*J. Limnol.*, *68(1): 122-132, 2009*

# Environmental factors that determine the occurrence and seasonal dynamics of *Aphanizomenon flos-aquae*

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*ABSTRACT*

*This study investigated the seasonal dynamics of two populations of* Aphanizomenon flos-aquae *Ralfs ex Bornet & Flahault var.* flos-aquae *and four populations of* A. flos-aquae *var.* klebahnii *Elenkin in eutrophic water bodies over 1 year from February 2006 to January 2007. The growth of* A. flos-aquae *var.* flos-aquae *was promoted at high temperatures even if in one case the biomass development was very low when other co-occurring cyanoprokaryotes (*Anabaena *spp. and* Microcystis *spp.) were abundant. In contrast, the highest density of the other population of* A. flos-aquae *var.* flos-aquae *was observed in August when the population density of* M. aeruginosa *(Kützing) Kützing reached an annual peak.* A. flos-aquae *var.* flos-aquae *usually bloomed in summer but could also tolerate low temperatures in the winter, and was present in relatively high densities. The populations of* A. flos-aquae *var.* klebahnii *observed in this study can be divided into three groups based on preferred temperature; three populations increased in winter, and the other increased in summer. Large biomasses of the low-temperature-adapted* A. flos-aquae *were observed mainly during winter when population densities of co-occurring cyanoprokaryotes (*Anabaena *spp.,* Microcystis *spp. and* Planktothrix raciborskii *(Woloszynska) Anagnostidis & Komárek) were relatively low or almost absent. The increase in or existence of co-occurring cyanoprokaryotes during the summer resulted in a decrease of the* A. flos-aquae *population density. It was revealed that high temperatures (20-25 °C) are suitable for maintaining* A. flos-aquae *var.* klebahnii *strains isolated from the study ponds, implying that low-temperature-adapted* A. flos-aquae *can grow over a wide range of water temperatures. The high-temperature-adapted* A. flos-aquae *var.* klebahnii *co-existed with* M. aeruginosa *during summer; however, its peak population density was significantly lower than those in previous years when* M. aeruginosa *was absent. Overall, analytical results imply a possible adverse impact of the existence of other cyanoprokaryotes, such as* Anabaena *and* Microcystis*, on both varieties of* A. flos-aquae*; however, its mechanism remains unknown. Nutrient concentrations and pH had no clear impact on the population density of* A. flos-aquae*. Based on these observations, progress of eutrophication and absence of other cyanoprokaryotes are likely the key factors determining the future expansion of* A. flos-aquae.

*Key words: cyanoprokaryote bloom, interspecific interaction, co-occurring cyanoprokaryotes*

## 1. INTRODUCTION

*Aphanizomenon* is a common bloom-forming cyanoprokaryotic genus existing in eutrophic freshwater ecosystems and recently the number of the reports on its appearance is growing (Pollingher *et al*. 1998; Tsujimura *et al*. 2001; Yamamoto & Nakahara 2005, 2006). *Aphanizomenon* has physiologically distinctive features, which seem to, at least partly, contribute to its expansion. It is worth notifying here, that *Aphanizomenon* produces akinetes in its life cycle. These cells arise from normal vegetative cells in response to environmental stresses, such as low phosphorus concentration, declines in temperature and low light intensity; they help therefore to promote survival in periods of unfavorable growth conditions (Lee 1999; Yamamoto & Nakahara 2007). Vegetative cells typically arise from akinetes when adequate conditions reappear. Therefore, akinetes can be regarded as resting spores; thus, *Aphanizomenon* can exist in a wide range of environments as akinetes. Moreover, the ability of *Aphanizomenon* to generate another differentiated cell type, called a heterocyte is also noteworthy considering the competitive strategy of *Aphanizomenon*. The $N_2$ molecules taken in by heterocytes are converted into $NH_4^+$, which is utilized for synthesis of amino acids (Lee 1999). Nitrogen often becomes a growth-limiting factor for photosynthetic plankters; thus, the ability to fix nitrogen contributes greatly to the propagation of general heterocystous cyanoprokaryotes.

*Aphanizomenon* has developed remarkable survival strategies and often forms blooms, especially at high latitudes. However, its appearance at lower latitudes is less frequent than that of other cyanoprokaryotes genera, such as *Microcystis* and *Anabaena*. *Microcystis* do not produce akinetes or heterocytes; however, this genus is very adaptable and frequently becomes the dominant organism as eutrophicasion progresses. *Microcystis* has developed effective nutrient-uptake capacities, such as a high affinity for dissolved inorganic nitrogen (Takamura *et al*. 1987), the storage of large amounts of phosphorus as polyphosphate (Jacobson & Halmann 1982), direct uptake of phosphorus from fish gut (Lewin *et al*. 2003) and attached bacteria (Jiang *et al*. 2007). Moreover, its

**Tab. 1**. Basic characteristics of the study fields and the occurrence of varieties of *A. flos-aquae.*

| | Surface area ($km^2$) | Depth (m) | | *A. flos-aquae* |
|---|---|---|---|---|
| | | maximum | mean | |
| Lake Biwa (southern basin) | 670 (52) | 104 (7) | 41.2 (3.5) | var. *flos-aquae* |
| Lake Yogo | 1.7 | 13.5 | 7.4 | var. *flos-aquae* |
| Hirosawa-no-ike Pond | 0.14 | 1.8 | 1.0 | var. *klebahnii* |
| Osawa-no-ike Pond | 0.038 | <3 | <2 | var. *klebahnii* |
| Benkei-ike Pond | 0.0053 | <2 | ~1 | var. *klebahnii* |
| KU Pond | 0.000026 | 1.7 | 1.7 | var. *klebahnii* |

