Research Report 4

Biofilm of the Mallee Tract

Bernard McCarthy[1]
Ben Gawne[2]
Shaun Meredith[1]

Murray-Darling Freshwater Research Centre
[1]P.O. Box 3428, Mildura 3502
[2] P.O. Box 921, Albury 2640

Final Report to MDBC

August 2004

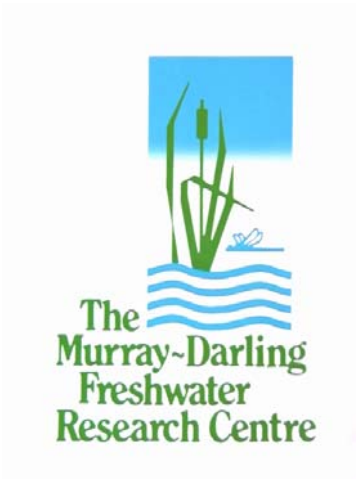

Table of Contents

Report Linkages

This individual Research Report forms a component of the larger report:

McCarthy, B., Gawne, B., Meredith, S., Roberts, J. and Williams, D. (2004). Effects of Weirs in the Mallee Tract of the River Murray. Murray-Darling Freshwater Research Centre, Mildura. Report to the Murray-Darling Basin Commission, Canberra.

Introduction

Biofilms are assemblages of algae, bacteria, fungi and protozoa embedded within a polysaccharide matrix (Lock, 1984) that establish on wetted surfaces. Biofilms also capture dead organic material (detritus) and inorganic material from the water column and incorporate these components into their structure. For this study, the term biofilm is restricted to the organic matter component (estimated as ash free dry mass) of the material that establishes on a substrate.

Biofilms are an important component in food webs, being a food source for many invertebrates. The palatability of biofilm to different species is influenced by its composition. The construction of weirs and the creation of weir pools is believed to have altered biofilm composition and contributed to the loss of some snails from the Lower River Murray (Sheldon and Walker, 1997) whilst promoting the atyid and palaemonid decapod crustaceans (Sheldon and Walker, 1998; Burns and Walker, 2000a). The stable water levels in the weir pools have resulted in the establishment of autotrophic biofilms (true algae and cyanobacteria dominance; Burns and Walker, 2000b) whilst biofilms prior to river regulation are speculated to have been heterotroph dominated as a result of changing water levels and fluctuating photic depths (Sheldon and Walker, 1997).

Several studies have examined biofilms in the Mallee Tract of the River Murray. In the Mildura weir pool, Mullen (1998) compared biofilm that established on UV resistant plastic strips and natural stems of *Juncus ingens* over time, and recorded viable algal biomass of around 8 µg Chl.cm^{-2} after 8 weeks of continual submergence. In the Wentworth weir pool, Cook (1999) recorded viable algal densities of up to 6 µg Chl.cm^{-2}, (more typically ca. 2 µg Chl.cm^{-2}) in biofilms that established on horizontal River Red Gum (*Eucalyptus camaldulensis*) substrates. Cook (1999) also examined macroinvertebrate colonisation of these substrates and found a significant positive correlation between macroinvertebrate density and biofilm dry mass, ash free dry mass and chlorophyll content. In contrast to these weir pool studies, Treadwell (2002) examined established biofilm on River Red Gum snags at a free-flowing reach of river at Hattah and recorded a mean viable algal density of 2.4 µg Chl.cm^{-2}.

Objectives

The Mallee Tract of the River Murray contains free-flowing reaches and weir pools that allow a direct comparison of the effects of weirs on biofilm characteristics. In this study, River Red Gum substrates were deployed at various depths in the water column for 6-9 weeks and the biofilms that established were sampled so as to:

1. Compare biofilm composition in weir pools and free-flowing reaches of the Mallee Tract of the River Murray.

2. Examine changes in biofilm composition with water depth in the Mallee Tract of the River Murray.

Methods

Details of the three weir pool (WP) and two free-flowing (FF) reaches along the Mallee Tract of the River Murray (Thoms *et al*., 2000) are provided in the Project Report. Biofilms from Boundary Bend (FF), Euston (WP), Iraak (FF), Mildura (WP) and Wentworth (WP) were examined from October 2001 – June 2003.

Sample Collection

Biofilm sampling was achieved by suspending weighted string lines in the water column that maintained small River Red Gum (*Eucalyptus camaldulensis*) blocks (10 x 10 x 75 mm) at set positions in the water column (depths of 0.05 m, 0.5 m, 1.0 m, 2.0 m and 3.0 m beneath the surface when first deployed) to allow for biofilm establishment. Samplers were deployed 0.5-1.0 km upstream of the Euston weir, 2-3 km upstream of the Mildura and Wentworth weirs, and over a 0.5 km reach of the free-flowing sites at Boundary Bend and Iraak. Six string lines were deployed at each of the five sites (three were spares in case of vandalism) and were positioned on available emergent River Red Gum snags where water depth exceeded 3 m at the time of first deployment. Each string line contained a weight to maintain the string line in a vertical position where it remained for the duration of the study so as to mimic snag conditions.

After 6-9 weeks the biofilm-encased blocks were collected from three string lines, placed in individual jars containing river water and kept cool and dark until refrigeration (4^{o}C) in the laboratory. Blocks from the remaining three string lines (spares) were removed but not analysed. Fresh blocks (all aged River Red Gum) were attached to all six string lines and redeployed (substrates were deployed continually for the two year study period).

Relative water level was measured at each site on each sampling occasion to allow linkage with daily stage data (MDBC) to document the daily depths of each substrate. Daily stage data was available for all sites except Iraak. The water level at Iraak was estimated by displacing and compressing the daily stage hydrograph at Hattah (51 km upstream) to fit the water level measurements obtained manually.

Sample Processing

In the laboratory each block was gently double rinsed in containers of fresh tap water to remove any entrapped phytoplankton, and biofilm from a 26 cm^2 area scraped from the block with a razor blade. The removed biofilm was halved and each portion rinsed with distilled water into a filtering apparatus containing (A) a pre-ashed and weighed GFC filter for dry mass (DM) and ash free dry mass (AFDM) determination (APHA, 1995) and (B) a GFC filter for chlorophyll determination (an index of algal biomass).

A further 5 cm^2 area of biofilm was scraped from the block and preserved in distilled water with Lugol's iodine solution prior to identification and enumeration of algae to species level (sometimes genera level for minor taxa) at a NATA-certified laboratory (WSL Consultants, Richmond). Periphyton samples collected on 13 August 2002 and 3 March 2003 (deployed predominately in winter and summer, respectively) at 0.5 m depth were analysed.

Total Biomass

The GFC filters containing biofilm for weight determination were dried to constant weight (overnight) at 80^{o}C, cooled and weighed. Filters were combusted in a muffle furnace at 550^{o}C for two hours, cooled and rewetted with distilled water (to restore the waters of hydration lost from the clay fractions). Filters were dried at 105^{o}C to constant weight (overnight), cooled and reweighed to determine the ash free dry mass (AFDM), which represents the organic matter component or the total biofilm biomass (inorganic matter excluded).

Algal Biomass

The GFC filter containing biofilm for chlorophyll analysis (13 cm^2 area) was placed in a plastic centrifuge tube containing 10 mL of 90% ethanol (20 mL for large biofilm samples), shaken well and

refrigerated overnight at 4^{o}C. Samples were heated for 5 min in a water bath (78^{o}C), shaken well, cooled and centrifuged at 2000 rpm for 15 mins. A 3 mL aliquot was analysed in a spectrophotometer at wavelengths of 665 and 750 nm to determine the total chlorophyll pigment (TCP) (Golterman and Clymo, 1971). The aliquot was then acidified with 0.1 mL of 0.1 M HCl and mixed well before analysing it at wavelengths of 665 and 750 nm after 90 s to allow the calculation of the viable and non-viable proportions of chlorophyll (APHA, 1995). A strict protocol of acidification was adhered to as the amount of acid added, degree of mixing, and time after acidification each strongly influence the results.

