# Potential for biofilms as biological indicators in Australian riverine systems

**By Adrienne Burns and Darren S. Ryder**

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**Summary** Biological indicators have been widely used in Australian riverine systems to assess the effectiveness of past and current management. The short generation time, sessile nature, responsiveness to environmental conditions and the availability of sound, quantitative methodologies make biofilms suitable as a monitoring tool in these systems. This paper describes biofilm structure, function and development through the processes of succession and disturbance. Biofilms are assemblages of algae, fungi and microorganisms which cover rocks, wood and sediments in aquatic systems. A review of biofilm collection and processing techniques using relevant Australian and international studies reveals a large literature on many structural and functional biofilm attributes. Studies using structural attributes such as biomass and diversity to examine water quality impacts and invertebrate grazers dominate the Australian literature. More recently, studies have used functional biofilm attributes such as metabolism and foodweb interactions. Monitoring programs that combine structural and functional biofilm attributes will allow the best assessment of impacts in riverine systems. Biofilm functional parameters provide an integrated, long-term measure of ecosystem function, with structural attributes such as biomass and diversity allowing historical comparisons with previously recorded datasets. Monitoring programs such as these with a well-founded scientific base and defined management outcomes will expand our knowledge of river function and contribute to the restoration of Australian river systems.

**Key words** *algae, bacteria, biodiversity, biofilms, bioindicator, ecological process, review.*

## Introduction

There is a current focus in Australia on the selection of biological indicators to determine the effectiveness of different approaches to river management. A large number of biological indicators have been adopted including: macroinvertebrates (e.g. Growns *et al.* 1995); fish (Harris 1995) and algae (Reid *et al.* 1995). These generally provide information on structural parameters (e.g. species distribution and abundance), but provide little understanding of ecosystem function, an increasingly important focus of river research throughout the world. While structural indicators are relatively easy to quantify and standardize, spatial and temporal variability within a river community may limit their use. Functional measures, on the other hand, can be used to integrate diverse communities into a few attributes, allowing easier comparison among different systems and within systems over time (Pratt & Cairns 1996). The combination of structural and functional information, at population, community and ecosystem levels, can be provided by the measurement of microorganisms (Veal *et al.* 1998) and offers even greater potential for ecologically meaningful analysis.

The most commonly cited microorganisms that respond to environmental change are the algae. There has been less focus, however, on the use of non-photosynthetic microorganisms (e.g. bacteria and fungi) as monitoring tools (Veal *et al.* 1998). Planktonic, attached and macrophytic algae have all been used to provide information on ecosystem condition. Algae have predictable responses to pollutants and are often used as early warning systems (Whitton & Kelly 1995). Algae have been successfully employed in biological surveys and monitoring programs providing both structural and functional information (McCormick & Cairns 1994). In their recent review, Veal *et al.* (1998) advocate the use of autotrophic (photosynthetic organisms: algae) and heterotrophic (consumers) microbial indicators for monitoring river health because these provide an assemblage that requires only small sample sizes to yield high species richness and functional diversity. Similarly, biofilms provide an assemblage with all the benefits of both algal and heterotrophic microbial organisms.

### *What are biofilms?*

Submerged surfaces in lakes and rivers are colonized by assemblages of algae, fungi, bacteria and unicellular animals in a matrix of polysaccharide exudates and detritus (Wetzel 1983). These are the 'biofilms' (or periphyton) which cover rocks, wood, sediment particles and other surfaces in aquatic systems (Fig. 1). They are sensitive to changes in environmental conditions, are abundant and cosmopolitan in their distribution, can be sampled rapidly and have a wide range of attributes which can be measured quantitatively (Steinman & McIntire 1990). Biofilms are species rich, partly due to highly efficient powers of dispersal of the microorganisms, and have wide tolerances to environmental conditions. Biofilm assemblages have short life cycles allowing a rapid response to changing conditions. They

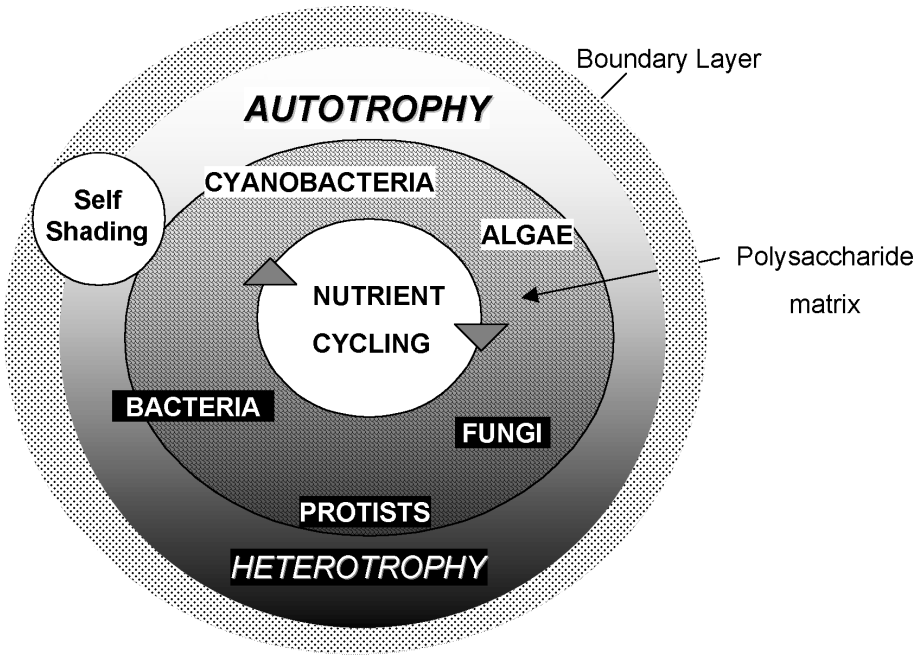


**Figure 1.** Diagrammatic representation of a biofilm and a flexible unit of autotrophs (Cyanobacteria and algae) and heterotrophs (bacteria, fungi and protists) in a polysaccharide matrix. The biofilm can also control its own microenvironment. Examples of this are illustrated as cycles within the main circle: self-shading, and nutrient transfer between heterotrophic and autotrophic cells. The boundary layer of the microenvironment created by the biofilm is depicted by the grey border.