preference for low concentrations of dissolved inorganic carbon (Nakano *et al*. 2003), tolerance of strong irradiance (Paerl *et al*. 1985), ability to adjust cell buoyancy in response to light intensity and occupy the best position for photosynthesis (Ibelings *et al*. 1991), negative impact on filter-feeding cladocerans (Nizan *et al*. 1986), and overwintering in the form of vegetative cells (with no need to produce resting spores) (Preston *et al*. 1980) are all important to the ecological superiority of *Microcystis*. Although such characteristics contribute to bloom formation of *Microcystis*, whether some of these characteristics serve as critical factors enabling *Microcystis* to exist in the water more commonly than *Aphanizomenon* remains unknown because available comparable data for certain physiological parameters of both genera are lacking. Conversely, *Anabaena* has the same ecological strategies as *Aphanizomenon*; a recent molecular biological study demonstrated that these two genera are extremely similar (Gugger *et al*. 2002). *Anabaena* is a cosmopolitan genus found from tropical to subarctic regions; thus, the higher prevalence of *Anabaena* than of *Aphanizomenon* probably is related to qualitative differences in physiology, such as nutrient uptake activity and stress tolerance. Kinetic studies showed that *Anabaena* sp. had a higher maximum specific growth rate than *Aphanizomenon flos-aquae* (L.) Ralfs and had a higher phosphorus uptake ability, whereas the ability to adapt to light-limiting conditions was higher in *Aphanizomenon flos-aquae* (De Nobel *et al.* 1997, 1998). Although Livingstone & Jaworski (1980) germinated 64-year-old *Anabaena* akinetes, whether the life span of *Aphanizomenon* akinetes is as long as those of *Anabaena* remains unknown, even though *Aphanizomenon* akinetes can survive for at least 18 years. The long-term viability of akinetes may provide opportunities to become a component in plankton community and, thus, the long-term viability of *Anabaena* would be more advantageous than that of *Aphanizomenon*.

Explaining the relative low frequency of *Aphanizomenon* blooms based on previous studies is indeed difficult. However, the first appearance of *Aphanizomenon* in several lakes (Jacobsen 1994; Breukers *et al.* 1997; Pollingher *et al.* 1998; Tsujimura *et al.* 2001) may be attributed to changes in the aquatic environment. Investigating the factors that provide a new ecological niche for *Aphanizomenon* is valuable based on the ecology of cyanoprokaryotes and predictions of the future expansion of *Aphanizomenon*. This study elucidates how environmental factors affect the occurrence of two populations of *A. flos-aquae* Ralfs ex Bornet & Flahault var. *flos-aquae* and four populations of *A. flos-aquae* var. *klebahnii* Elenkin that have appeared in six freshwater ecosystems.

## 2. METHODS

### 2.1. *Morphological characteristics of the two varieties of* A. flos-aquae

*A. flos-aquae* var. *flos-aquae* often exists as large fascicle-like colonies, typically composed of several dozen straight trichomes. In contrast, *A. flos-aquae* var. *klebahnii* exists as solitary trichomes and never aggregates. The single trichomes of these species are very similar; however, *A. flos-aquae* var. *flos-aquae* trichomes generally consist of large vegetative cells, akinetes and heterocytes (Komárek & Kováčik 1989; Watanabe 1991; Pechar 1992).

### *2.2. Study sites*

This study was performed at Lake Biwa, Lake Yogo, the Hirosawa-no-ike Pond, Osawa-no-ike Pond, Benkei-ike Pond and a small artificial pond on the grounds of Kyoto University (KU Pond). Lake Biwa and Lake Yogo are located in Shiga Prefecture, central Japan; the other water bodies are located in Kyoto Prefecture (Fig. 1, Tab. 1). The water level of Lake Yogo is reduced during summer for irrigation. Some of the water inflowing into Lake Biwa travels through the Yogo River. Conversely, Lake Yogo receives inflow from Lake Biwa *via* an artificial water pump system that refills Lake Yogo (Tsujimura 2004; Tsukada *et al.* 2006). The Hirosawa-no-ike Pond is used for irrigation and as a fish pond; its water level and water quality are markedly affected by the fishery. The pond is filled from mid-February to late November, and most pond sediment is exposed to air during other times. Water flows into the pond beginning in mid-February, and the Osawa-no-ike Pond is an important water source. The Benkei-ike Pond and KU Pond are closed systems, and serve as fishing pond and water for fire protection, respectively.

