



Utilization of fish gut analysis to elucidation of microcrustacean species composition (cladoceran and copepoda) in a shallow and vegetated lake (Jangcheok Lake, South Korea)

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Abstract

Structural heterogeneity results in different spatial distributions of microcrustaceans. Thus, in ecosystems with excessive macrophyte development, it may be difficult to determine the microcrustacean species composition. Given the importance of microcrustaceans in the food web, the elucidation of microcrustacean diversity is essential. In vegetated habitats, bluegill sunfish can prey on microcrustaceans, and therefore have a potential role as microcrustacean monitoring agents. In the present study, we compared microcrustacean species compositions in the field with those in the guts of bluegill, in Jangcheok Lake, South Korea. Our results showed that the number of microcrustacean species was higher in bluegill guts than in the field. Further, microcrustacean species, such as *Daphnia galeata*, *Graptoleveris testudinaria*, *Leydigia leydigii*, *Rhynchotalona* sp., and *Simocephalus exponisus*, were found only in bluegill guts. Our findings verify the validity of the fish gut analysis to monitor microcrustacean species compositions and to clarify spatial distributions of microcrustacean species in structurally heterogeneous ecosystems with excessive macrophyte development.

Key words: bluegill sunfish, fish gut analysis, macrophyte, microcrustacean species composition, shallow lake

INTRODUCTION

Elucidation of biological diversity within ecosystems is fundamental to our understanding of ecosystem structure and function. Biodiversity is mainly determined by the composition of resident species within the system. Species that are beyond the detection limits of conventional monitoring methods frequently cause under-estimation of diversity. Therefore, developing more effective monitoring processes is an urgent issue in biodiversity research. This is particularly important for ecosystems with highly sustained habitat heterogeneities and unusually large biodiversities.

Freshwater macrophytes have deterministic effects on the habitat structures of water, and result in the construction of heterogeneous mosaics on different scales (O'Hare et al. 2006, Smokorowski and Pratt 2007). Habitat heterogeneity may create additional niches and diverse ways of exploiting environmental resources for resident animals (Bazzaz 1975) by providing them with refuges from predators, suitable spawning, and foraging substrates, thereby resulting in a higher number of animal individuals (Vieira et al. 2007). Microcrustaceans depend largely on aquatic macrophytes and can effectively utilize macrophyte habi-

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tats (Burks et al. 2002, Kuczyńska-Kippen and Nagengast 2006). Habitat heterogeneity leads to differences in the species composition and spatial distribution of microcrustacean communities (Meerhoff et al. 2006, Thomaz et al. 2008). Given the importance of microcrustaceans in aquatic food webs (i.e., in the transfer of energy and materials), the precise determination of the species composition of microcrustacean communities is central to limnological research, but it is frequently hindered by excessive macrophyte development.

Planktivorous fish spend a large proportion of their time in foraging activity and therefore encounter a high number of microcrustacean species within an ecosystem. In general, the presence of macrophytes is believed to decrease prey capture by fish (Meerhoff et al. 2006, 2007); however, some fish species have developed specialized abilities to forage in vegetated habitats (Jacobsen et al. 1997). For example, the bluegill sunfish (*Lepomis macrochirus*) is a small fish species that can more effectively prey on microcrustaceans in vegetated habitats (Crowder and Cooper 1982, Paukert and Willis 2002). Therefore, fish foraging may have a potential role in the determination of microcrustacean species composition.

In the present study, we determined the microcrustacean species composition in an ecosystem with excessive macrophyte development, by using fish gut analysis. We hypothesized that the number of microcrustacean species would be higher in fish guts than in the field. To test our hypothesis, we compared the microcrustacean species compositions in the field with those in the guts of bluegill sunfish.