Statistical Analysis

Multivariate analysis of the periphyton community data was conducted with PC-Ord (MjM Software, v4.27). The six taxa counted as 50 µm filaments.mL^{-1} comprised 5.02% of the total abundance when combined and were included unmodified in the statistical analyses with the remaining taxa (cells.mL^{-1}). The community data set contained many zeros and was heavily right skewed, so was fourth root transformed to reduce the influence of abundant taxa. The Sorensen (or Bray-Curtis) distance measure was adopted in the classification analyses because it is robust and particularly suited to community data sets (both abundance and presence/absence) (Faith *et al*., 1987). The Flexible Beta Group Linkage Method (set at –0.1 due to its ability to recover known groups) was used (Belbin *et al*., 1992). Dendogram details are described in the Main Report.

Univariate analyses were conducted with SYSTAT v9 (SPSS Inc.) following fourth root transformation to shift the data closer to normal and to meet the assumptions of the test.

Results

Depth of Biofilm Substrates

Substrates at the free-flowing sites, particularly Boundary Bend, were subjected to a greater range of water depths (and desiccation for some substrates) than substrates in the weir pools (Figure 1). At the Mildura weir pool, substrates were not deployed during the complete drawdown and refilling events in 2002 and 2003 (Figure 1D). In 2002 samples were collected early (after four weeks colonisation and new substrates redeployed immediately following refilling), and in 2003 the biofilm was not sampled.

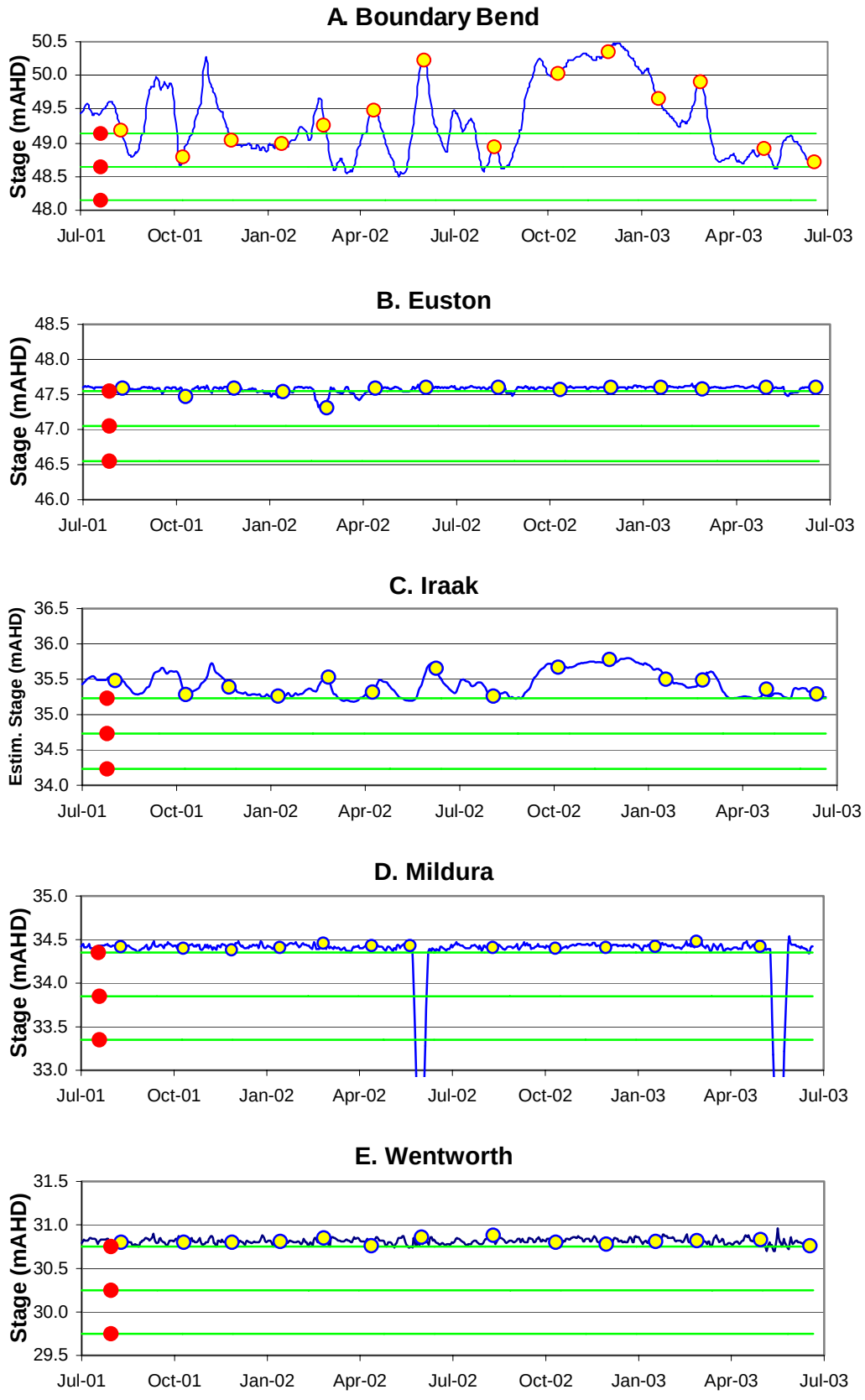

Figure 1. Daily stage at (A) Boundary Bend, (B) Euston, (C) Iraak (estimated stage), (D) Mildura and (E) Wentworth with depths of the 0.05m, 0.5m and 1m biofilm substrates highlighted (red dots). Yellow dots on hydrograph represent the days when substrates were collected and new substrates redeployed.

Biofilm Attributes

Total Biomass and Algal Biomass

Biofilms that established at the 0.05 m, 0.5 m and 1 m depths in weir pools were of greater total biomass (AFDM) than those at free-flowing sites. At the 2 m and 3 m depths, biofilm biomass was comparatively similar across sites (Figure 2). For the free-flowing sites, total biomass remained similar at all depths.

The high algal biomass in the weir pool biofilms at the 0.05 m, 0.5 m and 1 m depths is contributing to the greater total biomass at these sites. The free-flowing sites, in contrast, contained much lower algal biomass (Figure 2). The large decrease in algal biomass at the 2 m and 3 m substrates demonstrates light limitation of algal growth at these depths.