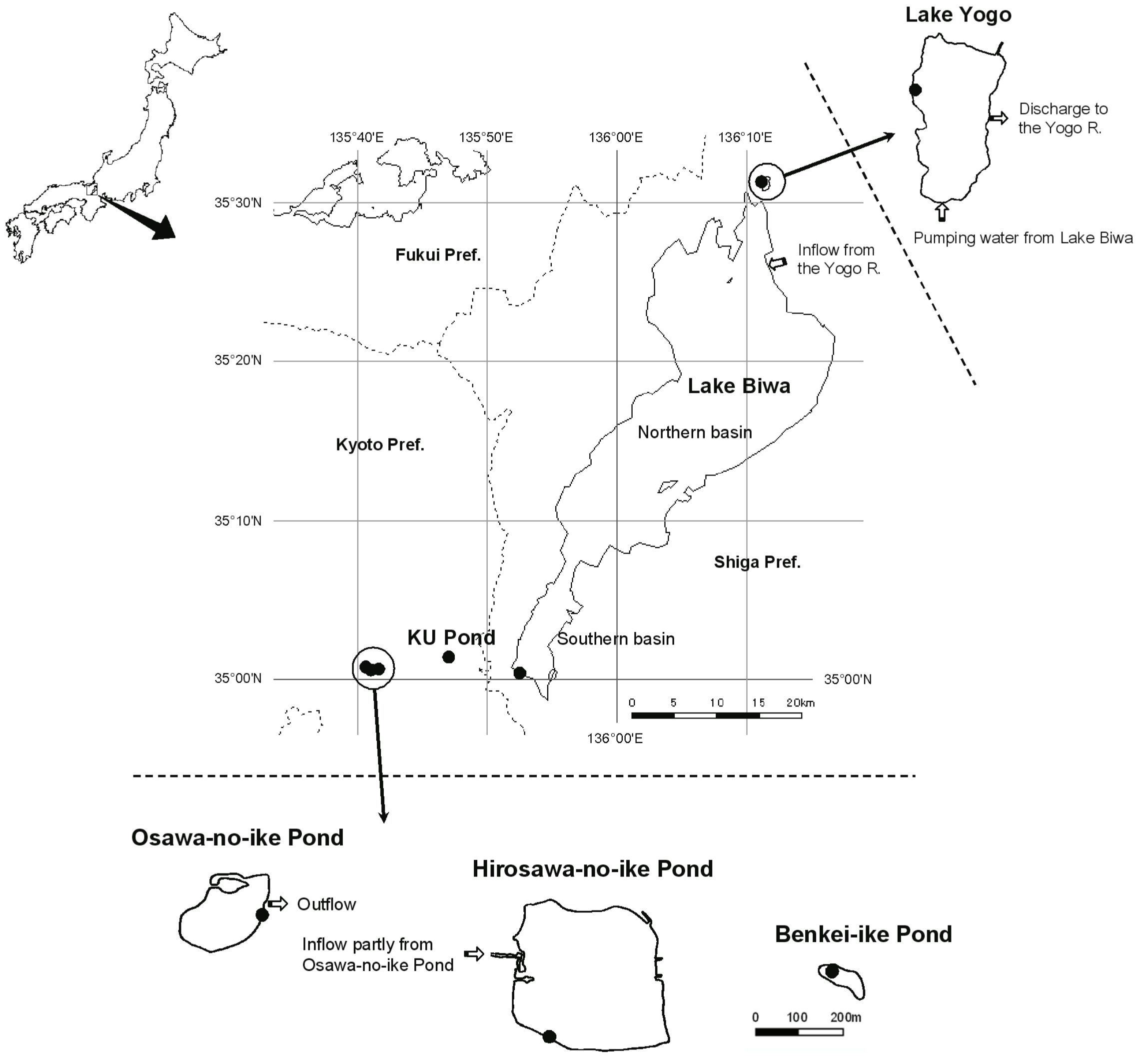

**Fig. 1**. Map showing the location of the study fields.

*2.3. Sampling and chemical measurements*

Water samples were collected monthly from Lake Biwa, Lake Yogo, the Benkei-ike Pond and KU Pond, and biweekly from the Hirosawa-no-ike Pond and Osawa-no-ike Pond, throughout seasons from February 2006 to January 2007. Temperature and pH were measured in situ using a mercury thermometer and a B-212 compact pH meter (Horiba, Ltd., Kyoto, Japan). The concentrations of $NO_3^-$-N, $NO_2^-$-N, $NH_4^+$-N, and $PO_4^{3-}$-P in samples filtered through a Whatman GF/C filter were measured with a spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) using the methods described by Mullin & Riley (1955), Bendschneider & Robinson (1952), Sagi (1966), and Murphy & Riley (1962), respectively. Dissolved inorganic nitrogen (DIN) was determined as the sum of $NO_3^-$-N, $NO_2^-$-N and $NH_4^+$-N. Chlorophyll-*a* concentrations in organisms trapped in filters were determined with a fluorometer (Turner Designs 10-AU 005, Sunnyvale, California, USA) after extraction in 90% acetone for 24 hours under darkness.

*2.4. Enumeration of organisms*

One hundred milliliter-water samples were fixed with Lugol's solution (final concentration 1%) and sedimented. After 48 hours, 90 mL of supernatant was removed and sedimented organisms were poured into a test tube and again sedimented for 24 hours by adding 1 drop of Lugol's solution. The trichome or colony densities of cyanoprokaryotes in concentrated samples (1–10 mL) were measured under an inverted microscope (Nikon ECLIPSE TE300, Tokyo, Japan) by diluting with distilled water when necessary. The biovolumes of *A. flos-aquae* were estimated by assuming their shapes were cylinders.

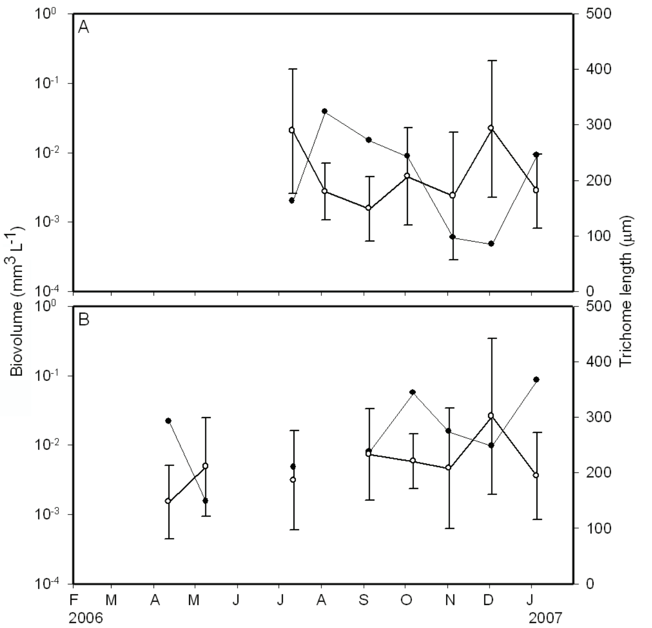

**Fig. 2.** Changes in biovolume (closed circles) and trichome length of *A. flos-aquae* (open circles, where error bars represent standard deviation) in Lake Biwa (**A**) and Lake Yogo (**B**).

## 3. RESULTS

### *3.1. Seasonal dynamics of* A. flos-aquae *var.* flos-aquae

In Lake Biwa, relatively long trichomes (289 ± 112 µm) of *A. flos-aquae* were first detected in July (Fig. 2A). Biovolume peaked in August (0.0383 $mm^3$ $L^{-1}$) with small trichomes (180 ± 50.9 µm). Biovolume decreased starting in September, and then rapidly increased in January.

*A. flos-aquae* appeared in April-January in Lake Yogo; however, it was not detected in June and August (Fig. 2B). Mean trichome length was 147-301 µm. *A. flos-aquae* was dominant in January (0.0854 $mm^3$ $L^{-1}$).

### *3.2. Seasonal dynamics of* A. flos-aquae *var.* klebahnii

*A. flos-aquae* existed in the Hirosawa-no-ike Pond in mid-February, and both biovolume and trichome length increased 2 weeks later (Fig. 3A). Biovolume markedly decreased through April, and was undetectable from May, but it reappeared in mid-October at a low density, and rapidly increased in November.

In the Osawa-no-ike Pond, *A. flos-aquae* also existed in February (Fig. 3B). The trichome length increased in mid-February, and then decreased at a rate of 1.38 µm $d^{-1}$ ($r = -0.995$, $n = 7$, $p < 0.001$) until mid-May. *A. flos-aquae* disappeared from plankton by the end of May, but reappeared in September and a high level persisted after November.

The biovolume of *A. flos-aquae* in the Benkei-ike Pond decreased from 0.0727 $mm^3$ $L^{-1}$ to 0.0422 $mm^3$ $L^{-1}$ from February to April (Fig. 3C). A rapid increase in May (0.520 $mm^3$ $L^{-1}$) was followed by a sharp decline, and *A. flos-aquae* disappeared from plankton in August. However, it reappeared in October; both biovolume and trichome length increased until December.

In contrast to the other ponds, *A. flos-aquae* in the KU Pond was first detected in May at a low density (7.88 × $10^{-4}$ $mm^3$ $L^{-1}$) (Fig. 3D). The biovolume increased sharply in June and a high level (0.382-3.67 $mm^3$ $L^{-1}$) was maintained until September. Mean trichome length was 85.5-253 µm. The *A. flos-aquae* density was always below the detection limit after October.

