Dewatering as a non-toxic control of nuisance midge larvae in algal wastewater treatment floways

Troy A. Keller and Emily M. Husted

ABSTRACT

Attached-algae floways have tremendous potential for use in wastewater treatment because natural algal communities show high nutrient removal efficiencies, have low operating costs, and are easy to maintain. Algal wastewater floways may also serve as a sustainable option for producing renewable energy because algae grow rapidly, are easily harvested, and can serve as a source of biomass for biofuel. However, pests such as chironomids (Diptera) colonize open channel periphyton floways and their larvae damage the biofilms. While pesticides can control midge larvae, little information is known about alternative, non-toxic controls. This study examined the effectiveness of periodic, short-term dewatering (4 hours every 9 days) on midge abundance and periphyton growth in 16 recirculating, outdoor floways (3 m long, 0.1 m wide). We compared midge abundance and algal accumulation (chlorophyll a, b, c, and pheophytin) among control ($n = 8$) and dewatered ($n = 8$) floways filled with secondarily treated wastewater (27 days, 10 hours of daylight). Dewatered flumes had 42% fewer midges and 28–49% lower algal productivity (as measured by chlorophyll a, b, c, and pheophytin pigments). Chlorophyll a production rates averaged (±1 SD) 0.5 ± 0.2 μg/cm^2/day in control floways compared to 0.3 ± 0.1 μg/cm^2/day dewatered floways. Short-term dewatering effectively reduced midges but also damaged periphyton. To maximize the recovery of periphyton biomass, operators should harvest periphyton from floways during dewatering events before periphyton is damaged by desiccation or direct exposure to sunlight.

Key words | algae, biofuel, chironomids, green technology, sustainability

Troy A. Keller
Department of Earth and Space Sciences,
Columbus State University,
4225 University Ave,
Columbus,
GA,
USA

Emily M. Husted (corresponding author)
Department of Biology,
Columbus State University,
4225 University Ave,
Columbus,
GA,
USA
Current address:
863, Carnellian Lane,
Peachtree City,
GA 30269
USA
E-mail: *husted_emily@columbusstate.edu*

INTRODUCTION

Because of their high nutrient removal efficiencies, low maintenance costs, and ease of application, algae have been incorporated into wastewater treatment systems (Hoffmann 1998). One promising approach treats wastewater by channeling effluent down shallow streams covered in periphyton (Adey *et al.* 1993; Craggs *et al.* 1996b). Algal treatment projects have been constructed and have performed successfully in treating agricultural runoff (Adey *et al.* 1993) and secondarily treated wastewater (Craggs *et al.* 1996a, b). The microbial biofilms that make up the periphyton are composed of naturally occurring phototrophic and heterotrophic species such as algae and bacteria and they have been used in bioremediation, aquaculture, energy production, and fertilizer creation (Roeselers *et al.* 2008). Biofuel created from algae-based wastewater treatment systems can reduce carbon emissions with the added benefit of generating cleaner effluent (Craggs *et al.* 2011). Growing algae for biofuel using wastewater has the potential to offset wastewater treatment plant energy use and save money (Heubeck *et al.* 2011).

Although periphyton treatment systems have many potential applications, their open system design makes them vulnerable to colonization by pests such as chironomids (Craggs 2001). Chironomids are non-biting Diptera whose larvae develop in aquatic ecosystems. Midge larvae can become pestiferous in water treatment facilities (Ali 1991) and can damage the structure of biofilms in periphyton treatment systems (Craggs 2001). When abundant, larvae decrease the efficiency of the floways for nutrient removal and biofuel production. Large swarms of adult flies emerge from these breeding grounds, becoming pests and potential disease reservoirs (Halpern *et al.* 2004). Midge populations can be controlled using insecticides and other chemical treatments (Ali 1991; Craggs *et al.* 2005), but chemical

doi: 10.2166/wst.2014.442

residues may contaminate wastewater effluent and negatively affect non-target species (Hayasaka *et al.* 2012). An alternative method of controlling chironomid larvae without the use of chemicals is to periodically dewater the system, a technique advocated by Craggs (2001).