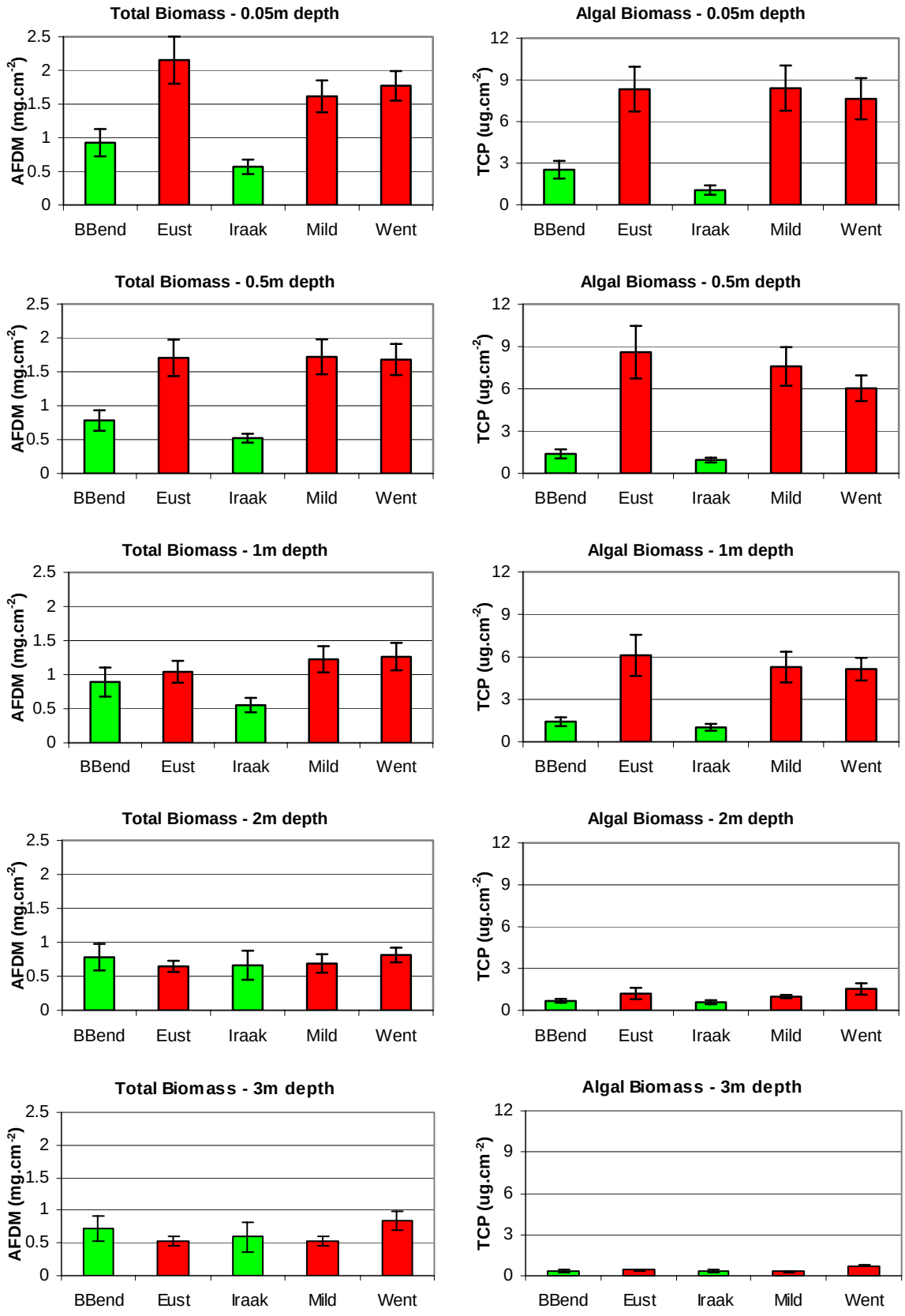

Figure 2. Mean biofilm total biomass (left column) and algal biomass (right column) for the two free-flowing (green) and three weir pool (red) sites at 0.05 m, 0.5 m, 1 m, 2 m and 3 m depths. Error bars = ± 1 S.E.

Chlorophyll Viability

The proportion of algal biomass that was viable was similar for the free-flowing and weir pool sites at a given depth, with algal viability decreasing with increasing depth (Figure 3).

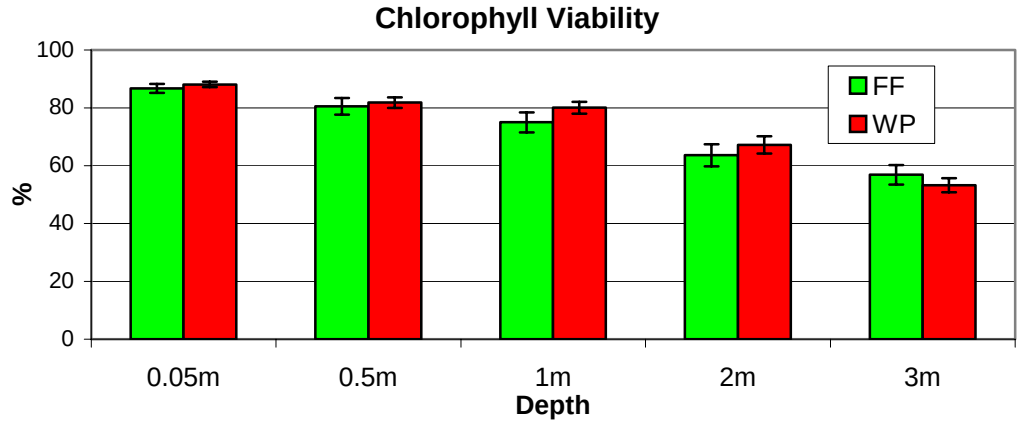

Figure 3. Viability (%) of the algal component of the biofilm for free-flowing (green) and weir pool (red) sites at 0.05 m, 0.5 m, 1 m, 2 m and 3 m depths. Error bars = ± 1 S.E.

Temporal and Spatial Patterns

Sites were grouped into weir pool or free-flowing to examine the temporal patterns of biofilm establishment. Details of biofilm from the 0.5 m and 2 m depths are presented.

For the 0.5 m depth, total biomass and algal biomass were consistently greater in the weir pools than the free-flowing sites for each sampling time, peaking in the warmer months of the year (Figure 4). These differences were significant both between sites and over time (Table 1). Algal biomass was lower in the second year for the weir pool sites, and may be due to lower available nutrients at this time (Research Report 2).

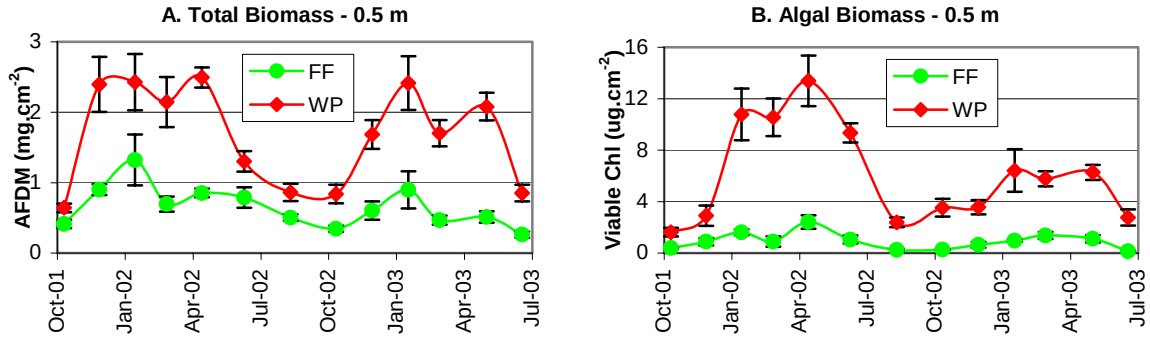

Figure 4. Biofilm (A) total biomass and (B) algal biomass (viable) at 0.5 m depth in weir pool (red) and free-flowing (green) sites from October 2001 – June 2003. Error bars = ± 1 S.E.