### *3.3. Physico-chemical conditions and co-occurring cyanoprokaryotes in waters in which* A. flos-aquae *var.* flos-aquae *appeared*

The water temperature in Lake Biwa peaked at 30.0 °C in August, and decreased to 6.3 °C in January. The pH was generally constant (6.4–6.8) from February to May, but increased to 9.3 in July, and then fluctuated at 6.5–7.6 after August. The DIN concentration was high in early spring, thereafter decreased gradually to the minimum of 2.5 µmol $L^{-1}$ in July. It rapidly increased to the maximum (22.6 µmol $L^{-1}$) in September. The concentration of $PO_4^{3-}$-P peaked in September.

Both *Anabaena* and *Microcystis* appeared in May and were abundant in July–September. *Anabaena* was composed of four species - *A. affinis* Lemmermann, *A. crassa* (Lemmermann) Komárkova-Legnerová & Cronberg, *A. flos-aquae* Brébisson ex Bornet & Flahault, and *A. macrospora* Klebahn - whereas the proportions of *A. affinis* and *A. macrospora* were always <10%, except for in September when the former and latter accounted

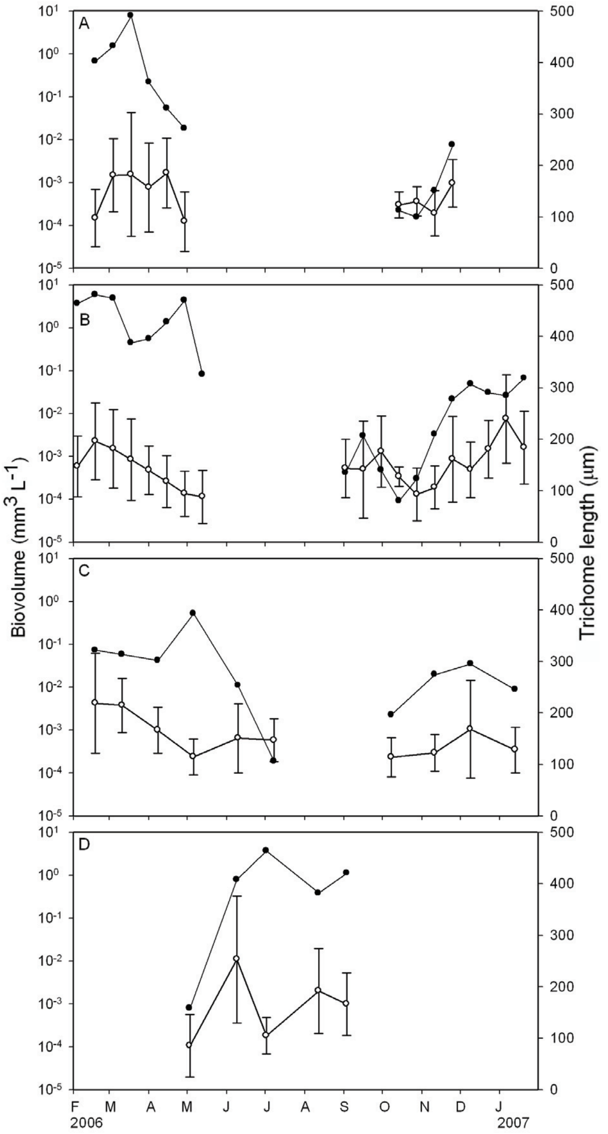

**Fig. 3**. Same as Fig. 2, but in Hirosawa-no-ike Pond (**A**), Osawa-no-ike Pond (**B**), Benkei-ike Pond (**C**) and KU Pond (**D**).

for 19.0% and 35.6%, respectively. *M. aeruginosa* (Kützing) Kützing was the dominant species and accounted for 60-100% of all *Microcystis* species throughout the study period; *M. wesenbergii* (Komárek) Komárek comprised the remaining percentage. *Oscillatoria kawamurae* Negoro increased from after first emerging in June and reached 29.3 trichomes mL$^{-1}$. Although densities were low, these cyanoprokaryotes were detected even in December.

The water temperature in Lake Yogo increased steadily and peaked at 29.7 °C in August. The pH fluctuated at 6.5-8.9. The DIN concentration in March was 18.6 µmol L$^{-1}$, but was below 2.9 µmol L$^{-1}$ after May. No clear seasonal variations existed in the concentration of PO$_4^{3-}$-P. *Anabaena* and *Microcystis* were detected for the first time in April at densities of 0.433 trichomes mL$^{-1}$ and 0.0167 colonies mL$^{-1}$, respectively. The *Anabaena* density declined to a minimum (0.130 trichomes mL$^{-1}$) in September, followed by a peak at 13.1 trichomes mL$^{-1}$, and high densities remained until January. *A. crassa* and *A. flos-aquae* accounted for >92% of *Anabaena* species, with the exception of June and July; *A. macrospora* appeared in June-December and accounted for 56.0% and 16.0% of all *Anabaena* species in June and July, respectively. *Microcystis* density peaked at 281 colonies mL$^{-1}$ in August. The *Microcystis* species composed of *M. aeruginosa* and *M. wesenbergii*; the former accounted for 75.0-99.6% of the whole *Microcystis*, with the exception of December, when the latter accounted for 75.0%. *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek existed during June-October intermittently with a maximum density of 0.15 trichomes mL$^{-1}$.

### 3.4. *Physico-chemical conditions and co-occurring cyanoprokaryotes in waters in which* A. flos-aquae *var.* klebahnii *appeared*

The water temperature in the Hirosawa-no-ike Pond peaked at 34.8 °C in August. The pH fluctuated at 6.9–9.8 from February to June; however, high values (>9.0) lasted in July-October. The DIN concentrations often remained low (<1.8 µmol L$^{-1}$); however, two rapid increases appeared in June (24.4 µmol L$^{-1}$) and July (43.8 µmol L$^{-1}$). The PO$_4^{3-}$-P concentrations were 0.176-5.44 µmol L$^{-1}$. *Anabaena* was present constantly from late June. The maximum density of *Anabaena* was 1.10 × 10$^4$ trichomes mL$^{-1}$, which was observed in early September. Four *Anabaena* species were identified: *A. crassa*, *A. flos-aquae*, *A. macrospora*, and *A. reniformis* Lemmermann. The mean proportions of *A. crassa*, *A. flos-aquae* and *A. reniformis* were 68.1%, 18.5% and 13.4%, respectively, and the proportion of *A. macrospora* never exceeded 0.02% despite its continual existence during July-September. The population density of *Microcystis* decreased from early March to early April, but typically exceeded 1000 colonies mL$^{-1}$ after late April. The main *Microcystis* species throughout the study period was *M. aeruginosa*; its proportion was 32.7-93.7% with an average of 71.5%. The mean proportions of *M. viridis* and *M. wesenbergii* were 12.6% and 15.9%, respectively. *Planktothrix raciborskii* (Woloszynska) Anagnostidis & Komárek appeared for the first time in early June, and continually existed until January. *Raphidiopsis mediterranea* Skuja was observed continuously in August-November.