The effects of drying on algal biomass need to be evaluated to determine the practicality of dewatering for periphyton treatment systems. Emersion negatively effects freshwater periphyton abundance in rivers (Bergey *et al.* 2010). Drying's direct negative effect on algal biomass could be offset by an indirect positive effect on algal growth caused by a reduction in grazers (Bergey *et al.* 2010). Desiccation can also shift algal community structure (Ledger *et al.* 2011). The complex architecture of the periphyton may provide a temporary refuge, protecting vulnerable algae from the detrimental effects of desiccation (Ledger *et al.* 2011). It is important to understand how dewatering to control midges affects algal biomass and community structure in periphyton treatment systems because changes in algal species composition could influence biomass quality for biofuel production (Chisti 2007; Brennan & Owende 2010).

This study was designed to determine whether dewatering effectively controls midge fly larvae, to ascertain whether drying alters algal biomass, and to assess whether drying shifts the composition of the algal communities. We hypothesized that dewatering would reduce the abundance of midges due to direct desiccation, enhance algal biomass by reducing midge destruction of the algal mat, and shift the algal community by selectively killing algae that are intolerant to desiccation. We tested these hypotheses in a short-term experiment that compared algal biomass and midge abundance between treatments dewatered for 4 hours every 9 days and treatments with constant flow. The 27-day experiment was conducted using replicated, recirculating artificial flumes filled with secondarily treated municipal effluent.

METHODS

This experiment was conducted at the Columbus Water Works (CWW), Inc. wastewater treatment plant in Columbus, Georgia, USA (32.4 °N, 85.0 °W) from 21 December 2012 to 17 January 2013. This facility treats up to 265,000 m^3/day of wastewater generated by roughly 65,000 households. Experiments were conducted in a replicated periphyton treatment system composed of two plastic head tanks (208 L), 16 flumes, and one sump (1,135 L) with a submersible pump to circulate water throughout the system

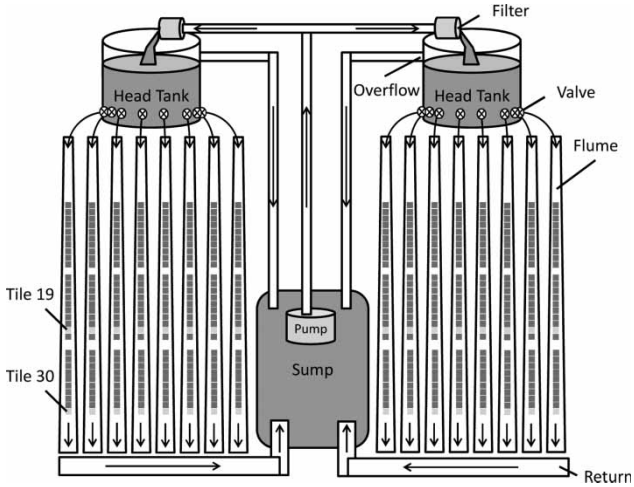

Figure 1 | Replicated floways ($n = 16$) used in these algal treatment system experiments.

(Figure 1). White vinyl rain gutters (width = 0.1 m, length = 3 m) were used as the replicated algal treatment floways (0% slope). To mimic substrates used in concrete periphyton systems, flumes were lined with unglazed gray ceramic tiles (5 × 5 cm). The source water for this experiment was filtered, secondarily treated effluent (not chlorinated or pH adjusted).

Experimental design and sampling protocols

The effect of dewatering on midges and algae was evaluated by comparing midge abundance and chlorophyll density from flumes with constant flow ($n = 8$, control) to those exposed to intermittent dewatering ($n = 8$, dewatered 4 hours every 9 days). These time intervals were chosen to create a reasonable schedule that could be implemented by wastewater treatment personnel and for their similarity to the 8-day optimal harvesting intervals reported by Adey *et al.* (1993). Four controls and four dewatering treatments were randomly assigned to each head tank. To establish algae and invertebrates, we operated the system without dewatering for 2 weeks prior to commencing the experiment. Flumes were dewatered on days 0, 9, and 18. The experiment was terminated on day 27. To quantify biotic responses, we collected tiles, placed them in Whirl-Paks®, transported them on dry ice, and stored them frozen (−20 °C) until analysis.

Dewatering effects on midge abundance

Midges were sampled at the end of the 27-day experiment when larval abundance peaked. Tiles were scraped before midges were counted at ×12.5 magnification. Because

some midges were damaged in the sample processing, only midge head capsules were counted. Treatment effects on midge abundance were evaluated using Pearson's chi-squared test of homogeneity.

