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# The composition and importance of the phytoneuston in two floodplain lakes in south-eastern Australia

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Abstract Normans Lagoon and 3-Gum pond are small floodplain water bodies adjacent to the Murray River, south-eastern Australia, and often have a visible film/sheen across their surface. Since few studies have provided quantitative comparisons of the surface and subsurface layer communities of shallow freshwater lakes, we determined the contributions of the surface and subsurface populations to overall algal biomass when a surface film was visible, and when it was not visible. We examined the algae and cyanobacteria present at the air-water interface of each water body, and compared the findings with those for the water immediately below the surface, and for the overall water column. The algal groupings Trachelomonas spp., other Euglenophyceae

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G. N. Rees (⊠) Murray-Darling Freshwater Research Centre, P.O. Box 991, Wodonga, Victoria 3689, Australia e-mail: Gavin.Rees@csiro.au (principally Euglena spp.), Chlorophyceae and Cyanobacteria usually comprised >95% of the measured biovolume within all samples. Samples from the air-water interface were considerably enriched (up to 200-fold) with respect to algal biovolume, whether or not a visible surface film was present, and elevated cell counts were observed within the air-water interface for motile organisms such as Trachelomonas spp. and green unicellular flagellates. The reverse was true for the cyanobacterium Planktolyngbya however, with greater concentrations occurring at depth. In terms of its contribution to the overall algal/ cyanobacterial populations within each water body, the surface layer was found to be responsible for <1-20% of the biovolume over the entire water column. Multivariate statistical analysis confirmed there were significant differences between the communities of the air-water interface and those of the water below, and that these differences occurred both in the presence and absence of a visible surface film/sheen.

**Keywords** Air-water interface · Algae · Cyanobacteria · Neuston · Lakes

#### Introduction

The air-water interface of aquatic ecosystems offers a special environment for the growth of

organisms. Studies on the chemical nature of the air-water interface have shown that organic carbon and inorganic nutrients readily accumulate within the surface layer (Parker & Barsom, 1970; Gladyshev, 1986; Kuznetsova & Lee, 2001; Kuznetsova et al., 2004), suggesting it could be an ideal site for colonization by various biota. However, several physical and chemical characteristics unique to the surface layer impose a range of potential stresses that make colonization difficult. The surface layer is subject to marked fluctuations in temperature, solar radiation (especially UV radiation), wind and rainfall (Norkrans, 1980; Estep & Remsen, 1985; Maki, 1993). It also suffers accumulations of heavy metals (Maki, 1993; and references therein), and the combined physical and chemical characteristics have lead Maki (1993) to suggest that the air-water interface should be considered an extreme environment.

Several of the studies on the chemical and physical properties of the surface layer have occurred in conjunction with studies on surfacelayer biota. Naumann (1917) first recognized a distinct surface-layer, or "neuston", community. Since then a number of studies have reported algae occurring within the neuston community (Petersen & Hansen, 1958, 1960; Hardy, 1973; Parker & Hatcher, 1974; Frølund, 1977). Albright (1980) reported that primary production was lower at the surface, as compared to subsurface waters, and suggested that cells in the surface layers were injured or inhibited in some way. However, Hardy & Apts (1984) showed that production by the phytoneuston could be considerably higher than by the phytoplankton of the waters beneath, and that methodological factors could lead to an apparent underestimate of photosynthetic activity of the phytoneuston. Further studies suggest that algae may not always be the dominant organisms within the surface layer, but on occasions may dominate in the water immediately below the surface layer (Estep & Remsen, 1985).

Coastal and oceanic waters have been dominant systems in neuston research (De Souza Lima & Chretiennot-Dinet, 1984; Hardy & Apts, 1984; Agogue et al., 2004), although Agogue et al. (2004) still suggest our knowledge of the marine neuston is still in its infancy. While a number of physical and chemical characteristics will be similar across all neuston communities, those of inland waters may well differ from those of coastal and oceanic systems, particularly for shallow lakes where relatively high organic inputs and high activities by sediment microbes have an effect on the water column and (potentially) the surface layer. Shallow floodplain lakes also are subject to high surface temperatures and this may also be to the advantage of surface communities.

