INTRODUCTION

Algal recruitment from sediments is considered to affect dominance patterns in a phytoplankton community in certain cases (Hansson, 1996); however, limited studies have attempted to quantify the resting cells of phytoplankton species in sediments. In temperate lakes and reservoirs, nuisance cyanobacterial blooms typically begin to form during summer, although there have been several reports of cyanobacterial blooms forming almost all year round (Zohary and Robarts, 1989). The process of the bloom formation by gas-vacuolate cyanobacteria can generally be divided into three parts: (i) recruitment of an overwintering population from sediment to the water column; (ii) growth proliferation in the water column; and (iii) surface accumulation of the population by their ability to regulate their buoyancy and advective transport following wind events.

The dependence on recruitment for bloom development seems to vary among species (Barbiero and Welch, 1992). There have been several reports of the sudden occurrence of cyanobacterial blooms in various water bodies (Messikomer, 1961; Tsujimura et al., 2001). For the first time, Aphanizomenon ovalisporum Forti was identified as the dominant species during an unusual bloom in Lake Kinneret, Israel, during the late summer and autumn of 1994. While Hadas et al. (Hadas et al., 1999) pointed out that the akinetes of A. ovalisporum left in the sediment could be a source for the next year’s bloom, there was no information on the invasion of this species in Lake Kinneret. These reports suggest that cyanobacterial blooms could be established without a massive recruitment from the sediment, since there was an insignificant overwintering population in the sediment before such cases of bloom formation.

Preston et al. (Preston et al., 1980) have shown that summer populations of Microcystis spp. in the water column originate from overwintering sediment colonies. It has been proposed, however, that intensive growth in

Development of *Anabaena* blooms in a small reservoir with dense sediment akinete population, with special reference to temperature and irradiance

SHIGEO TSUJIMURA* AND TAKUYA OKUBO
LAKE BIWA RESEARCH INSTITUTE, 1-10 UCHIDEHAMA, OTSU 520-0816, JAPAN
*CORRESPONDING AUTHOR: tsujim@lbri.go.jp
the water column is important for bloom formation of these organisms (Reynolds et al., 1981). In our previous study, we also demonstrated the importance of intensive growth in the water column for *Microcystis* blooms in Lake Biwa, Japan (Tsujimura et al., 2000). In this study, we also noted that the time of bloom formation is related to the quantity of *Microcystis* colonies in the sediment, i.e. if the quantity of new inoculum from the sediment is very large, the bloom will be formed earlier (Tsujimura et al., 2000).

Various observations have been made concerning the origin of blooms of *Anabaena* spp. Baker did not find the vegetative cells (filaments) of *Anabaena flos-aquae* Breb. ex Bornet et Flahault in the water column during winter and concluded that the summer population had originated entirely from the sediment (Baker, 1999). The same study showed that *Anabaena circinalis* Rabenh. ex Bornet et Flahault was found in the water column even during winter, suggesting that part of the summer population may originate from the residual winter population in the water column. Baker also documented the presence of a rich akinete population of *A. circinalis* in the sediment and estimated that these akinetes may represent an inoculum of 220 times that of the residual planktonic population at the commencement of seasonal growth (Baker, 1999). On the other hand, Head et al. used downward-facing traps to quantify the vertical movement of gas-vacuolate cyanobacteria (Head et al., 1999). They showed that although recruitment from benthic stocks of *Anabaena silaneae* Kleb. might play an important role in the initial development of this planktonic population, large summer populations of *A. flos-aquae* seemed to be dependent on a small overwintering population in the water column. Thus, the importance of an overwintering *Anabaena* population in sediment for water-bloom formation is still not clear.

In the present study, we observed cyanobacterial bloom formation in a small eutrophic reservoir where *Anabaena ucrainica* (Schkorb.) M. Watan. formed almost unialgal blooms during late spring and summer. The density of *Anabaena* akinetes in the sediment of the reservoir was also investigated. To evaluate the *Anabaena* development during seasonal increases in temperature, we examined the effects of temperature and irradiance on the akinete germination and growth of *A. ucrainica*.

