

## Heterotrophic bacterial production in the lower Murray River, south-eastern Australia

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**Abstract.** Bacterial production is important in aquatic carbon cycles because it represents a key component whereby dissolved and particulate carbon can be recycled back into food webs. Despite its acknowledged importance, few studies have examined bacterial production in lowland rivers. Since studies have suggested bacterial production is closely related to some carbon pools, we anticipated this to be the case in the Murray River, but that the timing and type of carbon inputs in the Murray River may lead to bacterial dynamics that differ from studies from other sites. Bacterial abundance and production were measured at three contrasting sites of the lowland Murray River, south-eastern Australia, over an 18-month period. Bacterial abundance varied across the three sites on the Murray River and was correlated with chlorophyll *a* concentrations but not with temperature, nutrients, particulate organic carbon and dissolved organic carbon concentrations. Bacterial production also varied across the sites. Lowest production was at the site most immediately downstream of a large reservoir, with production generally ranging from 0.88 to 8.00  $\mu\text{g C L}^{-1} \text{h}^{-1}$ . Bacterial production in a reach within a large forest ranged from 4.00 to 17.38  $\mu\text{g C L}^{-1} \text{h}^{-1}$ . Production at the reach furthest downstream ranged from 1.04 to 23.50  $\mu\text{g C L}^{-1} \text{h}^{-1}$ . Bacterial production in the Murray River was generally greater than in the European River Spree, reaches of the Meuse and Rhine without immediate impacts from major urban centres and the Amazon River, but was similar to the concentration measured in the Mississippi and Hudson Rivers. Bacterial production was closely correlated with chlorophyll *a* concentration and total phosphorus, but not with temperature, dissolved organic carbon, particulate organic carbon or inorganic nitrogen. Despite the differences in production and respiration measured at different sites across the Murray River, bacterial growth efficiency was very similar at the three sites. Bacterial populations in the Murray River appear to be influenced by reach-specific conditions rather than broad-scale drivers such as temperature, carbon and nutrient concentrations.

**Extra keywords:** bacterial abundance, carbon, rivers.

### Introduction

Organic matter in rivers can be derived from a range of autochthonous and allochthonous sources (Findlay and Sinsbaugh 1999). Autochthonous sources include production by algae and submerged macrophytes and allochthonous sources are highly variable and dependent on the characteristics of the catchment (Pollard 2004). Bacteria decompose organic matter in aquatic ecosystems and in the process respire some carbon to  $\text{CO}_2$  and assimilate some carbon into microbial biomass i.e. heterotrophic bacterial production.

Bacterial metabolism is now recognised as a key component of carbon processing in aquatic ecosystems, with secondary production by heterotrophic bacteria shown to contribute a significant proportion of overall production in several systems (Pomeroy 1974; Cole *et al.* 1988; Meyer 1994; del Giorgio *et al.* 1997). To date, much of our understanding of

the role of heterotrophic bacterial production has come from studies carried out on oceans, estuaries and lakes, with only very few studies examining lotic systems. The Hudson River is one of the few large rivers where bacterial production has been studied in detail (Findlay *et al.* 1991; Cole 1999). Bacterial production can exceed algal primary production in the Hudson River and macrophyte carbon is thought to be a major contributing factor (Findlay *et al.* 1991, 1992; Howarth *et al.* 1996). Heterotrophic bacterial production also can dominate in lowland rivers that receive significant input from industrial or urban sources because the latter often delivers a complex range of carbon and nutrients to the river (Servais and Garnier 1993).

Despite the growing body of work on bacterial production in general, the limited extent to which different lotic systems have been examined has meant that our knowledge

on heterotrophic bacterial production in lowland rivers is not as well developed as for other aquatic ecosystems. Importantly, little capacity exists for cross-system comparisons. We address this shortfall by examining bacterial abundance and production in the lower Murray River. The Murray River is a regulated river situated in south-eastern Australia and, like many lowland rivers, is of major ecological and economic importance. The Murray River differs from previously studied lowland rivers in several respects, including: hydrological regime, temperature conditions and the nature of its riparian and in-stream vegetation. Bacterial production is ultimately driven by carbon but will be affected by the physical and chemical properties within the river channel. Consequently, the role of heterotrophic bacterial production in the Murray River may not be consistent with other published studies. To address these knowledge gaps, we quantified bacterial abundance and production in the Murray River and examined which factors were related to bacterial abundance and production.