MATERIALS AND METHODS

South Korea is located in East Asia and has a temperate climate. Four distinct seasons lead to dynamic succession among biological communities inhabiting the country's freshwater ecosystems. In the present study, we monitored Jangcheok Lake, which is located in the southeastern part of South Korea (Fig. 1), where the mid- to low region of the Nakdong River flows. The surface water area is 0.5 km², and the average depth ranges from 0.6 to 1.4 m. The littoral area is characterized by macrophyte development from spring (May) to autumn (November). In the present study, we identified 8 species of macrophytes—*Ceratophyllum demersum*, *Hydrilla verticillata*, *Paspalum distichum*, *Phragmites australis*, *Salvinia natans*, *Spirodela polyrhiza*, *Trapa japonica*, and *Zizania latifolia*.

We investigated the species compositions of microcrus-

taceans and fish during spring (May), summer (August), and autumn (October) of 2012. Prior to collection of microcrustaceans, we considered habitat heterogeneity according to macrophyte presence. We created virtual grids over the map of the Jangcheok Lake and randomly selected 15 sampling points among the locations with macrophyte development. Using quadrat in each sampling point, we investigated macrophyte type. For microcrustacean collection and environment factors measurement, we collected water samples (10 L each) by using a 10-L column sampler at each sampling point. At a sampling point, we placed the sampler vertically into the water, in order to collect water sample from the entire water column. A dissolved oxygen (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; YSI Inc.) and Orion 250A pH meter (Orion Research Inc., Boston, MA, USA), respectively. The water samples were conveyed to the laboratory to measure the concentration of chlorophyll a and turbidity. Turbidity was measured by 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 (pore size, 0.45 µm) (A045A047A; Advantec MFS, Dublin, CA, USA), and the filtrate was used to determine the concentration of chlorophyll a based on Wetzel and Likens (2000). Microcrustaceans were collected through filtration of sampled water by using a plankton net (32-µm mesh), and the filtrate was preserved in formaldehyde (final concentration, approximately 5%). The microcrustaceans were identified and counted under an Axioskop 40 microscope (Zeiss, Oberkochen, Germany) at ×200 magnification, according to the classification key of Mizuno and Takahashi (1991).

After collection of microcrustaceans (i.e., approximately 30 min), we collected bluegill (*Lepomis macrochirus*) by using a cast net (pore size, 7 mm) and a scoop net (pore size, 5 mm). At each sampling site, the cast nets were cast for 30 min, and the scoop net was used for 20 min. We collected the fish in the area surrounding the microcrustacean sampling point (with 5 m radius). The dominant fish species collected was bluegill (approximately 95% of relative abundance). All of the fish individuals were fixed in methanol-formaldehyde solution immediately after catching, and stored at approximately 2°C to 3°C for further analysis of their gut content. We identified and counted all of the microcrustacean species in the gut contents of bluegill. To improve accuracy of data, we did not take broken or extensively digested individuals into

