



## Life Cycle of Cyanobacterium *Aphanizomenon flos-aquae*

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**ABSTRACT:** The life cycle of the cyanobacterium *Aphanizomenon flos-aquae* was elucidated based on data concerning the growth, formation and degradation of the bloom, as well as consideration of its mechanism. Abiotic factors such as water temperature, pH and day length are of critical importance in determining not only the growth in the water, but also germination and formation of akinetes. Besides these abiotic factors, biotic factors such as competition between coexisting phytoplankton and grazing by zooplankton should be taken into consideration – particularly at the initial and final stages of the bloom.

**KEY WORDS:** Akinetes, *Aphanizomenon flos-aquae*, day length, growth, life cycle, pH, water temperature.

### INTRODUCTION

Summer phytoplankton communities in eutrophic freshwater ecosystems are commonly dominated by cyanobacteria, which phenomenon has been a main research theme in limnology. Previous works have revealed that particular environmental conditions, such as low CO<sub>2</sub> concentrations (Paerl and Ustach, 1982; Shapiro, 1997) and a low ratio of nitrogen to phosphorus (Smith, 1983), are associated with many cyanobacterial blooms. A substantial amount of information is available on the ecophysiology of bloom-forming cyanobacteria. Nevertheless, elucidating the life cycle of certain species of cyanobacteria in a given field is difficult because relatively few continuous studies have been undertaken on ecophysiological aspects of the species.

*Microcystis* and *Anabaena* have been considered as the representative bloom-forming cyanobacteria genera in temperate regions; however, the number of reports of the first appearance of *Aphanizomenon flos-aquae*, which frequently form blooms in high-latitude regions, has been recently increasing (Yamamoto, 2009). An invasion of sub-arctic species into temperate regions is curious, given the recent trend toward global warming. Therefore, from the perspective of water management, understanding the ecological strategies of *A. flos-aquae* is important in considering the possibility of its future expansion, and knowledge of its life cycle is particularly important.

A continuous ecological survey was recently performed by targeting a specific population of *A. flos-aquae* var. *klebahnii* Elenk. This work elucidates the life cycle of this species.

### MATERIALS AND METHODS

#### Morphological characteristics of *A. flos-aquae*

*A. flos-aquae* exists as solitary trichomes with lengths from several tens to several hundreds of micrometers (Fig. 1A). The trichomes consist mostly of vegetative cells, and several heterocysts often appear at roughly regular intervals on a trichome. Akinetes begin to be produced in response to such degradation of their growth environment as a drop in temperature or a drop in irradiance. They then sink to the bottom and survive during the unfavorable period (Fig. 1B).

#### Field survey

A field survey was carried out in a small (surface area 25.6 m<sup>2</sup>), shallow (mean depth 1.7 m) artificial pond in Kyoto, Japan, from May 2002 to January 2007. Water samples were taken from the water surface weekly from May 2002 to April 2003, biweekly from May 2003 to October 2005, and monthly from February 2006 to January 2007, to measure temperature, pH, dissolved inorganic nitrogen (DIN) concentration, phosphate concentration and species composition of phytoplankton, with special reference to the dynamics of *A. flos-aquae*. DIN concentration was calculated as the sum of nitrate, nitrite and ammonium concentrations. The routine analysis methods were those described by Yamamoto and Nakahara (2005). Pearson's product moment correlation coefficient (Muto, 1995) was used to quantify the strength of the linear associations between the log-transformed trichome density of *A. flos-aquae* and environmental factors.

