

Seasonal and diel abundance and feeding patterns of *Chaoborus flavicans* in Sang-Chun reservoir

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To document the basic ecological aspects of *Chaoborus* species, which has never been reported in Korea, we attempted to identify the species, to monitor seasonal and vertical dynamics, and to elucidate trophic relations of the species in Sang-Chun reservoir. Using morphological characteristics, we identified the collected samples as *C. flavicans*. Also, we compared the distribution of *C. flavicans*, *Daphnia rosea* and chlorophyll *a* to observed seasonal dynamics. The increase of *C. flavicans* was observed 1–2 weeks after the increase of *D. rosea*. Survey of diel vertical migration patterns in the summer season showed that *C. flavicans* were in hypolimnion at daytime, but moved to the epilimnion at night. Finally, to determine trophic relationships in Sang-Chun reservoir, additional studies on the food web were undertaken by stable isotope analysis. *Chaoborus flavicans* I–II instars appear to be filter feeders based on carbon isotope values. Trophic levels of *C. flavicans* III–IV instars were shown to be higher than other zooplankton based on nitrogen isotope values.

Keywords: *Chaoborus*; *Daphnia*; seasonal dynamics; diel vertical migration; stable isotope analysis

Introduction

The larvae of the phantom midge *Chaoborus* are distributed worldwide with 14 described species in Nearctic and Palaearctic region (Sæther 1970). The long cylindrical body is transparent or semi-transparent and white or yellowish color (Figure 1). The key characters for identification are the two pairs of hydrostatic organs (air sacs or bladders), one on each side of the body (Živić and Marković 2006). *Chaoborus* larvae have four divided instars; instars I and II are planktonic, and instars III and IV show diel vertical migration between the water and the sediments (Stahl 1966; Halat and Lehman 1996). After over-wintering as III or IV instars, *C. flavicans* is known to pupate in the following year and after transforming into flying adults in July–August, the number of larvae in lakes becomes low (Meigen 1830; Hongve 1975). *Chaoborus* are omnivorous, gape-limited and ambush predators of small size preys such as rotifers and crustacean zooplankton, mainly *Daphnia* sp. (Swift and Fedorenko 1975; Pastorok 1981; Moore 1986; Moore et al. 1994; López and De Roa 2005). Also, they themselves are a food source for planktivorous fish. Therefore, they can play important roles in aquatic ecosystems (Xie et al. 1998).

Distributions of *Chaoborus* larvae are influenced by many environmental factors. For example, temperature is a predictor of the altitudinal and latitudinal distribution of *Chaoborus* (Barley et al. 2006). Some *Chaoborus*

sp. larvae need a critical water temperature to complete molting and will fail to come out of pupation below 12°C (Ouimet 2001). Also, many studies on *Chaoborus* larvae have focused on water columns with and without fish (Fedorenko 1975; Havens 1990). The primary factor limiting the presence and abundance of *Chaoborus* in lakes is the presence or absence of fish (Stenson 1978; Uutala 1990; Luoto and Nevalainen 2009). *Chaoborus flavicans* is one of a few species of *Chaoborus* that has a habitat preference for lakes with fish (Borkent 1981) and undergoes diel vertical migration (DVM), which reduces visual predation by fish (Pope et al. 1973; Bass and Sweet 1984; Lamontagne et al. 1994). They are very tolerant of low oxygen concentrations and can occupy the hypolimnion of lakes (Luecke 1986). *Chaoborus* species are used for paleolimnological studies (Sweetman and Smol 2006) and as an indicator species for fish populations (Schilling et al. 2009) and low oxygen and heavy metals (Ponton and Hare 2010). The purposes of the present paper are to give basic ecological information for *Chaoborus* sp. since *Chaoborus* species has been never studied in Korea. First, we monitored the seasonal dynamics patterns of *Chaoborus* sp. and *Daphnia rosea* in Sang-Chun reservoir for two years. In addition, we surveyed three times the DVM patterns of both species for a 24-hour period. Finally, we investigated trophic relations of *Chaoborus* sp. using both carbon and nitrogen stable isotope analysis.