**METHOD**

**Study site and sampling**

This study was conducted at Daimon-Ike, a small agricultural reservoir (surface area 5 ha, mean depth 1.8 m), situated in Shiga prefecture, Japan (Figure 1). The reservoir has experienced dense blooms of *A. ucrainica* since the summer of 1987, and local residents have reported the presence of musty odors (geosmin) at the reservoir. Two sampling sites (S1 and S2) were used to examine the seasonal variation of *A. ucrainica* in the reservoir. Sampling was conducted at these sites at roughly 8-day intervals from April to September during 1998 and 1999. Surface water was sampled using a bucket.

Temperature and nutrient concentrations in the reservoir, at the sampling sites, and in the inflowing and outflowing water during the study period, have been previously reported by Okubo et al. (Okubo et al., 2000).

In order to investigate the horizontal distribution of *A. ucrainica* filaments in surface water and akinetes in sediments, we took surface water and sediment samples from nine points (P1-P9) in the reservoir on 12 August 1998 (Figure 1). Sediment samples were taken from each point using a K.K. core sampler (Hashimoto Kagaku Co. Ltd, Kyoto, Japan) equipped with a plastic tube with a 5 cm internal diameter.

**Counting**

The density of *Anabaena* filaments in the water sample was determined using a Sedgewick–Rafter counting chamber under an inverted microscope (Nikon TMS). The net growth rate, \( \lambda \) (day\(^{-1}\)), was calculated for a period during the exponential rate of population increase:

\[
\lambda = \frac{\ln N_t - \ln N_0}{t}
\]

where \( t \) is time in days and \( N_0 \) and \( N_t \) are population densities at times \( t \) and 0.

Core samples of sediment were sectioned into 0–2 and 2–5 cm layers, and duplicate samples of each layer collected in plastic bags. An appropriate amount (~1 g wet wt) of sediment sample was suspended in distilled water at a concentration of 0.02 g ml\(^{-1}\). The *Anabaena* akinetes in 1.0 ml aliquots of the diluted sample were counted in a Sedgewick–Rafter counting chamber using...
showed that akinetes of *Anabaena* were observed. The volume weight of the sediment sample was measured separately and the density of akinetes was expressed as the number per centimeter cubed of sediment.

**Akinete germination experiments**

To study the effect of temperature on germination of *Anabaena* akinetes, we took a sediment sample using an Ekman-Birge Grab (Rigo Co. Ltd, Saitama, Japan) at S2 on 17 April 2000. The density of total akinetes in the sample was enumerated as stated above. The density of akinetes that could germinate was enumerated by the extinction dilution method under incubated conditions (Imai *et al*., 1984). The incubation experiment commenced on the day of sampling. Twenty milliliters of the sediment sample were suspended in 180 ml of sterile CT medium (Watanabe and Hiroki, 1997). After three serial 10-fold dilutions (10^{-6} to 10^{-9} fold dilution of the original sediment sample v/v) were made in the medium, 1 ml aliquots of diluted suspensions were inoculated into five replicate wells of a disposable sterilized tissue culture microplate (24 wells; Asahi Technoglass Corporation, Funabashi, Japan). Twenty microplates were prepared, with half of the plates wrapped with aluminum foil to shield from light. Each pair of plates (with and without illumination) was incubated at 10 different temperatures between 5 and 32°C at 3°C intervals. Plates without aluminum foil were incubated at 14 h:10 h light:dark cycle with a photon flux density (PFD) of 70 µmol photons m^{-2} s^{-1}. The appearance of trichomes or akinete germlings in each illuminated well was examined every other day for up to 20 days after incubation using an inverted microscope (Nikon TMS). Preliminary incubation experiments showed that akinetes of *A. ucrainica* would not germinate in total darkness; wells without illumination were only observed after 20 days incubation. Wells showing evidence of *Anabaena* filaments or germlings were scored as positive. The most probable number (MPN) of germinated akinetes was calculated according to the statistical table of Kadota and Taga (Kadota and Taga, 1985). The germination percentage of *A. ucrainica* akinetes at each temperature was measured by dividing the MPN by the total number of akinetes.