### RESULTS AND DISCUSSION

The trichome densities of *A. flos-aquae* rarely exceeded  $1.0 \times 10^4$  trichomes ml<sup>-1</sup> at water temperatures

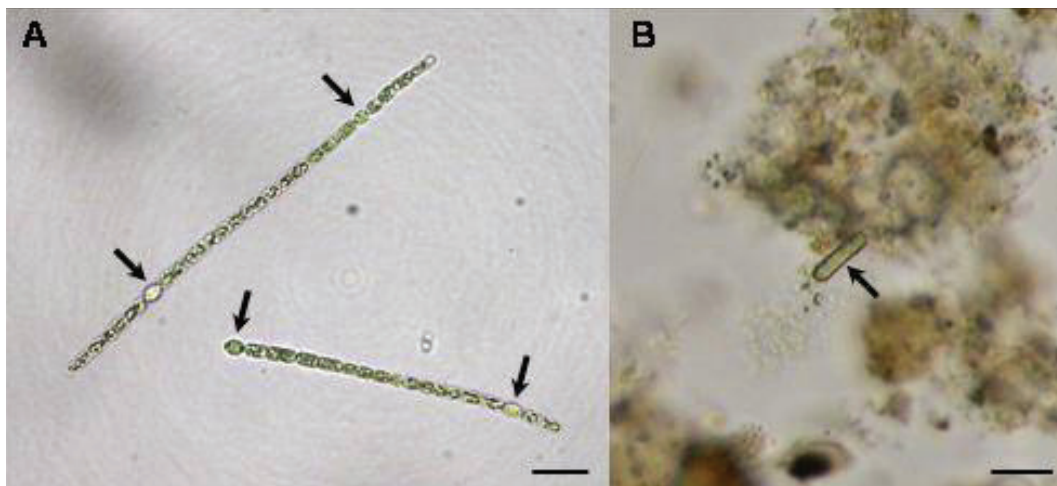


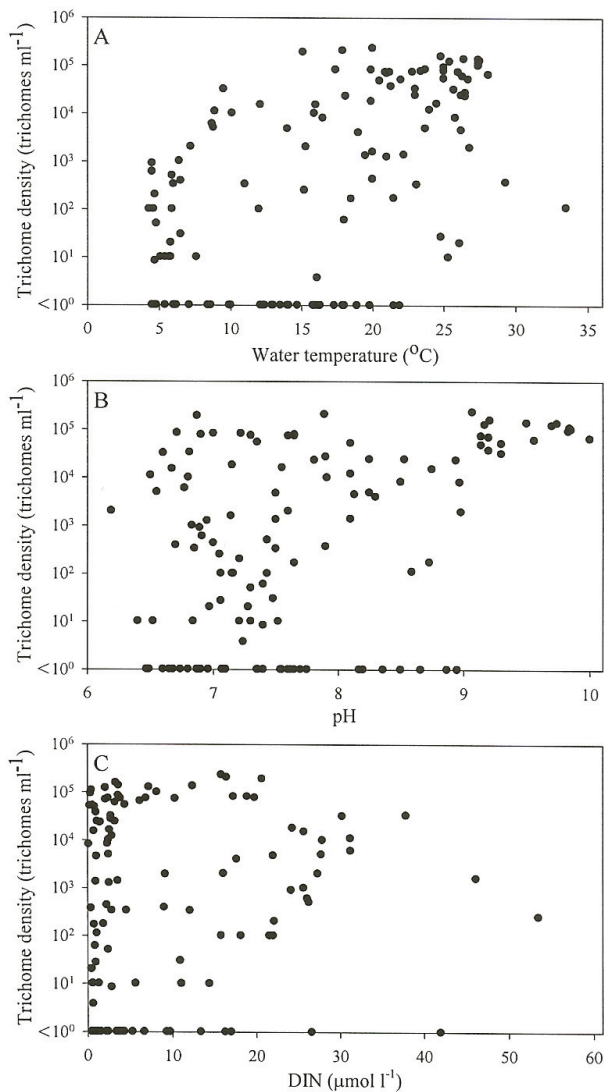
Fig. 1. Light micrographs of *Aphanizomenon flos-aquae*. A: Trichomes. Arrows indicate heterocysts. B: Akinete (arrow) in the sediment on 15 November, 2005. Scale bar = 20  $\mu\text{m}$ .