characteristically are the first organisms to respond to and recover from stress (Lowe & Pan 1996). Information about biofilms can therefore be collected, processed and analysed at time scales relevant to both scientific and management interests.

A recent publication by Reid and Brooks (1998) recommending indicators for aquatic studies in the Murray–Darling Basin, included biofilms as secondary indicators; commenting that further studies were required before biofilms could be included as key indicators of ecological change. We show that knowledge of biofilm function has rapidly advanced in the past few years. We discuss the attributes of biofilms that will be useful in monitoring Australian freshwater systems and draw together the large literature for biofilms both from Australia and worldwide. This review discusses the advantages and disadvantages of structural and functional attributes of biofilms that may by used within monitoring programs, examines the general structure and successional patterns of biofilms within an Australian context, and reviews methodologies for sampling biofilms.

## Biofilm structure and function

Where light prevails, biofilms are dominated by photosynthetic organisms (autotrophs), the algae (Lock *et al.* 1984), particularly Chlorophyta (green algae), Bacillariophyta (diatoms) and Cyanobacteria (Peterson 1996). Biofilms in low light environments are predominantly heterotrophic and dominated by bacteria (Fig. 1 e.g. Blenkinsopp & Lock 1994). The depth at which 1% of incident light remains indicates the depth of the photic zone and, by implication, conditions under which biofilms switch from autotrophy to heterotrophy (Lock *et al.* 1984). The balance between autotrophy and heterotrophy within the biofilm however, is not solely controlled by light availability. The dominance of algae over bacteria may be influenced by nutrient availability, and the types and abundance of algae are often determined by physical disturbances (Peterson *et al.* 1985; Peterson 1996).

Biofilms are a major autochthonous (instream) source of carbon, along with aquatic higher plants (macrophytes) and macro algae, but unlike allochthonous (terrestrial) inputs, they are not dependent on over bank flows. Biofilms provide a major energy source for aquatic food webs by contributing organic material to the water through leached exudates, sloughed dead and senescent material, and live cells (Lock *et al.* 1984; Rounick & Winterbourn 1986). Biofilm production may surpass that of catchment inputs in streams, lakes and wetlands (Minshall 1978), and may be important in combination with other instream material in large river systems (Thorp 1994). Although relatively low in biomass compared with aquatic macrophytes, the high turnover rate of biofilms is significant in aquatic productivity (Goldsborough & Robinson 1996). Consequently, biofilms form the base of food webs supporting grazers such as crustaceans, insects, molluscs and some fish (Lock *et al.* 1984; Rounick & Winterbourn 1986; Stevenson 1996). The role of biofilms within aquatic foodwebs varies, however, with their composition, density and productivity, all of which are dependent on the biofilms' successional state.

## Biofilm development: succession and disturbance

### *Biofilm succession*

The composition and productivity of biofilms are the consequence of multiple interactions between hydrological, chemical and biotic factors. Processes that control resources ultimately affect biomass accumulation with disturbances leading to losses (Biggs 1996). Succession of biofilms is driven by differential species performance in dispersal, survival and reproduction; through factors such as resource availability, ecophysiology, life history and disturbance (Pickett & McDonnell 1989). Nutrients, light and available substrata form the basic resources for biofilms. These can be modified by external factors, which ultimately regulate local resource availability (Fig. 2, modified from Stevenson 1996). Physical disturbances, such as flow and changes in water level, act as resource modulators for biofilms through changes to nutrient

