The response of phytoplankton and microlayer-adapted bacteria to monolayer application in a humic, eutrophic irrigation dam

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ABSTRACT

Repeat applications of artificial monolayers to farm irrigation dams to reduce evaporative loss may adversely affect water quality by enhancing populations of microlayer-adapted bacteria and blue-green algae. The microlayer, subsurface and water column of a 16 ha dam were monitored every two weeks for 18 months, to benchmark the seasonal dynamics of phytoplankton and microlayer-adapted bacteria prior to monolayer application. Results for Secchi depth, total P, total N, chlorophyll a, phytobiovolume and UV_{254} absorbance, characterise Logan's Dam as humic and hypereutrophic. Seasonal peaks in the cyanobacterial species $Microcystis$ $aeruginosa$ and $Anabaena$ sp. associated with regular thermal stratification periods over summer, exceeded the Queensland algal bloom alert level. Dissolved organic matter derived from aromatic char in the black soil used to construct the dam was the main substrate for microlayer-adapted bacteria. Intermittent monolayer application over seven weeks in late summer temporarily increased surface pressure, indicating a condensed monolayer had formed, with no increase in chemical oxygen demand or in populations of cyanobacteria or microlayer-adapted bacteria. The increase in dissolved organic carbon was well below the concentration recorded after a pump ingress event in late spring. In this humic hypereutrophic irrigation dam, repeat applications of the experimental monolayer formulation did not adversely affect water quality.

Key words | cyanobacteria, dissolved organic carbon, mono octadecyl ether, water quality

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INTRODUCTION

Of the estimated 7,000 GL of water stored in a million or so small (<10 ha capacity) Australian farm dams, up to 20% may be lost to evaporation (Craig *et al.* 2005). The installation of physical covers can reduce losses by up to 80%, but installation costs limit the adoption of this strategy to dams less than 5 ha in size. Artificial monolayers can be deployed over much larger dams (Barnes 2008), but prolonged monolayer application may adversely affect freshwater ecology (Hatcher & Parker 1974; McJannet *et al.* 2008). Farm dams are key reservoirs of aquatic biodiversity (Markwell & Fellows 2008).

An artificial monolayer forms when amphiphilic (with a hydrophobic head and hydrophilic tail), long carbon-chain molecules spontaneously spread across the water surface as a film one molecule thick (Barnes 2008). To retard evaporative loss, the surface pressure of the film must exceed

15 mN m^{-2} (a condensed monolayer). Ecologists are concerned water temperature may increase as latent heat loss reduces, increasing the risk of toxic cyanobacterial blooms. In subtropical Queensland, toxin-producing cyanobacterial species may dominate phytoplankton populations in dams over summer (Hunt *et al.* 2003). The maintenance of a condensed monolayer may also reduce the dissolved oxygen concentration by reducing gaseous exchange and by stimulating the activity of heterotrophic bacteria adapted for utilising monolayer compounds (McJannet *et al.* 2008).

Natural films occur on most water bodies, as long carbon-chain, amphiphilic molecules derived from bark and leaf litter (allochthonous), aquatic plants and/or algae (autochthonous) concentrate at the surface (the microlayer, Norkrans 1980). These chemically diverse compounds lack the uniformity required to reduce evaporative loss (Barnes

2008), but may increase surface pressure as wind and wave action compress the film (Pittaway & van den Ancker 2010). The microlayer may contain high concentrations of complex ultra violet (UV) light-absorbing aromatic compounds derived from allochthonous sources, that stain the water black or dark brown (Samios et al. 2007). These high molecular weight compounds resist microbial degradation, but are susceptible to photodegradation (Bertilsson & Allard 1996). Aquatic bacteria readily utilise low molecular weight photodegradation by products and allochthonous dissolved organic matter (DOM), stimulating microlayer populations 10 to 100 times above subsurface water populations (microlayer enrichment; Munster et al. 1998). The artificial monolayer compounds hexadecanol and octadecanol are susceptible to microbial attack but not to photodegradation (Pittaway et al. 2015a), whereas mono octadecyl ether resists microbial attack, but is susceptible to photodegradation.

In our study, the microlayer, subsurface and water column of a 16 ha farm irrigation dam was monitored over 18 months to characterise seasonal fluctuations in the concentration of UV-absorbing DOM, microlayer-adapted bacteria, and populations of phytoplankton in the water column. At the end of the monitoring period, an experimental monolayer formulation based on the compound mono octadecyl ether was applied during periods of low wind speed conducive to the formation of a condensed monolayer (less than 6 m sec^{-1}; Barnes 2008). Water quality parameters before, during and after monolayer application were compared to document the impact of repeat monolayer application on aquatic ecology.

