates (such as the stems and leaves of macrophytes) in complex microhabitats (Brönmark, 1985; Choi *et al.*, 2014); *Asplanchna* is not an effective predator of such rotifers. For this reason, vegetated zones are an unfavourable habitat for *Asplanchna*. As observed in our study, the prey rotifers preferred by *Asplanchna* include the pelagic rotifers *Keratella* and *Polyarthra*, which were abundant in open water zones. In addition, open water zones with large areas encouraged the proliferation of *Asplanchna*. Under these circumstances, it is advantageous for *Asplanchna* to occupy a habitat characterised by the presence of an abundant food source. Therefore, macrophytes are a very important factor affecting the distribution of rotifers, with consequent effects on the distribution of *Asplanchna*. Although a higher density of rotifers was

observed in vegetated zones than in open water zones, the relationship between this condition and *Asplanchna* density is unclear. One possible hypothesis is regarding scarce feeding activity of *Asplanchna* in vegetated zones. Relationship discovery between vegetation and predatory *Asplanchna* should be addressed in further research in order to examine food web structure and function more exactly.

In result of the stepwise multiple regression, the density of *A. priodonta* displayed strong relationship with the area of open water zones and the density of pelagic rotifers. Exclusion of other environmental parameters would be due to relatively smaller variation of the parameters among the study sites. It is expected that the sampling was conducted within short period to minimize seasonal variation of the investigated parameters, therefore, relatively more constantly maintained parameters would be recognized as responsible variable to the changes of *A. priodonta*. Kappes *et al.* (2000) suggested that seasonal changes of *Asplanchna* was depending on water temperature variation, however, in this study, the temperature variation was not large enough to bring out variation of *Asplanchna* density.

Tab. 4. Results of various types of simple regression analyses. The applications were aimed to investigate: i) influence of *A. priodonta* on different types of prey rotifer; ii) relationship between densities of prey rotifer observed in field and *A. priodonta* guts; and iii) relationship between densities of *Keratella* spp. observed in field and *A. priodonta* guts. For each comparison, five types of regression methods (linear, exponential, inverse, power, and logistic regressions) were adopted. Two degree of freedom (df) values are shown for each type of regression analysis, being the first the regression df and the second the residual df.

| Variables | | Value | Types of regression | | | | |
Explanatory	Response		Linear	Exponential	Inverse	Power	Logistic
A. priodonta	Pelagic rotifer	df	1,16	1,16	1,16	1,16	1,16
		F	11.83	41.56	30.48	81.64	41.57
		r^2	0.42	0.72	0.66	0.84	0.72
		P	0.00	0.00	0.00	0.00	0.00
	Epiphytic rotifer	df	1,16	1,16	1,16	1,16	1,16
		F	2.34	3.41	0.19	2.04	3.41
		r^2	0.13	0.18	0.01	0.11	0.18
		P	0.15	0.83	0.67	0.17	0.08
	Keratella spp.	df	1,16	1,16	1,16	1,16	1,16
		F	3.48	18.49	14.66	52.77	18.49
		r^2	0.18	0.54	0.48	0.76	0.54
		P	0.81	0.00	0.00	0.00	0.00
	Polyarthra spp.	df	1,16	1,16	1,16	1,16	1,16
		F	8.09	18.32	15.79	18.32	18.32
		r^2	0.34	0.53	0.50	0.62	0.53
		P	0.01	0.00	0.00	0.00	0.00
Prey rotifer in field	Prey rotifer in gut	df	1,16	1,16	1,16	1,16	1,16
		F	18.50	39.69	11.38	58.71	39.69
		r^2	0.54	0.71	0.42	0.79	0.71
		P	0.00	0.00	0.00	0.00	0.00
Keratella spp. in field	*Keratella* spp. in gut	df	1,16	1,16	1,16	1,16	1,16
		F	23.63	58.67	35.24	98.81	58.67
		r^2	0.60	0.79	0.69	0.86	0.79
		P	0.00	0.00	0.00	0.00	0.00

Even though there are some studies that have tried to distinguish rotifer into different habitat groups, there is still arguments about the separation. As shown in Supplementary Tab. 1, some species are under ambiguity. If precise information about the functional role of the *ambiguous* species is available, the interaction between prey rotifer and the predator (in this study, *Asplanchna*) will be more clearly understood.