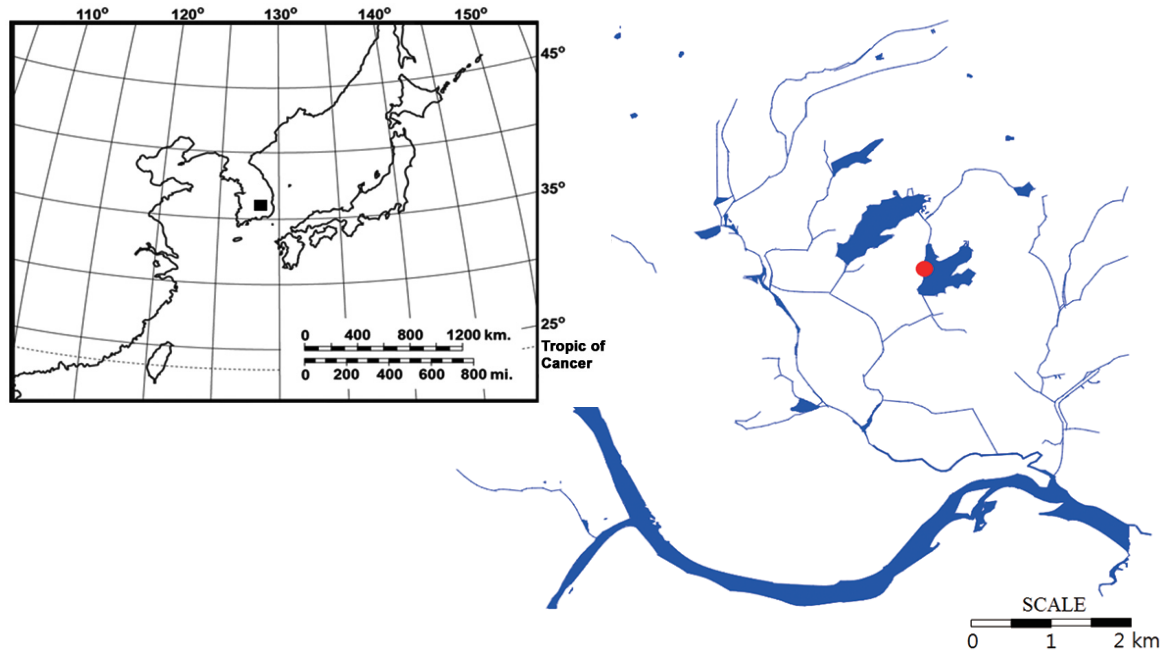


Fig. 1. Map of the study sites. The study sites are indicated as quadrangle (■) and located in the southeastern part of South Korea. The left map in the upper right-hand corner shows the Korean Peninsula, and the right map show the around of the lake include Jangcheok lake. Red circle (●) indicates the sampling point.

account for species identification in order to maintain identification consistency. Microcrustacean species that was broken or have only parts of body were not utilized in this study. We applied two-way ANOVA ($\alpha = 0.05$) to compare the species number of microcrustacean in fish gut with that of water samples. The major factors were location of microcrustacean (the fish guts and the water samples) and seasons (spring, summer, and autumn). For the statistical test, we used a statistical package SPSS for Windows ver. 14 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Results of physico-chemical parameters showed seasonal differences during study period. Water temperature exhibited the strongest seasonality; it was the highest in the summer and lowest in the winter. The percent (%) saturation of dissolved oxygen varied depending on temperature; summer and autumn showed lower saturation than spring and winter. The seasonal pattern of pH was also similar with % saturation of dissolved oxygen, lower in summer and autumn. Turbidity was the highest in autumn, but the average turbidity of each of the other seasons was similar. The chlorophyll a concentration was below 20 $\mu\text{g/L}$ from spring to autumn and was high in

winter.

During the study period, we found a total of 41 microcrustacean species: 24 species belonged to Branchiopoda; the remaining 17 species, Copepoda. The microcrustaceans showed different species compositions between each sampling point (Table 1). The differences are interpreted as a result of heterogeneous structure by different macrophyte species. Macrophyte habitat structure is an important factor to determine microcrustacean species composition (Manatunge et al. 2000, Kuczyńska-Kippen and Nagengast 2006). Sakuma et al. (2002) observed different microcrustacean species compositions between reed and submerged macrophytes zones, caused by different morphological structures between the 2 macrophyte types. In comparison with other macrophytes, submerged macrophytes are known to contribute considerably to the formation of complex habitat structure in water and to support higher density and greater species diversity of microcrustaceans (Jeppesen et al. 1998, van Donk and van de Bund 2002). Therefore, in the present study, a high number of microcrustacean species at a sampling point may be explicable by the presence of submerged macrophytes (Fig 2) whereas relatively lower species number of microcrustacean at a sampling point may be explained by the absence of submerged macrophytes. In particular, emergent macrophytes supported