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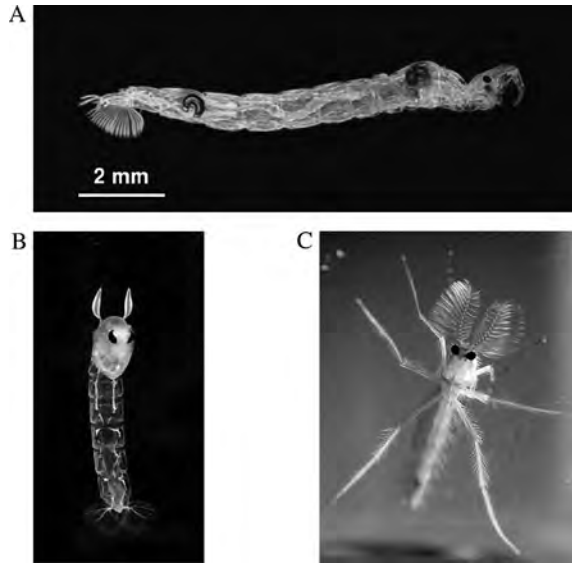


Figure 1. Various forms of *Chaoborus* through its life cycle. (A) *Chaoborus* larvae (instar IV) and (B) pupa, and (C) flying adult.

Materials and methods

Study site

Sang-Chun reservoir is located in the Gyeonggi-do of South Korea (37° 46' 05" N, 127° 28' 32" E, elevation: 151 m above sea level) near Lake Chungpyung and North-Han River (Figure 2). The reservoir has a maximum depth of 11 m and a surface area of 0.024 km². The site is located in woodland and thus has less anthropogenic influences, such as agricultural pesticides and fertilizers.

The fish community includes crucian carp, common carp, leather carp, bass and dark chub while the benthic

invertebrates include *Chaoborus* larvae and Chironomidae. The zooplankton populations include rotifers (*Asplanchna* sp., *Polyarthra* sp., *Keratella* sp. and *Kellicottia* sp.), cladocerans (*D. rosea* and *Bosmina longirostris*), and copepods (*Cyclop* sp. and calanoids) as dominant species. High species diversity of Sang-Chun reservoir has been observed compared to other small agriculture reservoirs (unpublished data).

Sample collection

All zooplankton samples were taken at a fixed sampling point where the depth was 9 m. Zooplankton were sampled almost every week in daytime (13:00–15:00) from January 2008 to December 2009 with a Schindler plankton trap (Wildco, USA, mesh size 61 µm, volume 12 L). Triplicate samples (duplicate between January and August 2008) were collected at four different depths (1, 3, 6, and 9 m). All samples were fixed with sucrose and 1% Lugol's solution in the field. Preserved samples were counted using a Bogorov counting chamber under a stereomicroscope (30 × magnification) in our laboratory. In addition, we collected *Chaoborus* larvae and *Daphnia* using by a zooplankton net (Wildco, USA, mesh size 74 µm, diameter 30 cm) for species identification.

Identification of *Chaoborus* larvae in Sang-Chun reservoir

In the morphological identification of *Chaoborus* larvae, the shape of the mandibles is the most important morphological feature. Mandibles of *Chaoborus* larvae were dissected with fine forceps under

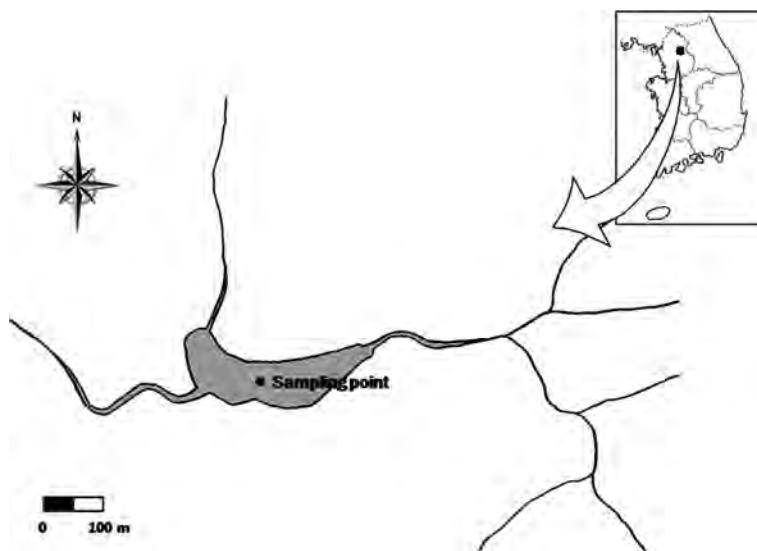


Figure 2. A map showing our sampling point in Sang-Chun reservoir.

a dissecting microscope, and identified under a dissecting microscope (Nikon, SMZ800, Japan) at 100 × magnification. Mandibles were identified according to the descriptions of Sæther (1970) and Uutala (1990).