below 14.7°C, but frequently exceeded  $1.0 \times 10^4$  trichomes  $\text{ml}^{-1}$  at and above 15.1°C (Fig. 2A). *A. flos-aquae* was always detected at water temperatures exceeding 22°C. The trichome densities of *A. flos-aquae* were significantly correlated with water temperature ( $r = 0.56$ ,  $n = 129$ ,  $p < 0.001$ ). The pH ranged between 6.19 and 10.0, with a mean of 7.68 (Fig. 2B). *A. flos-aquae* was present over a wide range of trichome densities ( $0$ - $1.9 \times 10^5$  trichomes  $\text{ml}^{-1}$ , with a mean of  $1.25 \times 10^4$  trichomes  $\text{ml}^{-1}$ ,  $n = 82$ ) at low pH, but frequently appeared with high trichome densities ( $0$ - $2.3 \times 10^5$  trichomes  $\text{ml}^{-1}$ , with a mean of  $4.44 \times 10^4$  trichomes  $\text{ml}^{-1}$ ,  $n = 47$ ) at high pH. *A. flos-aquae* always appeared in water with high pH ( $> 9$ ) at high trichome densities ( $> 3.15 \times 10^4$  trichomes  $\text{ml}^{-1}$ ). Consequently, the trichome densities of *A. flos-aquae* were also correlated positively with pH ( $r = 0.42$ ,  $n = 129$ ,  $p < 0.001$ ). Incubation experiments demonstrated that a water temperature of over 11°C and a pH of over 7.1 strongly facilitated the growth of *A. flos-aquae* (Fig. 3). Water temperature and pH explain not only the initial appearance of *A. flos-aquae* trichomes in a water column, but also the collapse of the bloom (Yamamoto and Nakahara, 2006a); therefore, these two factors each directly impacted the population dynamics of *A. flos-aquae* in the study pond.

The impact of light on the growth of *A. flos-aquae* is not as direct as those of temperature and pH, according to the incubation experiment; however, its growth is clearly suppressed when the photoperiod is short (Yamamoto and Nakahara, 2005), implying that the *in situ* growth performance of *A. flos-aquae* can be influenced by day length. Along with a high temperature, a long day length seems to contribute to the massive growth of *A. flos-aquae* in the study pond during the summer.

The phosphate concentration was almost always undetectable throughout the study period; on the other hand, DIN concentration was always detected. *A. flos-aquae* appeared even in waters in which the concentration of DIN remained low, but the frequency of appearance clearly increased with the concentration of DIN (Fig. 2C). A significant correlation existed between the trichome densities of *A. flos-aquae* and the concentration of DIN ( $r = 0.18$ ,  $n = 129$ ,  $p < 0.05$ ). *A. flos-aquae* can fix molecular nitrogen in heterocysts, and so can grow even in nitrogen-deficient environments. However, the positive correlation between its population density and the concentration of DIN suggests that *A. flos-aquae* prefers environments where DIN is available. In the pond, the DIN concentration generally remains low from early spring to early autumn. However, the trichome density of *A. flos-aquae* begins to increase from early summer (Yamamoto and Nakahara, 2006a, 2007). Therefore, the DIN concentration appears not to limit the growth of *A. flos-aquae*, at least in the pre-blooming period. Furthermore, the bloom of *A. flos-aquae* typically terminates in autumn when DIN is present at a high concentration (Yamamoto and Nakahara, 2006a), suggesting the absence of a clear relationship between the collapse of the *A. flos-aquae* bloom and the concentration of DIN.