The water temperature in the Osawa-no-ike Pond reached 32.7 °C in early August and gradually decreased to 7.0 °C in January. The pH varied at 7.0-9.3, with a mean of 7.9 ± 0.59 (SD). Although some sporadic increases in nutrient concentrations were observed, these increases were generally constant for the remainder of the sampling period. The concentrations of DIN and $PO_4^{3-}$-P exhibited no clear seasonal change. The concentration of DIN fluctuated between 0 and 5.3 µmol $L^{-1}$ with a mean ± SD of 0.95 ± 1.3 µmol $L^{-1}$. The concentration of $PO_4^{3-}$-P was typically <0.5 µmol $L^{-1}$, but sporadic increases (>1.3 µmol $L^{-1}$) were observed in April, September and December. *Anabaena* was observed during April-January, especially in April and late summer months (August and September). The species composition of *Anabaena* in the Osawa-no-ike Pond was identical to that in the Hirosawa-no-ike Pond, though the seasonal dynamics were different; the dominant species differed according to season, and each species became dominant at least once during the study period. *Microcystis* also occurred after May; however, its population density never exceeded 1 colonies $ml^{-1}$. *M. aeruginosa* was the dominant species in May-August, whereas *M. wesenbergii* was dominant at some times after September.

The water temperature in the Benkei-ike Pond fluctuated between 6.2 °C (February) and 28.9 °C (August). The pH peaked in May (8.7) and October (8.7). Concentrations of DIN exceeded 21 µmol $L^{-1}$ from February to April, but remained low (0.19–3.2 µmol $L^{-1}$) until October, and then increased from November. Mean concentration of $PO_4^{3-}$-P was 0.33 ± 0.034 (SD) µmol $L^{-1}$, with no clear seasonal variation. *Anabaena* (*A. crassa* and *A. flos-aquae*) was detected intermittently with a maximum density of 0.80 colonies $mL^{-1}$. In contrast, *Microcystis* was observed throughout the study period. The population density of *Microcystis* was 5.20-142 colonies $mL^{-1}$, with a mean of 44.3 colonies $mL^{-1}$. Notably, *M. aeruginosa* was often the most dominant species, accounting for 34.7-95.7% of all *Microcystis* species, followed by *M. wesenbergii*, which became dominant in October and November. Additionally, *M. viridis* comprised only a small proportion of *Microcystis* species (0-2.1%).

The water temperature in the KU Pond ranged from 2.8 °C (February) and 28.3 °C (August). A high pH (>8.6) lasted during April-August, followed by a reduction after October. The concentration of DIN peaked in July (9.2 µmol $L^{-1}$) and reached a minimum (4.3 µmol $L^{-1}$) in September. It increased from October and reached a maximum (16.3 µmol $L^{-1}$) in December. The $PO_4^{3-}$-P concentration was always below the detection limit. The sole co-occurring cyanoprokaryote was *M. aeruginosa*, which was detected in May-January; its maximum density was 75 colonies $mL^{-1}$ (September).

### 3.5. *Relationship between* A. flos-aquae *biovolume and environmental factors*

Figures 4 and 5 and table 2 show relationships between biovolume of *A. flos-aquae* and environmental factors. *A. flos-aquae* in Lake Biwa and Lake Yogo appeared over wide ranges of water temperature and pH. Biovolumes of *A. flos-aquae* in Lake Biwa and Lake Yogo were not significantly correlated with environmental factors, except for a positive correlation with the population density of *O. kawamurae* in Lake Biwa. Biovolumes of *A. flos-aquae* in Hirosawa-no-ike Pond and Osawa-no-ike Pond were negatively correlated with water temperature and the abundance of co-occurring cyanoprokaryotes such as *Microcystis* and *Anabaena*. In contrast, the biovolume of *A. flos-aquae* in KU Pond tended to increase with water temperature and the abundance of *Microcystis*, although the correlations were not statistically significant. *A. flos-aquae* appeared in the KU Pond at pH values of above 7.9, whereas *A. flos-aquae* was present in the other three ponds over a wide range of pH values. *A. flos-aquae* was present in Benkei-ike Pond almost independently of all environmental conditions. Overall, *A. flos-aquae* biovolumes were not clearly related to pH or nutrient concentration.

## 4. DISCUSSION AND CONCLUSIONS

All lakes and ponds in this study had water qualities safely classified as eutrophic, implying that eutrophication of freshwater ecosystems can provide *A. flos-aquae* with opportunities for growth, which is consistent with general beliefs. However, the dominant variety of *A. flos-aquae* differed among ecosystems; *A. flos-aquae* var. *flos-aquae* appeared in Lake Biwa and Lake Yogo, whereas *A. flos-aquae* var. *klebahnii* was the dominant variety in other aquatic systems. Notably, the waters in which *A. flos-aquae* var. *flos-aquae* appeared were considerably larger and deeper than those in which *A. flos-aquae* var. *klebahnii* existed. Water body depth is strongly related to the degree of thermal stratification, which is of particular importance when determining the vertical distribution of phytoplankton. However, severe thermal stratifications are unlikely to form in the water bodies in this study, due to relatively shallow depths, including the southern basin of Lake Biwa, and aeration system in Lake Yogo (Tsujimura 2004). An attempt to generalize the correlation between the occurrence of either variety of *A. flos-aquae* and size of a water body will, however, fail, as *A. flos-aquae* var. *flos-aquae* has been identified in water bodies with surface areas that are smaller than that of the Hirosawa-no-ike Pond and *A. flos-aquae* var. *klebahnii* has been observed in the water bodies with surface areas larger than that of Lake Yogo (Ganf 1983; Pechar 1992; Breukers *et al*. 1997). As a selective force in determining the dominant variety of *A. flos-aquae*, Pechar (1992) noted the role of large cladoceran *Daphnia*; they may change the underwater light environment by reducing the small-sized phytoplankton including solitary trichomes of *A. flos-aquae* var. *klebahnii*, which consequently provide an opportunity for development of the large aggregates of *A. flos-aquae* var. *flos-aquae*. Conversely, the absence of *Daphnia* is favored by *A. flos-aquae* var. *klebahnii.* Lynch & Shapiro (1981) and Ganf (1983) also pointed out the positive impact of *D. pulex* Leydig on the dominance of *A. flos-aquae* var. *flos-aquae*. Data presented