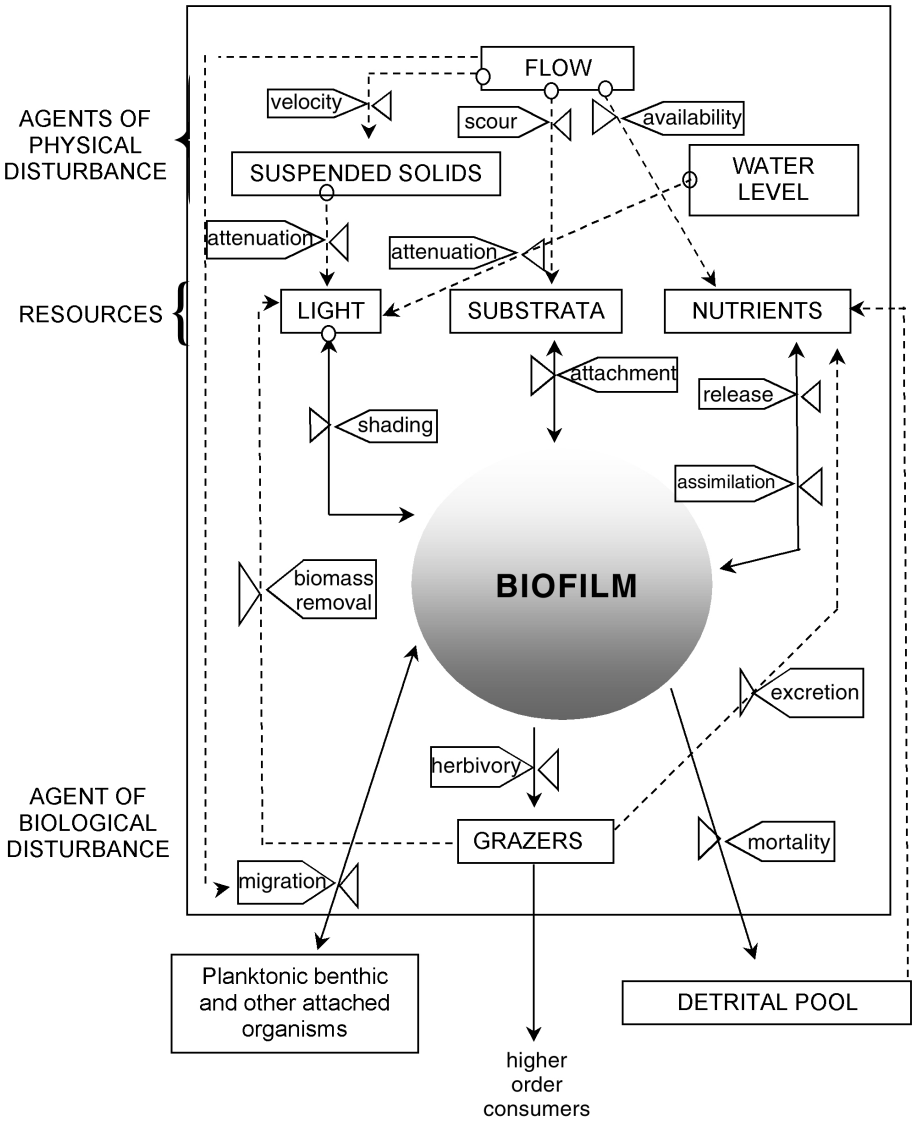


**Figure 2.** Components of community structure and function important in determining biomass, composition and physiology of riverine biofilms. Matter and energy flow are connected by solid arrows that indicate 'processes'. The 'modulators' of community function are indicated by arrows originating from small circles (---). Physical disturbances are derived from the community modulators such as flow (top) and grazers (below). Essential resources for biofilm growth are shown in the second layer of the diagram (resources and modulators derived from Stevenson 1996).

and light availability, and by clearing substrata through scouring and abrasion. Principally, flow regulates accrual of biofilm biomass in river systems. Currents directly affect biofilms through scour and substratum loss (which indirectly break boundary layers that impede nutrient uptake) and by increasing light attenuation through higher loads of suspended material. In the process, attached organisms are relocated and become available for colonization of new substrata (Fig. 2).

Grazing by aquatic invertebrates can have both indirect benefits and direct negative impacts on biofilm biomass and composition. Grazer action through the removal of sediment and senescent cells may increase light intensities within the biofilm (Fig. 2; Pringle *et al.* 1993). Grazing also enhances local nutrient supply through increasing turnover rates (Steinman *et al.* 1995) and leaching of dissolved organic material (McCormick & Stevenson 1991).

### *Substrata*

The nature of available substrata often differs between low-order streams and floodplain rivers. In the former, rocks, gravel and boulders are common and are subject to greater abrasion than the woody debris and macrophytes typical on riverbanks in semi-arid lowland rivers (cf. Uehlinger 1991). Biofilms which colonise rocks and boulders in fast currents are more resistant to abrasion than those growing on sheltered gravel and cobbles (Uehlinger 1991). The complexity of the substrate surface also affects resource availability through shading and shelter from direct current. Whether substrata are organic or inorganic influences pre-conditioning by bacterial enzymes. Biofilm enzyme activity ranged from 2–50-fold greater on wood than on cobble from a similar area, suggesting biofilms on inorganic substrata may be limited by carbon supply (Sinsabaugh *et al.* 1991). Wood is a common organic substratum for biofilm and invertebrate colonization in larger rivers systems both overseas (e.g. Hax & Golladay 1993), and in the Murray–Darling Basin, Australia (Scholz & Boon 1993a,b; Sheldon & Walker 1997; Burns & Walker 2000a). The suite of decomposers colonizing wood may also influence early biofilm succession. For example, microbial biomass accrual may be higher on wood than leaves (Hax & Golladay 1993). Scholz and Boon (1993b) hypothesized woody substrata provide an important site of colonization for biofilms in Australian riverine systems, and ultimately provide a food resource and a site of nutrient transformations.

### *Light*

Light is the principal factor determining whether biofilms will tend toward autotrophy (by algae and Cyanobacteria) or heterotrophy (by fungi and bacteria). The survival of algae under these varying light conditions is facilitated by structural, behavioural, physiological and chemical factors (Richardson *et al.* 1983), but little is known of the precise mechanisms.

Biofilms can be deprived of light if they fall below the photic zone, either through increased water depth, turbidity or being

covered by sediment. This directly affects species composition and productivity. Light deprivation caused by flood events is usually short-lived but prolonged darkness may occur for biofilms attached to overturned cobbles and boulders (Peterson 1996). The maintenance of algal viability under conditions of light deprivation has not been extensively studied but the ability to persist without light is likely to be species specific (Peterson 1996). Species that are capable of tolerating conditions of low light, such as that beneath late successional taxa, are likely to resist the stresses of light deprivation (Peterson 1996). Algae can resume photosynthesis on re-exposure to light, but decomposition of chlorophyll *a* during prolonged darkness limits algal viability (Wasmund 1989).