Dewatering effects on algal biomass

Periphyton was filtered onto glass fiber filters (0.7 μm pores, 47 mm diameter) and stored frozen (−20 °C) in foil pouches until extraction. Samples were analyzed after the freezer had thawed for an unknown length of time (1–7 days). Analyses were conducted despite this setback because all treatments were affected equally and studies such as Reuss and Conley (2005) found that storage at 20 °C had no negative effect on chlorophyll.

To measure algal abundance, chlorophyll pigments were extracted in 90% acetone following US Environmental Protection Agency (EPA) method 150.1 (spectrophotometric method) and were calculated as chlorophyll a, b, and c (uncorrected) and pheophytin based on equations described in *Standard Methods* by Eaton *et al.* (2005). Pigment densities were compared between desiccated and control tiles using an independent samples *t*-test. Separate *t*-tests were used to evaluate results for upstream (tile 19) and downstream (tile 30) tiles. To assess whether sample location influenced algal pigment density, upstream to downstream tiles (controls and treatments combined) were compared using paired samples *t*-tests. To determine whether dewatering shifted algal species composition, the ratio of chlorophyll b and c to total pigment density was calculated. Independent sample *t*-tests were used to compare chlorophyll b and c ratios between control and treatment (separately for upstream and downstream tiles). All analyses were conducted using SPSS Statistics 17.

RESULTS AND DISCUSSION

During the course of the experiment, temperature fluctuated by 24 °C ranging from 0.5 to 24.5 °C. The sump water temperature averaged 12.5 ± 5.9 °C (±1 SD). Air temperatures for Columbus, GA in December and January averaged 9.2 °C. Daylight lasted 10 hours 6 minutes at this latitude and lengthened only 15 minutes during this experiment.

Dewatering effects on midge abundance

We counted 68 midge larvae among all 32 tiles sampled (8.5 midges/100 cm^2). Midge larvae were 41.9% more abundant in control than dewatered floways at the end of the experiment ($X^2 = 3.56$, df = 1, $p = 0.007$). There existed no significant interaction between midge abundance and tile number ($X^2 = 0.83$, $df = 1$, $p = 0.36$) so data from both tiles were combined. Thus, short-term, periodic dewatering was an effective control of larval chironomids. Similar observations were reported by Craggs *et al.* (1996b), who documented declines in midge larval abundance in algal treatment systems after floways were dewatered and algae harvested. Chironomids have variable tolerance for desiccation. Temperate midge species have been shown to experience 50% or more mortality in as little as 1–2.5 hours of desiccation, but one species has been shown to survive for 8 hours (Suemoto *et al.* 2004). Because drying tolerance was directly correlated to these species' capacity to maintain internal moisture (Suemoto *et al.* 2004), those taxa who can resist internal moisture loss can survive prolonged episodes of drying.

Although effective, dewatering did not completely eliminate midge larvae in our experiments. Some midge larvae either survived our dewatering treatment or recolonized quickly. Refuges for midges likely existed in the interstices along the edges and undersides of tiles. Midge cases were observed in these refuges during regular sampling. To improve the effectiveness of dewatering for controlling midge larvae, it may be necessary to design periphyton treatment systems to minimize refuges for larvae. Regular harvesting of periphyton during dewatering will likely improve the control of midge larvae by removing midges living in algal tufts (Craggs *et al.* 1996b).

Chironomids have been effectively controlled in large-scale wastewater applications using chemicals such as methoprene (Craggs *et al.* 2005). Other pesticides (*Bacillus thuringiensis* var. *israelensis*, Fipronil, Pyriproxyfen and Diflubenzuron) have varying efficacy against chironomids (Ali & Lord 1980; Craggs *et al.* 2005). While chemicals exist to regulate midge populations, these pesticides cost money, need regular application (Craggs *et al.* 2005), and may affect non-target organisms living in receiving waters (Ali 1991; Hayasaka *et al.* 2012). In contrast, dewatering has low costs and leaves no residuals that could affect biota in receiving waters.