METHODS

Management of the irrigation dam

Logan's Dam (Lockyer Valley Southeast Queensland, 27° 34′ 26″ S, 152° 20′ 28″ E) was constructed within the last 15 years to augment declining groundwater supplies for commercial irrigated vegetable production. Water was pumped into the dam after high intensity storm events generated overland flow. The average volume of the dam was 509 ML (surface area 480 m × 350 m, maximum volume 700 ML, maximum depth 6 m). The energy flux and evaporative loss was monitored over an 18-month period (McJannet et al. 2013).

Shoreline, manual application of an experimental monolayer formulation based on the compound ethylene glycol monooctadecyl ether (Prime et al. 2012) occurred every second day over 15 consecutive days in late January–February (25 Jan. to 9 Feb. 2011). Application resumed during a second forecast period of calm weather from 23–30 March, from automated floating units. Further manual application occurred on 6, 13 and 18 April 2011. A total of 1,073 L of the monolayer formulation was applied.

Microlayer and subsurface water sampling

Water sampling occurred every two weeks within 2 h after dawn. The surface pressure of the microlayer within 2 m of the south eastern (SE) and north western (NW) shoreline of the dam was measured by applying indicator oils to the surface drop-wise, until the concentration of the indicator oil was equivalent to the spreading pressure of the microlayer (Barnes 2008). Microlayer and subsurface water was sampled manually (Pittaway & van den Ancker 2010) from an electrically powered dinghy within about 2 m of the SE and NW shorelines. Microlayer and subsurface water samples were equilibrated for at least 15 min in an insulated, dark container before dissolved oxygen, pH, conductivity and temperature were recorded (TPS 90 FL, TPS Pty Ltd, Springwood, Queensland, Australia). Filtered water samples (0.45 μm glass fibre) were measured for UV_{254} light absorbance (a surrogate for the concentration of aromatic organic compounds; Weishaar et al. 2003), using a spectrophotometer (matched quartz cuvettes, Cecil CE2021 spectrophotometer set at 253.7 nm).

After peak rainfall events, at the start and end of seasonally dry periods, and before and after artificial monolayer application, the population of microlayer-adapted bacteria was estimated by the most probable number method (MPN; Pittaway & van den Ancker 2010). From December 2010, dissolved organic carbon (DOC) was also monitored after key events, on a Shimadzu oxidative combustion-infrared detection TOC-V CSH Total Organic Carbon Analyser. Specific UV absorbance ($SUVA_{254}$) was calculated by dividing UV absorbance by DOC to indicate the ratio of aromatic to total DOC (Weishaar et al. 2003). The chemical oxygen demand (COD), biochemical oxygen demand (BOD) and non-ionic surfactants (CTAS method; Clesceri et al. 2005) were determined by a commercial laboratory.

Water column sampling

An integrated phytoplankton hose (clear plastic, 30 mm diameter) was used to sample the water column every two weeks to an average depth of 3.3 m, from an electrically powered

dinghy. Duplicate hose samples were taken from three locations across the dam to produce a composite sample. A 100 mL sample was preserved in an acidified Lugol's solution for microscopic analysis and the estimation of phytoplankton biovolume (Matveev & Matveeva 2005). Filterable reactive phosphorus, total phosphorus, ammonium, nitrate, total nitrogen and chlorophyll *a* were determined by a commercial laboratory. Water turbidity was measured down the water profile at the three locations with a Secchi disk, and with a YSI (Yellow Springs, Ohio, USA) turbidity probe.

Statistical analysis

Fortnightly monitoring data for subsurface and microlayer samples taken from the NW and SE shoreline were analysed separately using the Pearson correlation (SigmaPlot Version 12, Systat Software Inc.). Statistically significant correlations were further analysed using linear regression. A one-way analysis of variance and Pearson Correlation were used to analyse BOD, COD, MPN of microlayer-adapted bacteria, DOC and $SUVA_{254}$ data collected before, during and after the monolayer application period.