Table 1. Repeated measures ANOVA for 0.5 m biofilms (WP v FF)

Source		Biofilm Biomass			Algal Biomass (viable)		
BETWEEN	df	MS	F	P	MS	F	P
Type (WP v FF)	1	0.744	169.534	0.000	7.015	94.225	0.000
Error	6	0.004			0.074		
WITHIN							
Time	12	0.077	5.496	0.000	0.358	10.120	0.000
Time*Type	12	0.011	0.795	0.654	0.040	1.122	0.356
Error	72	0.014			0.035		

For the biofilms at the 2 m depth, total biomass was similar at the weir pool and free-flowing sites (Figure 5A) and not significantly different, although biomass was significantly different over time (Table 2). Biofilm biomass peaked in summer at both sites, with distinct gelatinous biofilms being recorded at the free-flowing sites - particularly Boundary Bend - at these times.

Algal biomass at 2 m depth was greater in the weir pools than the free-flowing site on all but one occasion (Figure 5B), and differences between sites and over time were significant (Table 2), although the interaction term was also significant indicating that algal biomass responded differently through time for the free-flowing and weir pool sites. The amount of algae growing at this depth is low compared to the substrates at 0.05 m, 0.5 m and 1 m depths due to limited light penetration to 2 m, but is greater than biofilm at 3 m depth, suggesting that the photic zone extended at least 2 m on occasion. Measurements of photic depth during the study period support this (Research Report 2). The chlorophyll peaks at 2 m depth in May 2003 are likely explained by the very low turbidities recorded at this time (Research Report 2).

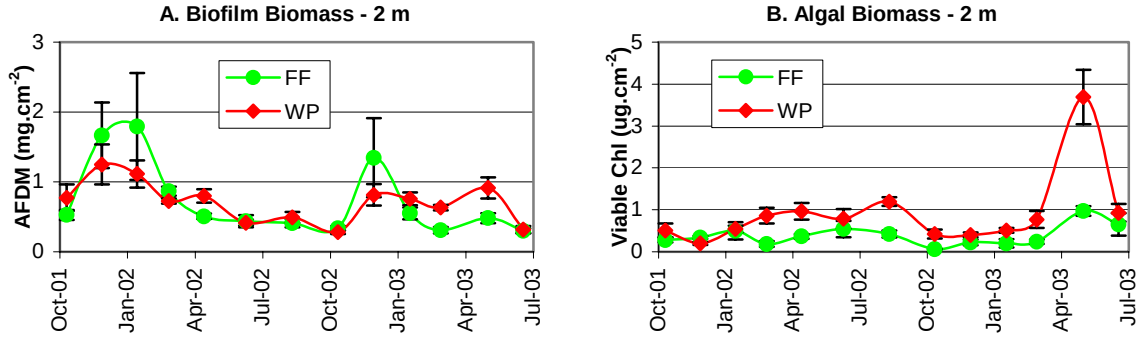

Figure 5. Biofilm (A) total biomass and (B) algal biomass at 2 m depth in weir pool (red) and free-flowing (green) sites from October 2001 – June 2003. Error bars = ± 1 S.E.

Table 2. Repeated measures ANOVA for 2 m biofilm (WP v FF)

Source		Biofilm Biomass			Algal Biomass (viable)		
BETWEEN	df	MS	F	P	MS	F	P
Type (WP v FF)	1	0.000	0.008	0.930	0.538	6.885	0.047
Error	5	0.024			0.078		
WITHIN							
Time	12	0.085	12.788	0.000	0.162	10.211	0.000
Time*Type	12	0.041	6.212	0.000	0.043	2.704	0.006
Error	60	0.007			0.016		

Autotrophic Index

The autotrophic index (AI) represents the ratio of the biofilm AFDM:viable chlorophyll (APHA, 1995) and provides a useful index for providing insight into the nature of the biofilm: biofilms with values below 400 are classified as autotrophic (algal dominated) and those with values above 400 are classified as heterotrophic (bacteria and/or fungi dominated) (APHA, 1995). Biofilms of the weir pools (0.5 m depth) are autotrophic compared to the biofilms of the free-flowing sites that are consistently heterotrophic (Table 3). Median values provide a more useful measure in this case due to occasional very high AI values (Wentworth WP > 5,400 on 27/11/01; Boundary Bend FF > 17,700 on 13/8/02) skewing the mean values considerably.

Table 3. Autotrophic Index of biofilms at 0.5 m depth

Site	Median	Mean (± S.E.)
Boundary Bend (FF)	1102	2552 ± 692
Euston (WP)	251	343 ± 54
Iraak (FF)	667	971 ± 115
Mildura (WP)	272	419 ± 67
Wentworth (WP)	318	598 ± 161

The autotrophic index (AI) of biofilms at 0.5 m and 2 m depths were lower in the weir pools than the free-flowing sites at all sampling times with the exception of November 2001 (Figure 6), and differences were significant between sites and over time (Table 4). The significant interaction term at the 0.5 m depth indicates that the autotrophic index responded differently through time for the free-flowing and weir pool sites (Table 4). The AI was lower at the 0.5 m depth compared to the 2 m depth on all but two occasions (Figure 6).

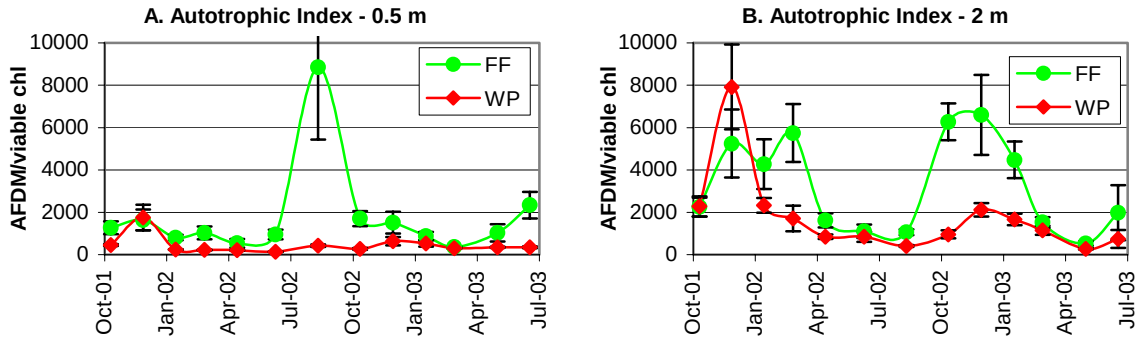

Figure 6. Autotrophic index (AFDM/viable chl) of biofilm at (A) 0.5 m and (B) 2 m depth in weir pool (red) and free-flowing (green) sites from October 2001 – June 2003. Error bars = ± 1 S.E.

Table 4. Repeated measures ANOVA: Autotrophic Index (WP v FF)

Source		0.5 m depth				2 m depth		
BETWEEN	df	MS	F	P	df	MS	F	P
Type (WP v FF)	1	74.994	79.82	0.000	1	29.243	16.168	0.010
Error	6	0.940			5	1.809		
WITHIN								
Time	12	7.323	14.118	0.000	12	13.292	13.613	0.000
Time*Type	12	3.975	7.663	0.000	12	1.249	1.279	0.255
Error	72	0.519			60	0.976		

Dry Mass

To facilitate a comparison with other studies, the proportion of organic material (biofilm) of the dry mass from 0.5 m depth was calculated for the study period (1/10/01 – 23/6/03). At the free-flowing and weir pool sites, biofilm comprised a mean 33.1% and 31.5%, respectively, of the dry mass of the scraped material (Table 5).

Table 5. Proportion of biofilm (AFDM) in dry mass at 0.5 m depth

	Mean	Median	Range
Free-Flowing	33.1 %	28.8 %	8.1 – 84.2 %
Weir Pool	31.5 %	30.1 %	12.7 – 77.4 %

The proportion of organic material in the dry mass (AFDM/DM) was lowest in the warmer months (Figure 7), despite algal biomass being highest at these times. This pattern was evident at both the free-flowing and weir pool sites, and may be explained by the greater algal biomass trapping higher amounts of inorganic material at these times.