The efficacy of midge control measures including dewatering may be limited because chironomids have been shown to rapidly colonize submersed hard surfaces (Churchel & Batzer 2006). Repeated dewatering will likely be needed to control midge populations in periphyton or other treatment systems. In this experiment, flumes were dewatered every 9 days for 4 hours. When growth regulators (i.e., chemical pesticides) were used to control midges,

populations have been suppressed for 10 days to 4 months (Ali & Lord 1980). Ledger *et al.* (2011) found that midge populations in artificial streams increased even when dewatered every 27 days. Thus, regular dewatering repeated every 10–14 days may be required to effectively control midge larvae in periphyton treatment systems. Further research is needed to identify dewatering schedules that effectively control midges but maintain optimal algal growth conditions.

Dewatering effects on algal biomass

Dewatering significantly reduced periphyton biomass as measured by changes in pigment density. For upstream (tile 19) chlorophyll a, b and pheophytin mean pigment densities were reduced by 31.6, 28.0, and 46.5%, respectively (Figure 2(a), *t*-test, $p = 0.031$, $p = 0.021$, and $p = 0.024$, respectively). Daily chlorophyll a productivities averaged (±1 SD) 0.5 ± 0.2 μg/cm^2/day for controls but only 0.2 ± 0.1 μg/cm^2/day for dewatered flumes. Emersion had no statistically significant effect on average chlorophyll c density on Tile 19 (Figure 2(a), *t*-test, $p = 0.064$). Similar patterns existed for downstream (tile 30) with chlorophyll a, b, c, and pheophytin showing 39.9, 38.3, 38.6, and 49.3%, respectively, greater mean densities in the control compared to the dewatered flumes (Figure 2(b), *t*-test, $p = 0.023$, $p = 0.028$, $p = 0.041$, and $p = 0.009$, respectively).

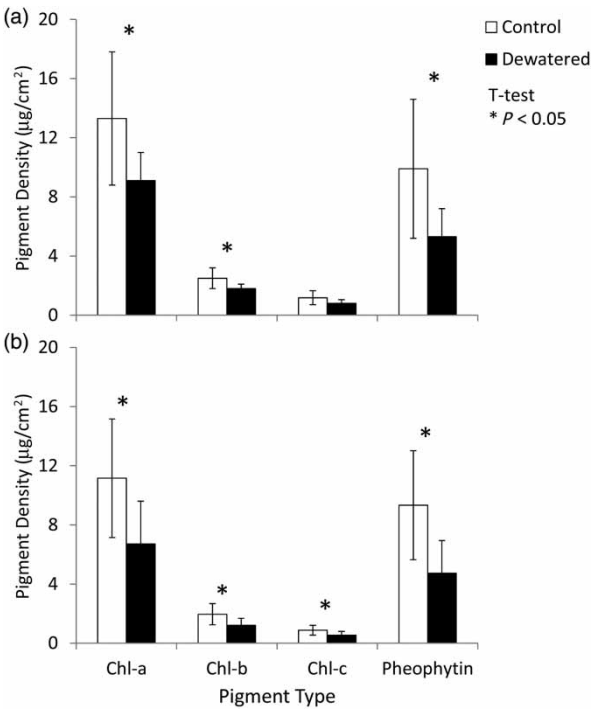

Figure 2 | Chlorophyll a, b, c, and pheophytin density (μg/cm^2) measured on tiles at the end of a 27-day experiment from periphyton treatment floways exposed to constant flow (open bars-controls, $n = 8$) or dewatered every 9 days (solid bars, $n = 8$). Results for (a) upstream and (b) downstream tiles are shown separately. Asterisks indicate significant differences determined using independent samples *t*-test ($p < 0.05$). Error bars represent ±1 SD.

Our results indicating that dewatering caused a decline in algal chlorophyll pigments were consistent with previous studies (Ledger *et al.* 2008; Bergey *et al.* 2010). Angradi and Kubly (1993) found that even short-term air exposure (2–8 hours) caused decreases in chlorophyll a in lotic periphyton. Emersion effects are generally stronger during daylight hours than at night (Bergey *et al.* 2010); thus, solar radiation may be damaging periphytic algae even before algae are completely desiccated. To minimize the effect of dewatering stress on periphyton, it would be advisable to dewater flumes at night when emersion effects on algae are less pronounced.