Although accumulations of algae at water surfaces are known, and indeed are quite often visible to the naked eye, comparisons between the surface layer algal community and the overall algal community have seldom been made. Since few quantitative data exist on surface layer communities of shallow freshwater lakes, we examined the surface film, subsurface layer and water column of two floodplain water bodies and identified the dominant algae. The contributions of the surface and subsurface populations to overall algal biomass were determined when a surface film was visible, and when it was not. The overall community patterns were compared to determine if a distinct phytoneuston was present on the two water bodies.

# Methods

# Study sites

Two contrasting freshwater bodies situated on the Murray River floodplain in south-eastern Australia were examined. 3-Gum pond was a small circular water body located within the Wonga Wetlands complex near Albury, New South Wales (146°51' E 36°04' S). The pond was approximately 15 m in diameter, had a maximum depth of 1.2 m and received its water through groundwater and rain. The southern side of the pond was verged by large River Red Gum trees (Eucalyptus camaldulensis), with several grass species comprising the ground cover. The northern side of the pond was covered by small eucalypt species and several species of grasses. Accumulations of the small floating fern Azolla filiculoides occurred across the water body. The

second site was Normans Lagoon, a freshwater lake lying adjacent to the northern edge of the Murray River. Normans Lagoon was some 700 m long and 40 m wide and between 2–6 m deep (Gribben et al., 2002). *E. camaldulensis, E. sphacelata* and populations of *Braesinia schreberi* are found along its shoreline. Beds of the rush *Eleocharis sphacelata* are found throughout the lagoon as is the small floating fern *A. filiculoides*. A section at the downstream end of the lagoon was selected as a study site for this project and was some 50 m long and 30 m wide, and had a maximum depth of approximately 2 m.

## Sampling

Sampling was conducted on three occasions during the Austral summer, on 14 November 2003, 24 December 2003, and 2 February 2004. A stratified random sampling approach was used to collect samples from the surface layer and subsurface water of each water body, with five samples being taken where there was a visible surface film present, five from where a visible surface film was not present (when this was possible) and one integrated depth sample from the water column. 3-Gum pond was completely covered with a visible surface film on the 24 December 2003 and 2 February 2004 sampling days and hence, a non-visible film-samples could not be obtained on these two occasions. Normans Lagoon was not sampled on 24 December 2003, due to a heavy rain event the previous day. Samples from surfaces with visible film, without a visible film and water column were obtained on all other occasions.

Surface layer samples were collected by placing a 100 mm by 100 mm glass plate on the surface of the water, removing it and transferring the adhering material to a 30 ml McCartney bottle. The glass plate was thoroughly rinsed with deionised water and dried between samples to prevent cross-contamination. In order to obtain an estimate of the amount of water collected, the glass plate was dried and weighed, placed onto the surface of a bucket of water and removed and reweighed. The process was repeated 30 times and the weights averaged to obtain a measure of the amount of water collected. The volume collected was then used to calculate the depth of the surface layer that we sampled.

Subsurface samples were collected from a depth of 5–10 mm using a 20 ml syringe and a narrow depth, purpose-made device constructed from a plastic filter holder. The device comprised two small disks held approximately 1 mm apart and connected to a syringe such that pressure applied to the syringe sucked water only from the perimeter gap between the disks. The apparatus was placed immediately beneath the pond surface and slowly moved laterally, with suction being applied to collect the sample. The sample was then transferred to a 30 ml McCartney bottle.

Water column samples were collected using a tube sampler consisting of a length of 30 mm diameter electrical conduit pipe. The tube was repeatedly lowered into the water, capped and the water collected placed into a bucket. This process was repeated until approximately 500 ml of water was collected.

#### Analyses

The samples detailed above were preserved using Lugol's iodine and stored in the dark until they were examined. Algal counts were conducted using a Lund cell and a Zeiss Axioskop light microscope. Initial observations of each sample were performed using a standard microscope slide and cover slip to make an assessment of the taxa present and to determine if the sample required concentrating (Hötzel & Croome, 1999).