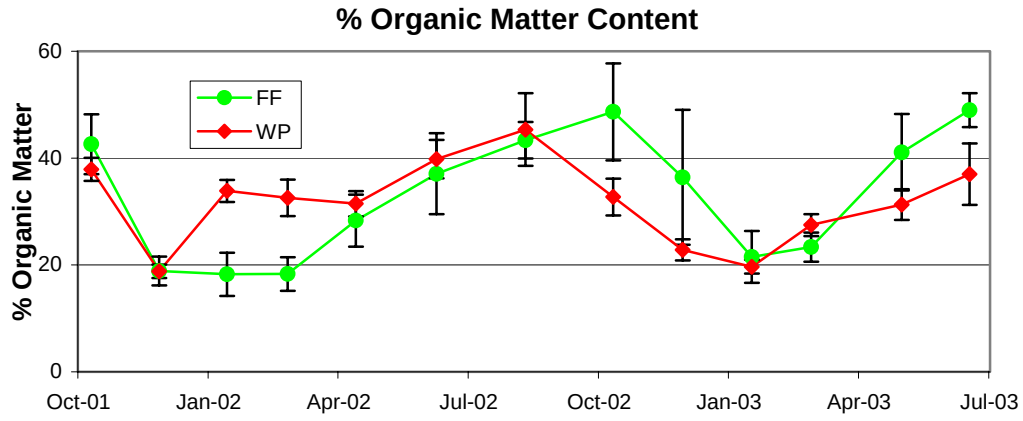

Figure 7. Ash free dry mass (AFDM)/Dry Mass (DM) of biofilm at 0.5 m depth in weir pool (red) and free-flowing (green) sites from October 2001 – June 2003. Error bars = ± 1 S.E.

Periphyton Taxa: Abundance and Richness

A total of 106 periphyton taxa were collected from the five sites along the Mallee Tract in August 2002 and March 2003 (listed in Appendix A). Bacillariophyceae (diatoms), Cyanophyta (cyanobacteria) and Chlorophyta (green algae) were the dominant periphyton groups in the 0.5 m biofilm and contributed over 99.9% of the periphyton cell abundance (pooled across all sites) (Table 6). Periphyton cell counts were greater in March 2003 than August 2002 and were consistent with the higher chlorophyll measurements at that time. Bacillariophyceae was the most abundant periphyton group, with mean abundance greater in March 2003 than August 2002 but remaining at a similar proportion of total periphyton abundance at each time.

Table 6. Periphyton abundance (mean no. cells.cm^{-2} or 50 µm filaments.cm^{-2})

Periphyton Group	Abundance (% of total)	
	August 2002	March 2003
Bacillariophyceae	117055 (60.3%)	252878 (62.7%)
Cyanophyta	30212 (15.5%)	112888 (28.0%)
Chlorophyta	46753 (24.1%)	37274 (9.2%)
Euglenophyta	36 (0.02%)	102 (0.03%)
Pyrrophyta	36 (0.02%)	39 (0.01%)
Cryptophyta	10 (0.01%)	23 (0.01%)
Chrysophyta	76 (0.04%)	20 (0.01%)
Total	**194178**	**403224**

Periphyton richness ranged from 13-48 taxa per sample, with a mean (± S.E.) of 35.1 ± 1.7 taxa. The diatom *Aulacoseira granulata* dominated periphyton assemblages, comprising over 55% of the total abundance (Table 7). This species is an important component of periphyton assemblages in addition to being consistently dominant in phytoplankton assemblages in the River Murray (Sullivan *et al*., 1988; Hotzel and Croome, 1996; Bormans and Webster, 1999). The high abundance of *Aulacoseira granulata* is consistent with another periphyton examination from a free-flowing site (Hattah) of the Mallee Tract (Treadwell, 2002).

Table 7. Most abundant periphyton taxa

Periphyton taxa	Contribution	Group
Aulacoseira granulata	55.7%	Bacillariophyceae
Anabaena torulosa	8.0%	Cyanophyta
Colothrix sp.	5.2%	Cyanophyta
Mougeotia sp.	5.2%	Chlorophyta
Tolipothrix distorta (50µm fil.cm^{-2})	3.2%	Cyanophyta

Multivariate classification of the abundance data (3 replicates for each site/time were pooled) revealed a distinct separation of the periphyton communities from August 2002 and March 2003 (Figure 8). The periphyton community at Boundary Bend in August 2002 was most different to all other sites and may be due to the substrate becoming dessicated for a brief period prior to collection (see Figure 1A). The periphyton communities in weir pools and free-flowing sites were most clearly separated in the summer period, and the separation was less distinct in winter when the Euston weir pool site more closely resembled the free-flowing Iraak site than the other weir pool sites (Figure 8).

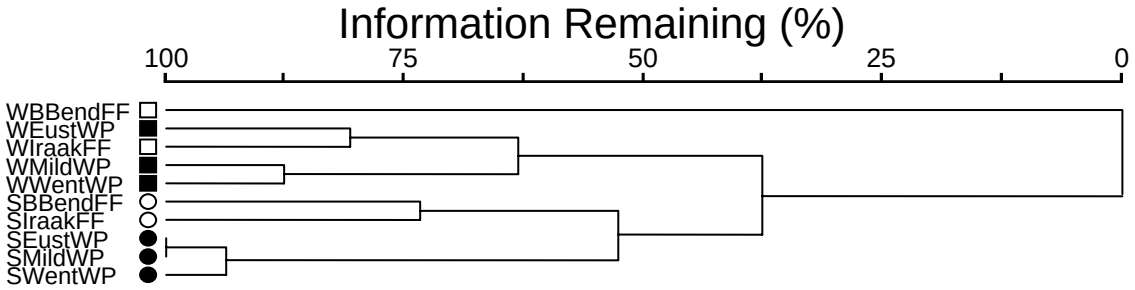

Figure 8. Dendogram of the periphyton communities (abundance data) across sites and times. W represents the winter samples (collected August 2002) and S the summer samples (collected March 2003).

Abundances of Bacillariophyceae, Cyanophyta and Chlorophyta were each consistently higher in the weir pools than the free-flowing sites for both August 2002 and March 2003 (Figure 9). Bacillariophyceae was the most abundant group, particularly in weir pools, due to the dominant *Aulacoseira granulata*. In August 2002, this species alone comprised 16% and 77% of the FF and WP cell counts, respectively, and in March 2003 comprised 48% and 63% of the FF and WP cell counts, respectively. Cyanobacteria and green algae were also more abundant in the weir pools than the free-flowing sites at each time. The abundance of green algae remained relatively consistent for a given site across time, whilst the blue green algae and diatoms were more abundant in March 2003 than August 2002.