RESULTS AND DISCUSSION

Water management

The study coincided with the end of a protracted drought, broken by record rainfall causing regional flooding in January–February 2011 (Figure 1(a)). The water depth exceeded the safe water storage capacity (105%; McJannet *et al.* 2013), but the dam did not or only marginally overtopped. The peak daily rainfall event was 224 mm, the total rainfall over the 18-month monitoring period was 1,761 mm. Water depth was not correlated with rainfall ($r = 0.122$, $P = 0.781$, $n = 42$; Figure 1), but was negatively correlated with water abstraction ($r = -0.528$, $P < 0.001$, $n = 42$) and periods of calm days ($r = -0.425$, $P = 0.006$, $n = 42$), characteristic of the seasonally dry winter when irrigation demand is greatest (Figure 1(b)).

Wind speed of less than $4 \, \text{m s}^{-1}$ occurred more than 80% of the time (McJannet *et al.* 2013). Water temperature profiles changed continuously from diurnal periods of thermal stratification, interrupted by periods of mechanical mixing (McGloin *et al.* 2014). A well-mixed water column prevailed during late autumn to winter with stratification dominating during late spring to summer, including during the monolayer application period. No evidence of
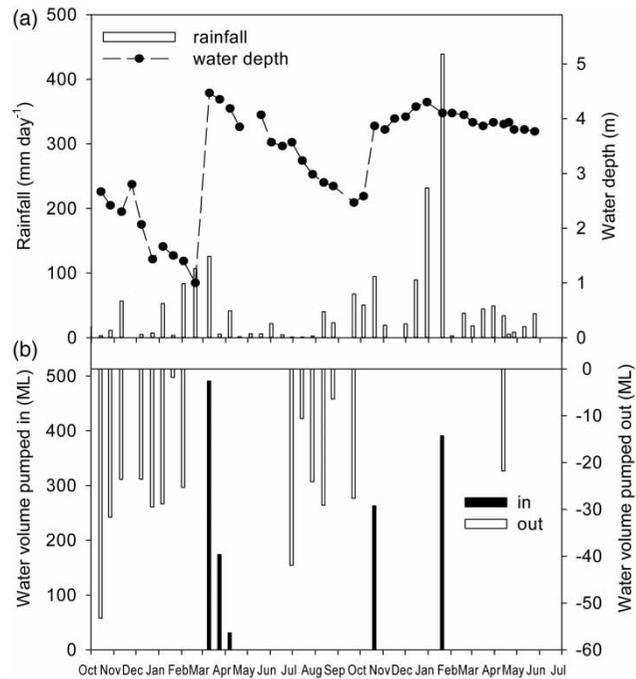

Figure 1 | Logan's Dam water depth and cumulative rainfall (a), and the volume of water pumped in and out of the dam (b) within the two weekly sampling dates from 14/10/09 to 25/5/11. Pump inflow sensors were not installed until March 2010.

a reduction in evaporative loss was recorded during the monolayer application period. The formation of a thermally stable surface film during periods of thermal stratification may have induced a resistance to evaporative loss that exceeded the resistance imposed by a condensed monolayer (Pittaway *et al.* 2015b). In the subtropics, water demand for irrigation is greatest during the seasonally dry winter (Figure 1(b)). The availability of the experimental formulation delayed monolayer application until the seasonally humid, wet summer. On this subtropical dam, monolayer application to a well mixed water column in winter may have been more effective in reducing evaporative loss.

Physical and chemical water quality

Phytobiovolume, total phosphorus, total nitrogen and Secchi depth readings characterised Logan's Dam as hypereutrophic (Wetzel 2001; Table 1). In common with most hypereutrophic lakes the dam is shallow, with turbulence resuspending sediment in the water column. A Secchi depth of consistently less than 0.25 m was recorded over the duration of the study (Table 1). Median surface pressure for the microlayer was $4 \, \text{mN m}^{-1}$, with sporadic, higher readings (highest reading $36 \, \text{mN m}^{-1}$) recorded (Figure 2(a)).

Table 1 | The trophic status of Logan's Dam benchmarked against published criteria for eutrophic lakes

	Secchi depth (m)	Total P (mg L^{-1})	Total N (mg L^{-1})	Chlorophyll a (μg L^{-1})	Phytobiovolume (mm^3 L^{-1})	Microlayer UV$_{254}$ (abs cm^{-1})
Logan's (2009–2011)	0.13 (0.1–0.2)	0.37 (0.21–0.48)	1.55 (1.0–2.2)	14.2 (2.0–90.0)	7.86 (0.06–107.5)	2.42 (1.39–3.04)
Wetzel (2001)	0.4–0.5	0.03 – >5.0 hyper-eutrophic	0.5–15.0 hyper-eutrophic	9.5–275 eutrophic	>10 hyper-eutrophic	
Tranvik & Bertilsson (2001)				>4.0 humic-eutrophic		>0.25 humic-eutrophic