Seasonal dynamics and diel vertical migration (DVM)

Chaoborus sp. and *D. rosea* were collected to study the seasonal dynamic patterns for nearly two years (2008–2009). The density of two species in the water column was determined from three replicate traps from 1, 3, 6, 9 m, then averaged into weighted means over the whole water column. Chlorophyll *a* concentration was measured using a spectrophotometer after extraction with 90% acetone from 47 mm GF/C filter (Whatman, UK) (Marker et al. 1980). Also, DVM patterns of both zooplankton were observed three times in October 2008 (depth: 1, 3, 6, and 9 m), June and August 2009 (depth: 1, 3, 5, 7, and 9 m). For each 24-hour survey, zooplankton were sampled six times at intervals of 4 hours. Likewise, all samples were fixed in the field and were counted in the laboratory.

Stable isotope analysis

The samples for stable isotope analysis were collected from the deepest point in June and August 2009. Zooplankton species were sorted into species using Pasteur pipettes under a dissecting microscope (Nikon, SMZ800, Japan). *Chaoborus* sp., zooplankton and fish samples were dried at 60°C in a drying oven, and ground to fine powders. Seston samples were collected on pre-combusted 47 mm GF/C filter (Whatman, UK) and dried at 60°C in the drying oven. Most zooplankton samples were composites of >5 individual samples. These samples were wrapped with flat tin disks (Perkin Elmer, USA) and compressed into a compact spherical shapes with a maximum dimension of 6 mm. Analysis was conducted by the Center for Stable Isotope Biogeochemistry at UC Berkeley using a Delta Plus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan, Germany). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were expressed as differences in parts per thousand (‰) between samples and standards: Peedee belemnite (PDB) marine limestone for ^{13}C and atmospheric nitrogen for ^{15}N as follows:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 10^3$$

$$R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}$$

Result

Identification of *Chaoborus* larvae in Sang-Chun reservoir

The subordinate tooth of the *Chaoborus* larvae collected in this study was short and was placed at the base of the medial tooth or halfway between the posterior and medial teeth, showing characteristics of *C. flavicans* or *C. crystallinus* (Louto and Nevalainen 2009) (Figure 3). Although *C. crystallinus* is morphologically close, its subordinate tooth is larger than *C. flavicans*, and is placed above the base of the medial tooth, which does not match with our samples. Most of all, *C. crystallinus* do not exist with fish and our study site has many fish species.

Seasonal dynamics and diel vertical migration (DVM) patterns

We compared distributions of *C. flavicans*, *D. rosea* and chlorophyll *a* to observe seasonal dynamics during two years. The relationship of *D. rosea* and chlorophyll *a* showed a well-known chlorophyll-*Daphnia* pattern (Figure 4A). A clear water phase occurred once in spring and autumn for two years. Also, abundances of chlorophyll *a* and *D. rosea* were observed in reverse

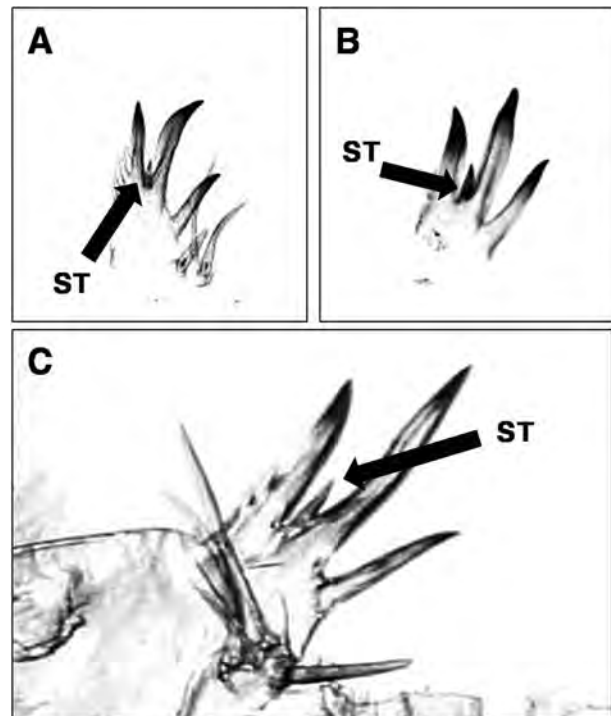


Figure 3. Extended focus images of mandibles of (A) *Chaoborus flavicans* from Alaska and (B) *C. flavicans* from Sweden (Dupuis et al. 2008) and (C) mandible sampled in Sang-Chun reservoir (ST, subordinate tooth).