## Materials and methods

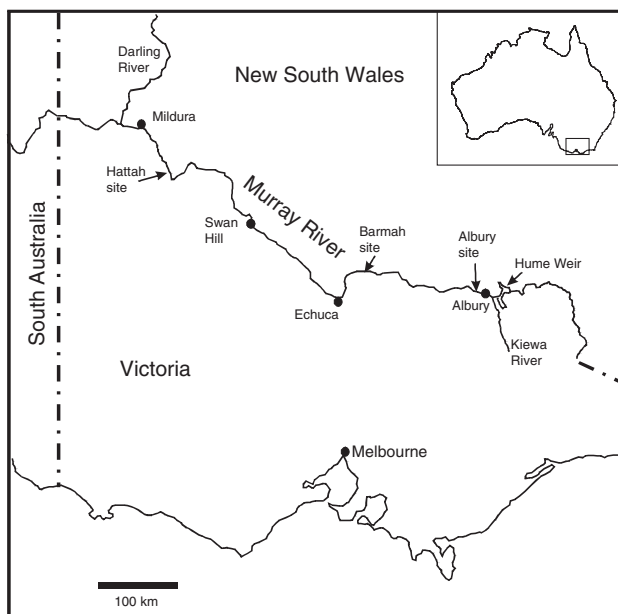
### Study sites

The Murray River rises on the western slopes of the Great Diving Range of south-eastern Australia and flows westward before turning southward and entering the southern ocean. We selected three sites at three contrasting reaches of the river (Fig. 1). The general characteristics of the river at the three sites are shown in Table 1. The Albury site (36°04'22"S, 146°49'24"E) is ~40 km downstream of a major dam and 20 km downstream of the combined cities of Albury–Wodonga, population ~70 000. At Albury, the Murray River is subject to high summer flows as a result of irrigation water releases from the dam. The Albury site also receives flow from an unregulated tributary. The Barmah site (35°49'33"S, 145°05'35"E) is situated 300 km downstream of Albury in the Barmah–Millewa forest, a large, managed, floodplain forest comprised principally of river red gum (*Eucalyptus camaldulensis* Dehnh.). Highest flow at Barmah occurs in summer. Many in-stream benches support growth of *Typha domingensis* Pers. and *Phragmites australis* (Cav.) Trin. Ex Steude and *Juncus ingens* N.A. Wakef. The Hattah site is a further 800 km downstream (34°34'34"S, 142°25'34"E). River red gum and black box (*E. largiflorens* F. Muell.) woodland with occasional patches of river cooba (*Acacia stenophylla* Cunn. Ex. Benth.) dominate riparian vegetation. Emergent and submergent macrophytes are not common at Hattah.

### Sampling and analytical methods

Six independent replicate water samples were collected from each of the three sample sites along the Murray River, every 4 months over an 18-month period from June 1998 to December 1999. For bacterial abundance measures, samples were preserved in formaldehyde, stored in the dark at 4°C and analysed within 7 days of sampling. Bacterial cells were collected by filtering the preserved samples onto black 0.2-µm-pore polycarbonate filters (Nucleopore, Pleasanton, CA). The filters were stained with acridine orange and examined using an Axioskop fluorescence microscope (Zeiss, Jena, Germany) (Hobbie *et al.* 1977). Five optic fields were examined for each sample, counting at least 300 cells.

Bacterial production was estimated from the rate of tritiated thymidine incorporation (Findlay *et al.* 1984), but measuring activity into bacterial macromolecules rather than in purified DNA. Fifty µL of 2-Ci mmol<sup>-1</sup> [methyl-<sup>3</sup>H] thymidine was added to 6 time-course series, each reaction containing 4 mL of water sample, which were incubated at *in situ* temperatures in the dark. Incubations generally were for 20–30 min, although 45-min incubations were required for the coldest water samples. Bacterial cells were collected on glass fibre filters (GF/F, Whatman, Maidstone, UK) that had been pre wet with a 5% (w/v) thymidine solution. GF/F filters were used to collect cells because the often-high concentrations of suspended solids in the river water blocked small-pore-sized membrane filters and made filtration unmanageable. In preliminary experiments, we filtered water samples through a GF/F filter then passed the filtrate through a 0.2-µm-pore polycarbonate membrane filter and measured isotopic activity on the GF/F and polycarbonate filters. The preliminary experiments demonstrated that loss of radioactive cells through the GF/F generally represented less than 5% of the total



**Fig. 1.** Location of the sampling sites along the Murray River, south-eastern Australia.

**Table 1.** General characteristics of the three sample sites examined along the Murray River

Field site	Characteristics					
	Catchment (km <sup>2</sup> )	Approximate annual rainfall (mm)	Discharge (m s <sup>-1</sup> )	Average channel width (m)	Temperature range (°C)	Annual average conductivity (µS cm <sup>-1</sup> )
Albury	17 350	600	20–254	70	8–24	35
Barmah	25 200	400	23–173	70	11–28	57
Hattah	275 150	300	28–220	110	11–31	205