Algal biovolumes were estimated by obtaining cell dimensions and relating each individual taxon to a known shape. The percentage contributions of the surface layer, the subsurface layer we sampled and remainder of the water column were calculated based on (1) the surface layer to be the 0.506 mm thick film collected by the glass plate sampler, (2), presuming the subsurface layer we sampled to be 10 mm thick and (3) calculating the "remaining water column biomass" as the biomass of the entire water column (determined from the tube sample) less that of the surface and subsurface layers.

Conductivity, pH, turbidity, dissolved oxygen and temperature were measured in situ approximately 200 mm below the water surface, using a Horiba (U-10 model) water quality meter and submersible multi-probe.

Multivariate statistics were used to examine different aspects of the algal community structure. Bray-Curtis similarities were determined for all data sets, calculated with un-transformed and fourth-root-transformed algal cell abundances. Ordination of community structures was carried out with non-metric multidimensional scaling (MDS) (Clarke, 1993; Clarke & Warwick, 2001). We used an Analysis of Similarity (ANOSIM) routine to test whether there were significant differences between the different layers within the water bodies (Clarke, 1993; Clarke & Warwick, 2001). Similarity percentage analysis (SIM-PER) was used to illustrate which groups of organisms were most responsible for any dissimilarity between layers when a visible was present and absent (Clarke & Warwick 2001). Analyses were carried out on a combined data set for occasions with a visible film and those without a visible film. Community analysis (MDS, ANO-SIM, SIMPER) was carried out for samples taken when a visible film was present, and when it was not visible. All statistical procedures were implemented within PRIMER v5 statistical software (PRIMER-e Ltd, Plymouth UK).

#### Results

The surface waters of both sites were relatively warm throughout the study period (Table 1). The pH and electrical conductivity at the two sites were similar, and were typical of previously reported values for Murray River floodplain lakes (Gribben et al., 2002). Dissolved oxygen values were extremely low in 3-Gum pond, reflecting high net respiration in the water column and/or sediments. The corresponding values for Normans Lagoon were considerably higher. Turbidity values were relatively high in both waters, but more so in Normans Lagoon.

# Algal and cyanobacterial biovolumes

For samples taken where a visible surface film was present, the air–water interface always contained the greatest concentrations and biovolume of algae. In Normans Lagoon for instance (Table 2) the algal biovolume within the visible surface film on 14 November 2003 was  $1.517 \text{ mm}^3 \text{ ml}^{-1}$ , while that of the sub-surface water was  $0.008 \text{ mm}^3 \text{ ml}^{-1}$ , and of the water column  $0.011 \text{ mm}^3 \text{ ml}^{-1}$ . The differences were less marked for the visible film samples from Normans Lagoon on 2 February 2004, but the same trend was apparent, just as it was for all three visible film samplings for 3-Gum pond (Table 3).

For samples taken where a visible film was absent, the air-water interface samples again showed the greatest biovolume of organisms. In Normans Lagoon on 14 November, for instance, a surface algal biovolume of  $0.026 \text{ mm}^3 \text{ ml}^{-1}$  was recorded, against  $0.012 \text{ mm}^3 \text{ ml}^{-1}$  for the subsurface, and  $0.011 \text{ mm}^3 \text{ ml}^{-1}$  for the water column (Table 2). The only sample with no visible film from 3-Gum pond (14 November 2003) had an algal biovolume at the surface of 0.439 mm<sup>3</sup> ml<sup>-1</sup>, while those of the subsurface waters and water column were 0.002 and 0.003 mm<sup>3</sup> ml<sup>-1</sup>, respectively (Table 3).

Varying results were obtained for those occasions when it was possible to compare algal biovolumes at the air-water interface where a

 Table 1 Water quality in 3-Gum pond and Normans Lagoon

Date	Temperature (°C)		Dissolved oxygen (mg l <sup>-1</sup> )		pН		Turbidity (NTU)		Electrical conductivity μS cm <sup>-1</sup>	
	3-Gum	Normans	3-Gum	Normans	3-Gum	Normans	3-Gum	Normans	3-Gum	Normans
14/11/03 24/12/03 2/2/04	20 21 20	24 NS <sup>a</sup> 23	0.7 0.6 0.6	6.5 NS <sup>a</sup> 5.0	7.6 7.7 7.7	7.8 NS <sup>a</sup> 7.6	4 42 23	72 NS <sup>a</sup> 91	65 64 77	72 NS <sup>a</sup> 81