Emersion effects on algal pigment composition

We assessed changes in algal community composition by examining shifts in the proportions of pigments. Analyses revealed no statistically significant treatment differences in the proportions of chlorophyll b or c density on the upstream (*t*-test, $p > 0.59$ for both) or downstream (*t*-test, $p > 0.4$ for both) tiles. Thus, it appears that major algal groups, as measured by pigment density, were equally sensitive to these emersion treatments. Dewatering has been shown to act as a disturbance that can alter algal species assemblages. For example, Ledger *et al.* (2008) described taxonomic shifts in algal assemblages in dewatered verses perennial artificial streams. Drying appeared to favor closely adhering taxa that were more desiccation tolerant (Ledger *et al.* 2008). Additional research is needed to determine how seasonal changes in temperature, humidity, and light intensity alter algal species, susceptibility to desiccation and recovery after disturbance.

Longitudinal gradients in algal biomass

Gradients in algal pigment density existed from upstream to downstream in these flumes. Chlorophyll a, b, and c were significantly lower (20.2–28.0%) on the downstream tiles than on the upstream tiles (paired samples *t*-test, $p = 0.031$, $p = 0.003$, $p = 0.006$, respectively). Unlike chlorophyll pigments, pheophytin density was similar across treatments (paired samples *t*-test, $p = 0.6$). Gradients in floway algal productivity have been reported over tens of meters; however, this study documented pigment differences across spatial scales of less than 1 m. Patchiness is common in

natural streams and is thought to derive from the highly unpredictable interaction between organisms and their environment (Cooper *et al*. 1997). In our study, we explicitly regulated flow and habitat variables to minimize this spatial variation in environmental conditions. In our recirculating system, it is possible that living algae are deposited upstream as they make their way from the flumes through the sumps and are pumped back into the floways. This process could explain why we found elevated algal pigment densities in the upper portion of the flumes.

There are alternative explanations for this pattern that are directly related to nutrients. One possibility is that nutrient uptake by upstream algae reduces downstream concentrations. Because the distances examined in this study are very small, the nutrient concentrations exceed algal requirements and flow rates are high, chemical gradients seem unlikely to exist. An alternative explanation is that water mixing rates differ upstream to downstream in the flumes. Injecting water into the upstream portion of the flumes (Figure 1) creates a region with highly turbulent flow. Turbulent flow reduces the thickness of the viscous sublayer, a thin layer around a solid substrate that has extremely slow flow. This sublayer transports molecules through the very slow process of molecular diffusion; thus, areas with thin sublayers are likely to have greater rates of chemical transfer from the water column to the algae. This could stimulate nutrient uptake and support rapid algal growth. As the water travels downstream, the turbulent energy would be dissipated and the viscous sublayer would thicken. A thicker sublayer would slow chemical transport to the algae because chemicals move only by diffusion through the viscous sublayer.

CONCLUSIONS

Biological treatment of wastewater using periphyton holds tremendous promise for nutrient removal from effluent and biomass production for biofuels. Since chironomid larvae infest these highly productive systems and reduce periphyton accrual, it is important to develop sustainable, environmentally friendly methods to control larval populations. Our experiments show that short-term dewatering (4 hours) of experimental periphyton treatment systems can be a cost-effective and environmentally sound approach for decreasing chironomid larval abundance, but dewatering also disturbs periphyton and reduces algal biomass. Before dewatering can become a standard practice, it will be important to optimize drying intensity and timing among seasons. Understanding the importance of these factors can help managers minimize drying's effect on algae while maximizing its control of midge larvae in algal treatment systems.

ACKNOWLEDGEMENTS

We recognize Columbus State University funding sources: Department of Earth and Space Sciences, Biology Department, College of Letters and Sciences Dean's Office, and faculty development grants. Support and access were generously provided by CWW. We appreciate the logistical and technical support provided by CWW employees, Dr Morton Reed, Chris Cornett, and William Kent. We thank Craig Husted and Jeremy Klein for their assistance with the field work.

REFERENCES

Adey, W., Luckett, C. & Jensen, K. 1993 Phosphorus removal from natural waters using controlled algal production. *Restoration Ecology* **1** (1), 29–39.

Ali, A. 1991 Perspectives on management of pestiferous Chironomidae (Diptera), an emerging global problem. *Journal of the American Mosquito Control Association* **7** (2), 260–281.

Ali, A. & Lord, J. 1980 Experimental insect growth regulators against some nuisance chironomid midges of Central Florida. *Journal of Economic Entomology* **73** (2), 243–249.