Average values (range is given in brackets) for water samples were recorded from November 2009 to May 2011.
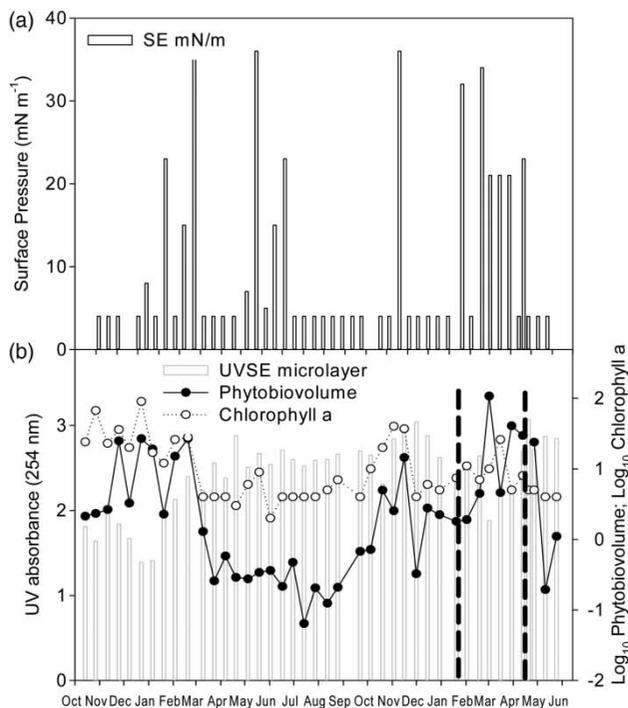

Figure 2 | Surface pressure (SE mN/m in (a)) and UV absorbance (UVSE in (b)) of the microlayer taken within 2 m of the SE shore, and phytoplankton biovolume (phytobiovolume) and chlorophyll a absorbance of water column samples (b) taken every two weeks from 14 October 2009 to 25 May 2011. Dashed lines indicate the start and end of monolayer application.

Periods of consistently high surface pressure (SE shore mean of 20.0 mN m^{-1}) coincided with monolayer application. Water temperature ranged from 13.0 °C and 11.5 °C for subsurface and microlayer samples in winter, to 27.9 °C and 27.2 °C, respectively, in summer. Microlayer pH, conductivity and dissolved oxygen ranged from 6.51 to 8.86 pH units, 209 to 295 μS, and 8.32 to 13.14 mg L^{-1} respectively, similar to other dams in South East Queensland (Markwell & Fellows 2008).

Microlayer UV$_{254}$ absorbance consistently exceeded 0.25 (Figure 2(b)), indicating Logan's Dam is humic

eutrophic (Tranvik & Bertilsson 2001). Absorbance was proportional to turbidity (SE subsurface UV$_{254}$ = 0.626 + (0.005 × turbidity), r^2 = 0.598, P < 0.001, n = 42), and correlated with water depth (r = 0.582 and r = 0.670 for microlayer and subsurface respectively, P < 0.001 n = 42), water abstraction (microlayer r = 0.440, P = 0.004, subsurface r = 0.504, P = 0.001; n = 42), and with calm conditions (microlayer r = 0.448, P = 0.003, subsurface r = 0.394, P = 0.042; n = 42). The rise in absorbance occurred before pumping in March 2010, increasing over the seasonally dry, calm winter when water abstraction and evaporative loss reduced water depth. No increase in absorbance for microlayer or subsurface water was evident during the monolayer dosing period. These results suggest the aromatic DOM in microlayer and subsurface water is derived mainly from the black soil sediment.

The COD of microlayer water sampled from the SE and NW shore margins was very similar (median values of 56.5 and 62 mg L^{-1}, respectively), and only marginally higher than the median values of subsurface water samples (46.5 and 46.0 mg L^{-1} for SE and NW shores, respectively). The peak in COD of 223 mg L^{-1} for the SE microlayer sample coincided with peak ingress pumping events after summer rain in February–March 2010 (Figure 1(b)). There was no corresponding spike in UV absorbance (Figure 2(b)), indicating the DOM was not aromatic. There was no increase in UV$_{254}$ absorbance or in the COD of microlayer or subsurface water samples during or after monolayer application (Table 2). Results for the concentration of non-ionic surfactants were typically below the lowest order of detection (<0.5 mg L^{-1}) before, during and after monolayer application, indicating the CTAS method did not detect the monolayer formulation or the concentration was below the detectable limit. No analytical testing of the monolayer formulation was possible, due to the confidential nature of the product.