<sup>a</sup> No samples were taken

	14 November 2003						2 February 2004					
	Visible film		No visible film		Water	Visible		No visible film		Water		
	Surface	Subsurface	Surface	Subsurface	column	Surface	Subsurface	Surface	Subsurface	column		
Biovolume $(mm^3 ml^{-1})$	1.517	0.008	0.026	0.012	0.011	0.889	0.229	0.726	0.241	0.278		
Percentage contribut	tion to bi	ovolume by										
Trachelomonas spp.	29	2	8	2	18	4	2	3	1	1		
Other Euglenophyceae <sup>a</sup>	11	1	6	1	1	41	1	10	3	9		
Chlorophyceae	56	96	81	96	81	48	6	77	8	9		
Cyanobacteria	0	0	0	0	0	5	91	8	88	80		
Bacillariophyceae	4	1	1	1	0	1	0	1	0	0		
Other <sup>b</sup>	0	0	4	0	0	1	0	1	0	1		

Table 2 Algal and cyanobacterial biovolumes in Normans Lagoon

<sup>a</sup> Principally Euglena spp

<sup>b</sup> Cryptophyceae, Chrysophyceae, unknowns

visible surface film was present, with those where a visible surface film was absent i.e. on both sampling occasions in Normans Lagoon, and once in 3-Gum pond. In Normans Lagoon of 14 November 2003 (Table 2) an algal biovolume of 1.517 mm<sup>3</sup> ml<sup>-1</sup> was recorded at the air-water interface where a surface film was visible, and a much lesser 0.026 mm<sup>3</sup> ml<sup>-1</sup> where no film was visible. On 2 February 2004 however, the corresponding figures were 0.889 and 0.726 mm<sup>3</sup> ml<sup>-1</sup>, respectively, showing little difference between the two areas. For 3-Gum pond, the sampling on 14 November 2003 actually showed a higher algal biovolume at the air-water interface where a visible film was absent  $(0.439 \text{ mm}^3 \text{ ml}^{-1})$  than where a visible film was present  $(0.344 \text{ mm}^3 \text{ ml}^{-1})$ (Table 3).

## Contributions to biovolume and cell numbers

The groupings *Trachelomonas* spp., other Euglenophyta (principally *Euglena* spp.), Chlorophyceae and Cyanobacteria usually comprised >95% of the algal + cyanobacterial biovolume, but differences in composition were apparent between the surface, subsurface and water column samples (Tables 2, 3). By way of example, in Normans Lagoon on 14 November 2003 the biovolume of the visible film surface sample was rich in *Trachelomonas* spp. (29%) and other Euglenophyceae (11%), and on 2 February 2004 there was dominance of the subsurface and water column samples by Cyanobacteria (80–91%), with the surface samples being dominated by other Euglenophyceae (up to 41%) and Chlorophyceae (up to 77%).

In 3-Gum pond on 14 November 2003 (Table 3) Chlorophyceae dominated in all samples but the visible film surface sample, where *Trachelomonas* spp. was also prevalent, marking a difference between the visible film and no-visible film surface samples on this occasion despite their similarity in biovolume. On 24 December 2003, other Euglenophyceae (i.e. principally *Euglena* spp.) contributed substantially to biovolume within the visible film surface sample, but were also prevalent in the water column. On 2 February 2004 Euglenophyceae contributed substantially to surface and subsurface biovolumes, making a lesser contribution to the water column.