**Growth experiments**

An axenic culture of *A. ucrainica* was isolated from the reservoir in July 1998 and was designated strain LBRI 17. The stock culture was maintained under standard conditions: 25°C, 14 h:10 h light:dark cycle and a PFD of 50 µmol photons m^{-2} s^{-1} in CT media. Incubation experiments using this strain were carried out at 10 temperature levels (every 3°C from 5 to 32°C) in combination with three PFDs (20, 50 and 150 µmol photons m^{-2} s^{-1}). Stock cultures were inoculated into new media each week and kept under the standard conditions. This was performed in duplicate. Cultures were then separated into 10 test tubes with new media and each tube incubated at the various temperatures under a 14 h:10 h light:dark cycle and a PFD of 50 µmol photons m^{-2} s^{-1}. After five days of pre-conditioning, the culture at each temperature level was inoculated into triplicate PP-capped test tubes (13 x 120 mm) containing 5 ml of media for each experimental regime. Three replicates were conducted for each experiment.

Growth was determined by measuring the in vivo chlorophyll a (Chl a) fluorescence using a Turner Designs Model 10-AU fluorometer (Brand *et al*., 1981; Yamaguchi *et al*., 1997). Fluorescence measurements were taken daily for 25 days. All measurements were carried out within 2–3 h after entering the dark period. Specific growth rates (µ; day^{-1}) were calculated using data from the exponential region of the growth curve by fitting a least-squares linear regression to the natural logarithm of fluorescence against time. The mean growth rate was calculated using three independent estimates of µ.

**RESULTS**

Temperature and nutrient concentrations in the reservoir are shown in Figure 2. Water temperatures increased from <20°C in late April to >30°C in July. A common trend during both years of the investigation was that the dissolved inorganic nitrogen (DIN) concentration was high in spring due to the effluent load from paddy fields linked to the reservoir. The DIN was also higher in 1999 than in 1998. The soluble reactive phosphorus (SRP) concentration in 1998 resembled that in 1999 in terms of the pattern of variation, except for some instability in April. Silicate levels showed a characteristic pattern, decreasing from April until depletion (<0.1 mg l^{-1}) in early June 1998 and again in mid-May 1999.

*Anabaena wenningii* formed blooms in both years investigated. We also observed other bloom-forming cyanobacteria such as *Anabaena planctonica* Brunnth. and *Microcystis* spp., but their densities were always low until the end of August, succeeding *A. ucrainica* as the dominant member of the cyanobacterial population. *Anabaena* sp. filament numbers began to increase exponentially in mid-May 1998 and in late April 1999 (Figure 2). Prior to this seasonal proliferation of *Anabaena*, a centric diatom...
Aulacoseira ambiguа (Grunow) Simonsen was dominant for both years of the study. Little difference was observed in the growth pattern and timing of proliferation for A. ucrainica sampled at either S1 or S2. The average in situ net growth rate during the exponential period of population growth was 0.18 day\(^{-1}\). The population of A. ucrainica in 1999 decreased abruptly in mid-August with the density falling below one filament per milliliter in September. After the extinction of the Anabaena bloom, Microcystis spp. dominated the reservoir.

Fig. 2. Seasonal variation in temperature, dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), silicate (SiO\(_2\)) concentrations and A. ucrainica population in surface water at S1 and S2. In situ net growth rate (\(k\); day\(^{-1}\)) during the exponential increase in population size is also indicated. Temperature and nutrient data were taken from Okubo et al. (Okubo et al., 2000).
The horizontal distribution of *A. ucrainica* filaments in the surface water and akinetes in the sediment was investigated on 12 August 1998. The density of *Anabaena* in surface water was >3.8 × 10³ filaments ml⁻¹ at all sampling sites, indicating the spread of the *Anabaena* bloom to all regions of the reservoir. Akinete numbers in the 0–2 cm uppermost sediment layer were >10⁴ cm⁻³ at seven of the nine sampling points with the lowest number of akinetes being 7.5 × 10² cm⁻³ at P7. Akinete numbers in the 2–5 cm layer were always lower than in the 0–2 cm samples with an average of 7.2 × 10¹ cm⁻³.