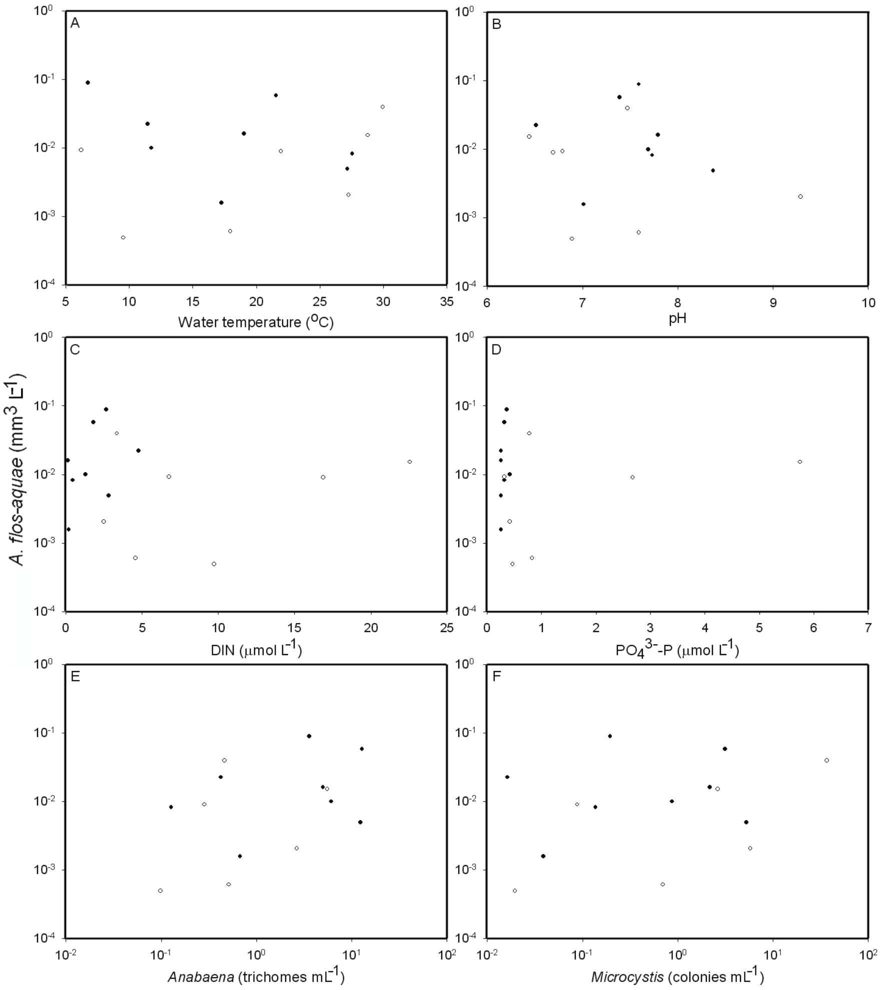

**Fig. 4**. Relationships between *A. flos-aquae* biovolume and water temperature (**A**), pH (B), DIN concentration (**C**), $PO_4^{3-}$-P concentration (**D**), trichome density of *Anabaena* (**E**) and colony density of *Microcystis* (**F**) in Lake Biwa (open circles) and Lake Yogo (closed circles).

by Breukers *et al*. (1997) showing the frequent appearance of *A. flos-aquae* var. *klebahnii* especially after the disappearance of *D. pulex* may suggest a negative impact of *D. pulex* on the dominance of *A. flos-aquae* var. *klebahnii*. Moreover, *D. pulex* can graze on *A. flos-aquae* var. *flos-aquae* as long as they exist as single trichomes or form small colonies <1.5 mm (Holm *et al*. 1983). These observation results suggest that the existence of large cladocerans can be a critical factor in selecting the variety of *A. flos-aquae* in waters and in regulating *A. flos-aquae* seasonal dynamics. However, the primary components of zooplankton communities in the aquatic systems in this study were rotifers and/or small cladocerans; large cladocerans like *Daphnia* were always undetectable in concentrated water samples (data not shown). Therefore, in this study, the direct impact of large herbivorous zooplankton on *A. flos-aquae* was negated and the role of small zooplankton in a cyanoprokaryotic community was probably negligible due to their low feeding activities.

Notably, *A. flos-aquae* var. *flos-aquae* existed in both Lake Biwa and Lake Yogo almost throughout the entire year with a relatively high biomass in winter. Tsujimura *et al*. (2001) examined the growth response of *A. flos-aquae* var. *flos-aquae* isolated from Lake Biwa in a wide range of water temperatures (5-32 °C), and demonstrated that the lowest temperature at which *A. flos-aquae* can grow is 8 °C; optimum temperature range was 23-29 °C. They also reported that *A. flos-aquae* cultured at 5 °C for 25 days retained the ability to grow, indicating the possibility that the high population densities of *A. flos-aquae* in Lake Biwa and Lake Yogo during winter was due to its low-temperature tolerance rather than low-temperature preference. This physiological feature enables *A. flos-aquae* to exist during winter in high densities by accumulating at the surface (Tsukada *et al.* 2006).

In Lake Yogo, the *A. flos-aquae* biomass was low during summer when other populations of cyanoprokaryotes, such as *Anabaena* and *Microcystis*, increased.