Cell numbers from the sampling on Normans Lagoon on 4 February 2004 and 3-Gum pond on 14 November 2003 are given in Table 4. In Normans Lagoon cell numbers at the surface where a visible film was present were much raised for *Trachelomonas* spp., other Euglenophyceae, Cryptophyceae, green unicellular flagellates and particularly Bacillariophyceae, compared to the

U	-											
	14 November 2003					24 Dece	mber 20	03	2 February 2004			
	Visible film		No visible film		Water	Visible film		Water	Visible film		Water	
	Surface	Sub surface	Surface	Sub surface	column	Surface	Sub surface	column	Surface	Sub surface	condinin	
Biovolume $(mm^3 ml^{-1})$	0.344	0.001	0.439	0.002	0.003	1.926	0.010	0.004	0.694	0.003	0.015	
Percentage contribut	tion to bio	ovolume	by									
Trachelomonas spp.	42	6	7	4	15	4	21	7	12	5	2	
Other Euglenophyceae <sup>a</sup>	4	1	2	1	0	83	31	68	55	51	24	
Chlorophyceae	49	92	90	94	81	12	46	24	29	40	73	
Cyanobacteria	1	0	0	0	0	0	0	0	0	0	0	
Bacillariophyceae	3	1	0	0	4	1	1	0	4	1	0	
Other <sup>b</sup>	1	0	1	1	0	0	1	1	0	3	1	

Table 3 Algal and cyanobacterial biovolumes in 3-Gum pond

<sup>a</sup> principally Euglena spp

<sup>b</sup> Cryptophyceae, Chrysophyceae, unknowns

deeper samples. For the cyanobacterium *Plank-tolyngbya subtilis* highest cell abundances were observed in the subsurface and water column samples. Even where no visible film was present, much higher concentrations of other Euglenophyceae (principally *Euglena* spp.) and Bacillariophyceae were observed at the surface, compared with deeper samples. Similar results are apparent for 3-Gum pond, with especially *Trachelomonas* spp., Bacillariophyceae and green unicellular flagellates being much more concentrated at the air–water interface.

Algal and cyanobacterial community analysis

The MDS ordination of algal communities (cells  $ml^{-1}$ , fourth-root transformed data) for those occasions where a surface film was present showed there were differences among layers, as well as there also being a difference between sites (Fig. 1a). Samples from the surface layer clearly separated from those of the subsurface layer and water column, but some overlap was apparent between the subsurface layer and water column. Pairwise comparisons showed the surface layer

	Normans Lagoon 4 Feb 2004						3-Gum pond 14 Nov 2003					
	Visible film		No visible film		Water	Visible film		No visible film		Water		
	Surface	Subsurface	Surface	Subsurface	column	Surface	Subsurface	Surface	Subsurface	column		
Trachelomonas spp.	12,000	660	7800	4600	310	33,900	10	5180	80	102		
Euglena spp.	26,000	420	5450	600	380	1060	1	530	2	NR		
Cryptophyceae	5300	450	1030	440	240	280	12	NR	NR	9		
Bacillariophyceae	20,400	2640	6900	600	80	9300	15	3700	8	144		
Green unicellular flagellates	734,000	6700	75,400	53,700	569,000	896,400	640	180,000	1400	13,700		
Anabaena spp. <sup>a</sup>	1400	460	NR	570	930	720	NR	NR	NR	NR		
Planktolyngbya sp.	131,000	648,000	164,300	661,000	766,000	NR	NR	NR	NR	NR		

<sup>a</sup> NR: None recorded

<sup>b</sup> Filaments ml<sup>-1</sup>



**Fig. 1** MDS ordination of algal and cyanobacterial abundances (fourth-root transformed) in the surface, subsurface and water column of 3-Gum pond and Normans Lagoon when (**a**) a surface film was visible and (**b**) without a visible surface film. Ordination stress for (**a**) and (**b**) are 0.14 and 0.07, respectively

community was significantly different from that of both the subsurface and water column (ANOSIM, P < 0.01 for both comparisons), but the subsurface community was not significantly different from that of the water column (P = 0.68). SIM-PER analysis of the cell count data obtained where a visible surface film was present showed that the organisms contributing most to the difference observed between the surface and subsurface layers were unicellular flagellated Chlorophyceae (28.1%) and other Chlorophyceae (22.5%), followed by Bacillariophyceae (13.3%), *Trachelomonas* spp. (10.3%), *Euglena* spp. (5.6%) and Cyanobacteria (4.4%). The organisms contributing most to the difference between the surface layer and the water column were unicellular flagellated Chlorophyceae (24.9%) and other Chlorophyceae (19.8%), followed by Bacillariophyceae (15.1%), *Trachelomonas* spp. (11.6%), *Euglena* spp. (7.5%) and Cyanobacteria (5.8%). The observed differences between the communities remained significant when the data were not transformed prior to analysis (data not shown).