Table 2 | COD, BOD, DOC, and MPN of microlayer-adapted bacteria in microlayer (Micro) and subsurface (Subs) water sampled from the SE shore before, during (in bold) and after monolayer application

Sampling date	COD (mg L^{-1})		BOD (mg L^{-1})		DOC (mg L^{-1})		MPN (log$_{10}$)	
	Micro	Subs	Micro	Subs	Micro	Subs	Micro	Subs
1/12/10	61	57	<2	<2	28.1	27.2	4.4	3.9
20/1/11	48	43	<2	2	10.0	10.4	4.0	3.0
2/2/11	**49**	**46**	**< 2**	**< 2**	**11.7**	**13.1**	**3.7**	**3.9**
30/3/11	**51**	**47**	**7**	**< 2**	**13.5**	**10.1**	**4.5**	**4.7**
20/4/11	126	46	13	<2	10.7	8.3	5.5	5.3
11/5/11	na	na	na	na	10.0	10.8	5.3	4.9

Biological water quality

Phytoplankton biovolume was proportional to subsurface water temperature (Phyto = $(0.129 \times$ °C Subs$)$ – 2.469; $r^2 =$ 0.645, $P \leq 0.001$, $n = 41$), with populations of the cyanobacterial species *Microcystis aeruginosa* and *Anabaena* sp. increasing towards the end of the monolayer application period when water temperature was highest (Figure 2(b)). Biovolume was not correlated with chlorophyll *a* absorbance over summer, as the cyanobacterial phycobilin pigment absorbs light at 620 nm and chlorophyll *a* utilised by green algae absorbs at 440 nm (Asai *et al.* 2001). The drop in phytobiovolume two weeks later may reflect the drop in water temperature. In summer, the total phytoplankton biovolume consistently exceeded the Queensland algal bloom alert level of 4 mm^3 L^{-1}. Algal blooms were evident as a thick blue-green scum on the shoreline. Total P and FRP, total N and nitrous oxides were consistently above limiting levels for phytoplankton growth (Wetzel 2001). Microlayer-adapted bacterial populations were proportional to UV$_{254}$ absorbance (log$_{10}$MPN = $0.32 + (1.4 \times$ UV$_{254})$, $r^2 = 0.573$, $P < 0.001$, $n = 16$) but not to phytoplankton biovolume ($r = -0.122$, $P = 0.653$, $n = 16$), suggesting aromatic DOM derived from the black soil was their main substrate. There was no evidence of an increase in microlayer-adapted bacteria with monolayer application (Table 2).

Subsurface BOD was proportional to subsurface COD (BOD = $(0.65 \times$ COD$)$ -28.1, $r^2 = 0.969$, $P < 0.001$, $n = 8$) but microlayer BOD was not ($r = -0.560$, $P = 0.149$, $n = 8$), presumably due to the higher concentration of microbially-resilient DOM in the microlayer (Samios *et al.* 2007). A spike in BOD (microlayer 150 mg L^{-1}, subsurface 125 mg L^{-1}) coincided with the spike in COD in March 2010, associated with pump ingress events (Figure 1(b)). DOC was highest before monolayer application (Table 2),

diluting SUVA$_{254}$ values (microlayer 11.2 m^{-1} mg^{-1} L^{-1}, subsurface 11.4 m^{-1} mg^{-1} L^{-1}). One month later, SUVA$_{254}$ had increased (23.5 and 24.5 m^{-1} mg^{-1} L^{-1}, respectively) as the DOC decreased (14.2 and 10.4 mg L^{-1}, respectively), suggesting the DOM pumped into the dam in late November 2010 (Figure 1(b)) was susceptible to microbial and/or photo-degradation. The DOC of microlayer and subsurface samples increased marginally during the monolayer application period (Table 2), with no increase in UV$_{254}$, COD or in microlayer-adapted bacteria. During this period, the water depth changed very little, with no pumping events (Figure 1).

CONCLUSION

In this 500 ML, 16 ha farm dam, the intermittent application of 1,073 L of an experimental monolayer formulation over seven weeks in summer did not increase the COD, or populations of microlayer-adapted bacteria, or cyanobacteria in the water column. The rise in DOC over the monolayer application period was less than the concentration recorded after a pump ingress event in late spring. In this humic, hypereutrophic dam, repeat monolayer application did not adversely affect aquatic ecology.

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