A shallow photic zone and variable water levels promote heterotrophs (Findlay *et al.* 1986), while biofilms at late succession, dominated by autotrophs, are rare in such variable conditions. In turbid, unregulated rivers, biofilms are comparable to those of blackwater rivers, where light penetration is limited by high levels of dissolved organic matter (Findlay *et al.* 1986). Flow regulation decreases the magnitude of water level fluctuations, stabilizes the photic zone and favours biofilms dominated by autotrophic rather than heterotrophic organisms (Sheldon & Walker 1997).

### *Nutrients*

Organic enrichment affects all forms of biofilms (Steinman & McIntire 1990) by decreasing the richness of algal taxa, and favouring filamentous algal species when there is sufficient light (Rosemund 1993). Biofilms, however, have the potential to recycle nitrogen and carbon, with the polysaccharide matrix serving as the primary carbon reserve during low nutrient conditions (Freeman & Lock 1995). Biofilms, also transform inorganic nutrients into organic forms that are readily available for secondary production (e.g. Lock *et al.* 1984). In billabongs in south-eastern Australia, rates of phosphate mineralization by biofilms were significant although nitrogen assimilation was low (Scholz & Boon 1993b). The presence of Cyanobacteria can enhance biofilm productivity in low nitrogen environments through conversion of atmospheric $N_2$ to ammonia and amino acids (Peterson & Grimm 1992). Biofilms can also form sinks for inorganic nutrients by buffering release into the water column (Wetzel 1996).

### *Disturbance regimes*

Disturbance in river systems resets biofilm succession by removing biomass and clearing substrata for colonization. Natural disturbances, such as grazing and floods, disrupt the structure and function of biofilms in riverine systems. To encompass all temporal scales, a definition of 'disturbance' as 'an unpredictable, discrete or gradual event (natural or man-made) that disrupts structure or function at the ecosystem, community, or population level' (Sparks *et al.* 1990, p. 700) is most appropriate for large river systems. Flood events and grazing commonly govern biofilm biomass in streams, often preventing the late successional development of filamentous algae in situations where light does not limit growth. In regulated rivers, disturbance is manifest as gradual changes in water levels, often resulting in shifts in biofilm composition. Composition of biofilms is, therefore, prone to vary with changes in the local environment. This is readily seen in environments subject to frequent disturbance. In streams subject to spates, for example, pioneering biofilm taxa such as bacteria and unicellular algae are preserved as floods 'reset' the process of succession (e.g. Fisher & Grimm 1988). In more stable hydrologic environments, like deep lakes and lowland rivers, biofilm communities are likely to reach a climax community of filamentous taxa.

### *Flow regulation*

Flow regulation operates through a variety of processes that may result in both disturbances or stresses to biofilms. For example, regulation can cause direct changes to the underwater light climate, availability of nutrients and the abundance of grazers through modification of natural flow and water-level regimes. In regulated systems, where changes occur over a long time, factors which initially act as stressors (e.g. frequent desiccation and changes in light climate) may ultimately change the composition of a community and thus are 'disturbances' (Peterson 1996).

Regulation creates disturbances which effect sustained changes to species density. Ultimately, the system moves to a new state as species adjust. In contrast, systems subject to instantaneous disturbances such as floods, return to their initial state once the disturbance is removed (Yount & Niemi 1990). Depending on the scale of the disturbance, it may increase or decrease heterogeneity within the environment. Pulse disturbances such as floods create space within the environment, consequently changing patterns of resource availability within the disturbed landscape. Habitat heterogeneity results from the varying impacts of disturbance at different sites. However, large or frequent disturbances, such as those caused by regulation, promote homogeneity (Denslow 1985). In the case of such prolonged disturbances, species that are specialists at colonizing bare patches are locally excluded if the disturbance is not frequent enough. Only after extensive desiccation or a large scouring event would biofilms return to early successional taxa. Precise duration and magnitude required for such disturbances have yet to be identified.

A comprehensive study of littoral biofilms in the highly regulated River Murray by Burns (1997), found that disturbances created by flow regulation resulted in homogeneity of biofilm composition. Regulation resulting in a shifting state of light deprivation and atmospheric exposure has led to altered successional patterns for biofilms (Burns & Walker 2000a). Stable water levels and light attenuation at depth, allowed biofilms to persist in late successional states in much of the lower river, with algal biomass peaking in areas of maximum light and sustained inundation.

### *Grazing*

There have been many studies examining biofilm response to second-order consumers and environmental conditions. Interactions have been demonstrated between grazing of biofilms, nutrient supply and irradiance, involving a wide range of aquatic insects (Steinman *et al.* 1991; Winterbourn *et al.* 1992; Rosemund 1993), gastropods (e.g. Bronmark 1992),

fish (e.g. Stewart 1987) and shrimps (Pringle *et al.* 1993), although few studies are in large rivers (cf. Sheldon 1994; Burns 1997).