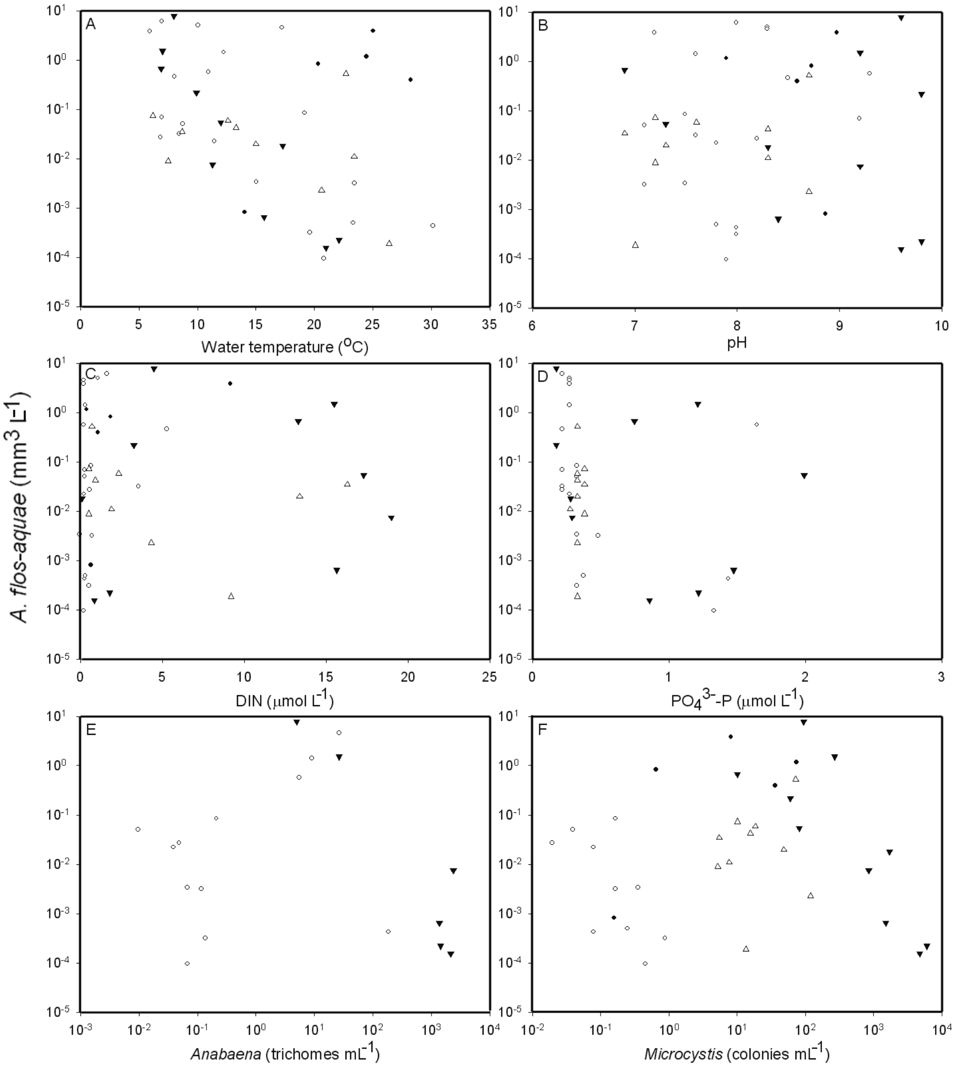

**Fig. 5.** Same as figure 4, but in Hirosawa-no-ike Pond (closed triangles), Osawa-no-ike Pond (open circles), Benkei-ike Pond (open triangles) and KU Pond (closed circles).

**Tab. 2**. Linear correlation coefficients between natural logarithm of *A. flos-aquae* biovolume and environmental factors in Lake Biwa (Biwa), Lake Yogo (Yogo), Hirosawa-no-ike Pond (Hiro), Osawa-no-ike Pond (Osa), Benkei-ike-Pond (Ben) and KU Pond (KU). Bold values are significant at the 5% level. The natural logarithms of the population densities of co-occurring cyanoprokaryotes are used to improve the fit.

| | var. *flos-aquae* | | var. *klebahnii* | | | |
|---|---|---|---|---|---|---|
| | Biwa | Yogo | Hiro | Osa | Ben | KU |
| Water temperature | 0.48 | -0.43 | **-0.91** | **-0.68** | -0.41 | 0.80 |
| pH | -0.28 | -0.09 | -0.19 | 0.20 | 0.27 | -0.25 |
| DIN | 0.27 | 0.42 | 0.19 | 0.21 | 0.29 | 0.49 |
| $PO_4^{3-}$-N | 0.40 | 0.37 | -0.30 | -0.36 | 0.15 | - |
| *Anabaena* | 0.38 | 0.30 | **-0.94** | 0.31 | - | - |
| *Microcystis* | 0.60 | 0.16 | **-0.82** | **-0.66** | 0.09 | 0.68 |
| *Oscillatoria* | **0.94** | - | - | - | - | - |
| *Planktothrix* | - | - | 0.48 | - | - | - |
| *Raphidiopsis* | - | - | 0.73 | - | - | - |

The maximum temperature of 29.7 °C in Lake Yogo is considered slightly higher than the optimum range for *A. flos-aquae* growth (Uehlinger 1981; Pechar 1992; Tsujimura *et al.* 2001). However, a decrease in the in situ population of *A. flos-aquae* had already occurred in May when water temperature was 17.3 °C, suggesting that high temperature itself is not a main causative factor for the disappearance of *A. flos-aquae* during summer. In fact, some studies reported the dominance of *A. flos-aquae* during summer (Jones 1979; Pechar 1992; Takano & Hino 2000). One possible factor determining the biomass of *A. flos-aquae* during summer is the existence of other cyanoprokaryotes. An inverse relationship between population densities of *A. flos-aquae* and other cyanoprokaryotes, such as *Anabaena*, *Microcystis* and *Oscillatoria*, during summer (Lynch & Shapiro 1981; Hino 1992; Shapiro 1997; Tsukada *et al.* 2006) suggests an existence of an interference interaction between *A. flos-aquae* and other cyanoprokaryotes. Due to the low-frequency survey and intermittent absence of *A. flos-aquae*, no significant correlation existed between population densities of *A. flos-aquae* and co-occurring cyanoprokaryotes in Lake Yogo. However, if the decrease in the *A. flos-aquae* population is related to the existence of other cyanoprokaryotes, they can suppress *A. flos-aquae* with very low densities; the population densities of *A. flos-aquae*, *Anabaena* and *Microcystis* in May, when the density of *A. flos-aquae* declined sharply, were 0.40 trichomes $mL^{-1}$, 0.69 trichomes $mL^{-1}$ and 0.040 colonies $mL^{-1}$, respectively.

In Lake Biwa, the peak biomass of *A. flos-aquae* var. *flos-aquae* occurred at the same time as those of *Anabaena*, *Microcystis* and *O. kawamurae*. This observation result is inconsistent with that for Lake Yogo. The population densities of *Anabaena* and *Microcystis* in Lake Biwa were usually lower than those in Lake Yogo, engendering a possibility that *A. flos-aquae* var. *flos-aquae* can grow without being affected severely by low densities of co-occurring cyanoprokaryotes. However, co-occurring cyanoprokaryotes likely had negative impacts on the *A. flos-aquae* population in Lake Biwa, as inferred by the rapid increase in *A. flos-aquae* in January when other cyanoprokaryotes were absent.