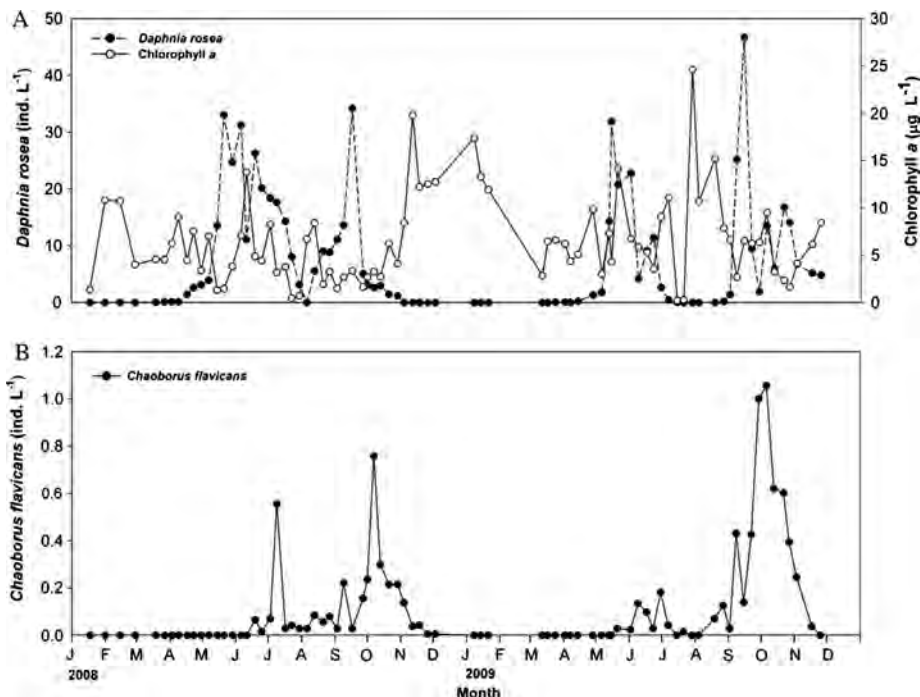


Figure 4. Seasonal dynamics monitored weekly from January 2008 to December 2009 (weighted means over the whole water column). (A) *Daphnia rosea* and chlorophyll *a*; (B) *Chaoborus flavicans*.

patterns. An increase of *C. flavicans* individuals occurred after an increase of *D. rosea* individuals in the water column. *C. flavicans* were mainly observed between June and November in the water column (Figure 4B).

Survey of DVM patterns showed clear patterns except for June 2009. In October 2008, distribution patterns of *C. flavicans* and *D. rosea* showed that *D. rosea* were in epilimnion, and *C. flavicans* were in hypolimnion only in daytime (Figure 5A). However, the patterns regarding depth and time were difficult to understand in June 2009 (Figure 5B). The patterns of October 2009 showed the clearest distribution between the two species (Figure 5C). We could observe that *D. rosea* were in epilimnion, and *C. flavicans* were in hypolimnion in daytime. At night, *C. flavicans* moved to the epilimnion.

Stable isotope analysis

In June 2009 (Figure 6A), we observed that *Chaoborus* I–II instars showed similar carbon isotope signatures to those of *D. rosea* and calanoids indicative of filter feeding on seston as food source. *Chaoborus* III–IV instars showed lighter carbon isotope values compared to filter feeders. Also, we observed that nitrogen isotope weights of *Chaoborus* III–IV instars were much greater than other zooplankton samples. With those results, *Chaoborus* III–IV instars were regarded as the highest-

level topmost predator among sampled zooplankton. The analysis in August 2009 (Figure 6B) showed that two fish species were higher-level predators with much heavier delta nitrogen isotope values compared to those of *C. flavicans*. The next order was *Chaoborus* III–IV instars and filter-feeding zooplankton. According to the results of carbon isotope values from August, seston appeared not to be an important carbon source of zooplankton, because the carbon isotope value of seston was not similar to that of other filter feeders.