activity and so filtering through polycarbonate filters was not routinely carried out. All time-course reactions were stopped by adding 1 mL unlabelled thymidine (5% w/v) then formaldehyde (2% v/v final concentration). Filters containing cells were washed successively with 1 volume (~5 mL) of unlabelled thymidine solution (5% w/v), 4 times with 5 mL saline citrate buffer (0.15 M NaCl/0.015 M tri-sodium citrate, pH = 7.0) and finally twice with 5 mL ice-cold 80% (v/v) ethanol. Macromolecules were extracted by treating filters with hot trichloroacetic acid (5% w/v) for 90 min. The radioactivity in extracted samples was counted in a Beckman scintillation counter. Isotope dilution experiments were included during measures of thymidine uptake. A cell carbon content of 6 fg C per cell was used to convert rate of cell production to rate of carbon production and was derived as described by Sharma *et al.* (1998) and Driessen (2001). Sedimentation field-flow fractionation was applied to river water, which separated the bacterial cells on the basis of their buoyant mass. The average bacterial biomass was determined on bacterial size distributions obtained from the separated bacterial cells (Sharma *et al.* 1998). Bacterial cellular carbon ratios have been a subject of debate (Findlay *et al.* 1986, 1991; Austin and Findlay 1989) and although our value represents a carbon content at the low end of those that have been described previously, it more realistically represents the carbon content of the cells in the system we studied.

Planktonic respiration was determined in dark-light bottles incubated *in situ* during each field trip. Algal respiration was subtracted from total community respiration assuming algal respiration was 10% of the maximum photosynthetic rate. The remaining respiration was attributed to microbial respiration and used to calculate bacterial growth efficiencies. Bacterial growth efficiency (BGE) was calculated according to the formula  $BGE = BP/(BP + BR)$ , where BP is bacterial production and BR is bacterial respiration (del Giorgio and Cole 1998).

For chlorophyll *a* measurements, water was filtered through GF/C filters (Whatman) and the filters were extracted at 70°C in 95% (v/v) ethanol for 5 min. Chlorophyll *a* concentration was determined spectrophotometrically, without phaeophytin correction, as described by ISO 10260 (ISO 1994).

Chemical analyses for filterable reactive phosphate (FRP), total phosphorus (TP), oxidised nitrogen species ( $NO_x$ ), total nitrogen (TN), dissolved organic carbon (DOC), fine particulate organic carbon (FPOC) and coarse particulate organic carbon (CPOC) were carried out at the analytical chemistry laboratory of the Murray-Darling Freshwater Research Centre, Wodonga, which is operated within national testing standards of quality control and quality assurance. Standard methods of analysis were applied for nitrogen and phosphorus species (APHA 1995). Samples analysed for dissolved organic carbon were filtered through 0.2- $\mu$ m-pore membrane filters, acidified with phosphoric acid to remove inorganic carbon and analysed using a total carbon analyzer (OI Analytical, College Station, TX). For fine particulate organic carbon measures, water samples were filtered through pre-ashed GF/F glass fibre filters and organic matter was estimated from the loss on ignition after combustion at 550°C for 1 h. For coarse particulate organic carbon, water was filtered through a 250  $\mu$ m net and the carbon content in the retained material measured using an OIA carbon analyzer.

#### Statistical analysis

Statistical analyses were performed using SYSTAT 6.0 (SPSS Inc., Chicago, IL). Regression analyses were used to identify relationships between variables. In particular, we explored whether any relationships could be determined between bacterial abundance and/or production and any water quality measures. In addition to linear regressions, we attempted to fit non-linear models to data where no linear relationships were apparent. Where necessary, log-transformations were carried out to remove heterogeneity of variances. Analysis of variance (ANOVA) was used to explore spatial and temporal variation among sites and times.

## Results

Bacterial abundance was different at each of the sites along the Murray River (Fig. 2). Of the three sites, the lowest bacterial cell numbers were recorded at the Albury site, where