When the germination experiment of *Anabaena* akinete in sediment was conducted, levels of *Anabaena* in the water column dropped to only several filaments per liter with the water temperature fluctuating between 10 and 15°C. The density of akinetes in the sample used for the germination experiment was 7.9 × 10² cm⁻³. Akinete germination of *A. ucrainica* did not occur in the absence of illumination. Under the light conditions used, the temperature of incubation appeared to alter the germination percentage (Figure 3). Akinete germination was strongly inhibited at low temperatures (5–8°C) and suppressed slightly at higher temperature (26–32°C). High germination percentages were seen at temperatures between 14 and 23°C.

In the growth experiments, three replicates of each condition showed experimental reproducibility, except under conditions which combined the two highest temperatures and the lowest PFD (Figure 4). Figure 5 shows specific growth rates at conditions which combine 10 temperature levels and three PFDs calculated from each growth curve. The culture experiments demonstrated that *A. ucrainica* could not grow at 5°C under all PFDs examined. *Anabaena ucrainica* was likewise unable to grow at a combination of 8°C and 150 µmol m⁻² s⁻¹, however, slight growth was noticed at lower PFDs at this temperature. Increasing specific growth rates responded to increasing temperatures up to 26°C with the maximum specific growth rate of 0.78 day⁻¹ seen at 26°C and a PFD of 150 µmol m⁻² s⁻¹. The specific growth rate fell slightly at the two highest temperatures examined.

**DISCUSSION**

We found that the akinetes of *A. ucrainica* spread out over the entire sediment of the Daimon-Ike reservoir with an average density of 1.5 × 10³ akinetes cm⁻³ in the 0–2 cm layer at nine sampling points. Huber (Huber, 1984) investigated *Nodularia* akinetes in the sediments of the Peel-Harvey Estuary in Western Australia during several bloom events and reported a maximum of 1.1 × 10⁴ viable propagules ml⁻¹ within the top 1 cm layer of the sediment. Head et al. (Head et al., 1998) observed 10⁴–10⁵ akinetes of *A. platensis* ml⁻¹ in the top 1 cm layer of sediment in Cauldshiels Loch, UK, where dense *Anabaena* blooms (>10⁴ mm³ l⁻¹ in biovolume) occurred. Baker (Baker, 1999) investigated the akinetes of *A. circinalis* and *A. flos-aquae* in the Murray River and in adjacent lagoons, reporting up to 1.1 × 10⁴ and 7.0 × 10³ g⁻¹ (wet wt) of akinetes in surface sediments (0–2 cm), respectively. It was difficult to compare the akinete population in Daimon-Ike reservoir with others in the literature due to the various methods employed for quantification; however, the order of 10⁴ akinetes cm⁻³ in the top layer of sediments seemed to be typical for cyanobacterial akinete densities in water bodies where dense blooms had occurred.

Several environmental factors have been implicated in initiating the germination of cyanobacterial akinetes. One example is the seasonal increase in water column and sediment temperature (Paerl, 1988). Our experiments only approximate germination percentages using the MPN method. These results show that akinete germination was temperature dependent. High germination percentages in the range of 14–23°C, observed in the present study, were comparable or lower than at 20–25°C for *Anabaena circinalis* (Fay, 1988; Baker and Bellifemine, 2000) and *Nodularia spumigena* Mert. (Huber, 1985). *Anabaena ucrainica* was first detected (>1 filament ml⁻¹) at the sampling sites on 20 May 1998 and 26 April 1999 for the 2 years of this study. At these times the water temperature was over 15°C. Thus, akinete germination in the reservoir was probably supported by temperature increases through late spring and early summer.