Discussion

In our results, *Chaoborus* larvae sampled in Sang-Chun reservoir were identified as *C. flavicans*, reporting an unrecorded species in Korea. Our results suggest that *C. flavicans* is related to *D. rosea* in a predator-prey relationship according to seasonal and vertical distribution. From the trophic relation survey, *C. flavicans* I–II instars appear to be filter feeders while the trophic positions of *C. flavicans* III–IV instars are ranked between fish and filter-feeding zooplankton.

Interestingly, within the genus of *Chaoborus*, the behavioral patterns and living habitats are known to be unique to each species (Pope et al. 1973; Borkent 1981; Uutala 1990; Louto and Nevalainen 2009). After observing the shape, size, location and the color (darkness) of the mandible, we suggest that the larvae captured in Sang-Chun reservoir are identified as the

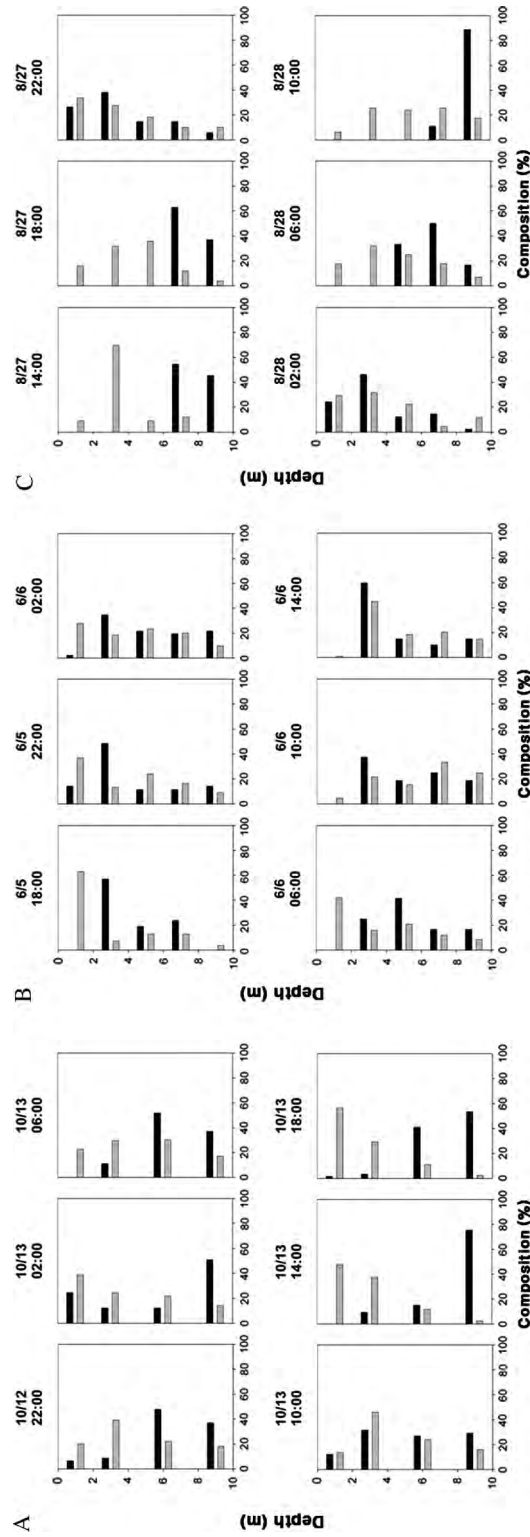


Figure 5. Diel vertical migration (DVM) patterns for a 24-hour period of *Chaoborus flavicans* larvae and *Daphnia rosea* on (A) 12–13 October 2008, (B) 5–6 June 2009 and (C) 27–28 August 2009. Black bar, *C. flavicans*; gray bar, *D. rosea*.