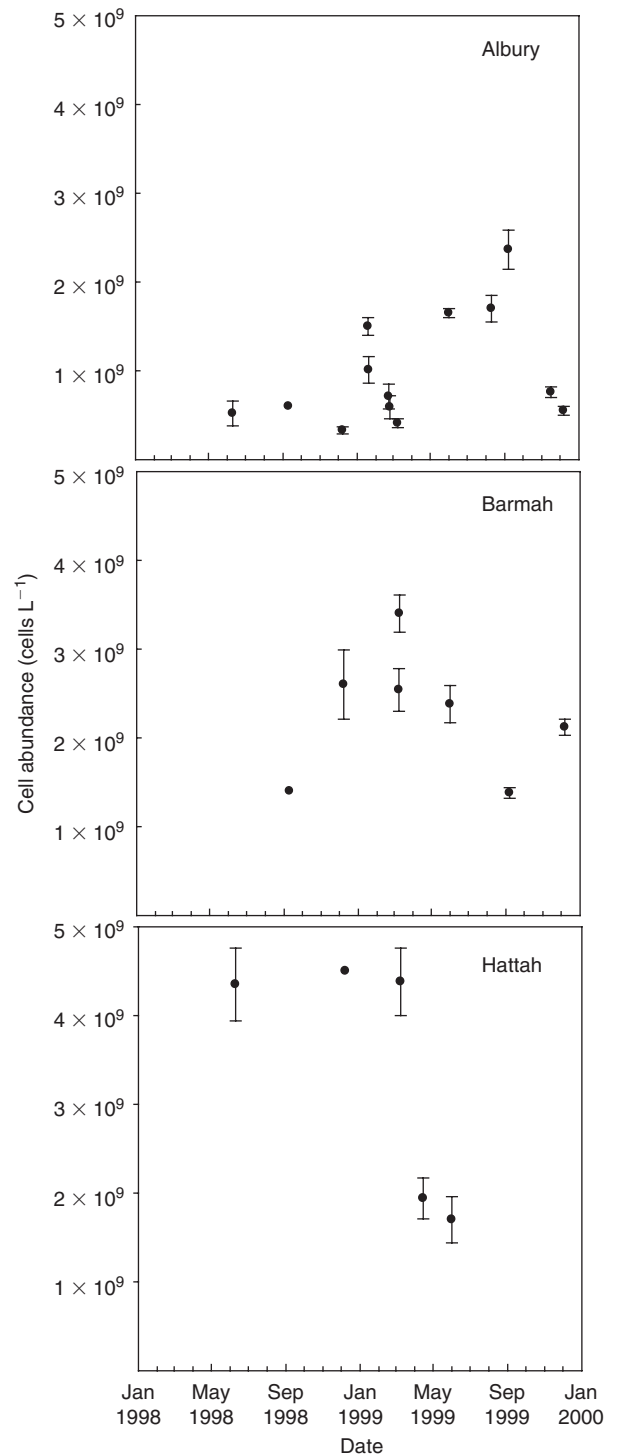


Fig. 2. Mean ( $\pm$  s.e.) bacterial cell abundance in the Murray River at the Albury, Barmah and Hattah sites.

cell abundances were generally between  $2 \times 10^8$  and  $1 \times 10^9$  cells per litre. No obvious seasonal trends in cell numbers were apparent at the Albury site. The highest cell abundance recorded at the Albury site was in September 1999, which coincided with rain higher in the catchment causing increased flow in an upstream tributary. Bacterial cell abundance at Barmah showed some seasonality, with the highest cell numbers occurring in summer and lowest during winter, but overall there was no significant relationship with temperature ( $r^2 = 0.09$ ,  $P = 0.55$ ,  $n = 17$ ). The highest cell numbers were recorded at Hattah, but no clear trends can be seen. Bacterial abundance across all sites was not correlated with FRP, TP,  $\text{NO}_x$ , TN, DOC, FPOC, CPOC concentrations or temperature, but bacterial abundance was significantly correlated with chlorophyll *a* concentration ( $r^2 = 0.52$ ,  $P < 0.001$ ,  $n = 19$ ).

Bacterial production was significantly different among sites and there was a significant difference between sites over time (ANOVA,  $P < 0.05$ ) (Fig. 3). Production at the Albury site was generally the lowest of the three sites, ranging between 0.87 and  $8 \mu\text{g C L}^{-1} \text{h}^{-1}$ . Lowest bacterial production at Albury was measured during the summer periods, with an unusually high value recorded at Albury during September 1999; the latter coincided with the site receiving increased flow from an unregulated tributary with the highest bacterial cell abundance. Bacterial production at the Barmah site ranged from 4 to  $17 \mu\text{g C L}^{-1} \text{h}^{-1}$ . The Hattah site, occurring furthest downstream, had highest bacterial production in summer and lowest production in winter and generally ranged from 1 to  $11 \mu\text{g C L}^{-1} \text{h}^{-1}$ . High bacterial production was measured at Hattah in September 1999, which date was also associated with highest variability between the replicate samples.

Bacterial production was not correlated with temperature, DOC, FPOC, CPOC, FRP,  $\text{NO}_x$ , or TN, but was correlated with TP ( $r^2 = 0.35$ ,  $P < 0.05$ ,  $n = 13$ ) and chlorophyll *a* ( $r^2 = 0.37$ ,  $P < 0.05$ ,  $n = 13$ ). Bacterial production across all sites was not correlated with discharge ( $r^2 = 0.001$ ,  $P = 0.88$ ,  $n = 16$ ) (Fig. 4). Bacterial production across all sites was correlated with bacterial abundance ( $r^2 = 0.49$ ,  $P < 0.05$ ,  $n = 12$ ).

Mean annual bacterial growth efficiencies ( $\pm$  s.e.) for the Albury, Barmah and Hattah sites were 0.35 (0.09), 0.38 (0.07) and 0.37 (0.07), respectively, indicating a high degree of similarity across the sites.