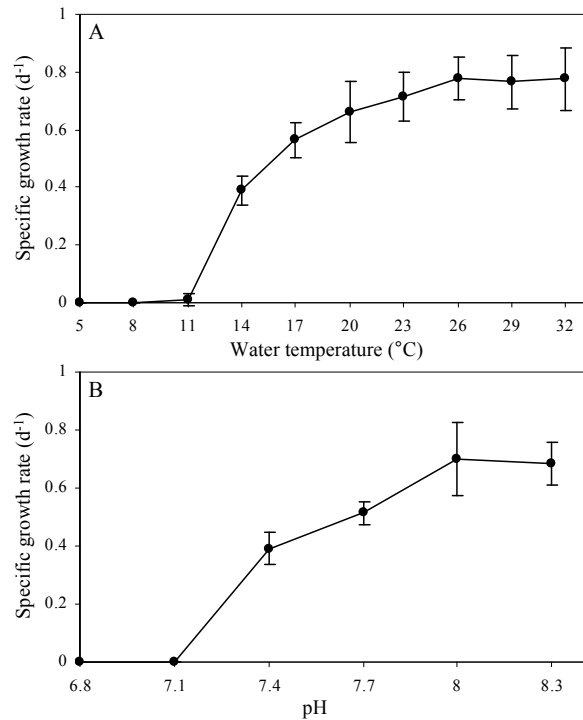
Following bloom collapse, various microorganisms are probably importantly involved in the decomposition of fragmented trichomes (Granhall, 1972; Caldwell and Caldwell, 1978; Sigee et al., 1999; Gons et al., 2002), which may then be consumed by zooplankton (Hanazato and Yasuno, 1987; Van Hannen et al., 1999). The population densities of rotifers increased rapidly after the collapse of the *A. flos-aquae* bloom in the late autumn (Yamamoto and Nakahara, 2006b). This observation



**Fig. 2.** Relationships between trichome densities of *A. flos-aquae* and water temperature (A), pH (B) and the concentration of DIN (C) throughout the study period.

seems to suggest that rotifers have a significant role in the elimination of *A. flos-aquae* trichomes from the water (Van Hannen et al., 1999).

The recruited benthic population of cyanobacteria is potentially important as the inocula of the planktonic population (Takamura et al., 1984; Rengefors et al., 2004), especially in the life cycle of nostocalean genera, in which akinete formation supports overwintering (Tsujimura and Okubo, 2003; Karlsson-Elfgren and Brunberg, 2004). A field survey revealed the formation of akinete on the trichomes of *A. flos-aquae* before the collapse of the bloom and in winter the trichome density in the water column ultimately fell below the detection limit (Yamamoto and Nakahara, 2007). Therefore, *A. flos-aquae* can be reasonably assumed to have remained



**Fig. 3.** Effects of water temperature (A) and pH (B) on the specific growth rate of *A. flos-aquae* isolated from the study pond. The standard experimental condition was 25°C, pH 8.2, and a photon flux density of 90  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  on a 14h: 10h light-dark cycle in CT medium (Watanabe and Ichimura, 1977). Error bars represent standard deviation, calculated from four replicate cultures. (modified from Yamamoto and Nakahara, 2005).

in the form of akinetes from winter to spring. In fact, the population density of *A. flos-aquae* in the initial period following their appearance was significantly lower at the surface of the water column than close to the bottom, suggesting that they overwintered in the form of akinetes, and that trichomes remained close to the bottom shortly following germination (Yamamoto and Nakahara, 2006b). The germination of *A. flos-aquae* akinetes was probably triggered by the increase in water temperature, as observed in many other species (Cmiech et al., 1984; Huber, 1985; Baker and Bellifemine, 2000; Tsujimura and Okubo, 2003). However, the growth of recruited *A. flos-aquae* trichomes is regulated by various environmental factors, such as water temperature, pH, irradiance and day length (Yamamoto and Nakahara, 2005). As well as these abiotic factors, a predominance of competitive species in the water column can negatively influence the growth of *A. flos-aquae*. The field survey suggested that the green alga *Ankistrodesmus falcatus* (Corda) Ralfs and the cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing can inhibit the growth of *A. flos-aquae* (Yamamoto and Nakahara, 2006a; Yamamoto, 2009).