The corresponding MDS ordination for the communities where a visible film was not present again showed the surface community was different from that of the subsurface (Fig. 1b). The pairwise analysis of similarity showed the surface and subsurface communities were significantly different (P < 0.01) and that there was some evidence of a difference between the surface layer and water column communities (P = 0.068), and also between the subsurface layer and water column communities (P = 0.066). SIMPER analysis of the cell count data obtained where a visible surface film was not present showed that the organisms contributing most to the difference observed between the surface and subsurface layers were unicellular flagellated Chlorophyceae (29.6%) and other Chlorophyceae (22.1%), followed by Bacillariophyceae (10.9%), Trachelomonas spp. (9.0%), Cyanobacteria (6.2%), and Euglena spp. (4.6%). Again, the observed differences between the communities were similar when the data were not transformed prior to analysis (data not shown).

Contribution by the surface film, subsurface layer and water column to total algal/ cyanobacterial volume

In Normans Lagoon, the algae/cyanobacteria of the water column always contributed 95% or more to total biovolume within the water body (Fig. 2). Contributions by the surface and subsurface layers were usually less than 1%, the exception being the surface layer contribution within the visible-film area on 14 November 2003, which was calculated to be 5% (Fig. 2e).

The contribution to total biovolume made by the surface layer in 3-Gum pond was more Fig. 2 Percentage contribution of surface layer, subsurface layer and the remaining water column to the overall algal and cyanobacterial biovolume in 3-Gum pond and Normans Lagoon. (a), (c), (d), (e), and (g) show contributions when a surface film was visible. (b), (f), and (h) show contributions when no surface film was visible



Percentage contribution

substantial, ranging from 1.9% on 2 February 2004 (Fig. 2d) to a surprising 20.2% on 24 December (Fig. 2c). On 14 November 2003, the surface film contributed 4.7–6.1%, the latter value being determined for an area of the pond where a surface film was not readily apparent to the naked eye. The surface layer contribution in 3-Gum pond always exceeded that of the subsurface layer, despite the difference in thickness of 0.506 versus 10 mm.

## Discussion

The air-water interface of a water body is a distinct microenvironment, characterized by high light levels and the presence of material arriving from both the atmosphere and water column. A discernible layer often develops at the surface of the water, comprising lipids, proteins and polysaccharides in a hydrated gel-like matrix in which humic materials and particulates also accumulate (see Maki, 1993). In smaller water bodies, sufficient material often accumulates in this layer so as to be seen with the naked eye as a sheen across the surface of the pond or lagoon. Wind has a major influence on the amount of surface material that can collect at the air-water interface (Estep & Remsen, 1985). Theoretical considerations, combined with experimental studies, have shown that surface layers can be remarkably resilient to mixing and that wind speeds greater than 3 m  $s^{-1}$ are required to mix floating algal cells from the water surface into the overall water column (Hutchinson & Webster, 1994; Webster & Hutchinson, 1994). Accumulation of a surface film at the air-water interface surface dampened wave activity which in turn reduced mixing (Hutchinson & Webster, 1994). Furthermore, algae trapped within the surface layer are likely to maintain and even possibly enhance the physical properties of the surface layer, brought about with the production of various metabolites and cell exudates that will certainly change the chemical nature of the surface film. Surface tension can show considerable spatial heterogeneity within a given water body, as well as seasonal trends, with higher values reported in winter than in summer (Hardy, 1973).

Any study on the surface layer of water bodies should consider the sampling techniques used to obtain surface layer samples as the actual sample, particularly the depth that is collected, will be defined by the method that is used. Such variation in sampling does mean that cross-study comparisons are difficult. In our study, one technique was used throughout and so variation due simply to method variability is not an issue and we can hold confidence in our results. The two sites in this study differed with respect to their surface accumulations. Normans Lagoon was sufficiently large that substantial areas without a visible surface film occurred throughout the study period. In the smaller 3-Gum pond, a film was visible over the entire surface during two of the three sampling occasions. Significantly, we showed a phytoneuston was present across the surface at each study site on each sampling occasion, even when parts of the water body were not covered by a visible surface film.