## Discussion

Bacterial populations in the Murray River appear to be influenced more by reach-specific conditions than broad-scale drivers such as temperature, carbon and nutrient concentrations. Unlike other studies, bacterial abundance in the Murray River did not always show marked seasonality, with seasonal patterns only apparent at the Barmah site. In a previous

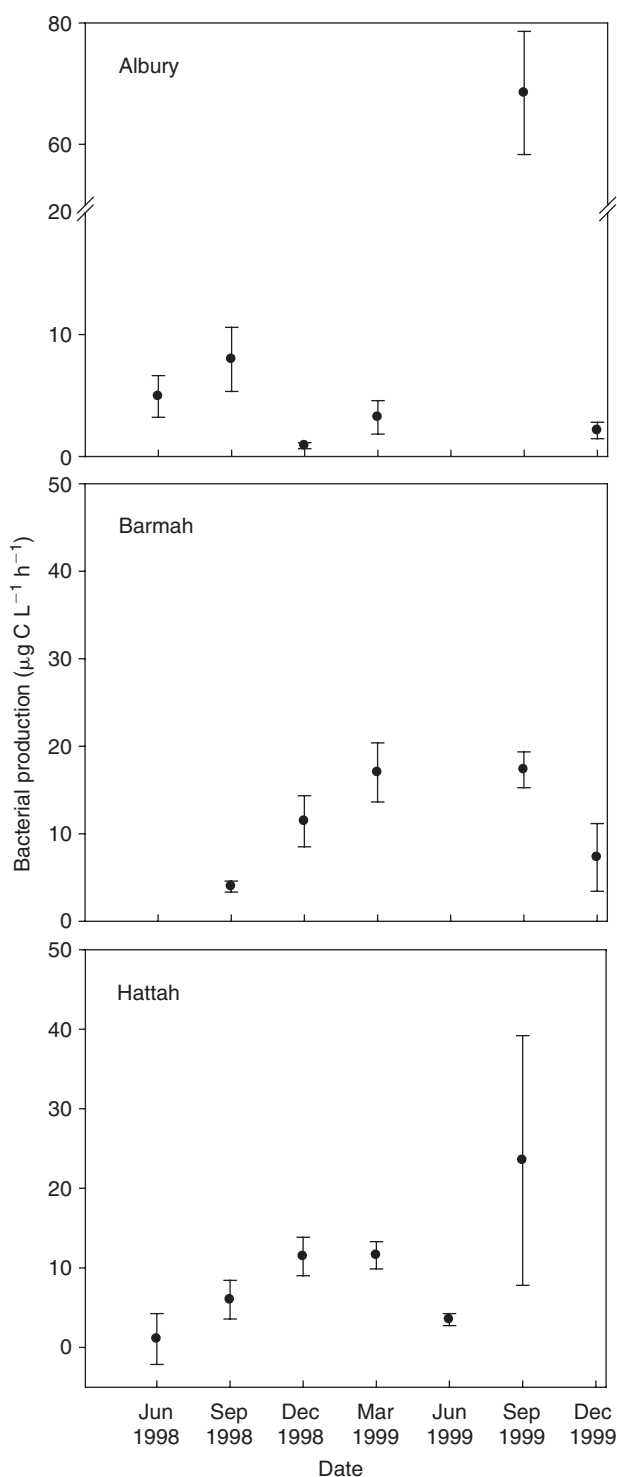


Fig. 3. Mean ( $\pm$  s.e.) bacterial production in the Murray River at Albury, Barmah and Hattah.

two-year study of the Murray River, there also was some evidence of a summer peak in bacterial numbers at Barmah in one year, but the seasonal peak was not evident in the second year of the study (Boon 1991). In contrast to our work,

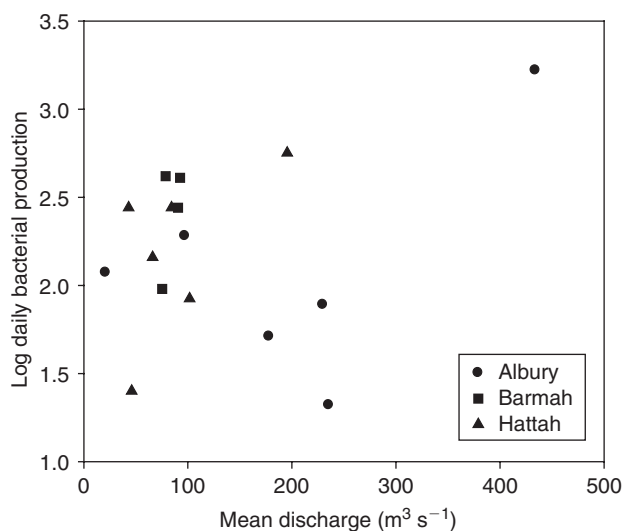


Fig. 4. Relationship between bacterial production and discharge in the Murray River.

studies often have reported clear seasonal patterns in bacterial abundance. For example, seasonal responses occurred in the Hudson River, even though no difference in bacterial numbers occurred between different sampling sites along the river (Findlay *et al.* 1991). Like several northern hemisphere rivers, the Hudson River has severe winter temperatures; so riverine bacteria experience temperatures well below their growth optima for an extended period of time. The range in temperature that bacteria in the Murray River experience may be similar to previous studies; however, the minimum and maximum temperatures in the Murray River generally are higher than those in the northern hemisphere. Absence of extreme cold may contribute to the limited occurrence of seasonality in bacterial abundance in the Murray River.