Angradi, T. R. & Kubly, D. M. 1993 Effects of atmospheric exposure on chlorophyll a, biomass and productivity of the epilithon of a tailwater river. *Regulated Rivers: Research & Management* **8** (4), 345–358.

Bergey, E. A., Bunlue, P., Silalom, S., Thapanya, D. & Chantaramongkol, P. 2010 Environmental and biological factors affect desiccation tolerance of algae from two rivers (Thailand and New Zealand) with fluctuating flow. *Journal of the North American Benthological Society* **29** (2), 725–736.

Brennan, L. & Owende, P. 2010 Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable & Sustainable Energy Reviews* **14** (2), 557–577.

Chisti, Y. 2007 Biodiesel from microalgae. *Biotechnology Advances* **25** (3), 294–306.

Churchel, M. A. & Batzer, D. P. 2006 Recovery of aquatic macroinvertebrate communities from drought in Georgia piedmont headwater streams. *American Midland Naturalist* **156** (2), 259–272.

Cooper, S. D., Barmuta, L., Sarnelle, O., Kratz, K. & Diehl, S. 1997 Quantifying spatial heterogeneity in streams. *Journal of the North American Benthological Society* **16** (1), 174–188.

Craggs, R. J. 2001 Wastewater treatment by algal turf scrubbing. *Water Science and Technology* **44** (11–12), 427–433.

Craggs, R. J., Adey, W. H., Jenson, K. R., St John, M. S., Green, F. B. & Oswald, W. J. 1996a Phosphorus removal from wastewater using an algal turf scrubber. *Water Science and Technology* **33** (7), 191–198.

Craggs, R. J., Adey, W. H., Jessup, B. K. & Oswald, W. J. 1996b A controlled stream mesocosm for tertiary treatment of sewage. *Ecological Engineering* **6** (1–3), 149–169.

Craggs, R., Golding, L., Clearwater, S., Susarla, L. & Donovan, W. 2005 Control of chironomid midge larvae in wastewater stabilisation ponds: comparison of five compounds. *Water Science and Technology* **51** (12), 191–199.

Craggs, R. J., Heubeck, S., Lundquist, T. J. & Benemann, J. R. 2011 Algal biofuels from wastewater treatment high rate algal ponds. *Water Science and Technology* **63** (4), 660–665.

Eaton, A. D., Clesceri, L. S., Rice, E. W. & Greenberg, A. E. 2005 *Standard Methods for the Examination of Water and Wastewater*. APHA, Washington, DC.

Halpern, M., Broza, Y. B., Mittler, S., Arakawa, E. & Broza, M. 2004 Chironomid egg masses as a natural reservoir of *Vibrio cholerae* non-O1 and non-O139 in freshwater habitats. *Microbial. Ecol.* **47** (4), 341–349.

Hayasaka, D., Korenaga, T., Suzuki, K., Sanchez-Bayo, F. & Goka, K. 2012 Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil. *Ecotoxicology* **21** (2), 421–427.

Heubeck, S., de Vos, R. M. & Craggs, R. 2011 Potential contribution of the wastewater sector to energy supply. *Water Science and Technology* **63** (8), 1765–1771.

Hoffmann, J. P. 1998 Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology* **34** (5), 757–763.

Ledger, M. E., Harris, R. M. L., Armitage, P. D. & Milner, A. M. 2008 Disturbance frequency influences patch dynamics in stream benthic algal communities. *Oecologia* **155** (4), 809–819.

Ledger, M. E., Edwards, F. K., Brown, L. E., Milner, A. M. & Woodward, G. 2011 Impact of simulated drought on ecosystem biomass production: an experimental test in stream mesocosms. *Global Change Biology* **17** (7), 2288–2297.

Reuss, N. & Conley, D. J. 2005 Effects of sediment storage conditions on pigment analyses. *Limnology Oceanography: Methods* **3**, 477–487.

Roeselers, G., van Loosdrecht, M. C. M. & Muyzer, G. 2008 Phototrophic biofilms and their potential applications. *Journal of Applied Phycology* **20** (3), 227–235.

Suemoto, T., Kawai, K. & Imabayashi, H. 2004 A comparison of desiccation tolerance among 12 species of chironomid larvae. *Hydrobiologia* **515** (1–3), 107–114.

First received 23 August 2014; accepted in revised form 23 October 2014. Available online 5 November 2014