Food source differentiation among *Asplanchna* species

We observed that the diet composition of the two predator species (*A. priodonta* and *A. herrickii*) examined in this study differed from each other. The former utilised both rotifers and phytoplankton and was therefore omnivorous. In contrast, the latter relied primarily on *Euglena*, mirroring what was reported by Chang *et al.* (2010). However, the greater dependency of *A. herrickii* on detritus may be explained by the hypothesis of Kappes *et al.* (2000) and Pociecha and Wilk-Wozniak (2008). The morphotypes and trophi morphology of *Asplanchna* have been suggested as important factors influencing species-specific feeding habits (Hampton and Starkweather, 1998). *Asplanchna priodonta* and *A. herrickii* have very similar rami morphologies (Jersabek *et al.*, 2003), but *A. priodonta* has more sharpened rami tips than *A. herrickii*. Relatively inefficient trophi morphology for active predation might lead the species to consume more diverse food items. Based on the above, *Asplanchna* species may exhibit species-specific feeding habits, and different species may play different ecological roles in the freshwater food web. Furthermore, food differentiation between the two *Asplanchna* species examined may reduce competition, leading to their mutual coexistence. In our study, *A. herrickii* was observed only at the three wetlands with the largest areas of open water. In addition, these wetlands were characterised by highly turbid water, a low density of prey rotifers, and low chlorophyll *a* concentration. The observed environmental conditions (*i.e.*, the presence of sufficient detritus) in those wetlands may be suitable for the growth and development of *A. herrickii*, but this requires further assessment.

Prey preference of *Asplanchna* spp.

Most predators experience difficulties in finding their preferred prey, which become scarce due to intensive elimination (O'Donoghue *et al.*, 1997). Generally, the feeding behaviour of *Asplanchna* depends on the rate at which they encounter food/prey sources in open water. Conde-Porcuna and Sarma (1995) confirmed that *Asplanchna* increased its consumption of prey species that were present at high densities, and attributed this phenomenon to an increased rate of predator-prey encounters, despite the fact that the presence of *Asplanchna* induces activation of certain protective mechanisms in prey rotifers (Iyer and Rao, 1995; 1996). In this study, *Polyarthra* and *Keratella*, which were largely consumed by *A. priodonta*, were the most frequent species in all of the examined wetlands during the study period (*i.e.*, open water zones). Gilbert (1985) suggested that *Polyarthra* exhibits effective escape mechanisms, such as rapid jumping movements, to avoid predators. This may explain why *Polyarthra* was less likely to be consumed than *Keratella*
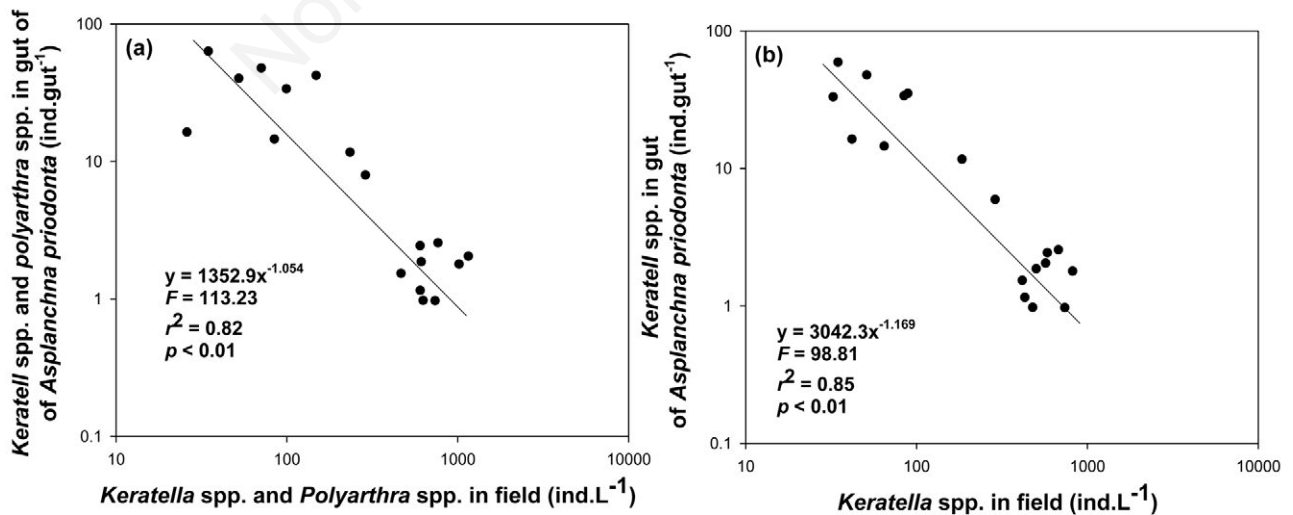
Fig. 6. The relationship between prey items (*Keratella* spp. and *Polyarthra* spp.) in the water samples and those in the gut contents of *Asplanchna priodonta* (regression df, 1; residual df, 16). (a) Density of two genera (summed) and number of trophi; (b) *Keratella* spp. density and its trophi number in the guts. n=18 for each panel.