The physico-chemical conditions (temperature, pH, dissolved oxygen, turbidity and electrical conductivity) within the water body showed little variation at each of the sample times, so it is difficult to infer how any of the physico-chemical factors may have affected the biological observations within sites. There were major and consistent differences in the DO between the two sites, which may have had a role in determining the subsurface communities in the two water bodies. Despite the lack of apparent physico-chemical changes at the scale we measured, major shifts occurred in the algal/cyanobacterial communities over time.

We measured 200-fold differences in algal/ cyanobacterial biovolumes between the surface, subsurface and water column samples of both Normans Lagoon and 3-Gum pond (Tables 2, 3). The groups *Trachelomonas* spp., other Euglenophyceae, Chlorophyceae and Cyanobacteria usually comprised >95% of the biovolume, but their relative contribution varied with sample type, with *Trachelomonas* spp. and other Euglenophyceae (principally *Euglena* spp.) often contributing more to the surface waters than to the water column as a whole. The most striking differences in cell counts between surface samples and subsurface/water column samples were recorded for *Trachelomonas* spp. (e.g. 33,900 versus 10–102 cells  $ml^{-1}$ , 3-Gum pond 14 November), green unicellular flagellates (896,000 versus 640–13,700 cells  $ml^{-1}$ , ibid.) and *Planktolyngbya subtilis* (131,000 versus 648,000–766,000 filaments  $ml^{-1}$ , Normans Lagoon 4 February).

To determine if there were significant statistical differences between surface and subsurface layers, we combined the algal and cyanobacterial abundances from both sites and all sampling times where a visible surface film was present, and where a visible film was absent. The combined data sets were used for the ordinations. Community patterns were examined based on fourth-root and non-transformed data. Fourth root-transformation of abundance data emphasizes the contribution of rarer species to sample or site similarities, so an ordination derived from fourth root-transformation predominantly reflects differences in overall community structure (Clarke & Warwick, 2001). For non-transformed data, no down-weighting occurs and large abundances have a large effect on similarity coefficients, so the resulting MDS ordination is heavily driven by abundances of individual taxa. We showed that even though there were different algal/cyanobacterial communities at the difference sites, there also were significant differences between the surface and subsurface layer communities, irrespective of whether a visible surface film was present or absent. The differences were apparent with both untransformed and transformed data, indicating that the differences in the community ordinations were driven not only by cell abundances, but also by community structure.

Given the limited number of studies on neuston communities, it is difficult to describe overall tends that may exist in neuston communities. For example, Parker and Hatcher (1974) showed differences in the algal communities between the surface and subsurface layer of ponds. There was considerable temporal variation, but flagellates tended to dominate in the surface layer. However, in a further study of a small water body, a chrysophyte was the dominant surface algae in the surface layer during summer, yet a bacterium (identified as *Leptothrix* sp.) was the dominant organism in the surface layer during spring (Frølund, 1977).

Although the concentrations of algae and cyanobacteria were generally highest in the surface layers, their percent contributions to the overall biovolume within the water body varied. In Normans Lagoon the surface contributions were generally less than 1%, which may not be surprising since the greater water depth means there is a much greater volume of water for the phytoplankton to be distributed through the water column. However, in the considerably more shallow 3-Gum pond, the surface layer could be responsible for up to 20% of the measured biovolume. The implication then for shallow lakes or ponds is the potential role of the surface layer to contribute to the overall production within the water body.

This study showed that a phytoneuston exists within shallow floodplain lakes, and on each sampling occasion the algal and cyanobacterial communities at the air-water interface were significantly different with respect to composition and abundance from those in the water immediately below the surface. Furthermore, these community differences were apparent both in the presence and absence of a visible surface film. Future studies should combine community structure analysis and estimates of production so that the role of phytoneuston communities in production within the overall system can be estimated. The surface layer not only represents an important site for algae and cyanobacteria, but also potentially for heterotrophic activity. The role of grazing organisms and microbial production within the surface layers also remain areas open for further research.