The most widely studied aspect of biofilms is their relationships and interactions with stream biota. The reciprocal nature of plant–grazer interactions dominates both the Australian and overseas literature (Steinman 1996). Biofilm growth and biomass have been quantified indirectly as part of macroinvertebrate species richness and abundance studies (Boulton & Lake 1992; Hurley *et al.* 1995), but are more commonly studied as direct grazer–consumer interactions. In Australian upland streams, dominant grazers significantly reduce biofilm biomass and influence taxonomic composition similar to overseas studies (Jordan & Lake 1996). Microhabitat architecture was a major factor in determining the magnitude of the impact by *Agapetus* (Trichoptera) on biofilm biomass in Australian temperate streams (Gawne 1995, 1997; Gawne & Lake 1995). Microhabitat architecture also influenced biofilm biomass more than macroinvertebrate grazers in riffle habitats in a Tasmanian river (Robson & Barmuta 1998). Interactions between grazers and biofilms in Australian lowland rivers have shown that prosobranch gastropods (Sheldon & Walker 1997) and the abundant decapod *Paratya australiensis* (Burns and Walker 2000b) are omnivorous feeders, which displayed a preference for some benthic algal groups. The current distribution of prosobranch gastropods in the Murray River, South Australia is in part restricted by the composition of littoral biofilms (Sheldon & Walker 1997). Grazing by *Paratya australiensis* reduced the biomass of Cyanobacteria and diatoms and enhanced green algal growth. The initial age of the biofilm influenced the final organic biomass after grazing, with biofilms at an intermediate successional stage most resilient to grazing (Burns 1997).

## Biofilms as indicators of disturbance

Effective indicators need to be applicable across a wide range of riverine habitats (main channel, tributaries, wetlands, floodplain habitats), have a wide range of quantitative attributes, respond to changes in disturbance regimes at spatial and temporal scales relevant to river management, have a scientific basis and be cost-effective. Biofilms possess all of these attributes: they have short generation times, responding rapidly to changed environmental conditions; are species rich; and are characteristically the first organisms to respond to, and recover from stress (Lowe & Pan 1996). Measures of the current state of biofilms can be obtained rapidly and at low cost through structural characteristics such as biomass or taxonomic and chemical composition. The determination of biofilm function can be done through measuring changes in system state using techniques such as biofilm metabolism, nutrient uptake and extracellular enzyme activity.

In Australia, biofilms have been used as indicators of natural and human-induced disturbances. Natural flow disturbances have been examined in the tropics (Mosisch & Bunn 1997) and regulated flows examined in south-eastern Australia (Sheldon & Walker 1997; Burns and Walker 2000a). Table 1 provides a summary of

**Table 1.** Australian freshwater biofilm studies indicating study location, biofilm substrata, and the processes and parameters quantified

| Author(s) | Location / habitat | | Substrata | Process | Parameters |
|---|---|---|---|---|---|
| Bunn & Boon (1993) | MDB | Billabongs | Macrophyte | Food web | Stable C and N isotopes |
| Bunn *et al.* (1999) | Qld / WA | Various | Inorganic | Biological indicators | GPP, respiration, stable C and N isotopes |
| Burns & Walker (2000a) | MDB | Floodplain / river | Wood | Flow regulation, desiccation, light | Chl *a*, organic weight, algal composition |
| Burns & Walker (2000b) | MDB | Floodplain / river | Wood | Food web | Stable C and N isotopes |
| Chessman *et al.* (1992) | Vic. | Various streams | Nutrient diffusing | Nutrient limitation | Chl *a*, composition |
| Chessman *et al.* (1999) | SE Aust. | Various streams | Various | Species composition | Diatom composition |
| Gawne & Lake (1995) | Vic. | Upland stream | Bricks | Microhabitat structure, grazing | Chl *a*, organic weight, bacterial abundance |
| Gawne (1995) | Vic. | Upland stream | Bricks | Grazer–epilithon interactions | Chl *a*, organic weight |
| Gawne (1997) | Vic. | Upland stream | Bricks | Grazer–epilithon interactions | Chl *a*, organic weight, bacterial abundance |
| Hurley *et al.* (1995) | N Aust. | Tropical reservoir | Glass slides | Grazer distribution | Biomass |
| Jordan & Lake (1996) | SE Aust. | Upland stream | Bricks | Grazer–epilithon interactions | Chl *a*, organic matter, diatom density |
| Mosisch & Bunn (1997) | N Aust. | Rainforest stream | Cobble | Flow disturbance | Chl *a*, organic weight |
| Mosisch *et al.* (1999) | SE Qld | Subtropical stream | Nutrient diffusing | Succession, nutrients, light | Chl *a*, organic weight, composition |
| Robertson *et al.* (1997) | MDB | Wetlands | Wood | Carp disturbance | Chl *a*, biomass accumulation |
| Robson & Barmuta (1998) | Tas. | Mountain river | Clay tiles | Microhabitat structure, grazing | Chl *a* |
| Scholz & Boon (1993a) | MDB | Billabong | Wood | Microbial activity | Extracellular enzyme activity |
| Scholz & Boon (1993b) | MDB | Billabong | Wood | Bacterial succession, light regime | Phospholipid fatty acids |
| Sheldon & Walker (1997) | MDB | Floodplain / river | Wood | Food quality, grazing | C and N, stable C and N isotopes, organic matter |

MDB, Murray–Darling Basin.

contemporary Australian biofilm studies used in this review indicating the study location, substrata type and parameters measured. Early research on Australian biofilms saw them used as indicators of water quality and nutrient enrichment in agricultural, urban and industrial areas (Chessman 1985; Chessman *et al.* 1992). Studies using structural attributes such as biomass and species composition of biofilms to examine the impacts of water quality and invertebrate grazers now dominate the Australian literature. Biofilm growth and biomass have been quantified indirectly as part of macroinvertebrate species richness and abundance studies (Hurley *et al.* 1995), but are more commonly studied as grazer–consumer interactions.