Our data suggest a trend of increasing bacterial numbers downstream, as has been demonstrated in the Rhine River on at least one occasion (Ietswaart *et al.* 1999). However, rather than simply a trend along the river continuum, we suggest this phenomenon probably reflects the localised conditions on the microbial populations in the Murray River. Cell numbers generally were lowest at Albury, which is ~40 km downstream of a large reservoir. Determining the impact of water release on microbial activity in the Murray River is beyond the scope of this work. However, we suggest that hypolimnetic releases of water with low bacterial density may in part account for the low bacterial abundance (and production – see below) measured downstream. The extent of urban and diffuse agricultural inputs, as well as hydrological retention within the channel and associated backwaters, is not known at our study site. Although the nature of these influences is unknown, it is reasonable to suggest the microbial populations at Albury have still been heavily influenced by the upstream reservoir. The only time the dam's influence appears to have been overridden was during a high rainfall event

(September 1999), when the upstream tributary had a major impact on discharge.

The second highest bacterial abundance occurred at Barmah. Abundance was not correlated with temperature, nutrients or carbon concentrations. We suggest instead that the high numbers may have been linked to a variation in the carbon supply, either through the contribution from algae or the surrounding forest. Alternatively, high bacterial abundance may reflect a decreased grazing pressure. Highest abundance occurred at Hattah; however, fewer samples were analysed from this site and the variability in abundance makes it difficult to determine the factors affecting cell abundances.

Bacterial production is fuelled by carbon, which can be derived from within the river channel or from external sources. A very strong linkage between chlorophyll *a* and bacterial production has been demonstrated (Cole *et al.* 1988; White *et al.* 1991), but the role of allochthonous carbon in some rivers has also been emphasised, with the strongly heterotrophic nature of the Hudson River thought to be fuelled by external carbon loading (Findlay *et al.* 1991; Howarth *et al.* 1996). Like other studies, bacterial production and abundance across all the study sites in the Murray River were correlated with chlorophyll *a*, and although production was greater than has been measured in South American and European systems, production was of a similar magnitude to that measured in some North American rivers. Annual average bacterial production at Albury, Barmah and Hattah were 14.6, 11.3 and 9.5  $\mu\text{g C L}^{-1} \text{h}^{-1}$  respectively. Production at each site on the Murray River was greater than the average 1.16  $\mu\text{g C L}^{-1} \text{h}^{-1}$  recorded in the Amazon River (Benner *et al.* 1995) and the range of production measured in the European lowland rivers Spree and Meuse (Servais 1989; Servais and Billen 1989; Fischer and Pusch 2001). Bacterial production during the ice-free period in the Hudson River was 10.25  $\mu\text{g C L}^{-1} \text{h}^{-1}$  (Findlay *et al.* 1991), which was very similar to production on the Murray River. Bacterial production in the Murray River also was similar to pool 19 of the Mississippi River (6.7–12.5  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ), where macrophytes were thought to be a major source of carbon for microbial activity (Henebry and Gorden 1989). It is noteworthy that the latter production estimates were derived from the frequency of dividing cells, which appears to give higher estimates for production than methods involving uptake of radioisotope-labelled substrates (Boon 1991).

Production was consistently lowest at the Albury site and, as with the cell numbers, may be reflecting the influence of the upstream reservoir. Our estimates of bacterial production at the Albury site were greater than the values reported in the literature for large standing water bodies with comparable chlorophyll *a* concentrations (Pace and Cole 1994; del Giorgio *et al.* 1997). However, since bacterial production was not measured directly in the upstream reservoir and no reports exist on bacterial production in Australian reservoirs, its contribution to downstream riverine production is speculative.

Whether riverine conditions between the upstream reservoir and our study site are likely to have had some influence on the production estimates remains to be shown. The major flow event that occurred at the Albury site during September 1999 coincided with bacterial production exceeding  $60 \mu\text{g C L}^{-1} \text{ h}^{-1}$ , a value in excess of many described in the literature. Values this high have only been associated with study sites downstream of urban impacts (Servais and Garnier 1993). The extremely high bacterial abundance and production that occurred during September 1999 demonstrate that high flows can greatly impact riverine microbial dynamics, presumably through delivery of allochthonous carbon to the water column.

A source of carbon clearly is fundamental for fuelling bacterial production. In rivers with floodplain connection, a supply of readily available carbon can be transported from the floodplain to the river channel during high-flow events (Benner *et al.* 1995) and such carbon delivery can be responsible for seasonal variation in bacterial production. Particulate organic carbon metabolism is thought to be an important component of benthic processing in flowing systems, particularly in streams (Webster and Meyer 1997; Fischer and Pusch 2001; Fischer *et al.* 2002). Although there is some evidence that supports a role for POC in bacterial production within river channels (Findlay *et al.* 1991; Grossart and Ploug 2000), dissolved organic carbon is often the major carbon source (Meyer 1994; Findlay *et al.* 1998). A major interest of riverine ecologists is to determine the ultimate sources of in-stream carbon. Our study did not reveal any clear relationship between dissolved carbon, fine or coarse particulate carbon and bacterial production. Although clear relationships were not apparent, this does not necessarily lessen the potential role for the different fractions, because our type of study, as with most previous studies, is based on the concentrations of carbon pools rather than the rate of production and consumption of the carbon pools. For example, algal exudates may be a major source of carbon for bacterial production, but its rapid use means that it is effectively never measured in the carbon pool.