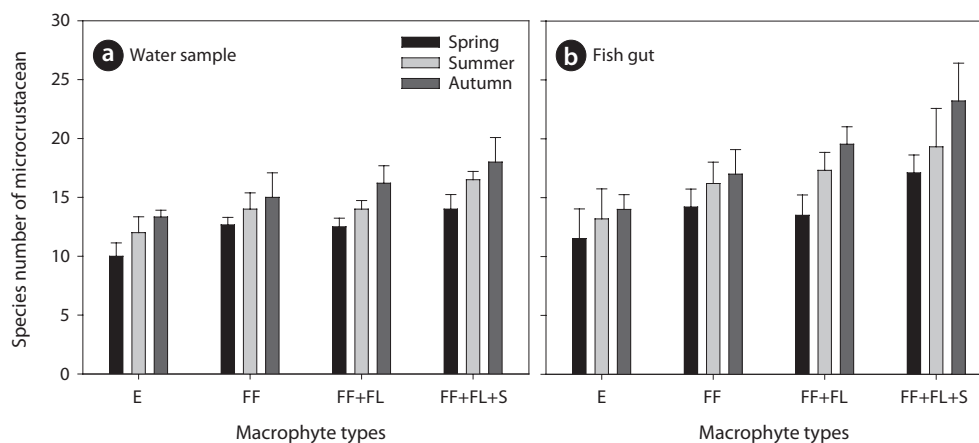


Fig. 2. Microcrustacean species number from (a) water sample and (b) fish gut in accordance with macrophyte types. E, emergent macrophyte ($n = 3$); FF, free-floating macrophyte ($n = 3$); FL, floating-leaved macrophyte ($n = 3$); S, submerged macrophyte ($n = 6$). Error bars represent standard deviation.

lowest species number of microcrustacean. Although we observed a higher number of microcrustacean species in sampling point with submerged macrophytes, some species (*Acroperus harpae*, *Alona guttata*, *Alona rectangularis*, *Graptoleveris testudinaria*, and *Pleuxus denticulata*) frequently appeared in sampling points without submerged macrophytes. These microcrustacean species are generally associated with surface-dwelling macrophytes (free-floating and floating-leaved macrophytes) and are termed “epiphytic microcrustaceans.” Moss et al. (1998) reported that epiphytic microcrustacean species utilize surface-dwelling macrophytes as their habitat. Furthermore, the species number of microcrustacean was also affected by seasons (Fig. 2). The number of species in autumn was relatively higher than spring and summer. We considered that seasonal growth of macrophytes could support more microcrustacean species. In autumn, interestingly, microcrustacean species showed a greater diversity in fish gut than in water sample (Fig. 2). Therefore, we considered that influence of fish foraging on microcrustaceans was higher in season with heterogeneous structures, thus particularly autumn was suitable season to find microcrustacean species by using fish.

In the present study, we analyzed total 85 bluegill gut (spring, 24; summer, 32; autumn, 29). We found that the number of microcrustacean species was higher in bluegill guts than in the field (Table 1 and 2). The species number of microcrustacean was not significantly different between fish gut and water samples (Two-way ANOVA, $df = 1$, $F = 0.23$, $P > 0.05$), but they were clearly different between seasons ($df = 1$, $F = 5.57$, $P < 0.05$). Fish foraging frequently declines in the vegetated zone (Bettoli et al. 1992, Warfe and Barmuta 2004); however, bluegill sunfish

have a high prey capture rate in habitats with a moderate plant density (Crowder and Cooper 1982). During the study period, we observed a high abundance of bluegill in vegetated zones; the size of these fish was relatively small (13 to 64 mm). In general, piscivorous fish are size-selective predators. The size of prey selectively ingested by piscivores is dependent on predator length (Hambright 1991). Smaller fish are more vulnerable to predation than larger fish (Werner and Gilliam 1984) and are therefore expected to use denser macrophyte beds. Thus, small fishes will have a greater influence on the abundance of small-sized microcrustaceans. Accordingly, we recorded a larger number of microcrustacean species and therefore higher species diversity, in bluegill guts than in the field. Further, some species (*Daphnia galeata*, *G. testudinaria*, *Leydigia leydigii*, *Rhynchotalona* sp., and *Simocephalus exponisus*) were found only in bluegill guts. Nowlin and Drenner (2000) reported that bluegill exerted strong effects on microcrustacean relative to fishless controls. Most juvenile fish are known to consume high amounts of pelagic microcrustacean species; interestingly, in the present study, we observed that bluegill frequently consumed not only pelagic species, but also epiphytic species.