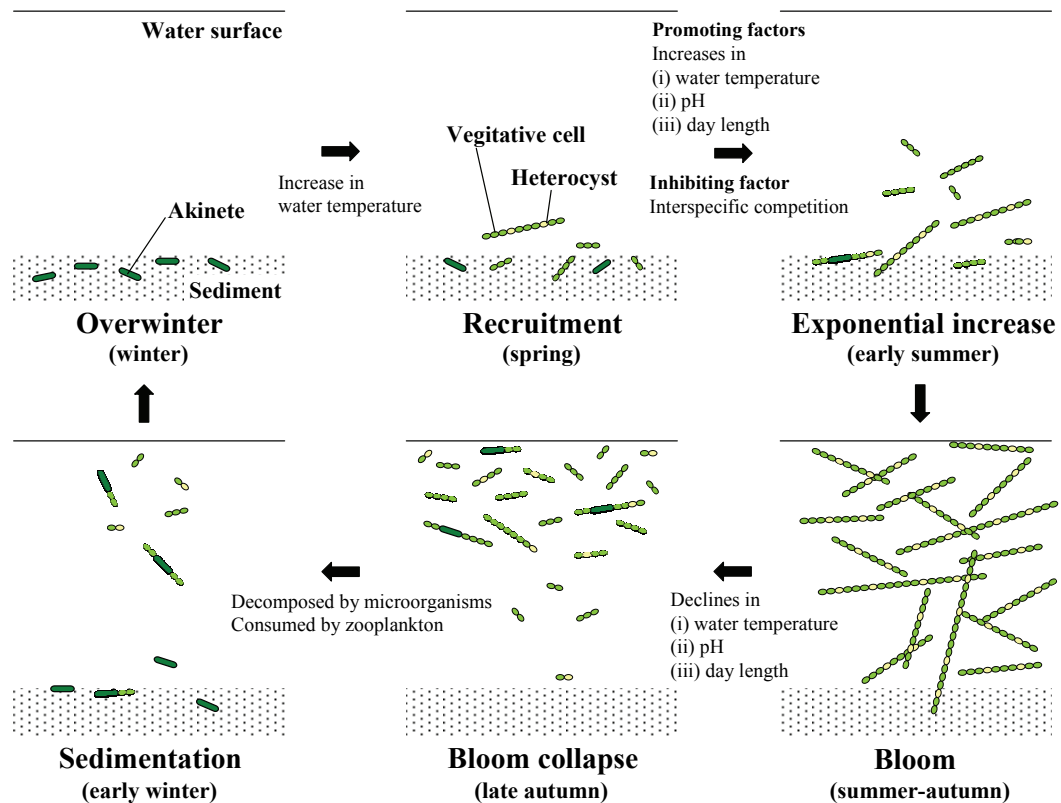


Fig. 4. Schematic view of the annual cycle of *A. flos-aquae* in the study pond.

The results of our survey imply the following life cycle of *A. flos-aquae*, presented in Fig. 4. *A. flos-aquae* overwinters in the form of akinetes in the sediment. In late spring, these akinetes germinate in response to the increase in water temperature and are recruited into the water column. The growth of vegetative cells, however, can be inhibited if other phytoplankton species already dominate the water column. Before reaching a bloom, most *A. flos-aquae* trichomes are present near the bottom, because of stratification that occurs in early summer. After they appear in the water column, increases in water temperature, pH and day length promote their growth, and they eventually form a dense bloom of large trichomes. Water mixing in early autumn enables the propagated trichomes to be distributed uniformly throughout the water column. As water temperature, pH and day length fall in late autumn, the environment becomes unfavorable for the growth of *A. flos-aquae*, instead favors akinete development. When water temperature or pH falls below the threshold for the growth of *A. flos-aquae*, they cease growing and the fragmentation of trichomes is accelerated. The fragmented trichomes, particularly those close to the bottom, are decomposed by microorganisms or consumed by zooplankton, establishing a gradient in the vertical distribution of *A. flos-aquae*: trichome density

declines with increasing depth at the terminal stage of the bloom (Yamamoto and Nakahara, 2006b). Uninfected akinetes remain in the bottom sediment until they germinate and provide inocula for future pelagic populations.

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## 藍綠藻水華束絲藻的生活史

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摘要：本篇文章依據水華束絲藻 (*Aphanizomenon flos-aquae*) 的生長、藻華形成及瓦解和其機制，闡明其生活史。水溫、pH 值及日照長度不僅是決定水華束絲藻生長的關鍵因素，而且還影響其厚壁孢子的形成和萌發。除了上述非生物因子的作用外，與不同浮游植物間的競爭及浮游動物造成的捕食效應也得考慮其中，尤其在藻華初期及結束階段。

關鍵詞：厚壁孢子、水華束絲藻、日照時間、生長、生活史、pH 值、水溫。