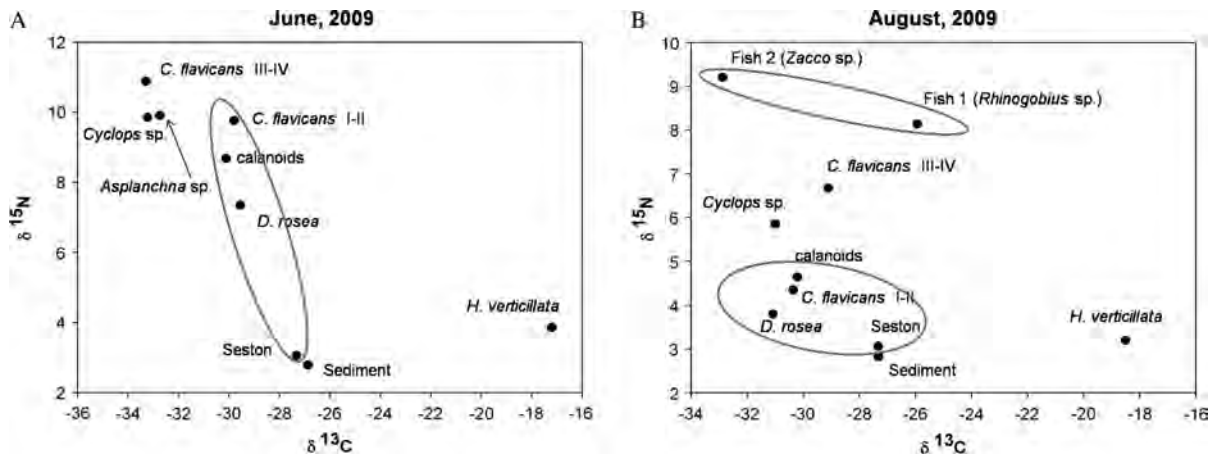


Figure 6. Stable isotope diagram of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for different components of the Sang-Chun reservoir food web collected in (A) June 2009 and (B) August 2009.

larvae of *C. flavicans*. In addition, the diel vertical migration behavior of the larvae observed in this study also supports the same conclusion (Louto and Nevalainen 2009). The increases of the *C. flavicans* population were observed one or two weeks after the increase of the *D. rosea* population. This might be interpreted as resulting from a prey-predator interaction between *C. flavicans* and *D. rosea* (Young and Riessen 2005). However, the interval is somewhat too long to suggest a trophic relationship between these two species. Other possible explanations include an indirect relationship with other planktonic members of the Sang-Chun reservoir community or a seasonal influence such as hatching after emergence of *C. flavicans* (Louto and Nevalainen 2009). However, the seasonal dynamics patterns do not appear to be related to emergence if we consider that the relative abundance of *C. flavicans* I–II instars were less than that of *C. flavicans* III–IV instars after September 2009 (unpublished data).

In an environment where fish, *Daphnia*, and *Chaoborus* coexist, *Chaoborus* is generally known to stay in the hypolimnion to avoid the preying pressure of fish and *Daphnia* stay in the epilimnion to escape from their predator, *Chaoborus*, during the daytime in summer (Hanazato 1997; Neill 1992; Lagergren et al. 2008). However, during the night, *Chaoborus* migrate to the epilimnion to prey upon *Daphnia* (Hanazato 1997; Bezerra-Neto and Pinto-Coelho 2007). This pattern of diel migration was especially noticeable in the months of August 2009 and October 2008 in daytime. However, the migration pattern was not detected during summer seasons (June 2009). A possible explanation for this would be much less fish predation on *C. flavicans* (Lagergren et al. 2008), which, unfortunately, could not be examined in this study due to lack of fish abundance data.

The stable isotope study indicates the trophic relations of *C. flavicans* in terms of both trophic levels and their energy sources. *C. flavicans* I–II instars appeared to be filter feeders because their carbon isotope value from June and August was similar to that of *D. rosea* and calanoids which are known as typical filter feeders. Considering nitrogen isotope signatures, *C. flavicans* III–IV instars are top-predators in June when no fish were sampled and are an intermediate trophic level linking fish and herbivores in August when fish were present, supporting the notion that *Chaoborus* show an ontogenic shift in trophic positions (Persaud and Dillon 2010). In addition, the stable isotope signatures suggest that *D. rosea* are one of the food sources for *C. flavicans* (Lee et al. 2002).

In conclusion, we were able to find a *Chaoborus* species native to Korea identified as *C. flavicans*. Also we found that *C. flavicans* had coupled seasonal dynamics patterns with *D. rosea* and typical diel vertical migration patterns in the studied reservoir. *Chaoborus flavicans* appear to show an ontogenic niche shift from filter feeding to predation. However, we do not know that they are a wide-spread species in the Korean peninsula at the present stage. If a few more habitats are found in Korea, it will be interesting to conduct comparative research to understand their ecological significance to the aquatic ecosystem. Also, those comparative studies would provide data for the population genetic structure of this species (Berendonk and Spitze 2006). From the results of long-term monitoring, we could confirm an increase and decrease pattern of population dynamics and a significant vertical migration pattern. Based on these data, we hope that further studies on this interesting freshwater species can be continued.

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