There is a consensus that light is a prerequisite for akinete germination of both gas-vacuolate (Huber, 1985; Fay, 1988; Barbieri and Kann, 1994; van Dok and Hart, 1997; Baker and Bellifemine, 2000) and non-gas-vacuolate cyanobacteria (Yamamoto, 1976; Rai and Pandey, 1997; Baker and Bellifemine, 2000) and non-gas-vacuolate cyanobacteria (Yamamoto, 1976; Rai and Pandey, 1997; Baker and Bellifemine, 2000).
1981), although several studies have shown that low photon flux densities are sufficient to trigger a return to the vegetative physiological state (Yamamoto, 1976; Huber, 1985; van Dok and Hart, 1997). Our study also confirmed the dependence of Anabaena akinete germination on irradiance, i.e. germination of akinetes did not occur in total darkness. Accordingly, germination is more likely to occur in environments where the euphotic zone...
The development of Anabaena blooms with dense sediment akinete population

In this study, we did not evaluate in situ germination frequency of Anabaena akinetes. Accordingly it was unclear how many akinetes actually germinated in the reservoir; however, only the Anabaena akinetes in the uppermost layer of sediments where the sunlight could penetrate in accordance with seasonal increases in temperature. Other viable akinetes may germinate given transport into the water column or to the uppermost sediment layer by turbulence.

In this study, we did not evaluate in situ germination frequency of Anabaena akinetes. Accordingly it was unclear how many akinetes actually germinated in the reservoir. However, the overwintering planktonic population of A. ucrainica was undetectable until April, suggesting an important role of akinete germination in the early development of Anabaena populations, as pointed out by Rother and Fay (Rother and Fay, 1977). The in situ net growth rate of A. ucrainica in the water column was 0.18 day⁻¹ on average and bloom development was steady over a long period for both years. If the rate of 0.18 day⁻¹ is applicable to the early Anabaena development, an order of magnitude increase of initial inoculum advances the bloom formation by 12.8 days. Thus, if the quantity of new inoculum from sediment is very large, the bloom will be formed earlier (Tsujimura et al., 2000). We also presume that the probability of bloom formation will be enhanced by the extent of sediment inoculations, since a larger benthic population may have a competitive advantage over other phytoplankton in the water column.

Before initiation of the Anabaena bloom, the diatom Anabaena ambigua dominated the reservoir in both years, although the density was not enumerated. Sherman et al. (Sherman et al., 1998) considered that the transition from Anabaena to Anabaena dominance began with the loss of Anabaena immediately upon the development of persistent stratification, when the heavy diatom would sink out of the water column. In the present study, it was possible that the same mechanism occurred, leading to the development of stratification accompanied by temperature increases. A decrease in silicate, however, would be more effective at suppressing growth of the dominant diatom A. ambigua at the study sites. Phytoplankton succession has frequently been shown to cause a decrease in the silicate levels of temperate lakes (e.g. Peripon and Harper, 1982; Hotzel and Croome, 1996]. The cessation of growth of A. ambigua under silica stress would give other phytoplankton a chance to grow; diatoms are typically fast growers. In this study, A. ucrainica appeared to outcompete other phytoplankton. The 3-week difference between initiation of the Anabaena blooms in 1998 and 1999 was well within the period of silicate depression. Exponential growth of A. ucrainica corresponded to a water temperature increase from ~20°C to ~25°C. This temperature increase also enhanced the growth rate of A. ucrainica in culture.

While the in situ net growth rate of A. ucrainica was 0.18 day⁻¹, the specific growth rate in laboratory cultures was 0.4–0.8 day⁻¹ at temperatures between 20 and 26°C, implying that the net growth rate was below the maximum growth rate in the range of temperatures tested. Since the specific growth rate in laboratory cultures was evaluated from in vivo Chl a fluorescence, the maximum growth rates might be overestimated because of photosaturation and increasing fluorescence per Chl a as nutrients were exhausted. Furthermore, the net growth rate is not the true growth rate but the instantaneous outcome of simultaneous conflicting processes, including several loss processes (Reynolds, 1984).