The appearance of *A. flos-aquae* var. *klebahnii* during winter was typical in the Hirosawa-no-ike Pond, Osawa-no-ike Pond and Benkei-ike Pond. This study did not investigate the response of these populations to various temperatures; however, temperatures of 20–25 °C are adequate for maintaining culture strains isolated from these three ponds (Yamamoto, unpublished data), suggesting that these *A. flos-aquae* populations can grow over a wide range of water temperatures. In the Hirosawa-no-ike Pond, a large number of viable *Microcystis* colonies existed from December to mid-February in the sediment with water content of >40% (Yamamoto, unpublished data); however, the existence of vegetative cells or akinetes of *A. flos-aquae* in pond sediment was unidentifiable by light microscopy. However, *A. flos-aquae* in the Hirosawa-no-ike Pond in February likely originated in the Osawa-no-ike Pond - this pond is a water source for the Hirosawa-no-ike Pond. Even though these two populations of *A. flos-aquae* have the same origin, their dynamics differed. In the Hirosawa-no-ike Pond, *A. flos-aquae* started to be excluded from plankton from April, and was absent from May to mid-October. In contrast, the *A. flos-aquae* population in the Osawa-no-ike Pond disappeared in mid-May-August. During these periods, other cyanoprokaryotes, particularly *Anabaena* and *Microcystis*, dominated, suggesting possible adverse effects of these genera on *A. flos-aquae*. The negative impact of *Anabaena* and *Microcystis* on *A. flos-aquae* was also verified by cyanoprokaryotes dynamics in the Osawa-no-ike Pond from late summer to autumn. Although *A. flos-aquae* reappeared in September, its density remained low until the end of October when either *Anabaena* or *Microcystis* existed in high densities. However, the population of *A. flos-aquae* increased from November when the density of *Microcystis* rapidly decreased. Similarly, *A. flos-aquae* in the Hirosawa-no-ike Pond reappeared in mid-October and increased rapidly when the population densities of *Microcystis*, *P. raciborskii* and *R. mediterranea* decrease. Based on the roughly constant density of *Anabaena* from mid-September to November and rapid increase in *P. raciborskii* in late November, the period when the *A. flos-aquae* density increased in Hirosawa-no-ike Pond in November corresponds to decreases of *Microcystis* and *R. mediterranea* densities. In the Benkei-ike Pond, the *Microcystis* density increased from summer to autumn and *A. flos-aquae* was almost undetectable during the same period. Moreover, when the *Microcystis* density decreased in November, that of *A. flos-aquae* started increasing. The decreases in the *Microcystis* density in these three ponds in late autumn are likely due to the intolerance of this genus to low water temperature (Robarts & Zohary 1987). However, the absence or low population densities of *A. flos-aquae* when *Microcystis* dominated were common in the three ponds, suggesting that *Microcystis* has a probable interference effect on *A. flos-aquae*.

Previous studies demonstrate that the *A. flos-aquae* var. *klebahnii* population in the KU Pond is adapted to high water temperatures (Yamamoto & Nakahara 2005, 2006). The biomass of *A. flos-aquae* peaked in July due to the remarkable increase after first appearing in May. Although *A. flos-aquae* sustained high population densities from June to September, the biomass observed during this period may have been suppressed by the co-occurring *M. aeruginosa*. In the KU Pond, *Microcystis* colonies were rarely detected in 2002-2005, and each summer bloom was composed solely of *A. flos-aquae*. Blomqvist *et al.* (1994) highlighted the importance of high $NH_4^+$-N with low $NO_3^-$-N for the development of non-nitrogen-fixing cyanoprokaryotes. The primary form of dissolved inorganic nitrogen in the KU Pond from spring to summer was $NH_4^+$-N since 2003 (Yamamoto & Nakahara 2006, 2007), indicating that the nutrient environment in the pond had changed to suit for the development of non-nitrogen-fixing species since 2003, though the $PO_4^{3-}$-P concentration remained at an undetectable level. In 2005, many goldfish *Carassius auratus* Linnaeus, 1758 and the submerged plant *Egeria densa* Planch were introduced into the pond several times; thus, *M. aeruginosa* colonies may have been introduced into the pond simultaneously. The maximum *A. flos-aquae* trichome densities ($mL^{-1}$) in the KU Pond in 2002, 2003, 2004, 2005 and 2006 were $2.3 \times 10^5$, $2.7 \times 10^4$, $7.8 \times 10^4$, $8.4 \times 10^4$ and $1.9 \times 10^3$, respectively (Yamamoto & Nakahara 2006, 2007). The factor(s) responsible for the extremely low population density of *A. flos-aquae* in 2006 remains unknown and, thus, the possible adverse impact of *M. aeruginosa* on *A. flos-aquae* cannot be excluded.

Based on the common phenomena among six aquatic systems, the patterns of future expansion or dynamics of *A. flos-aquae* are predicted as follows:

1) *A. flos-aquae* may appear as plankton in eutrophic waters where other cyanoprokaryotes are at almost undetectable levels. Marked reduction of cyanoprokaryotes, such as *Anabaena* and *Microcystis*, from the water can also open an opportunity for *A. flos-aquae* to appear.
2) Both seasonal dynamics and annual maximum biomass of *A. flos-aquae* are affected by co-occurring cyanoprokaryotes, especially *Microcystis*, and their increases or appearances lead to a decrease in the *A. flos-aquae* population.

The critical factor(s) determining which variety of *A. flos-aquae* will appear was unidentified in this study. Considerable evidence exists for the negative impacts of co-occurring cyanoprokaryotes, especially *Microcystis*, on *A. flos-aquae*; however, the mechanism remains mostly unknown. For instance, whether the decreases in *A. flos-aquae* var. *klebahnii* densities before the collapse of populations in Hirosawa-no-ike Pond and Osawa-no-ike Pond were due to the inhibitory effect of co-occurring cyanoprokaryotes is difficult to determine, because water temperatures during this period were likely too low for growth of *Anabaena* and *Microcystis* (Robarts & Zohary 1987). Although the temperature environment is considered adequate for growth of co-occurring cyanoprokaryotes, whether the density of *Anabaena* and *Microcystis* in Lake Yogo from spring to early summer was sufficiently high to suppress the *A. flos-aquae* var. *flos-aquae* population is open to debate. Additional research on the interspecific interaction between *A. flos-aquae* and other cyanoprokaryotes species, and the effects of general environmental factors is necessary to elucidate the in situ population dynamics of *A. flos-aquae*.

## ACKNOWLEDGEMENT

Ted Knoy is appreciated for his editorial assistance.

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*Received: July 2008*
*Accepted: December 2008*

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