More recently, studies have used biofilm functional attributes such as metabolism and food web interactions to go beyond parameter-based monitoring to use biofilms as indicators of ecosystem function (e.g. Bunn *et al.* 1999). In Australia, biofilms have been identified as an important component in riverine nutrient transformations (Scholz & Boon 1993a), in monitoring sources of pollution (Chessman 1985), and as carbon sources for herbivores (Gawne 1995; Robson & Barmuta 1998). Studies have used stable carbon and nitrogen isotopes to examine biofilms in aquatic food webs (Bunn & Davies 1999) and biofilm metabolism as a measure of ecosystem function (Bunn *et al.* 1999). Much of the work to date has been concentrated on riverine systems in south-eastern Australia and subtropical Queensland, encompassing biofilm growth on a wide range of organic and inorganic substrata. These papers test experimental effects of external nutrient supply, physical disturbance and grazing pressure on biofilm biomass and composition. Remaining studies examine the use of biofilms as a food for primary consumers, mostly in conjunction with other carbon sources.

## Biofilm collection and processing

In a review of methodology for studying biofilms, Aloi (1990) discussed the advantages and disadvantages of many field collection methods. Most studies examine biofilm biomass through scraping or brushing the substratum (Cattaneo & Roberge 1991). Such methods, although efficient in lakes, are inefficient in the removal of tightly attached alga common in streams and rivers. Davis and Gee (1993) have developed a simple field periphyton sampler for lotic systems utilizing a scouring pad. This method is cheap and easy to use, more efficient than brushing and scraping techniques, is small in size and facilitates replication. Scraping methods require the sampled surface area to be quantified. Colonizable rock surface area (CRSA) is a measure of the exposed surface area of the rock, and excludes the buried parts (Boulton *et al.* 1988). Of the many ways of measuring rock surface area, the easiest appears to be the plastic foodwrap technique used by Doeg and Lake (1981). Doeg and Lake (1981) state that aluminium foil is easily torn, ink pad squares underestimate surface area, and that latex moulds and digital image-analysis methods are time-consuming and expensive.

Artificial substrata are commonly used to sample biofilm colonization. Critical reviews such as that by Cattaneo and Amireault (1992) argue the ability of artificial substrata to reproduce natural substrata, concluding they often misrepresent both the quantity and composition of natural biofilms. Artificial substrata should be used with caution especially in inter-site and inter-season comparisons. Despite the limitations there are many benefits to using artificial substrata for measuring biofilm parameters. They can reduce the heterogeneity of the naturally occurring substrata, permit standardization of substrata between sites, and allow colonization when substrata may be limited (Cattaneo and Amireault 1992).

Once collected there is a comprehensive range of biofilm structural and functional attributes that can be quantified. The main structural and functional attributes of biofilms, the biological response time, processing time, costs and the overseas and Australian knowledge base for data comparison are summarized in Table 2. The following review describes in detail the range of quantifiable biofilm attributes and provides results and critiques from international and Australian studies using each method.

**Table 2.** Summary of biofilm structural and functional attributes outlining the biological response, field collection and processing times, sampling costs and the overseas and Australian knowledge base

| Biofilm attribute | Biological response time | Collection time | Processing time | Sampling costs | Overseas knowledge base | Australian knowledge base |
|---|---|---|---|---|---|---|
| Algal biomass | Days | Rapid | Moderate | Low | Good | Good |
| Total biomass | Hours | Rapid | Moderate | Low | Good | Good |
| Composition | | | | | | |
| Richness | Days | Rapid | Moderate | Moderate | Good | Moderate |
| Dominance | Days | Rapid | Long | High | Good | Moderate |
| Nutrients | Days | Rapid | Moderate | Moderate | Moderate | Poor |
| Primary productivity | Days | Slow | In field | Initial high | Good | Moderate |
| Metabolism | | | | | | |
| P:R | Hours | Slow | In field | Initial high | Good | Poor |
| Nutrient kinetics | Hours | Moderate | Moderate | High | Moderate | Poor |
| Enzyme activity | Hours | Moderate | Moderate | High | Moderate | Poor |
| Food web | Weeks | Slow | Long | High | Moderate | Moderate |

### *Structural attributes*

*Biomass* Biomass is measured per unit area of substrata as chlorophyll *a*, carbon (C), nitrogen (N) or phosphorus (P) content and inorganic or organic weight (Ash Free Dry Weight; AFDW). These are rapid, inexpensive methods for estimating biofilm biomass with a large literature base for comparison with other studies (Stevenson 1996). Chlorophyll *a* is most commonly used to estimate algal biomass. Many algae adjust their pigment concentrations relative to their light environment, thus the chlorophyll content may be inversely proportional to light intensity (Kirk & Tilney-Bassett 1969). Caution therefore needs to be exercised when extrapolating biomass results based solely on chlorophyll estimation. Determining biovolume from algal cell counts is a more accurate technique, but has the disadvantage of being time-consuming. The use of multiple methods for biomass estimation best facilitates comparison with other studies (Stevenson 1996).

In a review of aquatic biofilms, Goldsborough and Robinson (1996) suggested that chlorophyll loads in freshwater systems rarely exceeded 10 mg/m$^2$. In contrast, many measurements in larger river systems exceed 50 mg/m$^2$ and may exceed 900 mg/m$^2$ (Uehlinger 1991). In Australian floodplain wetlands, biofilm chlorophyll measurements are relatively low (0.2–42 mg/m$^2$) compared to adjacent river systems (<10–248 mg/m$^2$) (Scholz & Boon 1993a; Burns 1997; Robertson *et al.* 1997). This may result from different constraints on algal growth in wetlands and riverine systems. In wetlands, growth is dependent on the permanence of water in the wetland, macrophyte cover and nutrient loadings (Goldsborough & Robinson 1996). In rivers, the primary factor constraining algal biomass is discharge (e.g. Biggs & Close 1989; Uehlinger 1991). In Australian tropical streams, high discharge reduced both algal and overall biomass, however, these parameters recovered to pre-disturbance levels within 10–30 days of disturbance. Natural losses occurred after 60 days without a spate due to siltation and grazing (Mosisch & Bunn 1997). In lowland rivers, algal biomass peaked in areas of maximum light and sustained inundation (Burns & Walker in 2000a; D. S. Ryder unpubl. data).