A further question of interest arising from our study is the response time of microbes to changes in environmental conditions. We adopted a seasonal approach to look at broad-scale patterns in the Murray River. Given that microbes can respond rapidly to changes in carbon delivery, with doubling times in terms of hours, complimentary experiments that examine responses at these time scales would help in our understanding of how persistent any changes to microbial communities might be.

Sections of the Murray River presently are regulated to maintain minimum flow during winter and high flow in summer, a reversal of 'natural' conditions. River regulation also has reduced the frequency and extent of any connection of river channels with their floodplains. Before regulation, the major inundation of floodplains would have occurred during

winter and spring and considerably lower flow would have occurred during summer conditions. The impact of the carbon delivery to the river channel, occurring subsequent to floodplain inundation, is dependent on its relative bioavailability. Since litter from red gum forests is able to support extensive growth of bacterial populations (O'Connell *et al.* 2000), under pre-regulation conditions, bacterial production in the Murray River may have been more important during winter and spring when high flows increased delivery of organic matter. Flow reversal owing to river regulation has also affected water temperatures in particular, causing cooler temperatures in summer than would have occurred under 'natural flow' conditions. The strong linkage between temperature and bacterial production demonstrated in a cross-system review (White *et al.* 1991) was not apparent in the Murray River and may have been overridden by site-specific factors in the river, with carbon availability likely to be a major factor.

Although each of the sites on the Murray River varied in their bacterial production throughout the year, there was remarkable similarity in the bacterial growth efficiency (BGE) across all sites. The intermediate BGEs indicated the river is midway in terms of nutrient status, with oligotrophic systems typically having  $\text{BGE} < 0.1$  and eutrophic systems having BGE between 0.4 and 0.5. The factors regulating BGE are complex and although no clear patterns have been shown, the supply and nature of organic substrates can influence BGE (del Giorgio and Cole 1998). The high similarity in BGE across different sites of the river supports the notion that bacterial production in the Murray River currently is driven by a relatively constant supply in the types of carbon sources. Under current river management conditions, phytoplankton clearly play an important part in fuelling bacterial production. We suggest that the similarity in BGEs is driven by the combination of in-stream phytoplankton production and absence of floodplain-derived carbon inputs.

Our study indicates that bacterial production may represent an important component of the in-stream carbon cycle, yet the overall role of microbial processes in food webs remains unresolved. Thorp *et al.* (1998) showed the importance of algae in diets of riverine animals and suggested that terrestrial carbon is not important in riverine food webs. Furthermore, Thorp and Delong (2002) argued that a broad decomposition loop, which includes rotifers and ciliates, operates within rivers, but much of the carbon in the decomposer cycle remains within a decomposer loop. Since very little data exist on the diversity and functional characteristics of zooplankton communities in Australian lowland rivers, we are unable to gauge the overall importance of the transfer of organic carbon (detritus, algal exudates, etc.) through microbial carbon and the subsequent consumption of microbes by higher biota. Future food web studies that include bacterial production and consumption will help address this key knowledge gap.