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#### References

Agogue, H., E. O. Casamayor, F. Joux, I. Obernosterer, C. Dupuy, F. Lantoine, P. Catala, M. G. Weinbauer, T. Reinthaler, G. J. Herndl & P. Lebaron, 2004. Comparison of samplers for the biological characterization of the seas surface microlayer. Limnology and Oceanography Methods 2: 213–225.

- Albright, L. J., 1980. Photosynthetic activities of phytoneuston and phytoplankton. Canadian Journal of Microbiology 26: 389–392.
- Clarke, K. R., 1993. Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology 18: 117–143.
- Clarke, K. R. & R. M. Warwick, 2001. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation. (2nd ed.), Primer-E, Plymouth, UK.
- De Souza Lima, Y. & M. J. Chretiennot-Dinet, 1984. Measurement of biomass and activity of neustonic microorganisms. Estuarine Coastal Shelf Science 19: 167–180.
- Estep, K. W. & C. C. Remsen, 1985. Influence of the surface microlayer on nutrient, chlorophyll and algal diversity of a small eutrophic bog pond. Hydrobiologia 121: 203–213.
- Frølund, A., 1977. The seasonal variation of the neuston of a small pond. Botanisk Tidsskrift 72: 45–56.
- Gladyshev, M. I., 1986. Neuston of inland waters (a review). Hydrobiological Journal 22: 1–7.
- Gribben, D. L., G. N. Rees & R. L. Croome, 2002. Anoxygenic phototrophic bacteria and aerobic phototrophs in Normans lagoon, a "billabong' adjacent to the Murray River, south-eastern Australia. Lakes and Reservoirs: Research and Management 8: 95–104.
- Hardy, J. T., 1973. Phytoneuston ecology of a temperate marine lagoon. Limnology and Oceanography 18: 525–533.
- Hardy, J. T. & C. W. Apts, 1984. The sea-surface microlayer: phytoneuston productivity and effects of atmospheric particulate matter. Marine Biology 82: 293–300.
- Hötzel, G. & R. L. Croome, 1999. A Phytoplankton Methods Manual for Australian Freshwaters. Land

and Water Resources Research and Development Corporation, Canberra, Australia.

- Hutchinson, P. A. & I. T. Webster, 1994. On the distribution of blue-green algae in lakes: wind tunnel experiments. Limnology and Oceanography 39: 374–382.
- Kuznetsova, M. & C. Lee, 2001. Enhanced extracellular enzymatic peptide hydrolysis in the sea-surface microlayer. Marine Chemistry 73: 319–332.
- Kuznetsova, M., C. Lee, J. Aller & N. Frew, 2004. Enrichment of amino acids in the sea surface microlayer at coastal and open ocean sites in the North Atlantic ocean. Limnology and Oceanography 49: 1605–1619.
- Maki, J. S., 1993. The air-water interface as an extreme environment. In Ford, T. E. (ed.), Aquatic Microbiology: an Ecological Approach. Blackwell Scientific Publications, Oxford, 409–439.
- Naumann, E., 1917. Beiträge zur Kenntnis des Teichnannoplanktons. II. Über das Neuston des Süßwassers. Biologisches Zentralblatt 37: 98–106.
- Norkrans, B., 1980. Surface microlayers in aquatic environments. Advances in Microbial Ecology 4: 51–85.
- Parker, B. & G. Barsom, 1970. Biological and chemical significance of surface microlayers in aquatic ecosystems. BioScience 20: 87–93.
- Parker, B. C. & R. F. Hatcher, 1974. Enrichment of surface freshwater microlayers with algae. Journal of Phycology 10: 185–189.
- Petersen, J. B. & J. B. Hansen, 1958. On some neuston organisms I. Botanisk Tidsskrift 54: 93–110.
- Petersen, J. B. & J. B. Hansen, 1960. On some neuston organisms II. Botanisk Tidsskrift 56: 197–234.
- Webster, I. T. & P. A. Hutchinson, 1994. Effect of wind on the distribution of phytoplankton cells in lakes revisited. Limnology and Oceanography 39: 365–373.