*Taxonomic composition* Information on the taxonomic composition of biofilms can be summarized using measures of species richness (total numbers of species), diversity, or relative abundance. Recent Australian taxonomic publications on freshwater diatoms (Gell *et al.* 1999), Cyanobacteria (Baker & Fabbro 1999) and algae in general (Entwistle *et al.* 1997) assist in the identification of biofilm taxa. Other useful resources for taxonomic composition of Australian algae include Entwistle (1994) and Day *et al.* (1995). Taxonomic composition of attached diatom communities has recently been used by Chessman *et al.* (1999) to develop a predictive model for the rapid biological assessment of southeastern Australian river systems. Less accurate but more rapid techniques can be employed to infer taxonomic composition. These include autotrophic indices (e.g. chlorophyll α: AFDW), and pigment ratios to elucidate proportions of green algae, diatoms, and Cyanobacteria. Phospholipid fatty-acid profiles have also been used to quantify biofilm bacterial biomass and assemblage composition (Scholz & Boon 1993a).

*Chemical composition* Chemical composition of biofilms can be used as an indicator of nutrient uptake efficiency and food quality. Commonly, elemental ratios of nutrients, such as carbon and nitrogen (C : N) and nitrogen and phosphorus (N : P) are calculated (see food-web analysis below) (e.g. Biggs & Close 1989; Peterson & Stevenson 1992; Biggs 1995). Another commonly cited parameter is the phaeophytin : chlorophyll *a* ratio which can be used as a measure of algal senescence (Peterson & Stevenson 1992) within the biofilm.

Algal biofilms in Australian temperate (Chessman *et al.* 1992) and subtropical (Mosisch *et al.* 1999) streams were nitrogen limited. Nitrogen concentrations sufficient to increase algal growth in subtropical areas were above 0.055 mg/L, similar to thresholds in North American streams (Grimm & Fisher 1986). Total organic biomass of biofilms was less sensitive to changes in external nutrient supply than algal biomass in Australian systems compared to those overseas. Australian studies, however, are lacking in comprehensive seasonal coverage. This limits their potential in continental comparisons of the effects of nutrient supply on biofilm dynamics (Mosisch *et al.* 1999).

### *Functional attributes*

Recent studies have focused on biofilm function in aquatic systems by quantifying productivity, nutrient assimilation and their role in food webs (e.g. Guasch *et al.* 1995; Rier & King 1996). The measurement of the functional attributes of biofilms can be expensive, with high initial costs associated with the construction of chambers and food-web analysis requiring access to specialist equipment. The measurement of functional biofilm attributes, however provides an insight into ecosystem processes fundamental to river health that are not available through quantifying structural attributes.

*Production and respiration* Measures of metabolism can distinguish between autotrophic and heterotrophic dominance within biofilms. Gross productivity and respiration can be measured using changes in pH, dissolved oxygen or $CO_2$ concentration over time within *in situ* chambers on an area- or biomass-specific basis. Alternatively, uptake of $^{14}$C-labelled substrates within chambers can quantify algal and bacterial productivity (Neely & Wetzel 1995). These methods often rely on the use of colonized artificial substrata within chambers. Limitations caused by altered environmental conditions within chambers are evident in many studies (Bott *et al.* 1997). Recirculating chambers can alleviate some of these problems in lotic systems (e.g. Dodds & Brock 1998). Recent studies in Australian lowland rivers have developed fully automated, recirculating chambers, capable of venting chamber contents at regular intervals and floating at set depths to avoid problems associated with changing water levels (D. S. Ryder unpubl. data).

In Australian tropical and subtropical forest streams, biofilm productivity exceeds respiration (Bunn *et al.* 1999).

Similar patterns have been measured in Northern Hemisphere tundra ponds (Stanley & Daley 1976) and agricultural streams (Rier & King 1996). This seasonal shift in biofilm productivity was also noted in Mediterranean cobble streams (Guasch *et al.* 1995) and sediments in woodland streams (King & Cummins 1989). Heterotrophic metabolism dominates on woody substrata in upland (Tank *et al.* 1993) and blackwater streams (Fuss & Smock 1996), where overall metabolism is strongly heterotrophic (Fuss & Smock 1996). Sediment biofilms in Australian floodplain billabongs were also strongly heterotrophic (Robertson *et al.* 1997).

*Nutrient kinetics* Nutrient uptake or release from biofilms in chambers using radioactive labelled phosphate and measuring the accumulation of $^{32}P$ can also be used as a measure of biofilm function (Riber & Wetzel 1987). A simpler but more rapid measure of nutrient uptake is quantifying the loss of overlying water-column nutrients (Kim *et al.* 1990) or assessment of spiralling distance (Newbold *et al.* 1981). The Australian knowledge base dealing with biofilm nutrient kinetics is poor.