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## References

- APHA (1995). 'Standard Methods for the Examination of Water and Wastewater.' (American Public Health Association: Washington, DC.)
- Austin, H. K., and Findlay, S. E. G. (1989). Benthic bacterial biomass and production in the Hudson River estuary. *Microbial Ecology* **18**, 105–116.
- Benner, R., Opsahl, S., Chin-Leo, G., Richey, J. E., and Forsberg, B. R. (1995). Bacterial carbon metabolism in the Amazon River system. *Limnology and Oceanography* **40**, 1262–1270.
- Boon, P. I. (1991). Bacterial assemblages in rivers and billabongs of southeastern Australia. *Microbial Ecology* **22**, 27–52.
- Cole, J. J. (1999). Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. *Ecosystems (New York, N.Y.)* **2**, 215–225.
- Cole, J. J., Findlay, S., and Pace, M. L. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series* **43**, 1–10.
- del Giorgio, P. A., and Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics* **29**, 503–541. doi:10.1146/ANNUREV.ECOLSYS.29.1.503
- del Giorgio, P. A., Prairie, Y. T., and Bird, D. F. (1997). Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. *Microbial Ecology* **34**, 144–154. doi:10.1007/S002489900044
- Drissen, J. (2001). Microbial populations as indicators of river health. PhD Thesis, Monash University, Melbourne.
- Findlay, S., and Sinsbaugh, R. (1999). Unraveling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems. *Marine and Freshwater Research* **50**, 781–790. doi:10.1071/MF99069
- Findlay, S., Meyer, J., and Edwards, R. T. (1984). Measuring bacterial production via rate of incorporation of [<sup>3</sup>H]thymidine into DNA. *Journal of Microbiological Methods* **2**, 57–72. doi:10.1016/0167-7012(84)90031-9
- Findlay, S., Meyer, J. L., and Risley, R. (1986). Benthic bacterial biomass and production in two blackwater rivers. *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1271–1276.
- Findlay, S., Pace, M. L., Lints, D., Cole, J. J., Caraco, N. F., and Peierls, B. (1991). Weak coupling of bacterial and algal production in a heterotrophic ecosystem: the Hudson River estuary. *Limnology and Oceanography* **36**, 268–278.
- Findlay, S., Pace, M. L., Lints, D., and Howe, K. (1992). Bacterial metabolism of organic carbon in the tidal freshwater Hudson estuary. *Marine Ecology Progress Series* **89**, 147–153.
- Findlay, S., Sinsbaugh, R. L., Fischer, D. T., and Franchini, P. (1998). Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1**, 227–239.
- Fischer, H., and Pusch, M. (2001). Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. *Freshwater Biology* **46**, 1335–1348. doi:10.1046/J.1365-2427.2001.00753.X
- Fischer, H., Wanner, S. C., and Pusch, M. (2002). Bacterial abundance and production in river sediments as related to the biochemical composition of particulate organic matter (POM). *Biogeochemistry* **61**, 37–55. doi:10.1023/A:1020298907014
- Grossart, H.-P., and Ploug, H. (2000). Bacterial production and growth efficiencies: direct measurements on riverine aggregates. *Limnology and Oceanography* **45**, 436–445.
- Henebry, M. S., and Gorden, R. W. (1989). Summer bacterial populations in Mississippi River pool 19: implications for secondary production. *Hydrobiologia* **182**, 15–23. doi:10.1007/BF00006364
- Hobbie, J. E., Daley, R. J., and Jasper, S. (1977). Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology* **33**, 1225–1228.
- Howarth, R. W., Schneider, R., and Swaney, D. (1996). Metabolism and organic carbon fluxes in the tidal freshwater Hudson River. *Estuaries* **19**, 848–865.
- Ietswaart, T., Breebaart, L., van Zanten, B., and Bijkerk, R. (1999). Plankton dynamics in the River Rhine during downstream transport as influenced by biotic interactions and hydrological conditions. *Hydrobiologia* **410**, 1–10. doi:10.1023/A:1003801110365
- ISO (1994). 'Water Quality – Measurement of Biochemical Parameters – Spectrophotometric Determination of the Chlorophyll-*a* Concentration.' (International Organisation for Standardization: Geneva.)
- Meyer, J. L. (1994). The microbial loop in flowing waters. *Microbial Ecology* **28**, 195–199. doi:10.1007/BF00166808
- O'Connell, M., Baldwin, D. S., Robertson, A. I., and Rees, G. N. (2000). Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. *Freshwater Biology* **45**, 333–342. doi:10.1046/J.1365-2427.2000.00627.X
- Pace, M. L., and Cole, J. J. (1994). Primary and bacterial production in lakes: are they coupled over depth? *Journal of Plankton Research* **16**, 661–672.
- Pollard, P. C. (2004). Organic carbon mineralisation in a subtropical river. *Developments in Chemical Engineering and Mineralisation Processes* **12**, 505–514.
- Pomeroy, L. (1974). The ocean's food web, a changing paradigm. *Bioscience* **24**, 499–504.
- Servais, P. (1989). Bacterioplankton biomass and production in the River Meuse (Belgium). *Hydrobiologia* **174**, 99–110.
- Servais, P., and Billen, G. (1989). Impact of a nuclear power plant on primary production and bacterial heterotrophic activity in the river Meuse at Tihange (Belgium). *Archiv für Hydrobiologie* **3**, 415–430.
- Servais, P., and Garnier, J. (1993). Contribution of heterotrophic bacterial production to the carbon budget of the River Seine. *Microbial Ecology* **25**, 19–33. doi:10.1007/BF00182127
- Sharma, R., Edwards, R. T., and Beckett, R. (1998). Analysis of bacteria in aquatic environments using sedimentation field-flow fractionation: (I) biomass determination. *Water Research* **32**, 1497–1507. doi:10.1016/S0043-1354(97)00399-0
- Thorp, J. H., and Delong, M. D. (2002). Dominance of autochthonous carbon in food webs of heterotrophic rivers. *Oikos* **96**, 543–550. doi:10.1034/J.1600-0706.2002.960315.X
- Thorp, J. H., Delong, M. D., Greenwood, K. S., and Casper, A. F. (1998). Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* **117**, 551–563. doi:10.1007/S004420050692
- Webster, J. R., and Meyer, J. L. (1997). Stream organic budgets. *Journal of the North American Benthological Society* **16**, 3–4.
- White, P. A., Kalf, J., Rasmussen, J. B., and Gasol, J. M. (1991). The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microbial Ecology* **21**, 99–118.

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