Steinberg and Harmann (Steinberg and Harmann, 1988) noted that above a threshold of 10 µg l⁻¹ total phosphorus (TP), the development of cyanobacteria was dependent largely on physical factors, such as water column stability. The TP concentration always exceeded 25 µg l⁻¹ at this study site (Okubo et al., 2000). Furthermore, the DIN and SRP concentrations were seldom depleted during the study period (Figure 2), supporting the fact that nutrient levels are less important than physical factors with regards to Anabaena bloom development.
Several enclosure experiments with artificial mixing have shown the reduction in growth of Anabaena under a non-stratified regime (Reynolds et al., 1983; Nakano et al., 2001). According to Hawkins and Griffiths (Hawkins and Griffiths, 1995), artificial destratification in a small reservoir resulted in transition from a community dominated by cyanobacteria to that of diatoms as long as silica was available. Reynolds (Reynolds, 1984) also stated that net growth of Anabaena was suppressed during mixing, although the stock persisted well, enabling a resumption of growth during subsequent restratification episodes. Recent studies have indicated that fluctuation of light regimes, mostly in response to water column mixing, would be effective for the control of planktonic cyanobacteria growth (Nicklisch, 1998; Litchman, 2000; Nicklisch and Fietz, 2001). Moreover, Mitrovic et al. (Mitrovic et al., 2001) showed that buoyant populations of A. circinalis could gain an advantage over an evenly distributed population in terms of primary productivity. The present experiments also showed that low illumination decreased the growth of A. circinalis. Therefore, it seems reasonable to hypothesize that the true growth rate of Anabaena might be decreased temporarily under conditions of turbulent mixing, possibly induced by wind, during the development of the bloom. It should be considered that some disturbance may allow for other phytoplankton to outcompete the dominant Anabaena in the reservoir as predicted by the Intermediate Disturbance Hypothesis (Sommer et al., 1993). Simultaneously, however, it is plausible that such turbulent mixing may assist the upwelling of new inoculum from the dark sediment into the euphotic zone. The importance of resuspension events of resting cysts for the initiation of dinoflagellate blooms has been discussed by Nehring (Nehring, 1996). While the exponential growth of A. saximontana depended largely on water column physical stability, akinetes in the sediment function as new inocula during turbulent mixing, and the presence of large akinete populations in the sediment enhanced the relative probability for Anabaena blooms.

We consider that formation of cyanobacterial blooms is ultimately dependent on growth in the water column. Effective management to prevent planktonic cyanobacteria growing in the water column might include decreasing nutrient levels, control of physical conditions such as destratification, and the application of biomanipulation. We also suggest that removal of akinetes in sediment may contribute to a decrease in cyanobacterial blooms. Similar opinions have been expressed by Burchardt and Pataczkowa (Burchardt and Pataczkowa, 1987). Pousilíková et al. (Pousilíková et al., 1998) studied the influence of sediment removal on the structure and dynamics of phytoplankton communities in a small pond where Anabaena flos-aquae Ralß ex Bornet et Flahault and Microcystis spp. had dominated in the summer before sediment removal. They showed that Microcystis was absent until the end of the growing season of the third year after sediment removal. The removal of a Microcystis inoculum in the sediment top layer, by dredging, resulted in their absence in the water column for at least 2 years. Sediment removal contributes not only to the removal of phosphorus, as a source of internal nutrient loading, but also to the decrease of the seed population of planktonic cyanobacteria.

However, dredging projects are generally expensive and require a suitable clumping site for the sediment. This latter consideration is especially relevant in Japan. Another option for decreasing the size of the cyanobacterial inoculum is to desiccate the sediment periodically. According to Baker and Bellifemine (Baker and Bellifemine, 2000), desiccation of akinetes of A. circinalis for moderately short periods could impair significantly their capacity to germinate, and periodic drying of shallow wetlands has the potential for future algal bloom management.

ACKNOWLEDGEMENTS
We would like to express our gratitude to H. Tanaka for his assistance with the field surveys.

REFERENCES