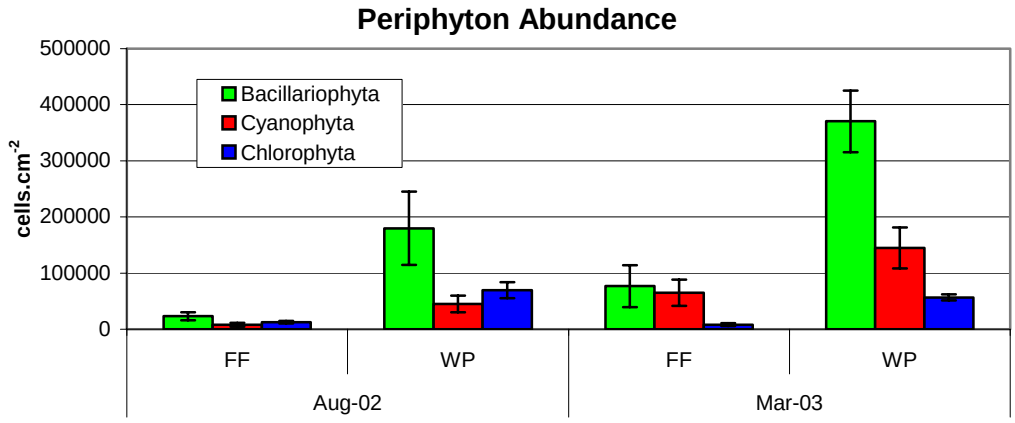

Figure 9. Mean abundance of the three dominant periphyton groups in free-flowing (FF) and weir pool (WP) sites in August 2002 and March 2003. Error bars = ± 1 S.E.

Multivariate classification of presence/absence data removes the influence of taxa abundance and highlights differences in taxa between sites and times (Figure 10). As with the classification based on abundance data, the communities from winter and summer were most different, and the community at Boundary Bend in winter was most different to all other sites. In each season, however, the periphyton taxa changed least between the Euston and Iraak sites (separated by 175 river km of free-flowing river) and exhibited longitudinal differences. This pattern is consistent with that of phytoplankton (Research Report 3) and suggests that the species colonising the River Red Gum substrates are being influenced by the sequence of weir pools.

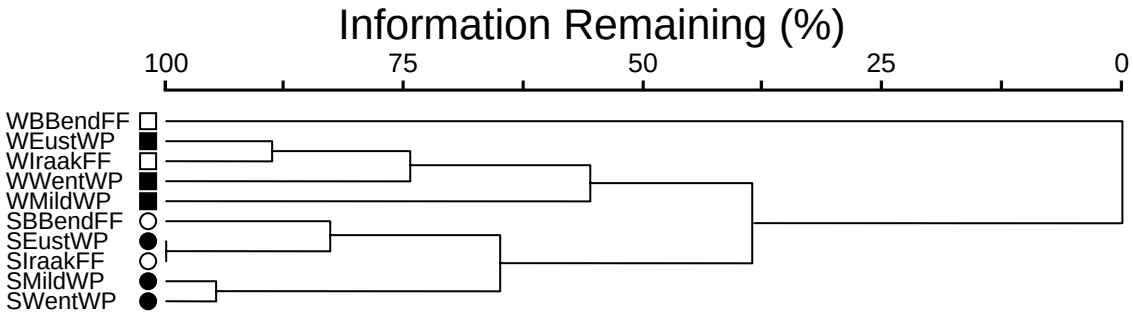

Figure 10. Dendogram of the periphyton communities (presence/absence) across sites and times. W represents the winter samples (collected August 2002) and S the summer samples (collected March 2003).

The periphyton assemblages were dominated by taxa from the Bacillariophyceae and Chlorophyta groups, with Cyanophyta also being well represented (Figure 11). The groups Crysophyta, Cryptophyta, Pyrrophyta and Euglenophyta contributed a combined mean of 1.5 taxa (range 0-4 taxa) to each sample.

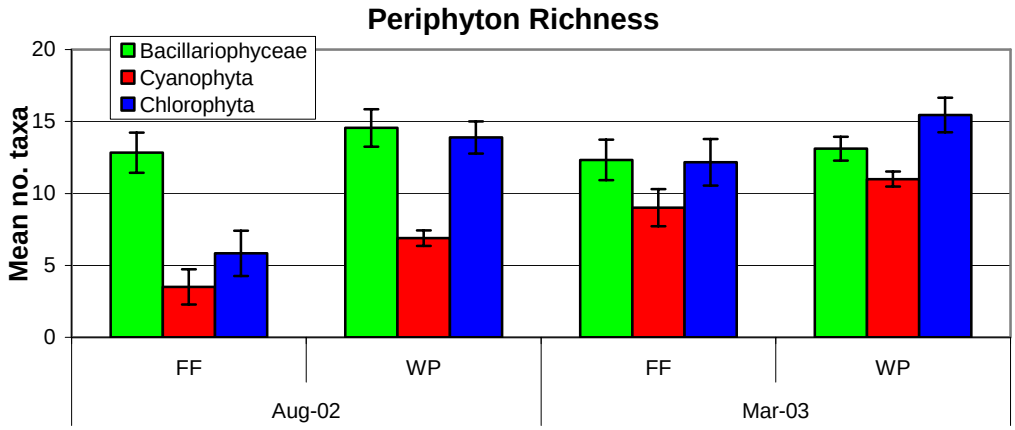

Figure 11. Mean number of taxa per sample for the three dominant periphyton groups in free-flowing (FF) and weir pool (WP) sites in August 2002 and March 2003. Error bars = ± 1 S.E.

Discussion

Effects of Weirs on Biofilm

Weir pools of the Mallee Tract favour the establishment of autotrophic biofilm in the photic zone (typically 0.05 m, 0.5 m and 1 m depths in this study), although biofilm remained heterotrophic at all sites where increased water depth (2-3 m) resulted in light limited algal growth. The dominance of autotrophs in biofilm growing in weir pools of the Lower Murray has been documented (Sheldon and Walker, 1997; Burns and Walker, 2000a), and attributed to the stable water levels. However, the lower turbidity in the Mallee Tract provides for a greater photic zone in this reach and highlights that water level change alone is not sufficient to explain the differences in biofilm characteristics between the free-flowing and weir pools sites. On several occasions water levels at the free-flowing sites remained relatively stable such that some substrates remained positioned within the photic zone throughout the period of deployment and the algal component of these biofilms remained consistently lower than for the comparable weir pool sites.

Low flow velocity is one factor that may account for the increased algal biomass in the weir pool biofilms in the Mallee Tract. Flow velocity in the weir pools was several times less than for the free-flowing reaches due to the greater channel cross-sectional area (Research Report 2). Lau (1995) showed that increased flow velocity (range 12.5 – 22.5 $cm.s^{-1}$) reduced rates of biofilm biomass accrual. Saravia *et al*. (1998) highlighted the importance of flow velocity in algal biomass accrual when they successfully modelled algal biomass fluctuations using flow velocity and nutrient concentrations as the driving variables. They showed that high flow velocity increased algal biomass initially due to increased immigration and diffusion of nutrients, but that these same velocities caused physical abrasion to the algae as biofilm size increased. These studies support the idea that flow velocity is limiting algal biomass accrual in the free-flowing reaches, and that weir pools have reduced the flow velocity and thereby hydraulic control of algal biomass accrual.

In the Lower Murray, the low flow velocity of the weir pools is likely a necessary condition for the development of the high algal biomass recorded in the photic zone. If the water level were stable and flow velocity high, the results of this study suggest that high algal biomass would not develop. This observation does not discount the role of water level change in controlling biofilm establishment and composition. The contribution of the turbid Darling River to the Lower Murray flows results in periods where the photic zone is much reduced compared to that of the Mallee Tract. Even small changes in water level and turbidity at these times may result in proportionately large changes in the photic depth in this region (Burns and Walker, 2000b), with light limitation and desiccation restricting algal growth. In the Mallee Tract, the lower turbidity and greater photic depths results in a greater area of littoral zone where biofilm may potentially develop where suitable substrate is available.