*Extracellular enzyme activity* Heterotrophic microorganisms form a key level in aquatic ecosystems, being supported by dissolved organic carbon (DOC). Bacteria shift their composition of extracellular enzymes in response to changes in DOC composition. These shifts can be attributed to changes in the availability of different sources of DOC such as from aquatic plants polysaccharides or proteinaceous compounds from different sources (Chròst 1991). Biofilm enzymatic concentrations can provide an indicator of physiological activity in response to nutrient availability. Measures such as phosphatase activity have been used to assess phosphate limitation (Biggs & Close 1989; Scholz & Boon 1993b). Sinsabaugh *et al.* (1991) examined the activity of selected biofilm bacterial extracellular enzymes as a measure of metabolism and buffering capacity to fluctuations in carbon supply in lotic systems.

*Food web analysis* Traditionally, food-web analysis has been achieved through gut contents analysis, laboratory or field-feeding observations or using radioisotope tracing (Rounick & Winterbourn 1986). Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ provide an alternative approach to elucidate carbon pathways and processes (Peterson & Fry 1987). The technique utilizes differences in the natural abundance of the stable carbon ($^{12}C$ and $^{13}C$) and nitrogen ($^{14}N$ and $^{15}N$) isotopes as tracers which move with little or predictable alteration through food chains (Peterson & Howarth 1987). Numerous food-web studies have demonstrated that the isotopic composition of animal tissue reflects that of the diet with only slight modification (e.g. Kwak & Zedler 1997). Stable isotopes possess the advantage over more traditional techniques of reflecting only the material actually assimilated and incorporated into tissues (Peterson & Fry 1987). Food-web analyses through the use of carbon and nitrogen stable isotopes offer opportunities to improve our understanding of freshwater food-web structure and to examine the trophic significance of carbon sources such as biofilms. This technique, however, is expensive and analysis by specialist equipment is required.

The assimilation of biofilms by invertebrate consumers has been successfully examined using stable-isotope analysis in Australian subtropical streams (Bunn *et al.* 1997), lowland rivers (Sheldon 1994; Burns & Walker 2000b) and floodplain billabongs (Bunn & Boon 1993). Gastropods and leptocerid caddisfly larvae in small floodplain billabongs obtained a mixture of carbon sources from epiphytes and their host macrophytes (Bunn & Boon 1993). Common grazers, however, including atyid shrimps were unlikely to derive all their carbon from biofilms in these habitats (Bunn & Boon 1993). Isotopic signatures of grazers were often inconsistent with signatures of dominant algal sources, indicating they were not reliant solely on biofilms as a food source. Changes in biofilm composition with river regulation have been speculated by assessment of dietary requirements of common grazers through the use of stable isotope studies. Sheldon and Walker (1997) suggested that long-term stability in water levels and photic zones associated with regulation have promoted Cyanobacterial and filamentous green algal growth, with a corresponding decrease in the heterotrophic components of biofilms. If this is correct, one consequence may have been to favour crustacean grazers over gastropods.

The composition of biofilms determines their food quality and consequently the food web they support. A widely used technique has been to infer food quality of different carbon sources from the carbon to nitrogen ratio (C : N), with a lower ratio indicating a more easily digestible food source (Steinman 1996). This technique has been used in freshwater wetlands (Royer & Minshall 1997) and lowland rivers (Sheldon and Walker 1997) to demonstrate a preference by invertebrate grazers and detritivores for food with lower C : N ratios. Steinman (1996) recognized algae as a dominant source of carbon in freshwaters and summarized the chemical composition of different algal groups, establishing C : N ranges for major algal orders.

## Conclusion

It is evident from this review that biofilms possess many attributes which make them useful ecological indicators in freshwater systems. Their short generation time, sessile nature, responsiveness to environmental condition and the availability of sound, quantitative methodologies for both structural and functional parameters make biofilms ideally suited as indicators of disturbance in riverine systems. The review of international and Australian studies demonstrates there is a large literature base for biofilms worldwide. The focus of Australian studies has been on specific scientific questions, such as nutrient limitations and biofilm–grazer interaction rather than on monitoring system changes. These data provide an excellent grounding for the use of biofilms in monitoring studies as many relationships between biofilms and environmental conditions have been established.

Structural biofilm attributes can be sampled and processed quickly at low cost. Sampling can be undertaken using sound, standardized and repeatable methodologies and the international

and local literature base is very good. Biofilms can be used to monitor short- and long-term changes in environmental conditions such as nutrient enrichment, pollution events and altered flow regimes at all spatial and temporal scales. The use of structural biofilm attributes is limited in current studies as the focus has primarily been on the algal component. Assessing environmental change through changes in biodiversity and system processes, therefore, requires measuring qualities of the total autotrophic–heterotrophic assemblage.

Functional attributes such as productivity, respiration and food-web analysis from biofilms are ideal measures of system integrity because they provide an integrated response to a broad range of disturbances. This allows the examination of long-term and cumulative impacts on aquatic communities from the base of the food chain. The main disadvantage in measuring functional biofilm attributes is the cost. The processing time is comparable to structural attributes but the literature base is relatively poor. The measurement of functional biofilm attributes, however, provides an insight into ecosystem processes fundamental to river health that is not available through structural attributes.

In designing a monitoring programme, the combination of both structural and functional biofilm attributes will allow the best assessment of impacts in riverine systems. Biofilm functional parameters provide an integrated, long-term measure of ecosystem function, with structural attributes such as biomass and diversity allowing historical comparisons from an excellent literature base. Monitoring programmes such as these, with a well-founded scientific base and defined management outcomes, will expand our knowledge of river function and contribute to the effective restoration of Australian riverine systems.

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