The results of our study verified the validity of fish gut analysis to monitor microcrustacean species compositions in ecosystems with excessive macrophyte development. The advantages of this method will be maximized when the habitat contains a large number of macrophytes because excessive macrophyte development frequently hinders the determination of microcrustacean species. In freshwater ecosystems, microcrustaceans are located at an intermediate level of the food web, where they play a key role in the transfer of energy and materials (Wetzel

Table 2. Species compositions of microcrustacean in water sample and in the fish guts in accordance with season

Taxa	Spring		Summer		Autumn		Taxa	Spring		Summer		Autumn	
	W	G	W	G	W	G		W	G	W	G	W	G
Branchiopoda							<i>Diacyclops crassicaudis</i>	+	+	+	+	+	+
<i>Acropenus harpae</i>	+	+	+	+		+	<i>Diacyclops bicuspidatus</i>	+	+	+	+		+
<i>Alona guttata</i>		+	+	+	+	+	<i>Diacyclops nanus</i>			+	+	+	+
<i>Alona rectangula</i>			+	+	+	+	<i>Ectocyclops phaleratus</i>			+	+		
<i>Bosmina longirostris</i>	+	+	+	+	+	+	<i>Eucyclops serrulatus</i>	+	+		+	+	+
<i>Bosminopsis deitersi</i>	+		+		+	+	<i>Eucyclops speratus</i>	+	+	+	+		+
<i>Camptocercus rectirostris</i>	+	+	+	+	+	+	<i>Eucyclops roseus</i>			+	+	+	+
<i>Ceriodaphnia reticulata</i>		+	+	+	+	+	<i>Macrocyclops albidus</i>			+	+	+	+
<i>Ceriodaphnia dubia</i>				+	+	+	<i>Mesocyclops leuckarti</i>	+	+	+	+		+
<i>Chydorus sphaericus</i>	+	+	+	+	+	+	<i>Mesocyclops pehpeiensis</i>	+	+	+	+	+	+
<i>Daphnia galeata</i>		+					<i>Mesocyclops dissimilis</i>	+	+		+	+	+
<i>Daphnia obtusa</i>	+	+					<i>Thermocyclops crassus</i>		+	+	+	+	+
<i>Diaphanosoma brachyurum</i>	+	+	+	+		+	<i>Thermocyclops taihokuensis</i>	+	+	+		+	+
<i>Graptoleveris testudinaria</i>				+		+	Total species number	22	25	27	34	27	35
<i>Iltocryptus spinifer</i>	+		+	+		+							
<i>Leydigia leydigii</i>				+		+							
<i>Macrothrix rosea</i>			+	+	+	+							
<i>Moina macrocopa</i>			+		+	+							
<i>Pleuxus denticulata</i>	+	+	+	+	+	+							
<i>Pleuxus laevis</i>	+			+	+	+							
<i>Rhynchotalona</i> sp.						+							
<i>Sida crystalina</i>			+	+									
<i>Simocephalus exponisus</i>		+				+							
<i>Simocephalus vetulus</i>	+	+			+	+							
<i>Scapholeberis kingi</i>	+	+	+	+	+	+							
Copepoda	+	+											
<i>Acanthocyclops vernalis</i>			+	+		+							
<i>Cyclops vicinus</i>	+	+		+	+	+							
<i>Cyclops kikuchii</i>		+	+	+	+	+							
<i>Diacyclops languidoides</i>				+	+	+							

W, water sample; G, fish guts; '+' indicates presence of microcrustacean species.

and Likens 2000). Thus, the use of a “biological monitoring agent,” such as bluegill gut analysis, will enable precise determination of species composition.

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