in this study. We considered that *Asplanchna* utilises primarily rotifers; however, the results of this study revealed that *Asplanchna* spp. simultaneously consumed diverse food sources, such as phytoplankton and protozoa, as well as prey rotifers. However, prey rotifers were the dominant item. In a previous study, *Asplanchna* utilised rotifers early during the growing season when rotifers were abundant, and then shifted their consumption patterns by favouring phytoplankton and protozoa later in the season (Kappes *et al*., 2000). Chang *et al*. (2010) also reported that *Asplanchna* actively fed on rotifers in wetlands when the average water temperature was 18°C, which may suggest that the period of this study was directly after the peak of rotifer consumption (Tab. 1; water temperature). The regression analyses of this study support the hypothesis that the density of prey rotifers (*i.e.*, *Keratella* and *Polyarthra*) in water samples was negatively related to their consumption by *Asplanchna*, and that a shortage of prey rotifers encouraged the ingestion of phytoplankton (*e.g.*, *Eudorina*, *Fragilaria* and *Phacus*) as alternative food sources; this was also indicated by Kappes *et al*. (2000). *Asplanchna* can feed on microscopic organisms, including bacteria, protozoa, and small rotifers (Iyer and Rao, 1996).

CONCLUSIONS

On the basis of our results, *A. priodonta* demonstrated preference for open water over vegetated zones. The two *Asplanchna* species examined in our study displayed different feeding habits. Open water zones were appropriate as habitat for *Asplanchna* because their food (mainly pelagic rotifers and phytoplankton) were more abundant in such zones. Additionally, the presence of a large area of open water supports a greater density of *A. priodonta*, and this relationship was also partially observed for *A. herrickii* in the three largest wetlands studied. These two *Asplanchna* species play different roles in the food web. With respect to the types of prey consumed, *A. herrickii* consumed primarily *Euglena*, whereas *A. priodonta* presented omnivorous character that consumed rotifers, phytoplankton, and protozoa. *Asplanchna priodonta* consumed only pelagic rotifer in open water, but had no effect on epiphytic rotifer densities. It can be expected that predation by *Asplanchna* may have an apparent impact on prey densities in open water zones. We conclude that the two aforementioned *Asplanchna* species play different roles in the food web, and that aquatic macrophytes significantly influence the distribution and feeding activity of *Asplanchna*.

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