Greater grazing pressure by macroinvertebrates in the free-flowing regions was also considered as a factor that may also account for the lower biofilm biomass and algal biomass at these sites relative to the weir pools. However, a study of snag-dwelling macroinvertebrates was undertaken concurrently with the biofilm study and found macroinvertebrate densities 3.5 times greater at the *weir pool* sites (Research Report 5). This pattern suggests that biofilms were determining macroinvertebrate densities rather than *vice versa*.

Biofilm Attribute Comparison

The high proportion of viable chlorophyll from 0.5 m depth biofilms in free-flowing sites in this study contrasts strongly with that of a comparable study by Treadwell (2002) examining established biofilm growing on submerged River Red Gum wood at the free-flowing site (Hattah) in the Mallee Tract (Table 8).

Table 8. Comparison of Biofilm Attributes

	Treadwell (2002) Hattah free-flowing site	This Study 0.5 m depth free-flowing sites
Viable:Non-viable Chlorophyll (%)	38%	81%
Viable Chlorophyll	24 mg Chl.m^{-2}	9.4 mg Chl.m^{-2}
AFDM	62 g.m^{-2}	6.7 g.m^{-2}
AFDM:DM (%)	18.3%	33.1%
Autotrophic Index	1587	1793

The lower algal viability recorded by Treadwell (2002) indicates that established biofilms growing on natural substrates contain higher proportions of the chlorophyll breakdown product phaeophytin (indicating algal cell degradation) than biofilms 6-9 weeks of age. The higher mean algal biomass, higher mean total biomass and lower proportion of biofilm in the established dry mass at Hattah likely reflect the age of the biofilm sampled, and suggest that algal and total biomass would have continued to increase beyond 6-9 weeks submergence. If this were the case, it may be argued that the biofilms at free-flowing sites may have developed at a slower rate than those in the weir pools, and final established biofilm may not necessarily differ between free-flowing and weir pool sites. However, this argument is weak given that the mean algal biomass in this study at 0.5 m depth across all weir pool sites and times was 62 mg Chl.m^{-2} (and potentially increasing as well) and well exceeded 24 mg Chl.m^{-2} in established biofilm recorded by Treadwell (2002) at the free-flowing Hattah site.

The clear gradient of decreasing algal viability with increasing depth, particularly at 2 m and 3 m, was not unexpected given the light limitation at these depths (Research Report 2). The result shows that whilst fewer algal cells are establishing at these depths, a higher proportion of those that are colonising the 2 m and 3 m substrates are degrading during periods of darkness when turbidity and/or water levels increase.

Cook (1999) found that in the Wentworth weir pool, the AFDM comprised around 10% of the dry mass. This is low compared to the results of this study (means exceed 30% in weir pools and free-flowing sites), and likely reflects the orientation of the substrates for biofilm establishment. Cook (1999) deployed wooden River Red Gum blocks that were maintained in a horizontal position, resulting in thick biofilm accumulations on the upper surface (ca. 1000 g.m^{-2}). Higher flows at the time of Cook's study also resulted in elevated turbidities throughout the period of deployment (51-300 NTU), and these high levels may account for the high accumulation of sediment on the substrates. A similar deposition of sediment was recorded on substrates in the Lower Murray during high discharge (Burns, 1996). The high algal biomass recorded by Cook (1999) in the Wentworth weir pool (up to 60 mgChl.m^{-2}) and by Mullen (1998) in the Mildura weir pool (ca. 80 mg Chl.m^{-2}) compare well with weir pool results of this study.

At the 0.5 m depth, biofilm (AFDM) comprised a greater proportion of the dry mass of material in the colder months. This result was surprising because it may be expected that the high algal biomass in summer/autumn would result in a greater proportion of biofilm at this time. A possible explanation is that the high algal biomass facilitates a greater capture and incorporation of inorganic material into the biofilm.

Periphyton Assemblages

The centric diatom *Aulacoseira granulata* was the most dominant algal taxa in the biofilm, comprising over 55% of the total abundance across all sites and times. This species is also the most dominant phytoplankton in the River Murray (Sullivan *et al*., 1988; Hotzel and Croome, 1996; Bormans and Webster, 1999) and was dominant throughout the study period (Research Report 3) but may colonise benthic surfaces where it can develop into long filaments (Barber and Haworth, 1981 cited in Treadwell, 2002). The high concentrations of this species in the phytoplankton would contribute to its establishment and dominance in the periphyton. This species also occurred in high abundance in established periphyton at Hattah (Treadwell, 2002). *Aulacoseira granulata* is much less prevalent in

the phytoplankton of the Lower Murray (Sullivan *et al*., 1988), which may account for its low abundance in the periphyton in this tract (Burns and Walker, 2000b).

Primary Production Implications

High benthic algal biomass and high photic depths (low turbidity) in weir pools of the Mallee Tract indicate high rates of primary production. Algal production on submerged wood is limited by available substrate given extensive desnagging of sections of the River Murray in the past (Treadwell, 2002). However, the banks and particularly the high abundance of aquatic macrophytes (Research Report 6) provide large amounts of substrate for benthic algal production. As such, it is predicted that the contribution of benthic algae to gross primary production will be greater in these reaches than the free-flowing reaches where the algal biomass of biofilm is lower and aquatic macrophytes are fewer. Similarly, primary production from aquatic macrophytes is predicted to be greater in the weir pools than the free-flowing reaches. At the time of this study, however, aquatic macrophyte abundance had increased markedly in the free-flowing reaches, most likely due to the low flows in the Mallee Tract since 1997 (Research Report 1).

In-stream primary production likely played an important pathway for carbon inputs into the food web in the Mallee Tract over the study period, and accords well with the Riverine Productivity Model (Thorp and Delong, 1994). Treadwell (2002) recorded relatively high production rates at Hattah during summer and autumn, consistent with the higher algal biomass and temperatures (Research Report 2) in this study. These inputs of carbon are likely important given the low flows and stable water levels in the weir pools which limit inputs of terrestrial carbon both laterally (as emphasised in the flood pulse concept; Junk *et al*., 1989) and longitudinally (as highlighted in the River Continuum Concept; Vannote *et al*, 1980). Direct leaf and branch fall from overhanging riparian vegetation, and wind-blown inputs of organic material from storm events, may provide important injections of terrestrial-derived organic carbon during times of stable flow, although their contribution relative to in-stream macrophyte, benthic algae and phytoplankton (and longitudinal allochthonous organic carbon transport) requires further research.

References

APHA (1995). Standard methods for the examination of water and wastewater. American Public Health Association, 19th Edition.

Barber, H.G. and Haworth, E.Y. (1981). A guide to the morphology of the diatom frustule with a key to the British freshwater genera. Freshwater Biological Association Scientific Publication No. 44.

Belbin, L., Faith, D.P. and Milligan, G.W. (1992). A comparison of two approaches to beta-flexible clustering. *Multivariate Behavioural Research* **27**, 417-433.

Bormans, M. and Webster, I.T. (1999). Modelling the spatial and temporal variability of diatoms in the River Murray. *J. Plankton Res*. **21**, 581-598.

Burns, A. (1996). Chapter 5: Activity of Biofilms. pp 66-69 In: Ecological effects of shallow Winter-Spring flooding in the Lower River Murray, 1995. Departments of Zoology and Botany, University of Adelaide, Adelaide. Report to Murray-Darling Basin Commission.

Burns, A, and Walker, K.F. (2000a). Biofilms as food for decapods (Atyidae, Palaemonidae) in the River Murray, South Australia. *Hydrobiologia* **437**, 83-90.

Burns, A. and Walker, K.F. (2000b). The effects of weirs on algal biofilms in the River Murray, South Australia. *Regulated Rivers: Research and Management* **16**, 433-444.

Cook, R. (1999). The effects of shading on biofilm bimass, macroinvertebrate density and macroinvertebrate community structure in the Murray and Darling Rivers at Wentworth. Honours Thesis, La Trobe University, Wodonga.

Faith, D.P., Minchin, P.R. and Belbin, L. (1987). Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio* **69**, 57-68.

Golterman, H.L. and Clymo, R.S. (1971). Methods for Chemical Analysis of Fresh Waters. IBP Hankbook No. 8, Blackwell Scientific.

Hotzel, G. and Croome, R. (1996). Population dynamics of *Aulacoseira granulata* (HER.) Simonson (Bacillariophyceae, Centrales), the dominant alga in the Murray River, Australia. *Archiv fur Hydrobiologie* **136**, 191-215.

Junk, W.J., Bayley, P.B. and Sparks, R.E. (1989). The Flood Pulse Concept in River-Floodplain Systems. In *Proceedings of the International Large River Symposium* (ed. D.P. Dodge). *Canadian Special Publication of Fisheries and Aquatic Sciences* **106**, 110-127.

Lau, Y.L. (1995). Relative importance of mean velocity and bed shear on biofilm accumulation in open-channel flows. *Wat. Sci. Tech*. **32**, 193-198.

Lock, M.A., Wallace, R.R., Costerton, J.W., Ventullo, R.M. and Charlton, S.E. (1984). River epilithon: toward a structural-functional model. *Oikos* **42**, 10-22.

Mullen, C. (1998). Comparison of biofilm growth on artificial substrata in static and fluctuating water levels in a weir pool in the Murray River. Honours Thesis, La Trobe University, Wodonga.

Saravia, L.A., Momo, F. and Boffi Lissin, L.D. (1998). Modelling periphyton dynamics in running water. *Ecological Modelling* **114**, 35-47.

Sheldon, F. and Walker, K.F. (1997). Changes in biofilms induced by regulation could explain extinctions of aquatic snails in the lower River Murray, Australia. *Hydrobiologia* **347**, 97-108

Sheldon, F. and Walker, K.F. (1998). Spatial distribution of littorol invertebrates in the lower Murray-Darling River system, Australia. *Marine and Freshwater Research* **49**, 171-182.

Sullivan, C., Saunders, J. and Welsh, D. (1988). Phytoplankton of the River Murray, 1980-1885. Water Quality Report No. 2, Murray-Darling Basin Commission, Canberra.

Thoms, M.C., Suter, P., Roberts, J., Koehn, J., Jones, G., Hillman, T. and Close, A. (2000). Report of the River Murray scientific panel on environmental flows: River Murray - Dartmouth to Wellington and the Lower Darling River. Murray-Darling Basin Commission, Canberra.

Thorp, J. H. and Delong, M.D. (1994). The riverine productivity model: a heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos* **70**, 305-308.

Treadwell, S. (2002). Patterns in community metabolism and biomass of biofilms colonising large woody debris along an Australian Lowland River. PhD thesis, Monash University, Melbourne.

Vannote, R.L., Minshall, G., Cummings, K.W., Sedell, J.R. and Cushing, C.E. (1980). The river continuum concept. *Can. J. Fish. Aquat. Sci*. **37**, 130-137.

Appendix A. Periphyton of the Mallee Tract

Numbers represent % contribution to total

Cyanophyta	
Anabaena affinis	0.222
Anabaena aphanizomenioides	0.146
Anabaena circinalis	0.828
Anabaena flos-aqua	0.121
Anabaena solitaria	0.079
Anabaena spiroides	0.141
Anabaena torulosa	8.018
Anabaena sp.	0.508
Aphanisomenon issatchenkoii	0.205
Aphanocapsa sp.	0.066
Colothrix sp.	5.237
Geitlerinema sp. [50um fil.cm^2]	0.280
Heteroleibleinia sp.	2.516
Lyngbya sp. [50um fil.cm^2]	0.681
Nostoc sp.	0.342
Oscillatoria sp. [50um fil.cm^2]	0.233
Phormidium sp.1 (Semploca sp.) [50um fil.cm^2]	0.593
Phormidium sp. [50um fil.cm^2]	0.050
Plactonema sp.	0.089
Planctolyngbya contorta	0.009
Planktolyngbya subtilis	0.032
Pseudanabaena limnetica	0.280
Stichosiphon sp.	0.013
Tolipothrix distorta [50um fil.cm^2]	3.183
Unident BGA 1	0.082

Euglenophyta	
Euglena sp.	0.016
Strombomonas sp.	0.002
Trachelomonas sp	0.005

Pyrrophyta	
Peridinium inconspicuum	0.006
Peridinium sp.	0.007

Cryptophyta	
Cryptomonas sp.	0.005
Rhodomonas sp.	0.001

Chlorophyta	
Actinastrum aciculare	0.096
Ankistrodesmus falcatus	0.041
Ankistrodesmus fusiformis	0.092
cf. *Ankyra lanceolata*	0.015
Audouinella sp.	2.958
Chlorohormidium sp.	0.240
Closterium sp.	0.005
Coelastrum sp.	0.045
Cosmarium granatum	0.314
Cosmarium sp.	0.484
Crucigenia sp.	0.009
Euastrum sp.	0.003
Kirchneriella obesa	0.018
Legerheimia sp.	0.001
Monoraphidium sp.	0.001
Mougeotia sp.	5.191
Nephrocytium sp.	0.009
Oedogonium sp.	0.298
Oocystis sp.	0.002
Pediastrum duplex	0.845
Pediastrum tetras	0.262
Pleurotaenium sp.	0.008
Quadrigula sp.	0.022
Scenedesmus acuminatus	0.579
Scenedesmus dimorphus	0.055
Scenedesmus opoliensis	0.105
Scenedesmus sp.	0.062
Spirogyra sp.	1.968
Staurastrum excavatum	0.003
Staurastrum playfairi	0.022
Staurastrum subpinnatum	0.009
Staurodesmus extensus	0.007
Tetraedron sp.	0.011
Treubaria triappendiculata	0.002
Zygnema sp.	0.121
Green 2	0.034
Green 3	0.061
Green 4 (*Conyugales*)	0.068

Bacillariophyceae	
Acanthoceras sp.	0.008
Aulacoseira granulata	55.666
Cyclostephanos tholiformis	0.003
Cyclotella meneghiniana	0.788
Melosira sp	0.273
Urosolenia eriensis var. *morsa*	0.001
Small Centric	0.187
Amphora sp.	0.010
Asterionella sp.	0.026
Cocconeis sp.	0.004
Encyonema sp.	0.020
Epithemia sp.	0.008
Eunotia sp.	0.045
Fragilaria sp.1	0.002
Gomphonema parvulum	0.005
Gomphonema sp.	0.012
Gyrosigma sp.	0.061
Navicula radiosa	0.029
Navicula sp.	0.681
Neidium sp.	0.003
Nitzschia acicularis	0.028
Nitzschia agnita	0.057
Nitzschia cf. *filiformis*	0.281
Nitzschia cf. *lorenciana*	0.006
Nitzschia palea	0.080
Nitzschia vermicularis	0.020
Nitzschia sp. 2 (star)	0.069
Nitzschia sp.	0.516
Pinularia subcapitata	0.044
Rhopalodia sp.	0.094
Surirella minuta	0.007
Synedra acus	2.848
Tryblionella sp.	0.044

Chrysophyta	
Centritractus sp.	0.001
Dinobrion sp.	0.014
Mallomonas sp.	0.001